Structure Activity Relationships of novel and selective β_1-adrenoceptor ligands

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ABSTRACT

Of the numerous β-blockers clinically available to treat conditions such as *angina pectoris*, hypertension and heart failure, none possess antagonist activity specific to the β_1-adrenoceptor. Those described as 'cardioselective', such as nebivolol and bisoprolol, generally show less than 50-fold β_1/β_2-selectivity, which can be lost at higher doses. Others, such as propranolol and sotalol are actually more β_2-selective. Overall, a degree of concomitant β_2-adrenoceptor blockade (risking compromised respiratory function and loss of peripheral vasodilatation) by current therapeutic agents precludes their use in patients with disorders such as asthma and peripheral vascular disease.

This project aims to develop novel molecules with much improved β_1/β_2-selectivity over current β-blocker therapy as well as improving knowledge of ligand-receptor interaction at the β_1-adrenoceptor, through an analogue-based drug discovery approach. A highly selective or specific β_1-adrenoceptor antagonist is likely to cause fewer side-effects and be suitable for use in previously contraindicated disease states.

This thesis reports the design, synthesis and pharmacological data (provided by Dr. Jillian Baker) of a library of novel ligands for the β_1-adrenoceptor, based upon the lead compound LK 204-545. LK 204-545 was selected based on reported high potency at the β_1-adrenoceptor as well as good β_1/β_2-selectivity.

Modification of various motifs on structures derived from LK 204-545 allowed the generation of new structure-activity relationships and ultimately afforded the highly β_1-adrenoceptor selective compound, 1-(2-(3-(4-(2-(cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropyl amino)ethyl)-3-(4-hydroxyphenyl)urea hydroformate **(125c)**. This compound acted as a highly-selective β_1-adrenoceptor antagonist in a pilot *in-vivo* study in the regional hemodynamic rat model (carried out by Prof. Sheila Gardiner).

ACKNOWLEDGEMENTS

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The medicinal chemistry work carried out in this project would have been of limited value without a robust set of pharmacological assays to subject them to. With this in mind I would particularly like to thank Dr. Jillian Baker for carrying out all the *in-vitro* cell-based assays and related data processing, and Prof. Sheila Gardiner for carrying out the *in-vivo* rat assays. Additionally both Jill and Sheila made time in their busy schedules to enlighten me in the practical and theoretical aspects of the respective assays.

I also thank members of the medicinal chemistry group who have offered their knowledge, experience, encouragement and most importantly; their time. A special mention goes to Dr. Richard Middleton, Dr. Luke Adams, Dr. Christophe Rochais and Dr. Cedric Lion for invaluable assistance in general chemistry; and Dr. Mike Hargreaves, Ross Fitzgerald, Claire Dale and Sarah Spencer for enduring my woes and offering timely inspiration!

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Last, but by no means least, the backing and encouragement provided by my parents and brother, have been a most welcome comfort in times of need, and I would not be where I am today, without them.

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ABBREVIATIONS

1°	primary
4°	quaternary
Ar	aromatic ring
ATP	adenosine triphosphate
β1AR	β_1-adrenoceptor
β2AR	β_2-adrenoceptor
β3AR	β_3-adrenoceptor
βARK	β-adrenergic receptor kinase
BBSRC	Biotechnology and Biological Sciences Research Council
Boc	*tert*-butyloxycarbonyl
Boc_2O	di-*tert*-butyloxy dicarboxylate
Bn	benzyl
br	broad
calcd	calculated
cAMP	cyclic adenosine monophosphate
CAN	ceric ammonium nitrate
Cbz	carboxybenzyl
CGP 12177	4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-one
CNGs	cyclic nucleotide-gated ion channels
CoMFA	comparative molecular field analysis
conc	concentrated
COSY	correlation spectroscopy
CRE	cAMP response element
CREB	CRE binding protein
CYP	cyanopindolol
d	doublet
DCC	*N,N'*-dicyclohexylcarbodiimide
DCI	dichloroisoprenaline
DCM	dichloromethane

dd	doublet of doublets
DBAD	di-*tert*-butyl azodicarboxylate
DEAD	diethyl azodicarboxylate
def	deformation
DEPT	distortionless enhanced polarisation transfer
DIAD	diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	*N,N* - Dimethylformamide
DMSO	dimethyl sulphoxide
DMSO-d_6	deuterated dimethyl sulphoxide
DPPA	diphenylphosphoryl azide
dt	doublet of triplets
Epac	exchange protein activated directly by cAMP
eq	molar equivalents
ES	electrospray
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
FA	formic acid
FCC	flash column chromatography
FT-IR	fourier transform - Infrared
G-protein	guanine nucleotide binding protein
GABA	γ-amino butyric acid
GAPs	GTPase activating proteins
GPCR	G-protein coupled receptor
GDP	guanine diphosphate
GEFs	guanine nucleotide exchange factor
GTP	guanine triphosphate
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum correlation
ICI	Imperial Chemical Industries PLC

ISA	intrinsic sympathomimetic activity
J	coupling constant
J_{CF}	carbon-fluorine coupling constant
lit	literature
LK 204-545	1-(2-(3-(2-cyano-4-(2-(cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea
m	multiplet
m	*meta*
MAPK	mitogen-activated protein kinase
MeCN	acetonitrile
MeOH	methanol
Mp	melting point
MS	mass spectrometry
MSA	membrane stabilising activity
MW	microwave
m/z	observed ion
NMR	nuclear magnetic resonance spectroscopy
o	*ortho*
p	*para*
PDE	phosphodiesterase
PE	petroleum ether 40-60
Phth	phthalimido
Piv	pivaloyl
PKA	protein kinase A
PLC	preparative layer chromatography
PMA	phosphomolybdic acid
PMB	*para*-methoxybenzyl
ppm	parts per million
PPTS	pyridinium *para*-toluenesulphonate
^{c}Pe	cyclopentyl
^{c}Pr	cyclopropyl
QSAR	quantitative structure activity relationships
q	quadruplet

rac	racemic
R_t	retention time
s	singlet
SAR	structure-activity relationships
str	stretch
SPAP	secreted placental alkaline phosphatase
t	triplet
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TM	transmembrane
TMS	tetramethylsilane
TOF	time of flight

All amino acids are referred to by standard IUPAC nomenclature, using three letter or single letter codes.[1]

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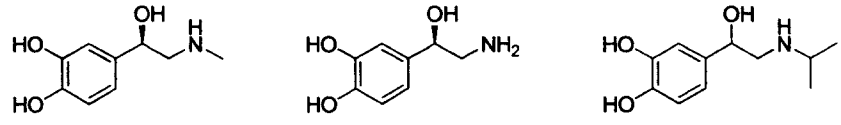
1. INTRODUCTION

1.1 β-adrenoceptors and the sympathetic nervous system

The sympathetic, parasympathetic and enteric nervous systems collectively form the autonomic nervous system, which in general regulates smooth muscle activity, certain hormonal secretions, metabolic activities and the heart rate and force.[2]

The well-known symptoms of the 'fight or flight' response are controlled by the sympathetic nervous system, through a series of receptors differentially distributed through tissues. It is the varying response of these 'adrenoceptors', to the hormone adrenaline **(1)** and neurotransmitter noradrenaline **(2)**, that results in a range of physiological effects.



1 **2** **3**

Figure 1-1: Classical catecholamines

Endogenous sympathetic agonists: (*R*)-adrenaline **(1)** and (*R*)-noradrenaline **(2)** and synthetic β-adrenoceptor selective agonist isoprenaline **(3)**. Biosynthesis of **1** and **2** from the metabolism of tyrosine results in formation of the active (*R*)- enantiomer.

The two adrenoceptor types: α and β, can be further sub-divided. In general, activation of α_1-adrenoceptors causes vasoconstriction, relaxation of the smooth muscle in the gut, and glycogen breakdown (glycogenolysis) in the liver; whereas α_2-adrenoceptors can pre-synaptically inhibit release of neurotransmitters, affect platelet aggregation and inhibit the release of insulin.[2]

The β-adrenoceptors can also be further classified into β_1, β_2 and β_3 receptors.[2, 3] The major concentration of the β_1-adrenoceptor (β1AR) is in the heart, and activation causes an increase in the heart rate (chronotropy), and force (inotropy) of contraction. In the kidneys, β1AR

activation leads to the release of renin and subsequent increases in blood pressure through activation of the renin-angiotensin-aldosterone system.

β_2-adrenoceptors (β2ARs) are also present in the heart (in a lower number), but are more localised to the airways and peripheral vasculature. Activation causes opening of the airways and vasodilatation. In the liver, β_2-adrenoceptor activation results in glycogenolysis, whereas in skeletal muscle, activation increases the speed of contraction.[2]

β_3-adrenoceptors (β3ARs) are predominantly found in adipose tissue and are involved in thermogenesis and lipolysis.[2]

Although the endogenous catecholamines **1** and **2** activate all adrenoceptors, the synthetic catecholamine isoprenaline **(3)** displays a much more selective action. It is a selective and more potent agonist of the β-adrenoceptor sub-type than **1** and **2**.[2, 4-6]

1.2 β-adrenoceptor structure

Adrenoceptors are rhodopsin-like/family A GPCRs (G-protein coupled receptors), belonging to the superfamily of 'serpentine' or 'seven transmembrane' receptors, that share a common general structure.[7]

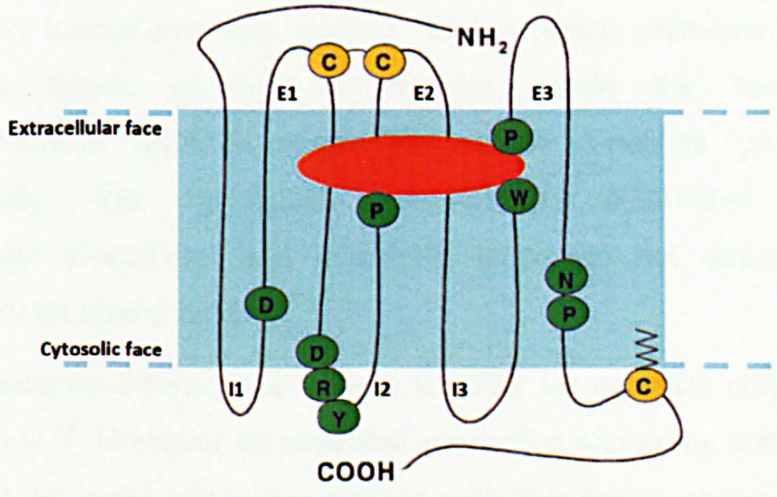


Figure 1-2: Schematic of rhodopsin-like/family A GPCR target for small molecules

Source: Adapted, originally from Jacoby *et al*, 2006[7]

E1/E2/E3: extracellular loops; I1/I2/I3: intracellular loops; BLUE: cell membrane; ORANGE: ligand binding site.

The receptor structure consists of a single polypeptide chain,[3] and is situated in the cell membrane with the *N*-terminus exposed to the extracellular environment and the *C*-terminus in the cytosol (**Figure 1-2**). The seven hydrophobic transmembrane (TM) α-helix[3, 7-9] regions are joined by intracellular and extracellular loops, and generally possess a disulfide bridge between cysteine residues on **E1** and **E2**.[7, 9] The transmembrane regions are not linearly positioned, and group to form the ligand binding domain, which is situated in this transmembrane bundle. A pseudo fourth intracellular loop is formed by anchoring of a palmitoylated cysteine residue to the cell membrane.[3, 7-9] In the β2AR, this appears important for activation of adenylyl cyclase downstream in the signalling cascade (see **section 1.2.3**).[3]

Sequencing and comparison of the three β-adrenoceptor sequences reveals a number of similarities.[3] The β1AR is a 477 amino-acid sequence, whereas the β2AR and β3ARs are 413 and 408 residues in length respectively.[3, 10] The β1AR and β2AR share 48.9% sequence homology, in comparison, the β3AR has 50.7% homology with the β1AR and 45.5% homology with the β2AR.[11]

Most of the information regarding residues in the sequence important for ligand binding, has arisen from studies involving site directed mutagenesis of the human β2AR.[11-13] Studies involving deletion of postulated transmembrane regions, and receptor chimeras, indicate that the ligand binding domain lies within the hydrophobic transmembrane regions where the seven α-helices group.[13, 14] Additionally, the hydrophilic extracellular *N*-terminal domain, intracellular *C*-terminal and inter-helix loops do not appear to be necessary for ligand binding.[11, 15]

Ligand-receptor interactions appear to differ for agonists compared to antagonists.[15] However an essential interaction shared by both types of ligand, is an ionic salt-bridge formed with the amino group of the β-amino alcohol motif common to all ligands. This key interaction is thought to take place with the acidic Asp^{113} on TM3 of the β2AR (see **Figure 1-3**).[3, 14, 16]

The catecholamine motif present on **1**, **2** and **3** appears to be responsible for agonist activity of these molecules. These phenolic groups are thought to interact via hydrogen-bonding with Ser^{204} and Ser^{207} on TM5 of the β2AR.[3, 14, 17] Later studies found Ser^{203} to be of importance also.[18]

Further interaction with the aromatic ring of catecholamine agonists is thought to take place with Phe^{289} and Phe^{290} on TM6.[19] It is postulated that interaction with Phe^{290} by the aromatic group on the ligand, may cause rotational movement of Phe^{290} and nearby residues, which may contribute to relative movement of TM3 and TM6, thus breaking ionic interactions stabilising the inactive state of the receptor.[20] This cascade of events may be important in GPCR activation and is described as a 'rotamer toggle switch'.[20]

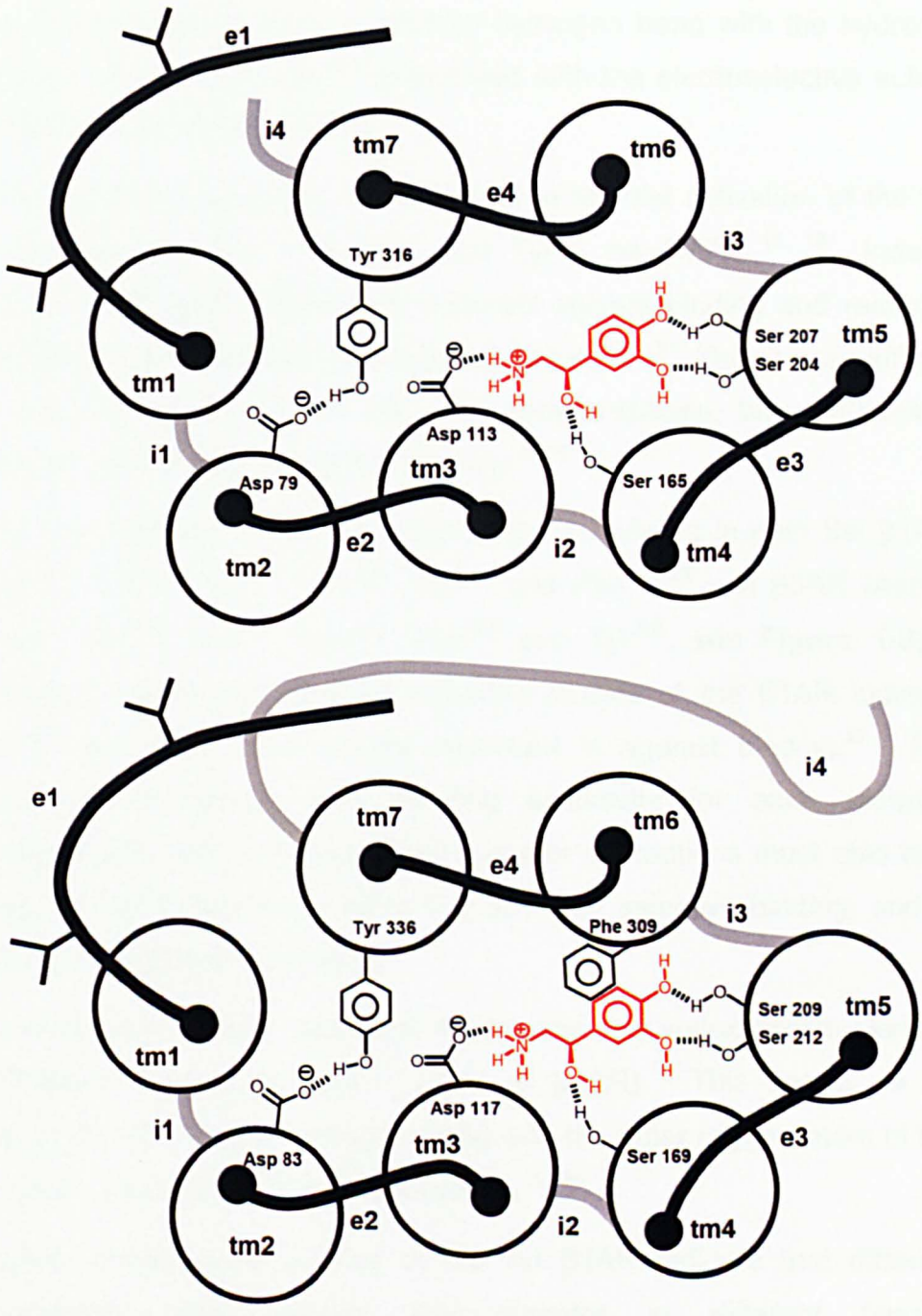


Figure 1-3: Interactions of the agonist noradrenaline with the β2AR (top) and β3AR (bottom)

Adapted from: β2AR - Strosberg 1993[3], β3AR – Strosberg 1997[10]

e1: extracellular *N*-terminal domain; e2-4: extracellular loops; i1-3: intracellular loops; i4: intracellular *C*-terminal domain; tm1-7: transmembrane regions. Although both agonists and antagonists share the essential interaction with Asp on tm3 at each receptor, antagonists rely on a variety of other interactions that are described below.

Computer-based modelling of the β2AR (see below) indicates that Ser^{165} may form an important interaction with the β-hydroxyl group of ethanolamine agonists, however mutagenesis studies show that Asn^{293}

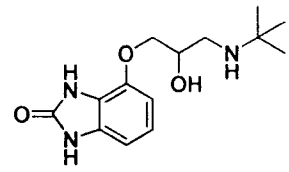
is a key residue, forming a potential hydrogen bond with the hydroxyl group.[21] Additionally Asn^{293} is involved with the stereoselective action of ligands (see **section 1.5.3**).[21]

Other residues thought to be important in agonist activation of the G-protein include; Asp^{79} on TM2, and Tyr^{316} on TM7.[3, 15, 16] Indeed mutations of Asp^{79} differentially reduced agonist binding and receptor activation, without affecting antagonist binding.[15, 16] The diverse effects of deletions on Asp^{79} and Asp^{113} suggest separate, but overlapping sites for agonist and antagonist binding.[15, 16]

The key residues found in the β2AR are conserved in both the β1AR (Asp^{104}, Asp^{138}, Ser^{228}, Ser^{229}, Ser^{232} and Phe^{341})[22] and β3AR (Asp^{83}, Asp^{117}, Ser^{208}, Ser^{209}, Ser^{212}, Phe^{309} and Tyr^{336}, see **Figure 1-3**)[10]. However, recent site directed mutation studies of the β1AR indicate Asp^{104} and Phe^{341} are not so important in agonist binding.[22] The existence of ligands with differing selectivity for each receptor demonstrates that, differing ligand-receptor interactions must also take place at each receptor to allow this sub-type selective binding, and in the case of agonists, activation.

An additional residue important for binding the aryloxypropanolamine antagonist ligands, is Asn^{312} (TM7 of β2AR). This seems to be important in forming a hydrogen bond with the ether oxygen atom of the general aryloxypropanolamine structure.[23, 24]

Further mutagenesis studies of the rat β1AR indicate that different antagonists may occupy the receptor in different binding conformations.[25, 26] Differences in binding conformations, may induce different conformational states at the level of the receptor, with the potential to activate diverse signalling cascades. Some evidence now exists to suggest that the β1AR and β3AR may support at least two binding sites or conformations.[27, 28]



4

Figure 1-4: 4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-one (CGP 12177)

CGP 12177 displays antagonist activity at lower concentrations, but agonist activity at higher concentrations at the β1AR.

An example of this is CGP 12177 **(4)** which binds to the β1AR at low concentrations as an antagonist.[27] At higher concentrations, **4** exhibits agonist activity, furthermore blockade of this activity is relatively difficult with classical β-blockers.[27, 29, 30]

1.2.1 Adrenoceptor modelling and crystallography

1.2.1.1 X-ray crystallography

The ability to co-crystallise protein targets with corresponding agonist or antagonist ligands, allows x-ray diffraction experiments to be carried out, in order to identify the ligand binding site. The coordinates obtained can be translated into a three-dimensional image of the tertiary structure of the protein, revealing the site of ligand interaction, and allowing visualisation of likely binding sites.

Adrenoceptors, belonging to the GPCR family, possess attributes that are undesirable in relation to the classical techniques employed in crystallising proteins.

The transmembrane serpentine structure (see above) requires the cell membrane for support, and is generally unstable in detergent solutions[31]. Consequently, not only is isolation and crystallisation of the pure protein difficult[32], the purified product may not share the same folded structure as would be found on the membrane.

Until recently, (see below) the only GPCR to be successfully crystallised, was bovine rhodopsin, with no ligand bound, i.e. in the inactive state.[33]

1.2.1.2 Computational studies

In the absence of a crystal structure of the desired receptor, computational techniques such as homology modelling and quantitative structure-activity relationships (QSAR) are available.

Homology modelling for GPCRs has traditionally been based upon the crystal structure of bovine rhodopsin in its inactive state.[34] The technique involves initial alignment of the amino acid sequence for the desired receptor, with that of bovine rhodopsin, based upon highly conserved residues amongst family A receptors.[34] Once aligned, the folding pattern of bovine rhodopsin can be used to predict that of the desired receptor.[34]

Although all family A GPCRs resemble rhodopsin in their general structure, the actual sequences are often vastly different. The β1AR, β2AR and β3AR share only 16%, 15% and 18% sequence homology with bovine rhodopsin respectively (comparison of UniProtKB sequences for each human receptor with sequence for bovine rhodopsin using CLUSTALW2).

Homology models generally include only the transmembrane areas of the receptor, with the assumption (see above) that this is where ligand binding occurs.[34] In addition, use of the inactive state of rhodopsin limits modelling of activated receptor conformations.[34] Overall, this means the modelling of β-adrenoceptors based on bovine rhodopsin, is unlikely to provide an accurate prediction of detailed ligand-receptor interaction.

Problems with receptor-based approaches, such as homology modelling, means ligand-based computational approaches can potentially offer valuable information about the nature of the target. Three dimensional QSAR methods, such as comparative molecular field analysis (CoMFA) require a training set of molecules that display a desired attribute (e.g. binding to or activation of a receptor), but have varying structures.[35] CoMFA assumes a common binding site or orientation for all molecules in the training set, based upon a bioactive

conformation.[35] The bioactive conformation can be derived from a co-crystal structure, or in the case of GPCRs, a ligand docked into a homology model. Based on this, a three-dimensional map of each molecule considering charge and steric distribution is generated, and measurements of theoretical interactions with various 'atomic probes' are taken across the molecule.[35] The results of these interactions are correlated with pharmacological data (e.g. binding affinity). Once analysed, contour maps can be generated showing areas around molecules that favour/disfavour electronegative and electropositive substituents, as well as steric bulk.[35]

1.2.1.3 Recent developments

Towards the end of 2007, Rasmussen *et al*[31] and then Cherezov *et al*[36] published crystal structures for the human β2AR bound to carazolol (an inverse agonist) – the first successful GPCR crystal structures since that of bovine rhodopsin in 2000[33].

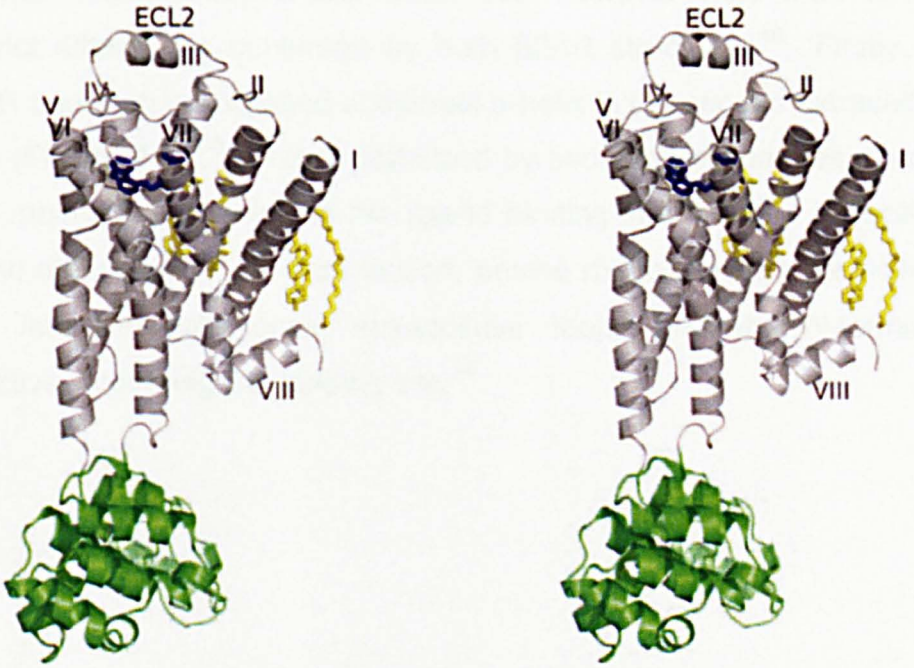


Figure 1-5: Stereo view of the folded human β2AR-T4 lysozyme complex with carazolol bound[36]

Source: Cherezov *et al*, 2007[36]

GREY: β2AR; GREEN: T4 lysozyme; BLUE: carazolol; YELLOW: lipid molecules bound to the receptor; ECL2: extracellular loop 2. Transmembrane helices are numbered I-VII, an additional α-helix VIII runs parallel to the cell membrane.

Although modifications to the receptor were required to produce stable receptors and allow crystallisation, these modifications were shown not to affect ligand binding.[31, 36, 37]

The initial structure was stabilised with an antibody fragment bound to the third intracellular loop of the receptor.[31] This structure gave only 3.4Å/3.7Å resolution, and much of the extracellular face was not visible.[31] A much higher 2.4Å resolution structure was obtained by replacing intracellular loop 3 (deemed to be highly flexible and conferring conformational instability on the receptor), with T4 lysozyme (a soluble protein that improves crystallisation properties) as shown in **Figure 1-5**.[36, 38] Additionally, both modified receptors had residues removed from the intracellular *C*-terminus.[31, 36]

Both structures were found to be very similar to each other, with the T4 lysozyme variant offering more detail on the extracellular face of the receptor also.[39] In relation to the original bovine rhodopsin model, the general folding structure was conserved, however there were several distinct differences confirmed by both β2AR structures.[40] Firstly, the β2AR bears an unexpected additional α-helix in the second extracellular loop (**Figure 1-5**).[40] This is stabilised by two disulfide bridges, leading to a maintained opening to the ligand binding site, thought to facilitate ligand diffusion.[37, 40] In comparison, bovine rhodopsin bears a β-sheet, that interacts with other extracellular loops and the *N*-terminus, effectively covering the binding site.[40]

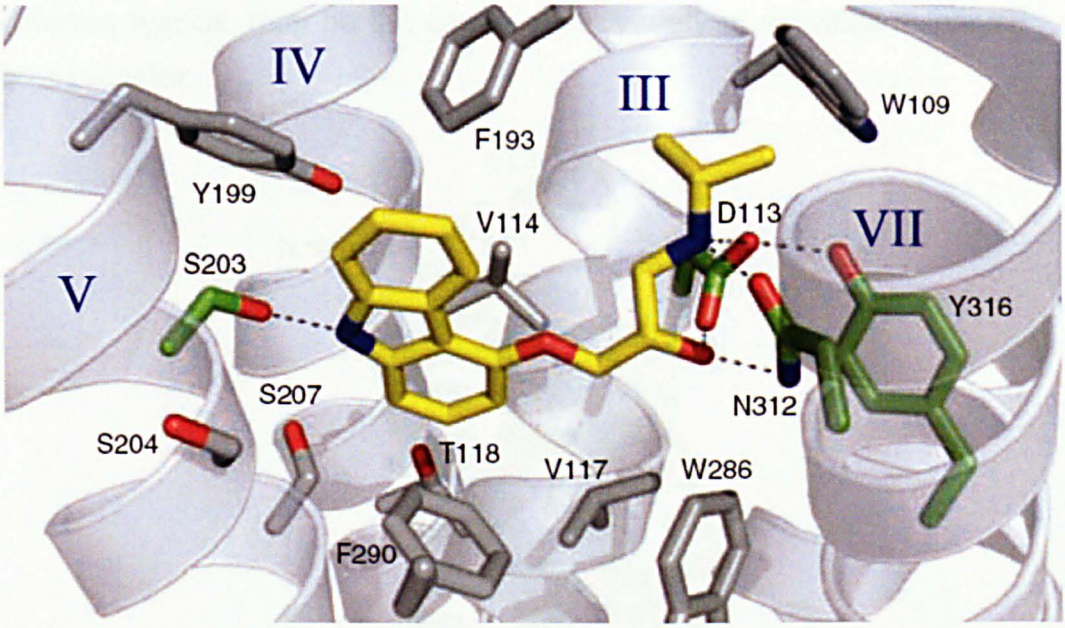


Figure 1-6: Ligand binding pocket of the human β2AR-T4 lysozyme complex co-crystallised with carazolol[38]

Source: Rosenbaum *et al*, 2007.[38]

YELLOW: carazolol; RED: oxygen; BLUE: nitrogen. Stick structures are shown for all residues within 4Å of carazolol, except for A200, N293, F289 and Y308. Structures in green are able to form polar contacts with carazolol (within 3.5Å).

Based upon the structure of bovine rhodopsin, the inactive conformation of family A GPCRs was thought to feature an 'ionic lock', where Arg^{135} (TM3) and Glu^{247} (TM6) form strong ionic and hydrogen bond interactions.[40] It appears that activation of bovine rhodopsin involves breakage of the ionic lock.[33] In the case of the human β2AR, the equivalent residues; Arg^{131} and Glu^{268} are 6.2Å apart; too far to form the strong ionic interactions implied.[40] Although such a difference may be due to the presence of the T4 lysozyme, the original β2AR-antibody complex displays an even larger distance of 10.58Å between the residues.[40] It is possible that the binding of carazolol to the β2AR may result in a receptor conformation with a broken ionic lock, but it still displays inverse agonist activity due to changes in downstream signalling pathways. It is difficult to imagine complex structures such as GPCRs, relying on disruption of a single interaction during receptor activation, and the disruption of differing interactions on binding of

different ligands, may be the cause of the variety of activities displayed by a receptor.

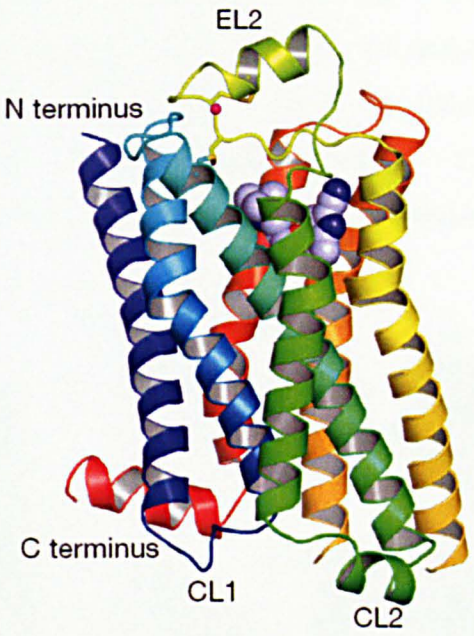


Figure 1-7: Ribbon structure of the mutated turkey β1AR co-crystallised with cyanopindolol (CYP)[41]

Source: Warne *et al*, 2008.[41]

BLUE: *N*-terminus; RED: *C*-terminus; EL2: extracellular loop 2; CL1/2: cytoplasmic loops 1 and 2; PINK: sodium cation. Cyanopindolol is shown as a space-filling model. Disulfide bridges close to EL2 are shown in yellow.

An examination of the ligand binding site of the β2AR in the presence of carazolol (**Figure 1-6**) confirms many key interacting residues as revealed by site-directed mutation studies (see above).[38] As expected, Asp^{113} and Asn^{312} are able to form polar interactions with the amino and hydroxy groups of the oxypropanolamine backbone respectively.[38] Although Tyr^{316} does not interact with the ligand directly, mutational studies have identified it as important. Interestingly, Ser^{203} was found to be important for agonist interaction with the receptor in mutational studies,[18] and also forms a hydrogen bond with the heteroatom in the carazolol aromatic ring.[38]

Phe^{290}, Val^{117} and Phe^{193} surround the aromatic group of carazolol forming a multitude of hydrophobic interactions.[38]

The most recent GPCR crystal structure to be published, is that of a turkey β1AR bound to the high affinity antagonist cyanopindolol (CYP),

by Warne *et al*[41] (**Figure 1-7**). This was chosen over the human β1AR due to better stability as a crystallisation target. Additional stability was achieved by removal of sequences from the *N*-terminus, *C*-terminus, and third intracellular loop.[41] Furthermore, mutations to the sequence, shifted the receptor to the inactive state, to which antagonists have theoretically higher affinity (see **section 1.2.2.1**).[41] These modifications, allowed a structure with 2.7Å resolution to be obtained.[41]

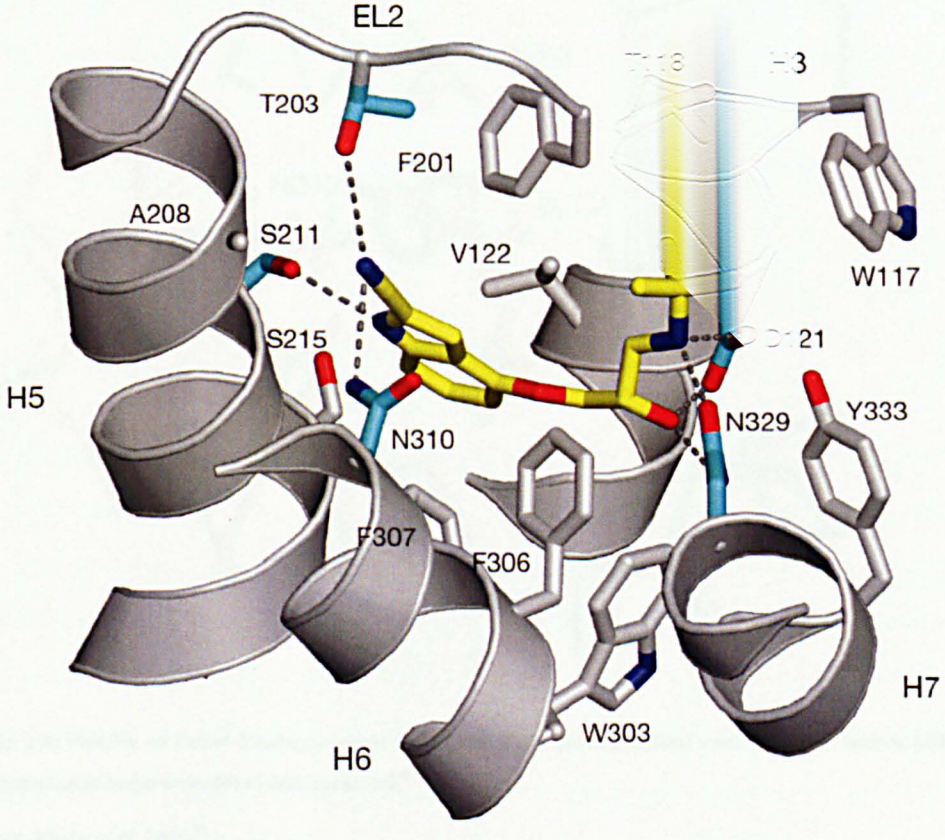


Figure 1-8: Ligand binding pocket of the mutated turkey β1AR co-crystallised with CYP[41]

Source: Warne *et al*, 2008.[41]

YELLOW: CYP; RED: oxygen; BLUE: nitrogen; GREY: Stick structures for residues forming non-polar contacts with CYP; AQUAMARINE: Stick structures for residues forming non-polar contacts.

The sequence homology between the human and turkey β1ARs is very high – around 82%, thus the obtained structure is still highly relevant to the human β1AR.

Similarly to the β2AR, Arg^{139} and Glu^{285} of the turkey β1AR (human β1AR residues Arg^{156} and Glu^{319} respectively) do not form the ionic lock

in this inactive receptor conformation, further questioning its importance beyond the interactions seen in the bovine rhodopsin structure.[41]

In addition, the second extracellular loop of the β1AR bears a helix stabilised by two disulfide bridges, as in the β2AR.

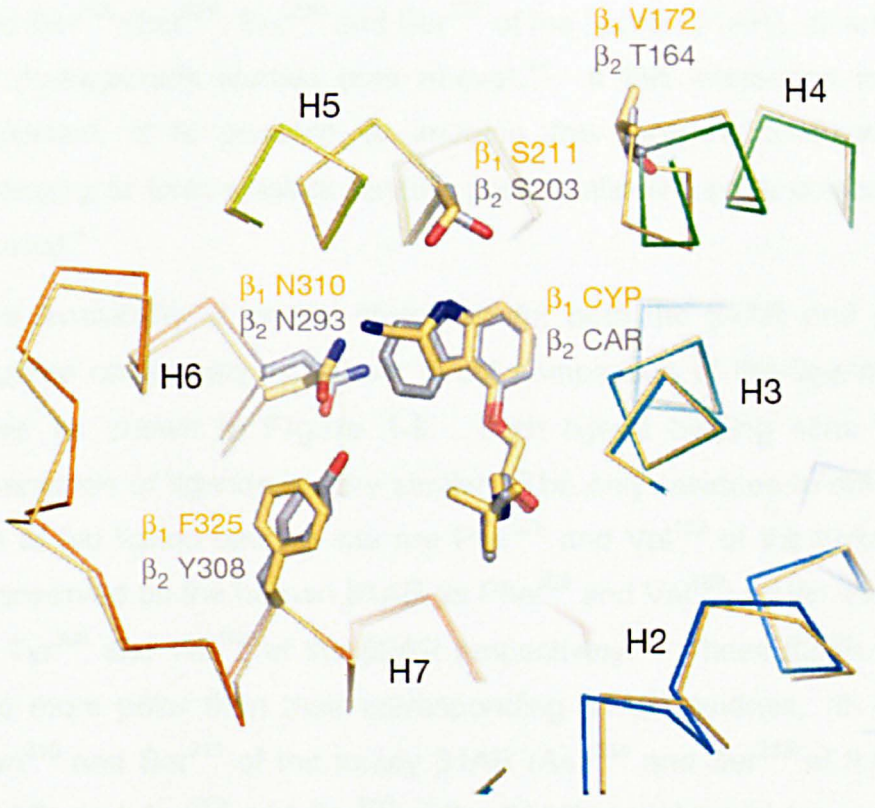


Figure 1-9: Overlay of ligand binding pockets for turkey β1AR (co-crystallised with CYP) and human β2AR-T4 lysozyme complex (co-crystallised with carazolol)[41]

Source: Warne *et al*, 2008.[41]

BLUE: nitrogen; RED: oxygen; YELLOW: β1AR residues; GREY: β2AR residues; CYP: CYP; CAR: carazolol. The ligand binding sites of the two receptors display very high sequence homology, however, within 8Å, differences occur at F325 and V172 of the β1AR (equivalent residue of the β2AR are Y308 and T164 respectively). Other residues shown are identical in nature, but appear rotated in their conformational orientation.[41]

Figure 1-8 shows the ligand binding pocket of the mutated turkey β1AR bound to CYP. The ligand binding pockets of both receptors share conserved key residues, and thus CYP interacts with the same corresponding residues in the β1AR as carazolol does in the β2AR.[41] Additionally, Thr^{203} of the turkey β1AR (human β1AR residue Thr^{221}) is able to hydrogen bond with the cyano group of CYP.[41]

With regards to agonist interaction with the ligand binding pocket, modelling of adrenaline **(1)**, with the inactive mutated turkey β1AR structure by Warne *et al*[41], suggests that when the essential interaction with Asp^{121} (Asp^{138} of the human β1AR) is present, the catechol moiety is too distant to form the postulated hydrogen bonds with Ser^{211}, Ser^{212} and Ser^{215} (Ser^{228}, Ser^{229} and Ser^{232} of the human β1AR), as suggested by mutagenesis studies (see above).[41] If this interaction is indeed important, it is possible to imagine the receptor conformationally changing to form a tighter binding pocket, allowing polar contacts to be formed.[41]

The availability of crystal structures for both the β1AR and β2AR in inactive conformations, allows direct comparison of the ligand binding sites as shown in **Figure 1-9**. Both ligand binding sites and the orientation of ligands is very similar. The only residues to differ within 8Å of the ligand binding site are Phe^{325} and Val^{172} of the turkey β1AR (conserved on the human β1AR as Phe^{359} and Val^{189}) which correspond to Tyr^{308} and Thr^{164} of the β2AR respectively.[41] These β2AR residues are more polar than their corresponding β1AR residues. In addition, Asn^{310} and Ser^{211} of the turkey β1AR (Asn^{344} and Ser^{228} of the human β1AR, and Asn^{293} and Ser^{203} of the β2AR respectively) appear rotated in their conformation between the receptors.[41] These small differences between the ligand binding sites may contribute to the differences in selectivity displayed by numerous ligands (see **section 1.7.1**).

Although these recent advances in crystallising both the β1AR and β2AR bring a wealth of information regarding the structure of these adrenoceptors and other GPCRs in general, it is important to remember they represent a snapshot of a complex receptor with the propensity to adopt multiple conformations. The structures reinforce many of the mutational studies that have been carried out previously, and future structures with a variety of ligands as well as continued mutational work, may provide more detail on the complex sequence of events involved in activation and guanine nucleotide binding protein (G-protein) coupling.

1.2.2 Receptor activation

1.2.2.1 Cubic ternary complex model

The cubic ternary complex model (**Figure 1-10**) describes the different complexes that can exist when interaction between agonist, receptor and G-protein are considered.

In this model, the receptor is able to exist in two states, either active ($\mathbf{R_a}$) or inactive ($\mathbf{R_i}$). Only $\mathbf{R_a}$ is able to activate the G-protein (**G**). The agonist (**A**) has affinity for both $\mathbf{R_i}$ and $\mathbf{R_a}$, but affinity is higher for $\mathbf{R_a}$ (in the case of inverse agonists, affinity would be higher for $\mathbf{R_i}$).[42] Additionally, binding of **A** to $\mathbf{R_i}$, to form $\mathbf{AR_i}$, promotes conversion to $\mathbf{AR_a}$ (i.e. activation of the receptor). Finally, **G** can be activated when $\mathbf{AR_aG}$ is formed (agonist binding and receptor activity), or when $\mathbf{R_aG}$ is formed (representing constitutive activity).[42]

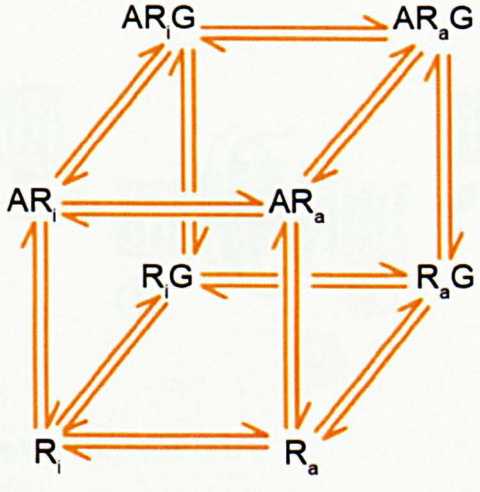


Figure 1-10: Cubic ternary complex model of GPCR activation

Source: Adapted, originally from Kenakin 2002[42]

A: agonist; G: G-protein; R_i: inactive form of receptor; R_a: active form of receptor.

A more complex extension to the model postulates different receptor active conformations, for which different ligands have varying affinity, and which may be able to signal via differing G-proteins and signalling cascades.[43]

1.2.2.2 G-protein activation cycle

As a superfamily, serpentine receptors can be activated by a variety of stimuli, including photons (rhodopsin), ions (calcium receptors), odorant molecules (olfactory receptors), gustatory molecules (bitter/sweet/savoury receptors), peptides (endothelin receptors, CCK receptors), proteins (latrophilin receptor) and biogenic amines (adrenoceptors, histamine receptors).[7-9]

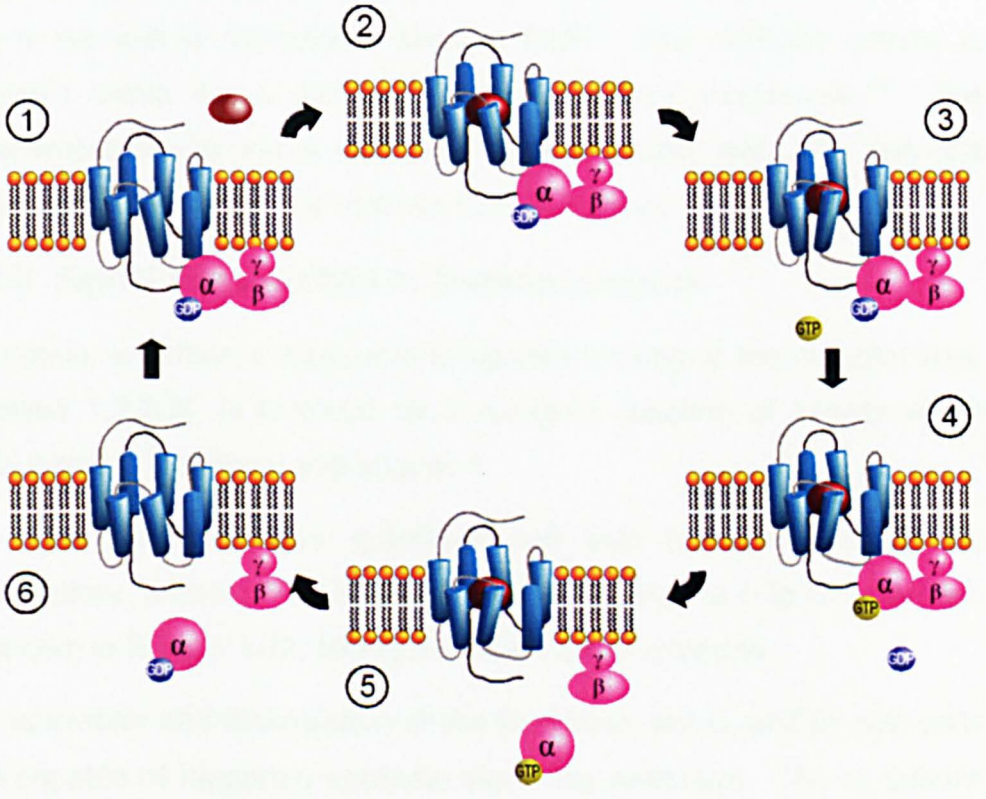


Figure 1-11: G-protein activation by GPCRs

Source: Sven Jähnichen, 24.04.2006.

1. The GPCR in the inactive conformation is bound to the heterotrimeric G-protein. The G-protein α-subunit in this inactive complex is bound to guanine diphosphate (GDP).

2 and 3. The agonist binds to receptor, which undergoes a conformational change.

4. The activated receptor causes the exchange of GDP for guanine triphosphate (GTP) on the G-protein α-subunit.

5. Binding of GTP causes the G-protein α-subunit to dissociate from the βγ subunit

6. The G-protein α-subunit possesses intrinsic GTPase activity causing hydrolysis of GTP to GDP. The GDP-bound α-subunit can then re-associate with the rest of the G-protein and GPCR in the inactive state.

In the case of the β-adrenoceptors, activation is initiated by the endogenous catecholamines adrenaline **(1)** and noradrenaline **(2)**,[3] see

section 1.1. On binding of the ligand, the receptor undergoes a conformational change, causing activation of the bound G-protein (**Figure 1-11**).[12] The inactive G-protein is bound to GDP and is comprised of α, β and γ sub-units that are associated together.[12] The conformationally changed receptor results in displacement of GDP by GTP.[11, 12] Consequently, the G-protein fragments into an α sub-unit and a βγ sub-unit. These are then able to activate other protein targets (see below). The activation cycle is terminated when the GTP bound to the α sub-unit is hydrolysed back to GDP. This GTPase activity is inherent within the α sub-unit as a self-control mechanism.[44] The deactivated α sub-unit is then able to re-associate with a βγ sub-unit and receptor to reform the inactive GPCR complex.

1.2.3 Signal transduction in β-adrenoceptors

G-protein activation subsequent to agonist binding to the receptor (see **section 1.2.2.2**), is followed by a complex cascade of events which both transfer the signal and amplify it.

All three β-adrenoceptor sub-types are able to signal via the G_s (stimulatory) protein. A depiction of events downstream to G_s activation is shown in **Figure 1-12**, taking the β2AR as an example.

On activation and dissociation of the G-protein, the α_s and βγ sub-units are capable of triggering separate signalling pathways. The α_s subunit causes activation of adenylyl cyclase, resulting in conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP).[45] Signal amplification arises from multiple molecules of cAMP being produced by activation of each adenylyl cyclase, as well as in earlier steps of the cascade, where each α_s sub-unit can activate multiple adenylyl cyclases, and each activated receptor can in turn activate several G-proteins.

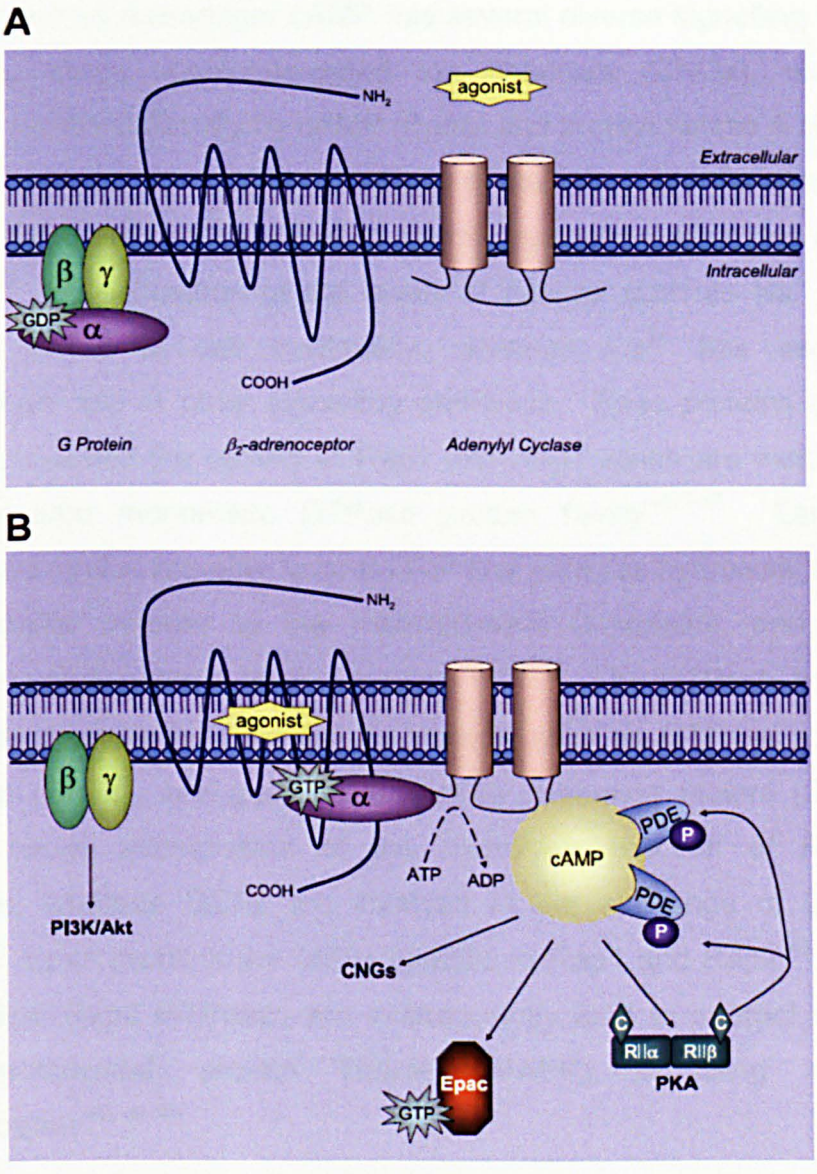


Figure 1-12: β2AR signalling pathway[45]

Source: Lynch and Ryall 2008[45]

ADP: adenosine diphosphate; Akt: protein kinase B; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; CNGs: cyclic nucleotide-gated ion channel; Epac: exchange protein activated directly by cAMP; GDP: guanine diphosphate; GTP: guanine triphosphate; PDE: phosphodiesterase; PI3K: phosphoinositide-3-kinase; PKA: protein kinase A – comprising of two regulatory (RIIα and RIIβ) and two catalytic subunits.

A: The β2AR and adenylyl cyclase are transmembrane proteins. Prior to activation, the G-protein exists as the heterotrimer bound to GDP and is associated with the cell membrane.[45]

B: Binding of agonist (e.g. adrenaline) to the β2AR cause G-protein activation and dissociation of the α and βγ sub-units (see section 1.2.2.2). The βγ sub-unit is able to activate the PI3K/Akt pathway, whereas the α sub-unit (in the case of a G_s protein) activates adenylyl cyclase. Adenylyl cyclase converts ATP to cAMP – the secondary messenger. cAMP is able to activate a number of targets such as CNGs, Epac and PKA. Signal control is achieved by activation of PDEs by PKA that terminate the secondary messenger signal by hydrolysing cAMP.[45]

The secondary messenger cAMP has several diverse signalling targets, including cyclic nucleotide-gated ion channels (CNGs), exchange protein activated directly by cAMP (Epac) and protein kinase A (PKA).[45, 46] CNGs are cation channels made up of multiple sub-units arranged to form a pore, and can be activated directly by cyclic nucleotides such as cAMP.[45, 47] Modulation of the levels of cations such as Na^+ and K^+ allows control of cell excitability, whereas Ca^{2+} has secondary messenger role in other signalling pathways. Epac proteins (Epac 1 and 2) modulate the activity of Rap1 and Rap2 which are members of the Ras-type monomeric GTPase protein family.[45, 47] Essentially GTPase proteins are able to bind GTP and catalyse hydrolysis to GDP; in a similar manner to the heterotrimeric G-proteins, and act as signalling molecules in their own right.[45, 48, 49] The GTPase activity of Ras-type proteins is facilitated by proteins such as GTPase activating proteins (GAPs), and guanine nucleotide exchange factors (GEFs).[47] GAPs cause acceleration of the hydrolysis reaction of Ras-type proteins, whereas GEFs are involved in the exchange of GDP for GTP.[47] Epac proteins are GEFs specific to Rap1 and Rap2.[45, 47] The Rap1 and Rap2 GTPases are subsequently able to interact with the mitogen-activated protein kinase (MAPK) signalling cascade downstream.[45, 48, 49]

The main cAMP effector is PKA, a heterotetrameric protein comprising of two catalytic and two regulatory sub-units.[45] Binding of cAMP to the regulatory sub-units induces a conformational change in the protein which unmasks the catalytic sub-units.[45] Once exposed, PKA is able to phosphorylate a number of cytosolic and nuclear targets.[45] Diffusion of the active catalytic PKA sub-units into the nucleus can result in phosphorylation of cAMP response element binding protein (CREB) and subsequent binding of phosphorylated CREB to cAMP response element (CRE) promoters on DNA, thus initiating gene transcription.[45]

With regards to heart function, PKA phosphorylates L-type voltage-dependant calcium channels, phospholamban and troponin I which are involved in the cardiac muscle contraction.[50]

In addition, PKA can phosphorylate the β2AR, causing a switch in the signalling system from G_s to G_i.[11, 49, 51-53] The α_i sub-unit of the G_i protein, in general has actions opposing those of the α_s sub-unit; i.e. inhibition of certain adenylyl cyclases[11], a reduction in cAMP production and consequent dampening down of any cAMP-mediated signalling. Interestingly, during G-protein coupled receptor (GPCR) coupling to G_i, the βγ sub-unit appears to be able to activate the MAPK cascade.[11, 49, 52]

The βγ subunit may also have a role in activating the PI3K/Akt pathway which is involved in a variety of roles involving protein synthesis.[45] The exact significance of this interaction is yet to be elucidated.

The complex interplay between a variety of signalling systems means it is difficult to interpret the exact outcomes of β-adrenoceptor activation. Although the immediate effects of receptor activation are evident (e.g. increases in heart rate/force of contraction on stimulation of the β1AR via coupling of the cAMP signalling system to the cardiac contractile muscle machinery)[50], the longer term effects are less clear.

It is known, for example that extensive exposure to agonists such as **3** cause further phosphorylation (via β-adrenergic receptor kinase (βARK) recruitment by βγ sub-units[11]) of the β2AR (beyond the PKA-mediated desensitisation) allowing targeting by arrestins.[12, 54] The arrestins can bind to the G-protein binding domain preventing further G-protein activation, as well as facilitating internalisation of the receptor itself into clathrin-coated pits.[54]

The MAPK, PI3K/Akt, and theoretically other signalling cascades,[11] have the potential to affect almost all aspects of the cell cycle and cell activity. The actual outcome is likely to be a fine-tuned response reliant on cell type, length of receptor activation, agonist residency period as well as the nature of the agonist itself. Indeed extensions of the cubic ternary complex model (**section 1.2.2.1**), suggest that different agonists may induce different activated receptor conformations, that in turn have the potential to activate different signalling cascades, or activate signalling cascades to different degrees.[43]

1.3 History and development of β_1-adrenoceptor antagonists

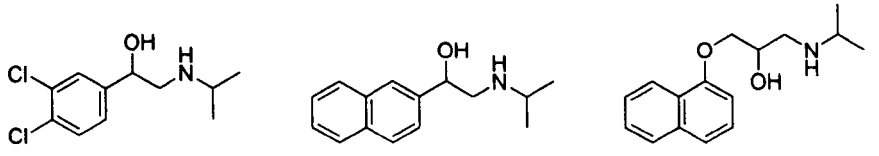
The possibility that adrenoceptors might exist in two sub-types was first suggested by Raymond Ahlquist in 1948, when a tentative classification of α and β adrenoceptors was made.[4] Subsequently, in 1958, a young James Black began work with Imperial Chemical Industries PLC (ICI) with a clear objective in mind:

"...I wanted to find a β-receptor antagonist. I expected this to reduce pulse rates at rest and during exercise and hoped that it would decrease the susceptibility of patients to angina pectoris." [55]

Remarkably, the notion that the β-adrenoceptors could be further sub-divided into β1AR and β2ARs did not surface until 1967[56] - some time after efforts to develop a β-adrenoceptor antagonist to treat cardiac diseases had already been initiated.

1.3.1 First generation β-blockers

The initial breakthrough in developing a β-adrenoceptor antagonist came in 1959, from the Lilly research laboratories with dichloroisoprenaline (DCI) **(5)**.[57] An analogue of isoprenaline **(3)**, DCI had poor efficacy and retained partial agonist activity.[55, 57-59]



5 **6** **7**

Figure 1-13: First generation compounds

Dichloroisoproterenol (5), pronethalol (6) and propranolol (7).

The naphthyl analogue of DCI was investigated as a replacement for the dichlorophenyl group, and became later known as pronethalol **(6)**.[55, 58] Although the undesirable intrinsic sympathomimetic activity or partial agonist effects were considerably lower, **6** was found to produce thymic tumors in rats.[58] Further studies aimed at eliminating any potential carcinogenicity, led to insertion of a methoxy linker before the ethanolamine moiety.[60] Lack of availability of 2-naphthol at the time

prompted the use of 1-naphthol instead, subsequently in 1964, propranolol **(7)** – the first clinically relevant β-blocker was reported.[2, 58, 61] When the 1-naphthol analogue was eventually synthesised it was found to be a poorer antagonist than **7**.

Propranolol was reported to be around 10-fold more active in blocking the actions of isoprenaline compared to pronethalol, had fewer side effects, and did not show any signs of carcinogenicity.[61]

The versatility of propranolol is evident from its continual use in a variety of disorders in the current clinical setting, including; hypertension, portal hypertension, phaeochromocytoma, angina, arrhythmias, thyrotoxicosis, anxiety, and migraine prophylaxis.[62]

With the observations published by Lands in 1967[56], indicating the existence of at least two different types of β-adrenoceptor came the realisation that propranolol was actually a non-discriminatory antagonist. The unsuitability of propranolol for patients suffering from chronic obstructive airways disorders had previously been identified.[63] Consequently, attention shifted to the development of cardiac-selective antagonists, which were less likely to induce bronchospasm.

Earlier compounds such as **6** and **7** were also found to possess an undesirable 'membrane stabilising activity' or local anaesthetic activity.[64] By affecting the conduction of nerve impulses, these drugs were found to have a direct depressant activity[64, 65] on the heart unrelated to their β-adrenoceptor antagonist ability. This activity appeared to be related to lipophilicity[65], thus subsequent approaches were also aimed at reducing lipophilicity.[59]

1.3.2 Second generation β-blockers

Practolol **(8)** was developed in 1968, and became the first compound available displaying selectivity towards the β1AR.[66] It differs from propranolol by replacement of the napthyl group with an acetanilide structure; the acetamide group being positioned *para*- to the oxypropanolamine. This *para*-substitution was found to be important in conferring selectivity (see **section 1.5.1.3**).[67] Although less potent than

propranolol as a β1AR antagonist,[64, 67] practolol was considered clinically superior, due to its improved selectivity profile and lack of membrane stabilising activity (MSA)[64, 66]. Unfortunately, practolol was found to cause oculomucocutaneous syndrome resulting in mucosal ulceration, conjunctivitis, drying of the eyes, and in some cases loss of vision.[68] These unacceptable side-effects resulted in the withdrawal of practolol several years later.

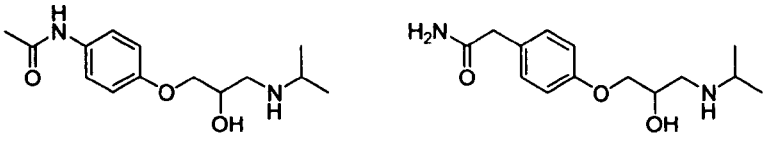


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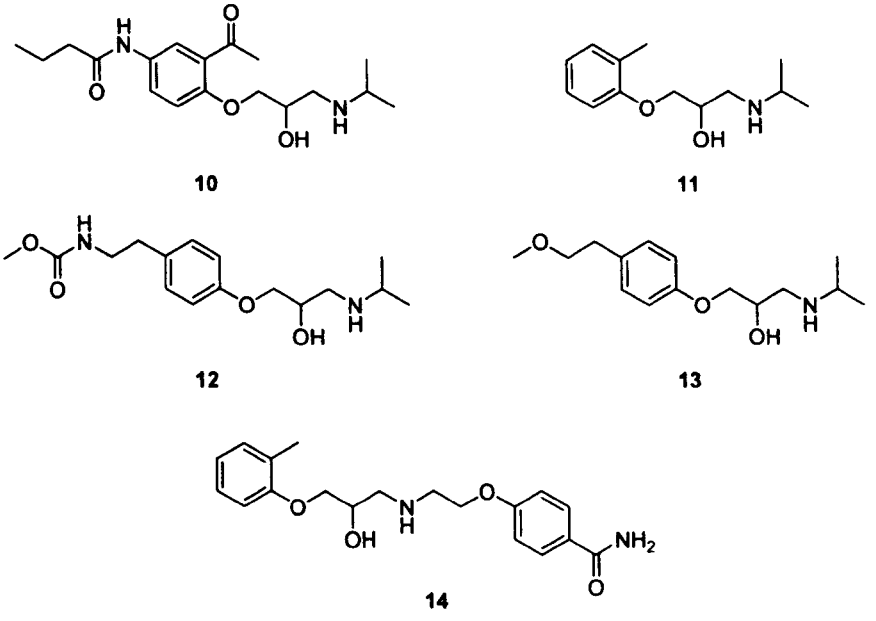
Figure 1-14: Pioneering second generation compounds

Practolol (8) and atenolol (9).

Manipulation of the acetamide structure of **8** eventually led to the discovery of atenolol **(9)** in 1973, as a compound offering good activity as a β1AR antagonist, cardioselectivity and without intrinsic sympathomimetic activity (ISA) or MSA[69]. Selectivity was maintained by reversal of the amide configuration in practolol, however it was the insertion of a methylene linker that was found to remove the ISA.[59]



10 11 12 13 14

Figure 1-15: Second generation compounds with reported cardioselectivity discovered during the 1970's

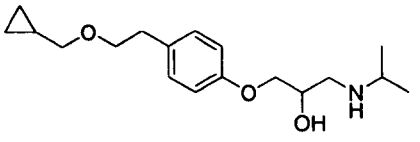
Acebutolol[70] (10), bevantolol[71] (11), pamatolol[72] (12), metoprolol[73] (13) and tolamolol[74] (14).

During the 1970's, numerous examples of β1AR antagonists with differing selectivity and activity profiles were synthesised. Those reported to be cardioselective[59] are shown in **Figure 1-15**.

Many of these older compounds, although relatively more cardioselective than their precursors, are still associated with a large number of adverse effects (circulatory problems, male impotence, hypoglycaemia) [58, 62] mediated by blockade of the β2AR.

1.3.3 Third generation β-blockers

In addition to further improving selectivity, the third generation of compounds resulted from a need to tackle the problem of metabolism with compounds such as acebutolol **(10)** and metoprolol **(13)**.[59] These drugs require twice or three times daily doses to maintain levels in the plasma,[62] leading to potentially irregular therapy and patient compliance issues.



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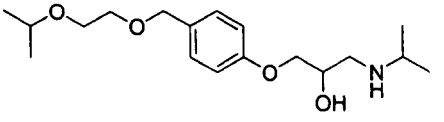

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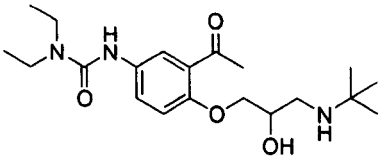

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Figure 1-16: Third generation compounds with improved pharmacokinetic profiles

Betaxolol (15), bisoprolol (16) and celiprolol (17).

The problems surrounding metabolism were addressed by increasing the bulk of the substituent *para* to the oxypropanolamine group.[59] Consequently, betaxolol[75] **(15)**, bisoprolol **(16)** and celiprolol[76] **(17)** became clinically available during the 1980's.[59]

Both bisoprolol and celiprolol are used in a once-daily dosing regimen.[62]

1.3.4 Nebivolol

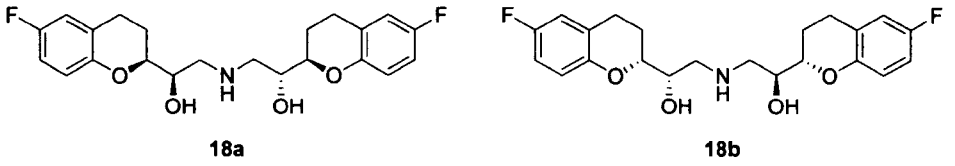
Nebivolol **(18a + 18b** as a racemic mixture**)** is the latest addition to the range of clinically available β-blockers. It differs from previous compounds in both structure and activity. In animal studies (guinea pig atrial and lung assay), nebivolol displays 300-fold[77] β_1/β_2-selectivity and is marketed as a highly selective β-blocker. In human ventricular membrane assays, the selectivity was found only to be around 40-fold.[78, 79] However, in isolated whole cell preparations expressing single human receptor sub-types, selectivity was found to be only around 14-fold (see **Table 1-1**)[80].

		Binding affinity, log K_D (M)		Selectivity	
		β_1-adrenoceptor	β_2-adrenoceptor	β_1	β_2
18a+18b	Nebivolol	-9.04 ± 0.03	-7.89 ± 0.03	14.1	
16	Bisoprolol	-7.83 ± 0.04	-6.70 ± 0.05	13.5	
15	Betaxolol	-8.21 ± 0.07	-7.38 ± 0.06	6.8	
9	Atenolol	-6.66 ± 0.05	-5.99 ± 0.14	4.7	
10	Acebutolol	-6.46 ± 0.03	-6.08 ± 0.07	2.4	
13	Metoprolol	-7.26 ± 0.07	-6.89 ± 0.09	2.3	
19	Labetolol	-7.63 ± 0.05	-8.03 ± 0.07		2.5
27	Carvedilol	-8.75 ± 0.09	-9.40 ± 0.08		4.5
7	Propranolol	-8.16 ± 0.08	-9.08 ± 0.06		8.3
20	Sotalol	-5.77 ± 0.11	-6.85 ± 0.09		12.0
26	Timolol	-8.27 ± 0.08	-9.68 ± 0.02		25.7

Table 1-1: Binding affinities and selectivity for human β-adrenoceptors of nebivolol (18a+b) and other β-blockers in clinical use[80, 81]

K_D for a given compound is the concentration which displaces 50% of specifically bound radioligand – ^{3}H-CGP12177. Selectivity refers to the relative ratios of affinity for the β1AR and the β2AR. I.e. nebivolol is 14.1-fold more selective for the β1AR over the β2AR, whereas timolol is 25.7-fold more selective for the β2AR over theβ1AR.

Nebivolol also exhibits a vasodilatory activity which appears to be mediated by nitric oxide,[79] though the clinical relevance of this is yet to be established.[62] Additionally, the racemic preparation of nebivolol appears to have beneficial effects on left ventricular function.[79]



18a **18b**

Figure 1-17: Nebivolol – marketed as the racemate

(*S,R,R,R*)-nebivolol (18a) and (*R,S,S,S*)-nebivolol (18b)

As with previous compounds, the oxypropanolamine motif is retained, however nebivolol has two of these in an almost-symmetrical arrangement around the nitrogen atom. Of the possible ten stereoisomers, **18a** is the most active.[77, 82] Intriguingly, the spacial configuration at both of the hydroxyl-bearing carbons in **18a** is opposite to that found in the active stereoisomers of other β-blockers (**section 1.5.3**).[82] Indeed the enantiomer **18b**, has around 175-fold lower affinity for the β1AR.[82] Interestingly, nebivolol is marketed in the racemic form and although **18a** is the more active β-blocker[83], **18b** appears to be responsible for the vasodilatory[84] effects.[82] It is only the racemic form that appears to display beneficial activity for the left ventricle, indicating that **18a** and **18b** may have a synergistic effect.[79]

The unexpected β-blocking activity of **18a** with respect to other compounds (i.e. activity lies with the *R*-configuration at the carbinol group, as opposed to the *S*-configuration), may be explained by an alternative binding conformation of **18a** at the receptor. The chromane-based structure of **18a** confers a relatively rigid oxypropanolamine group in comparison to other compounds. This rigidity may mean the molecule is held in a conformation that also allows favourable interaction with the receptor.[82]

1.3.5 Non-cardioselective β-blockers

The mainstream focus on developing more cardioselective β-blockers has also produced a multitude of non-cardioselective molecules[59] (**Figure 1-18**). Some of these compounds are available clinically despite being essentially non-selective between the β1AR and β2ARs, often, their *in-vitro* profile indicates selectivity towards the β2AR.[81]

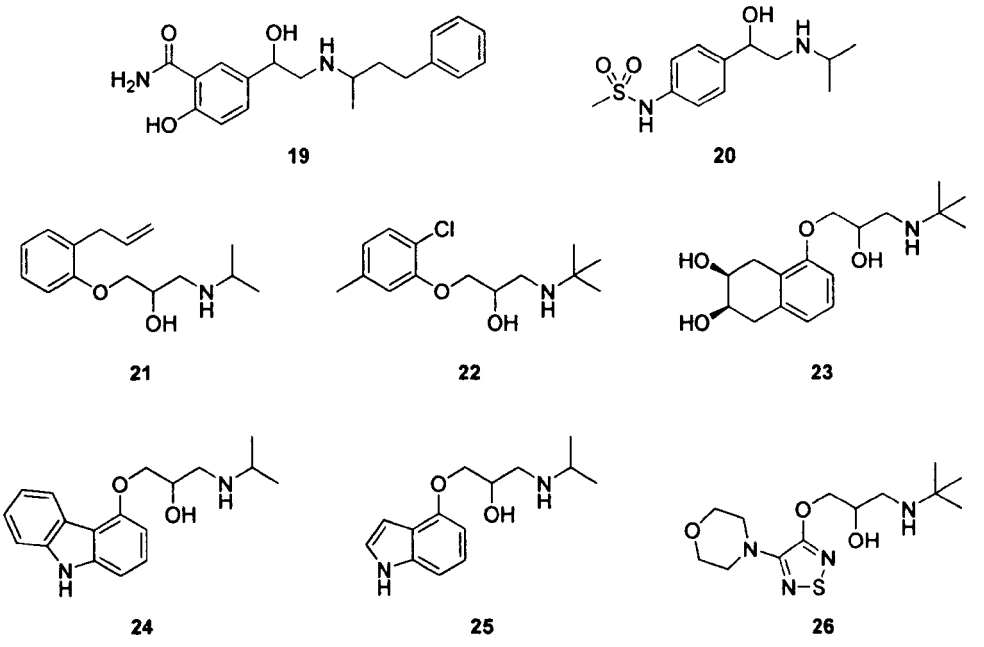


Figure 1-18: β-Blockers with greater selectivity towards the β2AR

Labetolol (19), sotalol (20), alprenolol (21), bupranolol (22), nadolol (23), carazolol (24), pindolol (25) and timolol (26).[80, 81]

It is important to remember that compounds exhibiting low selectivity towards either receptor in *in-vitro* assays, may be clinically non-selective compounds, in terms of the pharmacological effect they exert.

Often, these compounds have extremely high affinity for the β-adrenoceptors, and have proven to be useful drugs where cardioselectivity is not important, as a much lower dose is required for β-blocking activity.

1.4 Therapeutic indications for β_1-adrenoceptor antagonists

Although initially intended to treat *angina pectoris*,[55] there are numerous conditions for which β-blockers are now indicated.

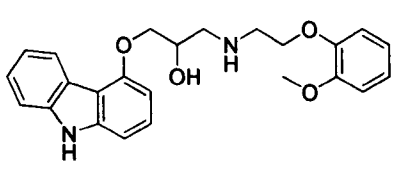
Angina pectoris describes the chest pain experienced due to insufficient blood supply to the heart. This is usually brought about by progressive occlusion of the vessels supplying blood to the heart. With stable angina, this pain is usually precipitated by exertion, whereby the demand for oxygen by the heart cannot be met by the reduced supply.[62] As the disease progresses, the occlusive atherosclerotic plaques can

rupture, resulting in clots and pain even at rest (unstable angina) with high risk of myocardial infarction.[62] β-Blockers control the heart rate and force of contraction and so reduce the oxygen requirement of the heart, thus providing control of angina symptoms.[62, 85]

Although the exact mechanisms are unclear[85], β-blockers are useful in hypertension.[62] They are often used additionally, or second-line to diuretic agents, or other antihypertensive agents such as ACE-inhibitors.

β-Blockers have been shown to reduce the risk of recurrent infarct and improve mortality,[85, 86] and their use is advised in the secondary prevention of myocardial infarction.[62, 87]

Arrhythmias are a complex group of conditions of varying aetiology, but generally result in irregular beating of the heart, quite often due to abnormalities in electrical conduction. The anti-arrhythmic effect (Class II) of β-blockers as a class of compounds, is due to their ability to dampen the sympathetic nervous system, in addition to controlling the rate and force of heart contraction[62, 88]. In particular, sotalol **(20)** has additional 'Class III' anti-arrhythmic properties, based on its ability to block potassium ion channels, allowing it to be effective in the treatment of more specific types of arrhythmia.[88]



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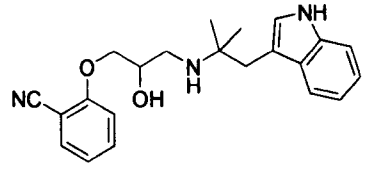

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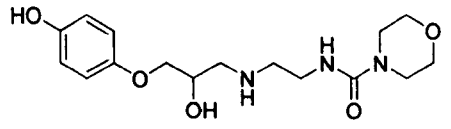


29

Figure 1-19: β-Blockers used in heart failure

Carvedilol (27), bucindolol (28) and xamoterol (29). Xamoterol was withdrawn 2000[89].

In heart failure, the cardiac muscle has reduced ability to pump blood around the body. As the disease progresses, structural changes in the heart muscle and enlargement, lead to a further increase in oxygen requirement and reduced functional efficiency. The initial contra-indication of β-blockers in treating heart failure, was based upon the logical assumption that further depression of an already failing heart would be detrimental.[85] However in more recent years, certain compounds, such as carvedilol **(27)**, bisoprolol **(16)** and metoprolol **(13)** have been shown to reduce mortality.[85] It is of interest to note that these compounds are devoid of ISA. Compounds such as bucindolol **(28)** and nebivolol **(18a + 18b)**, with known ISA, do not appear to cause a significant reduction in mortality.[85] Indeed xamoterol **(29)** was developed as a partial agonist with significant agonist activity[90] but was actually found to increase mortality. Consequently, xamoterol was withdrawn from the market.

β-Blockers are also used to treat the glaucoma, the symptoms of anxiety, as well as prophylactically in migraine sufferers. The diverse spectrum of indications for β-blocker usage, and the fact that the mechanism of action in many diseases is yet to be elucidated, is likely to be due to the differing activity profile of each individual agent. Apart from differences in receptor selectivity, ISA, MSA and lipophilicity, there may be other effects exerted by these compounds. As mentioned above, sotalol **(20)** has the ability to block potassium channels. Compounds such as nebivolol **(18a + 18b)**, labetolol **(19)**, celiprolol **(17)** and carvedilol **(27)** are able to cause arteriolar vasodilatation[62] though the long-term clinical implications of this are not clear.

An understanding of the structural foundations of these different activities, may allow design of compounds tailored to a particular disease state, and potentially minimise side-effects through unwanted activity. Indeed, the underlying mechanisms in these disease states may also become more apparent.

1.5 Known structure-activity relationships (SAR) for β_1-adrenoceptor antagonists

Often, a comprehensive idea of SAR cannot be formed due to lack of a complete set of molecules for comparison, or multiple changes being made to a compound. In addition, studies were published from various institutions utilising different, often incomparable, methods of pharmacological analysis, and through a time period when pharmacological techniques were also developing rapidly.

Much of the known SAR for β1AR antagonists has arisen from an intense programme of research into the area by ICI from the late 1960's to the 1980's.[59]

Taking the aryloxypropanolamine as a core motif, various modifications can be made to the molecule, resulting in improved affinity for the β1AR and better cardioselectivity. A summary of observations reported in the literature is provided below (**Figure 1-20**)

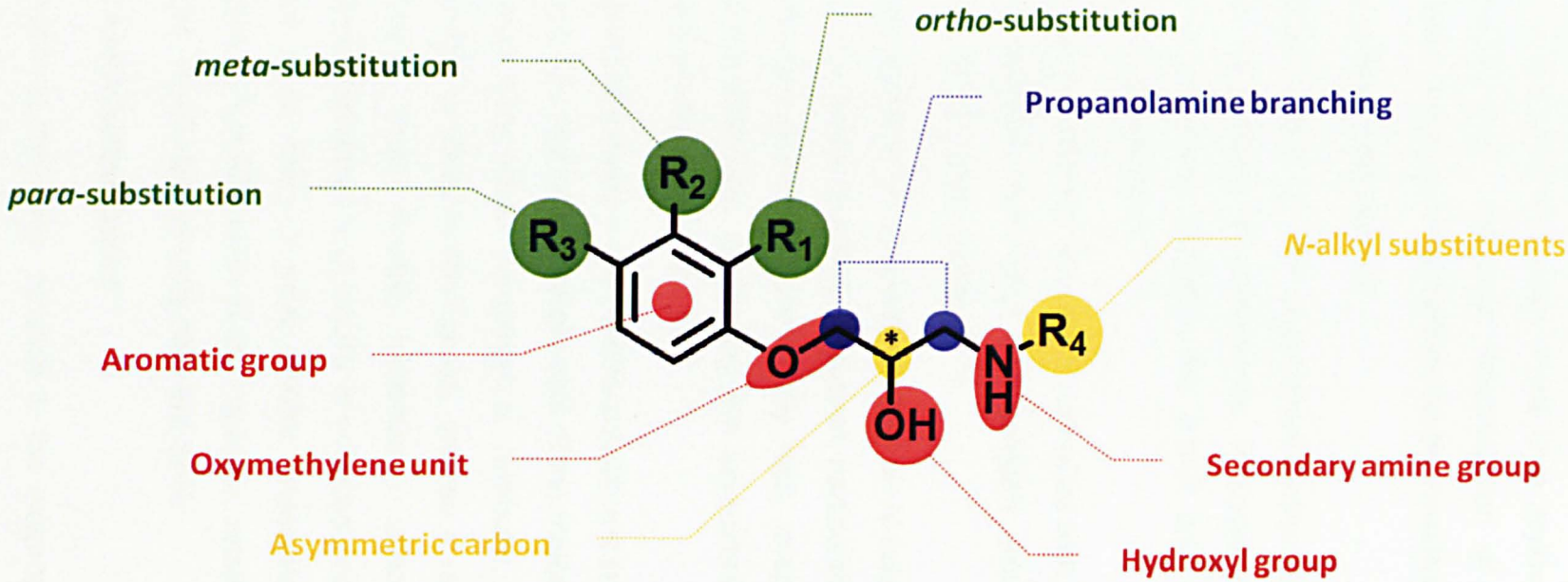


Figure 1-20: Structure activity relationships map of the core aryloxypropanolamine structure

Components in red represent the core essential structure of a β1AR antagonist. Synopsis of SAR relating to modification of each area of the molecule is detailed in the following pages.

1.5.1 The core aromatic ring

Numerous investigations into the type of substituent, and substitution pattern, of the aromatic group of the core aryloxypropanolamine have been carried out. Although interpretation of the results is often complicated, some general trends can be visualised.

1.5.1.1 *ortho*-substitution

Substitution *ortho*- to the oxypropanolamine backbone appears to increase the potency of compounds, however without increasing the degree of selectivity between the β1AR and β2AR, i.e. improving binding at both receptors.[91-96]

The increase in potency appears to correlate with increasing lipophilicity of the substituent, with alkyl and halogen groups conferring higher potencies within a given series.[93, 94]

A discrete series of compounds bearing an *N*-substituted oxyacetamide group in the *ortho*- position displayed particularly raised potencies.[95] The potencies remained consistently high, despite changes to other parts of the molecule, suggesting the oxyacetamide moiety promotes favourable receptor interactions.[95]

The general observation of an *ortho*-substituent improving potency, may be sensitive to changes in other parts of the molecule. Changing the *N*-alkyl group from either *iso*-propyl or *tert*-butyl, to a more extensive phenoxyalkyl or alkyloxyalkyl group, causes a reversal of this trend.[97] With these more flexible molecules, *ortho*-substitution of the aryloxypropanolamine ring results in reduced potency.[97] This may be indicative of an ability of more flexible molecules to adopt a different orientation, or conformation in the receptor, relative to the less flexible molecules, resulting in slightly different SAR.

1.5.1.2 *meta*-substitution

Substituents in the *meta*- position to the oxypropanolamine backbone have been reported to improve potency in a manner similar to those with comparable *ortho*- substituents (i.e. without increasing

selectivity).[91, 92] However, in comparison to the *ortho*-substituents, the increase in potency is not as marked.[91, 92]

1.5.1.3 *para*-substitution

Early investigations into β-blocker structure did not benefit from the knowledge of multiple β-adrenoceptor targets. Because of this, substitution *para*- to the oxypropanolamine backbone was not considered of particular interest, due to reported reduction in antagonist potency.[92] After Lands's paper on the existence of at least two different types of β-adrenoceptor in 1967,[56] and the development of differential pharmacological techniques, substitution at the *para*- position was investigated with renewed interest.

In general, *para*-substitution resulted in reduced antagonist potency, but was found to confer cardioselectivity; i.e. selectivity for the β1AR over the β2AR.[67, 73, 74, 91, 98-102]

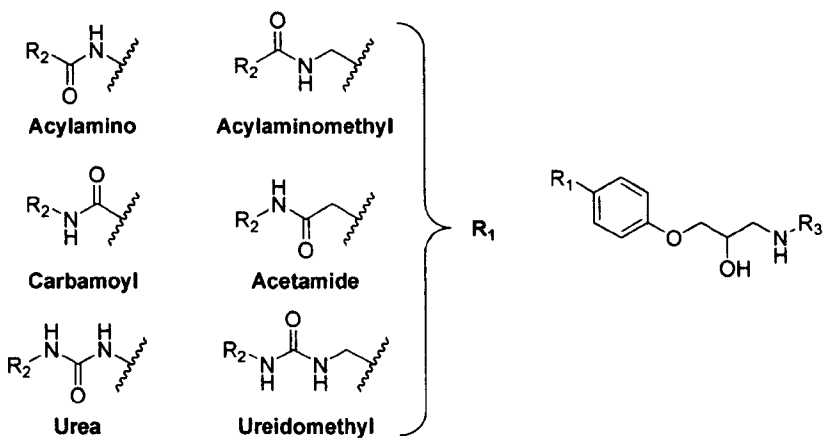


Figure 1-21: Amidic-based *para*-substituents

R_2 is either H or an alkyl group; R_3 is generally *iso*-propyl, *tert*-butyl or an aralkyl group.[67, 93, 94, 96]

Initial focus on the *para*-acylamino group (Figure 1-21) led to the discovery of **8** (see **section 1.3.2**). Further investigation into isosteric groups has shown *para*-urea groups are also tolerated, and offer improved cardioselectivity over the acylamino moeity.[94] Insertion of a methylene group between the ring and carbamoyl precursors, led to the discovery of **9** and was found to improve cardioselectivity.[93, 96]

However, when the same technique was investigated with the corresponding *para*-urea and acylamino substituents (**Figure 1-21**), a notable decrease in potency was observed.[93]

Although initial increases in length of the *para*-substituent caused a reduction in potency, [93, 103] further extension of the substituent causes a return of potency, with retention of cardioselectivity.[102, 104-106] This indicates the β1AR is able to accommodate a more extensive *para*-substituent than the β2AR.[103]

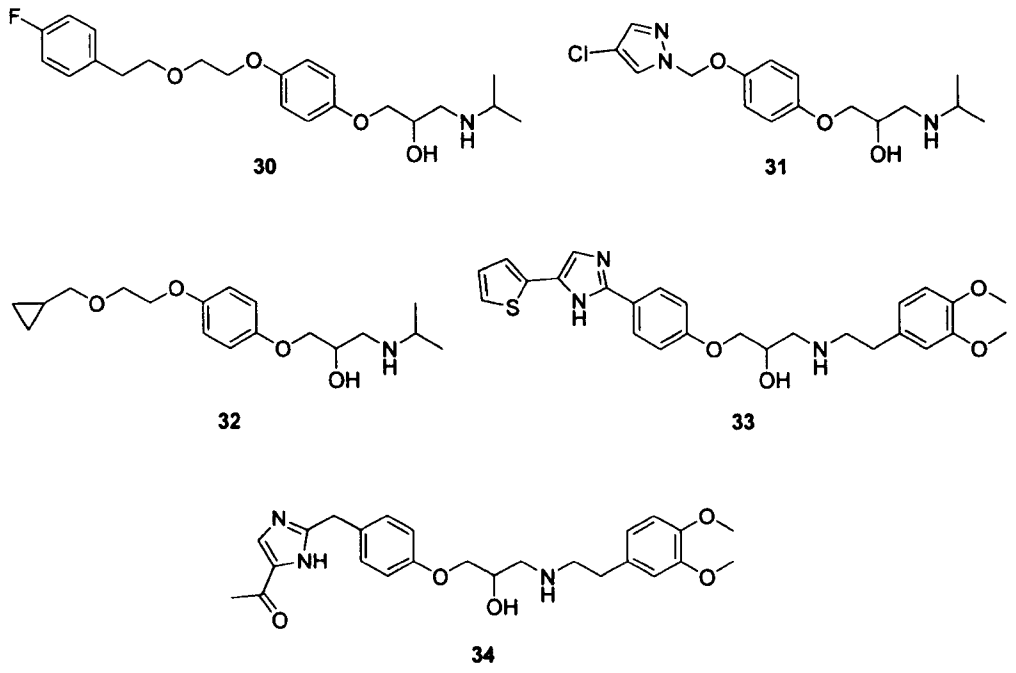


Figure 1-22: Compounds with high selectivity for the β1AR bearing extensive substituents *para*- to the oxypropanolamine backbone[98-100, 106, 107]

RO 31-1118/flusoxolol (30); Compound from Roche Products Ltd (31); cicloprolol (32); Compounds from Merck Sharp & Dohme Research Laboratories (33, 34).

Investigation into bulkier or more extensive *para*-substituents has led to two major types of compound being developed; those containing alkyl or aromatic groups, attached to the aryloxypropanolamine by means of an ether-based linkage,[98, 99, 106] and those linked more directly to heterocyclic aromatics[100, 107] (**Figure 1-22**).

Although the nature of the *para*-substituent varies considerably in **30-34**, and in other examples,[106] including **8**, **9**, **12**, **13** and **15**, the presence of a heteroatom spaced at 2-4 atoms from the aryloxypropanolamine motif is common. The presence of a heteroatom such as oxygen or nitrogen may be important in forming an interaction conferring selectivity towards the β1AR.

During investigations into the nature of the *para*-substituent, insight was gained into the property of ISA displayed by many molecules. It appears this may be linked to the presence of a heteroatom, such as oxygen or nitrogen attached to the *para*-position of the ring.[98] Such a heteroatom may be able to emulate the ligand-receptor interaction possible with catecholamine agonists **1**, **2** and **3**, and known partial agonists such as **29**.[98] Further evidence supporting this lies in the activity of two particular analogues of **30**. These compounds had the same structure as **30**, but were fluorinated in either the *ortho*- or *meta*-position relative to the oxypropanolamine backbone.[98] The *ortho*-compound retained ISA, whereas the *meta*-compound was devoid of ISA. It would appear that the powerful electron-withdrawing effect of the fluorine atom in closer proximity to the *para*-substituent, is able to reduce the ability of the nearby oxygen atom to interact with the receptor and produce agonist activity.[98]

This role of the heteroatom in the *para*-position is further substantiated when a methylene spacer is inserted, thus removing it from direct attachment to the ring. Such modifications result in molecules with much reduced or no ISA (**33** and **34**).[99, 107]

1.5.1.4 Replacement of the aromatic group

The ability of the β1AR to accommodate change to the type of aromatic group present in the core arylethanolamine or aryloxypropanolamine motif, is evident from the range of available β-blockers. The aromatic group can be varied from phenyl (as in betaxolol **15**, bisoprolol **16** and celiprolol **17**) to naphthyl (propranolol **7**), carbazolyl (carvedilol **27**), indolyl (pindolol **25**) and thiodiazolyl[108] (timolol **26**) to name but a few.

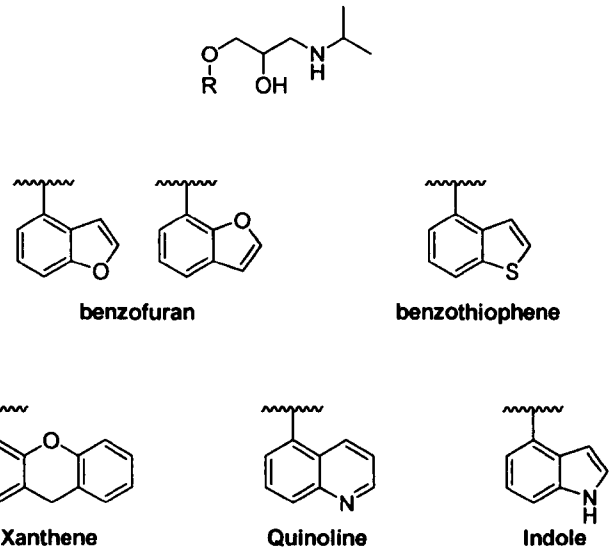


Figure 1-23: Analogues of propranolol bearing heterocyclic ring systems in exchange for the naphthalene group

Exchange of the naphthalene ring of **6** and **7**, with a variety of heterocyclic and non-heterocyclic ring systems, resulted in compounds with similar potency to the parent compounds.[67, 109-111] In the case of propranolol analogues, those where the oxypropanolamine was adjoined in the α-position were more potent (**Figure 1-23**).[110]

Attempts to incorporate larger aromatic or bulkier groups (anthracene, phenanthrenes, *N*-benzylindole), were reported to cause reduced potency.[112] Although anthracene and xanthene are of a similar size, the aromatic nature of anthracene confers coplanar geometry to the carbon atoms in the ring. In comparison, xanthene does not have a planar structure, whereas the parent naphthalene does. This indicates, that larger planar structures such as tricyclic aromatics may not be tolerated well at the β1AR, where as the kinked structure of xanthenes and the smaller naphthalene are. Additionally, the presence of a heteroatom seems important as both **24** and **27** (bearing a carbazole nucleus), are high affinity ligands for the β1AR[80, 81], however the naphthalene structure of **7** bears no such heteroatom.

Overall it appears a combination of factors is important regarding interaction with the receptor, including size and shape of the group, as well as heteroatom presence. These comparisons rely on the

assumption that a common binding orientation is shared between these molecules, which may not be the case. Without detailed computational work into the nature of the active site using x-ray crystal structures or accurate homology models, a more conclusive interpretation of the data is not possible.

1.5.2 Oxymethylene unit

Arylethanolamines such as **5** and **6**, were essentially derived from the natural catecholamine structures of **1** and **2**. Attempts to remove the carcinogenic activity of **6** led to the discovery of the aryloxypropanolamine class of molecules and ultimately **7**.[60] The aryloxypropanolamines as a class, redefined the core structure of β1AR antagonists, and are the basis for most of the clinically successful molecules developed since **7**.

In comparison to the arylethanolamines, the aryloxypropanolamines bear an oxymethylene linker unit between the aromatic and hydroxyl groups (**Figure 1-24**).

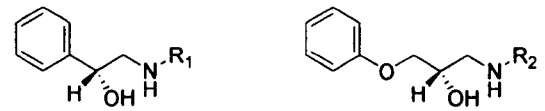
Chain extension studies involving the arylethanolamine, where extra methylene units were inserted adjacent to the aromatic ring to generate arylpropanolamine, arylbutanolamine, arylpentanolamine and aryl hexanolamine analogues have also been conducted.[113] The most potent antagonists were the arylbutanolamines (isosteric to the aryloxypropanolamines).[113] Furthermore, conversion of the arylbutanolamine to the corresponding aryloxypropanolamine (by replacing the methylene adjacent to the aromatic ring with an oxygen atom) resulted in even more potent antagonists.[113, 114]

The oxymethylene group seems optimal as a linker. Insertion of a further methylene unit to form the oxybutanolamine analogue of **7** caused a total loss of activity.[60]

1.5.3 The asymmetric carbon

Inherent to the general alkanolamine structure is a chiral centre at the carbinol. Resolution of the *R* and *S* enantiomers of a variety of

arylethanolamine-based and aryloxypropanolamine-based β1AR antagonists, and their subsequent testing has been carried out in several studies[74, 98, 115, 116]. Results show in each case that only one of the stereoisomers holds the majority of the antagonist activity at the β1AR.



R-arylethanolamine *S*-aryloxypropanolamine

Figure 1-24: General structure of active enantiomer of arylethanolamines and aryloxypropanolamines.

As is evident from the general structures (**Figure 1-24**) of the two classes of β1AR antagonist, both share the same spatial orientation at the chiral centre. The difference in nomenclature arises from the group assignment used in the standard Cahn-Ingold-Prelog rules for absolute configuration of chiral compounds.[117, 118]

In comparison to the *R*-arylethanolamines and *S*-aryloxypropanolamines, their respective enantiomeric counterparts need to be applied in much higher concentrations to see the same affects as β1AR antagonists.[74, 98, 115, 116, 119] In the case of propranolol **(7)**, the *S*-enantiomer is approximately 100-fold more active than the *R*-enantiomer.[116, 120] In other aryloxypropanolamines, the *S*-enantiomer has been reported as 33 to 530 times more active than the *R*-enantiomer, though different studies were carried out in different animal models.[120]

The naturally occurring catecholamine agonists **1** and **2** are also arylethanolamine-based, and share the same *R*-configuration at the chiral centre.[82] It therefore appears that the interaction with the β1AR is stereoselective.

The exception to the above trend is (*S,R,R,R*)-nebivolol **(18a)** as discussed in **section 1.3.4**. In this case the activity resides in the *R*-aryloxypropanolamine. An explanation for the apparent anomaly may lie in the rigid structure of **18a**, as the aryloxypropanolamine motif is

anchored into a chromane-based structure. The unusual pharmacology of nebivolol is further exemplified by studies demonstrating that vasodilatory effects are mediated by the (*R,S,S,S*)-enantiomer **18b**.[84]

Interestingly, the majority of β-blockers are clinically administered as the racemic mixture. The potential for the 'inactive' enantiomer to exert other pharmacological effects, as with **18b**, suggests a need to investigate these molecules independently to ascertain the appropriateness of administering a racemate.

1.5.4 The hydroxyl group

The importance of the free hydroxy group as a core component of the β1AR antagonist structure is evident from attempts to functionalise this moiety.

Replacement of the hydroxyl group of pronethalol **(6)** with isothiourea, thiol, amine, methylamine, and methoxy groups resulted in much reduced antagonist potency.[114] A similar reduction in potency was reported with the corresponding analogues of propranolol **(7)**.[114]

Conversion of the alcohol to an acetate ester or oxazolidine (with the amine group) also reduced the antagonist potency of these molecules.[92, 114]

Overall, the presence of the free hydroxyl appears essential for receptor interaction. This is likely to be due to a hydrogen bonding interaction in the receptor.[21]

1.5.5 Branching of the propanolamine

In general, the unbranched propanolamine backbone is the best-tolerated, and branching results in reduced antagonist ability.[121, 122]

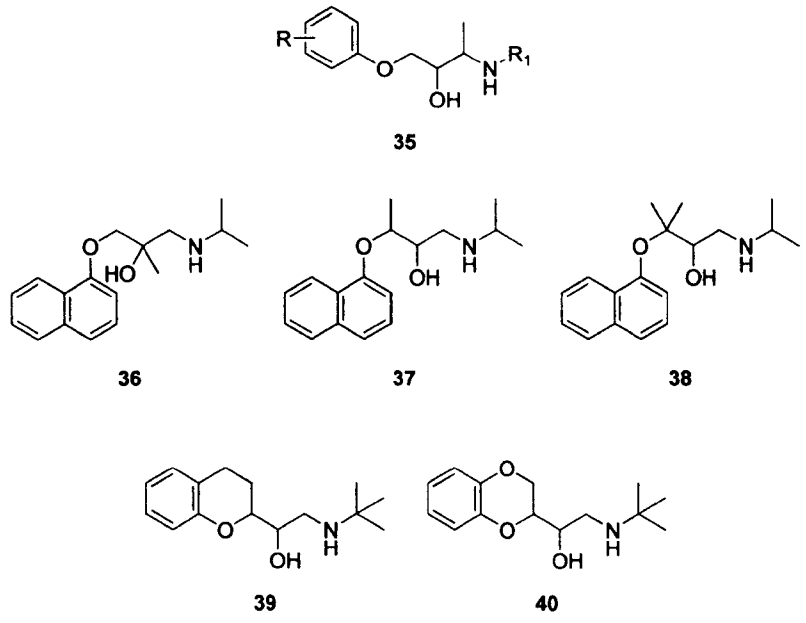


Figure 1-25: Examples of branching to the propanolamine backbone

35: General structure of aryl-substituted oxypropanolamines with methylation α to the nitrogen atom; 36: tertiary alcohol analogue of propranolol; 37 and 38: analogues of propranolol methylated adjacent to the naphthyloxy group; 39: chromane-based aryloxypropanolamine; 40: benzodioxane-based aryloxypropanolamine.

Investigations involving aryloxypropanolamine analogues bearing a methyl group in the α-position to the nitrogen atom were carried out (general structure **35**), with variation of the aromatic R-substituent and the *N*-alkyl group.[122] All analogues showed a reduction in antagonist potency relative to corresponding compounds lacking the α-methyl group; indeed many of the compounds were inactive.[122] Previous studies had shown that where R_1 is *tert*-butyl (**Figure 1-25**), potency is greater than the corresponding *iso*-propyl compounds (see **section 1.5.6.2**).[94, 96] However, insertion of the α-methyl group causes a total loss of activity with the *N-tert*-butyl compounds, indicating that excessive steric bulk in this region is not tolerated.[122]

Analogues of **7** bearing various methylations along the propanolamine backbone have also been synthesised (**36**, **37** and **38**).[121] Methylation of the carbon adjacent to the naphthyloxy group is better tolerated, though **37** and **38** remain poorer antagonists relative to **7**.[121]

A series of chromane **(39)** and benzodioxane **(40)** analogues showed that branching of the propanolamine backbone at the carbon adjacent

to the aryloxy group is tolerated.[123] In fact, in the case of benzodioxane **40**, antagonist potency was five to ten-fold that of **7**.[109, 123] Chromane **39** had similar activity to **7**. Overall, the benzodioxane compounds were five to ten-fold more potent than their corresponding chromane analogues.[123]

The raised potency of **40** relative to **7** could be due to several reasons. Firstly, ring-locking of the oxypropanolamine structure generates a more rigid structure, potentially holding the molecules in a favourable conformation in the receptor. Secondly, the benzodioxane-based oxypropanolamine emulates the structure of the general *ortho*-substituted aryloxypropanolamine, which is known to improve potency (see **section 1.5.1.1**).

Interestingly, the individual diastereoisomers were not isolated and individually tested in the chomanyl/benzodioxanyl study.[123] In light of the unusual activity of nebivolol **(18a)**, and its chromane-based structure, it seems prudent to re-evaluate the chromanyl and benzodioxanyl class of compounds using a more refined stereochemical approach.

1.5.6 Alkylation of the amine

1.5.6.1 Secondary amines

The amine group is thought to form an essential salt-bridge interaction with an acidic aspartate residue in the receptor.[16] The sensitivity of this interaction to modification of the amine is evident in numerous studies carried out, involving alkylation of the nitrogen to the tertiary amine.[60, 92, 108, 109, 124] The tertiary amine analogues were found to have much lower ability to act as β-blockers (measured as % inhibition of tachycardia in animal studies) than their secondary amine counterparts.[60, 92, 108, 109, 124] Doses required to elicit even low activity were often much higher, and in some cases the compounds were inactive.[60, 92, 108, 109, 124]

In addition the few examples of primary amine compounds synthesised also show poorer antagonist potency than secondary amine equivalents.[60, 109, 124]

The presence of a secondary amine appears to allow optimal interaction with the receptor. It may be the case that the more sterically hindered tertiary amines are less able to form this required interaction than their secondary amine analogues.

1.5.6.2 *N-iso*-propyl and *N-tert*-butyl derivatives

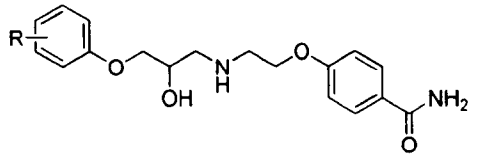
The *N-iso*-propyl group was initially found to confer selectivity towards β-receptors over α-receptors. In fact, it was the differential activity profile of **1** and **3** in different organs and vascular beds that prompted Ahlquist to suggest the existence of the two different classes of adrenoceptor.[4]

The majority of early compounds developed, and many that are currently clinically available bear either an *N-iso*-propyl or *N-tert*-butyl alkyl substituent. In comparison to a variety of *N*-alkyl groups, those that were branched with three or four carbons were found to offer better antagonist potency in general.[60, 67, 92, 101, 108, 110, 123, 124]

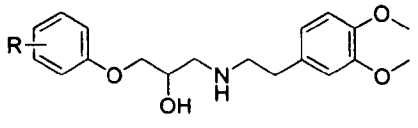
Generally the *N-tert*-butylated compounds display higher potency than the corresponding *N-iso*-propylated compounds.[94, 96] However the *N-iso*-propyl group may confer a higher degree of β_1/ β_2-selectivity.[93]

1.5.6.3 Extensive *N*-alkylations

The *N-iso*-propyl and *N-tert*-butyl groups were used extensively in the ICI research programme up until the early 1970s. Further investigations into the nature of the *N*-alkyl substituent revealed that an *N*-alkyl chain terminating in an aromatic ring often increased potency compared to the corresponding *N-iso*-propyl or *N-tert*-butyl derivatives,[124] though the extent of the increase may be assay-dependent[102].



N-(4-carbamoyl)phenoxyethyl



N-(3,4-dimethoxy)phenethyl

Figure 1-26: *N*-substituents terminating in an aromatic ring conferring improved cardioselectivity/potency

The *N*-(4-carbamoyl)phenoxyethyl[74] group and *N*-(3,4-dimethoxy)phenethyl[91, 100] groups were found to confer improved potency and β_1/ β_2-selectivity (**Figure 1-26**).[97]

The ether based linker in compounds with the general structure in **Figure 1-27** seems important as a determinant of cardioselectivity.[97, 125] Replacement of the oxygen atom with a variety of different groups offers a range of potencies and selectivities between receptors.[125, 126]

Where **X** is a sulphur atom compounds have reduced potency relative to the parent ethers, however sulfoxide-based compounds were similar in activity to the parent oxygen-containing compounds.[125] In comparison the sulfone-based analogues had very poor potency.[125]

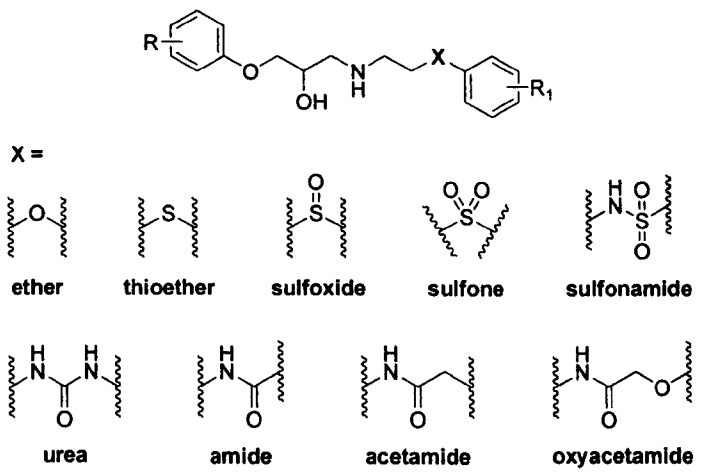


Figure 1-27: Variations in the group adjacent to the aromatic ring of *N*-aralkyl substituted aryloxypropanolamines

R and R_1 are either H or a variety of non-aromatic substituents (e.g. alkyl, halogen or heteroatom in nature).

In general, groups of the amido type (urea, amide, acetamide, oxyacetamide and sulfonamide) appear to improve potency and selectivity (**Figure 1-27**).[126, 127] Potency appears to be lower where the amide is linked directly to the aromatic ring, as benzamide-based compounds were reported to be less potent than the phenylacetamides, and phenoxyacetamides.[126]

Although the urea moiety was found to proffer relatively higher cardioselectivity than other amide-related isosteres, it was also found to be less potent.[126] The sulfonamide group appears to be tolerated, though trends are less easy to discern.[126]

In addition to their findings regarding amidic isosteres, Large and Smith[126] found the presence of an aromatic group attached to **X** is not absolutely necessary, and can be replaced with alkyl groups to give compounds with good potency and cardioselectivity.[126] Where an aromatic group was present, studies involving substituent R_1 were not extensive enough to draw strong conclusions.

The ethylene linker was found to be of optimal size in acting as a spacer between **X** and the nitrogen atom of the aryloxypropanolamine group. Additionally, branching at the α-carbon to the nitrogen with methyl or dimethyl groups (to give *iso*-propylene or *tert*-butylene linkers) was also found to improve potency.[124, 126]

1.6 Problems with current β-blocker therapy

Although there are many clinically available β-blockers, there is as yet no β1AR specific antagonist available.[2, 58] Many, such as **8**, **9**, **10**, **13**, **15**, **16**, **18a+b** and **29** are described cardioselective, but generally show less than 50-fold β_1/β_2-selectivity;[80, 81] with others such as **4**, **7**, **19**, **20**, **26** and **27** being more selective for the β2AR (see **Table 1-1**).[81] For example, atenolol **(9)** is an established clinically available drug, regarded as being cardioselective. *In-vitro* testing on human receptors shows it to have similar affinity for both the β1AR and β2AR, being only around 5-fold β_1-selective[81].

The relatively low cardioselectivity of currently available drugs, is thought to be lost at higher doses that are routinely used in patients[128]. This means there are unwanted effects arising from the concomitant blockage of β2ARs, such as bronchospasm (leading to compromised respiratory function), loss of peripheral vasodilatation (leading to cold extremities) as well as metabolic disturbances. Consequently, all current β-blocker therapy, even that described as cardioselective, is

contra-indicated in patients with asthma and peripheral vascular disease.[62]

Figures from the British Heart Foundation suggest that around 5.9% of the UK population (7.4% of men and 4.5% of women) suffer from coronary heart disease (including angina, heart failure and those that have suffered a myocardial infarction).[129] These are patients that are likely to require β-blocker therapy, but statistics do not take account those suffering from hypertension or any of the other conditions for which β-blocker therapy is indicated.

Overall, this means that patients that require regular β-blocker therapy may also experience adverse effects, due to concomitant β2AR blockade. In addition, patients suffering from both cardiovascular and respiratory diseases are unable to benefit from the potentially life-prolonging action of β-blockers.

1.7 Research aims

1.7.1 Selection of a lead molecule

Due to the afore-mentioned difficulties in designing ligands for GPCR targets (see **section 1.2.1**); an analogue-based approach was adopted.

This involved selection of an appropriate lead compound; namely an antagonist with a combination of high affinity for the β1AR and low affinity for the β2AR (i.e. good selectivity).

An evaluation of the literature reveals further drug development attempts that have yielded compounds with reportedly good cardioselectivity. Compounds such as **41-44**, are the products of research programs focussing on the development of the *para*-substituent, and *N*-alkyl substituent of the core aryloxypropanolamine structure (**Figure 1-28**).

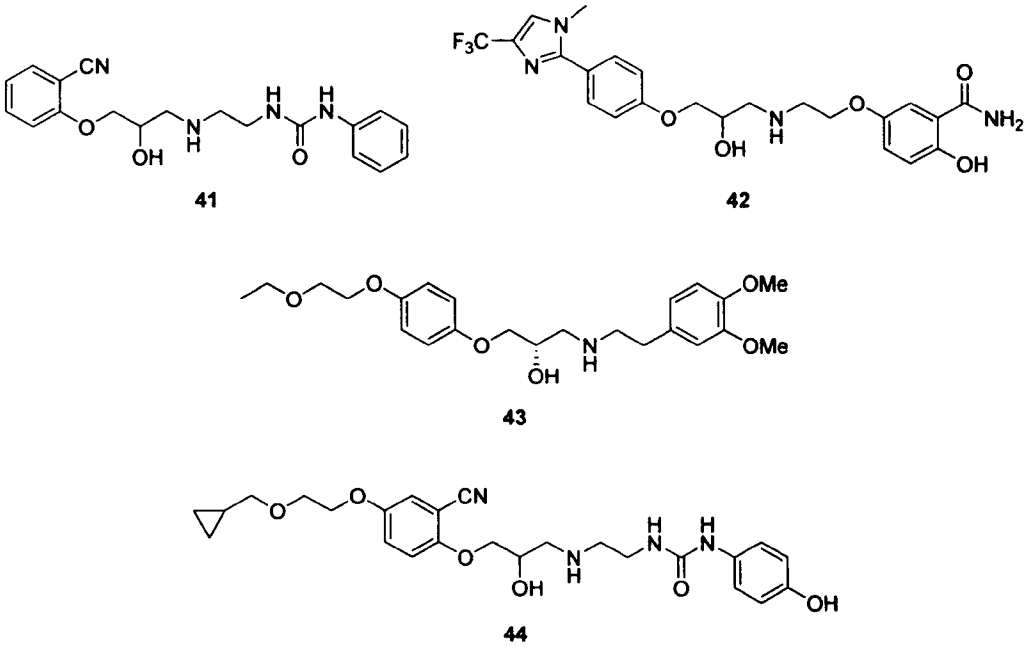


Figure 1-28: Compounds with high cardioselectivity

ICI 89406 (41); CGP 20712A (42); D140S (43); LK 204-545 (44).

The available data for binding affinities at the β1AR and β2AR and selectivity for the β1AR is displayed in **Table 1-2**. Binding data are based on displacement of a radioligand from human receptors transfected into Chinese hamster ovary cells.[105, 130, 131] In the case of **41** and **42**, ^{3}H-CGP 12177[130] was used as the radioligand, whereas *Louis et al* use ^{125}I-CYP[105, 131].

		Binding affinity, log K_D (M)		β_1/β_2 selectivity
		β_1-adrenoceptor	β_2-adrenoceptor	
41	ICI 89406	-8.91 ± 0.09	-7.07 ± 0.06	69
42	CGP 20712A	-8.81 ± 0.03	-6.11 ± 0.05	501
43	D140S	7.92 ± 0.01 (pK_i)*	**	**
44	LK 204-545	8.52 ± 0.12 (pK_i)*	5.27 ± 0.08 (pK_i)*	1778*

Table 1-2: Binding affinities and selectivity for human β-adrenoceptors of 41-44

Values for ICI 89406 and CGP 20712A from *Baker 2005*;[81] * values reported previously in the literature by Louis *et al*[105, 131]; ** no binding data to determine selectivity.

Due to lack of a β2AR binding affinity value for **43** in the literature, an accurate evaluation of its β_1/β_2-selectivity is not possible. However,

based upon functional *in*-vitro experiments on rodent atrial and tracheal preparations, values for potency (β1AR pA_2 = 8.15 ± 0.22, β2AR pA_2 < 4.5) are published to give β_1/β_2-selectivity > 4400.[132] In comparison, using the same pharmacological methods, the β_1/β_2-selectivity of **44** is quoted as 6300,[132] whereas for **42** it is 13183[105]. Based on this data, the selectivity of **43** at the human receptor is likely to be high, but still lower than **44**.

The *in-vitro* rodent model indicates compounds have a much higher selectivity than found at human receptors. This disparity raises the question of relevance and viability of previous studies that used other animals. However it appears that although the human and rodent β-receptors show differences in magnitude of binding affinity, the general trends are the same.[105, 131]

With the exception of **41**, the remaining compounds in **Figure 1-28** all conform to the general structural requirements outlined to confer cardioselectivity and potency for the β1AR (see **section 1.5**). These include *para*-substitution of the aryloxypropanolamine motif, with a heteroatom positioned two to four atoms from the ring, and alkylation of the amino group with a two carbon linker terminating in an aromatic ring.

In comparison to the other compounds, the combination of the ureido group, and a *para*-substituent to the oxypropanolamine backbone bearing an ethylene glycol based linker on **44**, seems to provide the best balance of high β_1/β_2-selectivity and good potency.

Further evidence for the importance of the ethylene glycol moiety is demonstrated when the classical *N*-isopropyl-propanolamine is retained (i.e. no extended aromatic N-alkyl group), and only the para-alkyl group is modified. With these compounds, the ether or diethylene glycol linkage to a bulky cyclic or aromatic structure, consistently resulted in augmented β_1/β_2 selectivity in the rodent model when compared to various other groups.[106]

Based on available data, and comparison to other molecules published in the literature, **44** was selected as a suitable lead compound.

1.7.2 Aims

As discussed above (see **section 1.6**), the low β_1/β_2-selectivity of clinically available β-blockers means their use in several disease states is contraindicated. In addition, they possess a number of adverse effects which are mediated by concomitant blockade of the β2AR.

This project aims to generate molecules with a much improved β_1/β_2-selectivity, using **44** (with reported high potency and selectivity) as a lead compound.

Additionally, the problems associated with designing ligands for GPCR targets (see **section 1.2.1**), means there is much to be learnt regarding interactions at the active site of the β1AR. As such, a systematic approach to drug design will undoubtedly improve understanding of the nature of the ligand-receptor interaction at the β1AR.

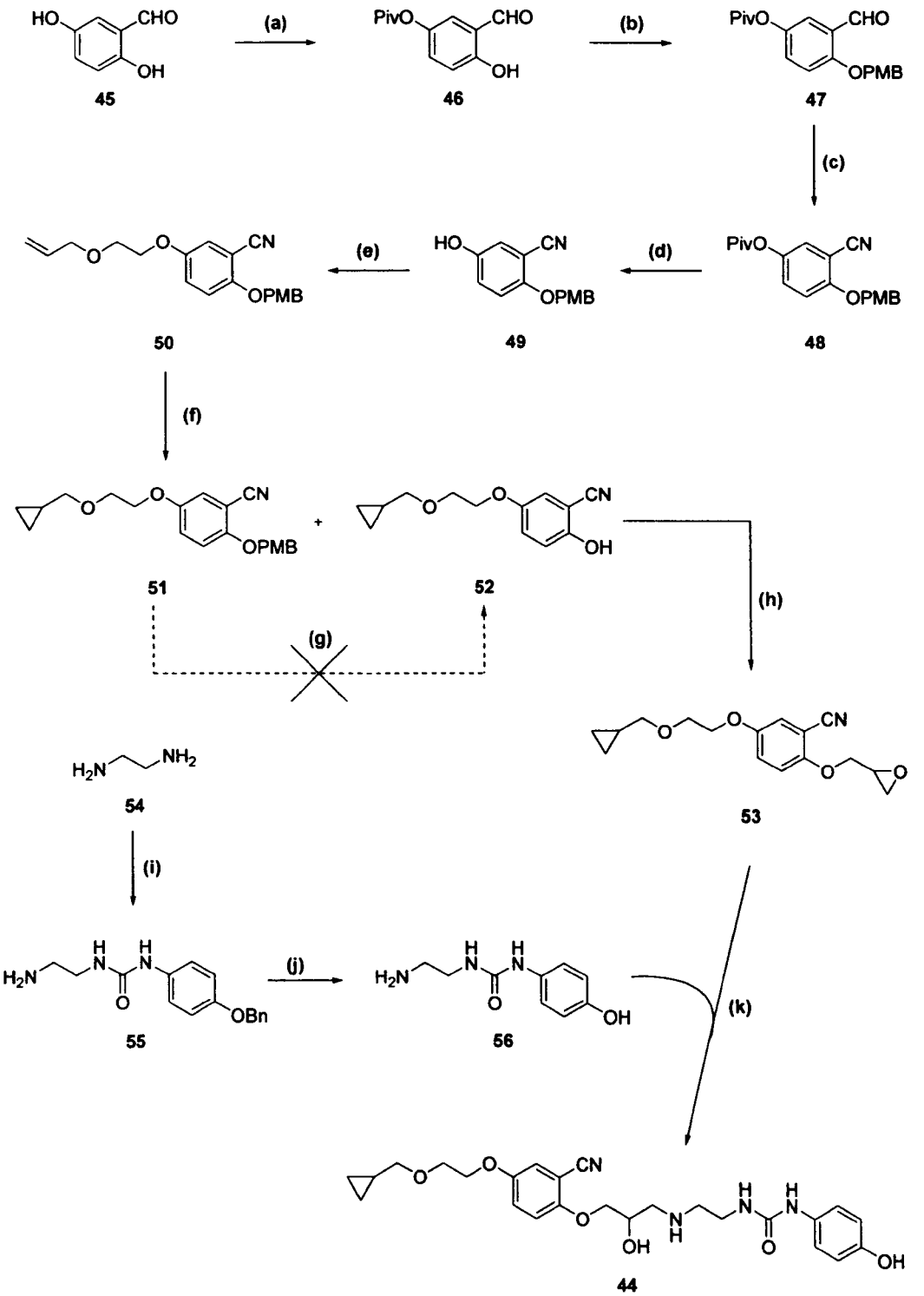
The following chapters report the approach adopted in modifying various motifs on the structure of **44**, the synthetic route adopted, pharmacological data (provided by Dr Jillian G Baker) and a description of new trends and SAR.

2. SYNTHESIS AND EVALUATION OF LK 204-545

The reported high β1AR affinity and β_1/β_2-selectivity of LK 204-545 **(44)**, rationalised its selection as a lead compound.[131] Due to limited available data on the synthesis and analysis of **44** in the literature, it was prudent to synthesise the described molecule, and establish an independent activity profile of this compound. Not only did this define the benchmark pharmacological profile of the lead compound using our own pharmacological techniques, but also allowed evaluation of the general synthetic route encountered with this type of molecule.

2.1 Synthetic route employed

The synthesis of LK 204-545 is not described in the literature, however the structure of the final compound and pharmacological activity are.[131, 133] A synthetic route to the related compound,1-(2-cyano-4-(2-cyclopropylmethoxyethoxy)phenoxy)-3-(2-(3-phenylureido)ethylamino)-2-propanol, is broadly described as an example of the class in patent literature[133, 134], however this involves introduction of the cyano group using cuprous cyanide. Rather than engaging in a potentially hazardous cyanide addition, alternative synthetic pathways were investigated. Since 2,5-dihydroxybenzonitrile was not commercially available, suitable functional group interconversion was considered. The ability to easily convert an aryl aldehyde to its corresponding nitrile,[135] allowed 2,5-dihydroxybenzaldehyde **(45)** to be selected as an appropriate starting material (Scheme 2-1).



Scheme 2-1: Synthesis of LK 204-545 (44).

Reagents and conditions: (a) triethylamine (TEA), pivaloyl chloride, *N,N*-dimethylformamide (DMF), 0 °C → rt, 72%; (b) i. NaH, DMF; ii. *p*-methoxybenzyl bromide, 0 °C → rt, 33%; (c) 37% $NH_{3\,(aq)}$, iodine, tetrahydrofuran (THF), 98%; (d) sodium *tert*-butoxide, methanol (MeOH), 34%; (e) allyloxyethanol, diisopropyl azodicarboxylate (DIAD), PPh_3, dichloromethane (DCM), 100%; (f) diethyl zinc, diiodomethane, toluene 0 °C → rt, 29% **51** and 25% **52**; (g) ceric ammonium nitrate, water, acetonitrile (MeCN); (h) TEA, *rac*-epichlorohydrin, 80 °C, 100%; (i) 4-(benzyloxy)phenylisocyanate, DCM, 94%; (j) i. concentrated HCl; ii. 2M $NaOH_{(aq)}$, neutralisation, 73%; (k) propan-2-ol, reflux, 2%.

Literature procedure for the selective mono-protection of the 5-hydroxy group of **45**, using pivaloyl chloride to generate pivaloate ester **46**, was followed.[136] The regioselective formation of the pivaloate ester, is possible by attack of the less encumbered 5-hydroxy group on the sterically hindered carbonyl group of pivaloyl chloride.

The remaining hydroxy group then required orthogonal protection to the pivaloate ester, and the *para*-methoxybenzyl (PMB) group was selected for this purpose. The relatively low yield of **47** obtained, may be a reflection of the poorly nucleophilic hydroxy group *ortho* to the aldehyde.

The ability to cleave a PMB ether under oxidative conditions, using agents such as ceric ammonium nitrate (CAN)[137] was anticipated, since this allowed selective removal of the group in the presence of the nitrile group later on. When the equivalent synthesis was attempted using the benzyl ether in place of PMB, hydrogenolysis of the benzyl group concomitantly reduced the nitrile to the corresponding primary amine (confirmed by MS and TLC analysis).

Once the orthogonally protected pivaloate ester **47** was obtained, conversion from the benzaldehyde to the corresponding benzonitrile **48** was achieved cleanly in near quantitative yield. This reaction is believed to proceed via oxidation of the intermediate imine.[135, 138]

After deprotection of the pivaloate ester, the corresponding phenol **49** underwent Mitsunobu coupling with 2-(allyloxy)ethanol to afford **50**. Cyclopropanation of **50** using Simmons-Smith chemistry[139] gave a mixture of products. The desired PMB-protected product **51** was isolated and subjected to CAN-mediated oxidative PMB group cleavage. Unfortunately, this did not yield the desired phenol **52**, possibly due to instability of the molecule to CAN, or oxidative dealkylation of the aralkyl ether[140]. Re-examination of products from the previous Simmons-Smith cyclopropanation revealed that these conditions had serendipitously caused partial deprotection of the PMB group. Consequently, phenol **52** was recovered, albeit as a side-product and in low yield.

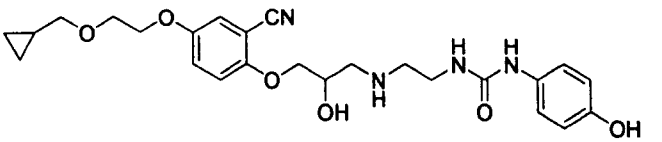
Alkylation of phenol **52** was conducted using TEA as a hindered base, and refluxing in *rac*-epichlorohydrin to obtain epoxide **53**. This was used without further purification.

Reaction of ethylenediamine **(54)** with 4-(benzyloxy)phenylisocyanate delivered the benzyl-protected compound **55**. Poor solubility of **55** in most solvents meant standard Pd(0) catalytic hydrogenolysis progressed very slowly. Consequently, benzyl deprotection was achieved cleanly in concentrated (conc) HCl to give aminophenol **56** after neutralisation.

LK 204-545 **(44)** was obtained by refluxing epoxide **53** and amine **56** in propan-2-ol.

2.2 Pharmacology data

Radioligand binding studies were carried out by Dr. Jillian Baker (Institute of Cell Signalling, Queens Medical Centre, Nottingham) on Chinese hamster ovary (CHO-K1) cells stably expressing either human β_1 or β_2-adrenoceptors.[27, 81, 141] Binding affinities were determined by competitive displacement of ^3H-CGP 12177.



	Binding affinity, log K_D (M)		β_1/β_2 selectivity
	β_1-adrenoceptor	β_2-adrenoceptor	
Literature data	8.52 ± 0.12 (pK_i)*	5.27 ± 0.08 (pK_i)*	1778*
Experimental data	-8.09 ± 0.04**	-5.20 ± 0.03**	776**

Table 2-1: Human β_1 and β_2-adrenoceptor binding affinities and receptor selectivity of LK 204-545 (44).

**$n \geq 8$ for all assays. * values reported previously in the literature by Louis et al[131]. The pK_i value is comparable to the log K_D with inversion of sign.

The K_D is the dissociation constant for the ligand and gives a measure of receptor affinity. This corresponds to the concentration of applied ligand which displaces 50% of specifically bound radioligand. High

affinity ligands will displace the radioligand at lower concentrations, giving larger, negative log K_D values.

Table 2-1 shows the binding profile of **44** at the β1AR and β2AR. Although a very high affinity ligand for the β1AR, with excellent selectivity, the β1AR quoted in the literature is somewhat higher.[131] This is likely to be due to experimental differences. In-house radioligand binding assays conducted by Dr. Jillian Baker use ^{3}H-CGP 12177 as the radioligand, whereas Louis *et* al use ^{125}I-CYP[131]. According to the cubic ternary complex model of receptor-ligand interaction (see **Section 1.2.2.1**), different ligands are able to stabilise different conformations of the receptor. Thus, if ^{3}H-CGP 12177 and ^{125}I-CYP are stabilising different receptor conformations, the ligand applied in the assay (in this case **44**), may possess different affinity for each stabilised conformation. Overall, the different assays might give different binding affinities for the same ligand.

The differing results obtained, highlight the value in the re-evaluation of LK 204-545 using in-house pharmacology. With this data, a more accurate, experimentally relevant comparison with further analogues can be made.

The more hydrophilic nature of ^{3}H-CGP 12177 compared to ^{125}I-CYP, means it is more suitable as a radioligand in binding assays with respect to much reduced non-specific binding.[142] Additionally, ^{3}H-CGP 12177 is considered a much safer radioligand than ^{125}I-CYP, and is also more practical to use with regards to the extra safety precautions required when using ^{125}I-CYP.

2.3 Modifications to the core LK 204-545 (44) structure as a lead compound

Synthesis of **44** was achieved in poor yield over multiple steps. The need for orthogonal protecting groups extended the synthesis. With the need to generate many analogues in a parallel synthetic manner, the complications afforded by the cyano group were deemed unreasonable.

Removal of the cyano group provided a more facile synthetic route with fewer steps and less prevalent use of protecting groups. Additionally, *ortho* substitution of the aryloxypropanolamine structure is known not to be essential for binding to the β1AR, though may modulate it[92, 126, 143]. Finally, the synthesis of these types of molecules would fall into previously unexploited chemical space.

Previous studies involving *para*-substituted *N*-isopropylphenoxypropanolamines[106] compared several motifs for β1AR and β2AR affinity. Of these *para*-substituents, the 2-(cyclopentyloxy)ethoxy- group was found to have comparatively higher affinity for the β1AR compared to the 2-(cyclopropylmethoxy)ethoxy group present in LK 204-545. Synthesis of this group was also reported as a one step reaction with reasonably high yield.[144]

Overall, our new target lead pharmacophore was 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea **(57)**; illustrated in **Figure 2-1**.

2.4 Areas for investigation on lead compound

The new lead pharmacophore was designed to be amenable to a more parallel synthetic approach. Modifications to several key motifs on the core pharmacophore were planned in order to build up clear structure activity relationships.

As previously described (see **Section 1.5**), the core aryloxypropanolamine structure is essential for binding to the β1AR. However, modifications to other areas of this particular pharmacophore have not been systematically investigated.

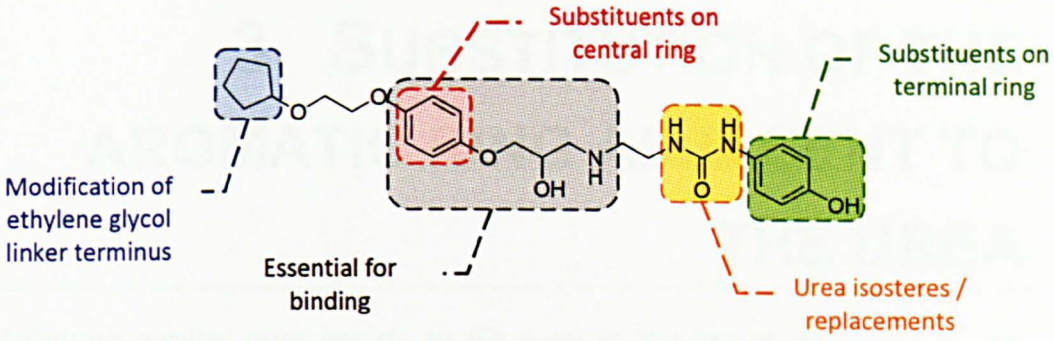


Figure 2-1: Areas for investigation on core pharmacophore: 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea (57).

The modifications described in **Figure 2-1** will be detailed in the following chapters. These include modifying the nature and position of substituents on the aromatic ring adjacent to the urea (**Chapter 3**), investigation of urea isosteres or replacement groups (**Chapter 4**), modification of the ethylene glycol linker terminus and varying the substituents on the central ring (**Chapter 5**).

3. SUBSTITUTION OF THE AROMATIC RING ADJACENT TO THE UREA

Although similar compounds to **57** exist in the literature[131, 133, 134], an investigation into substitution of the aromatic ring adjacent to the urea, had not previously been carried out in a logical or systematic fashion.

3.1 Principles behind synthetic strategy

Taking **57** (derived from LK 204-545, **Chapter 2**) as an initial target compound, it was envisaged that a series of analogues with differing substituents on the aromatic ring adjacent to the urea, could be synthesised with relative ease.

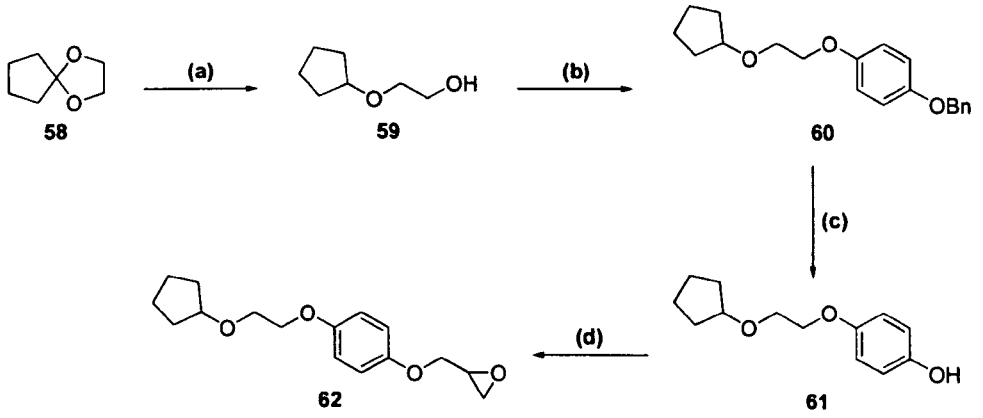
The classical route to aryloxypropanolamines involves aminolysis of the corresponding glycidyl aryl ether[59] in either neutral or basic conditions, to effect opening of the epoxide via the least hindered carbon.[145] On this basis, the strategy employed required separate syntheses of the epoxide and amine fragments, before a final convergent epoxide opening step.

3.2 Phenyl mono-substituted compounds

In order to evaluate the positional, steric and electronic effects of ring substituents, it was necessary to compare a range of *ortho-*, *meta-* and *para-* derivatives. Accordingly, the methyl, methoxy, fluoro, chloro, bromo, trifluoromethyl and hydroxy analogues were all synthesised alongside the unsubstituted phenyl urea.

3.2.1 Synthesis of the epoxide fragment

Generation of epoxide **62** was relatively straightforward, and high yielding, via four steps from commercially available starting materials (**Scheme 3-1**).



Scheme 3-1: Synthesis of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane (62).

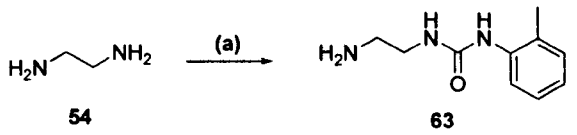
Reagents and condition: (a) $NaBH_4$, $ZrCl_4$, THF 0 – 5 °C, 81%. (b) PPh_3,4-(benzyloxy)phenol, di-*tert*-butyl azodicarboxylate (DBAD), DCM, 75%; (c) H_2, 10% Pd/C, ethanol (EtOH), 100%; (d) i. NaH, DMF, 0°C → rt; ii. *rac*-epichlorohydrin, 84%.

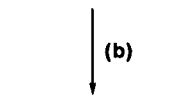
Reductive cleavage of cyclopentanone ethylene ketal **58** to 2-(cyclopentyloxy)ethanol **59** was achieved via literature procedures.[144] The alcohol **59**, was then coupled directly to 4-benzyloxyphenol using Mitsunobu chemistry.[146] DBAD was selected as the azodicarboxylate for reported acid lability and subsequent ease of removal on workup.[147-149] The benzyl (Bn) protected phenol **60** was subjected to standard hydrogenolysis conditions over Pd(0) to yield phenol **61**. Finally, formal deprotonation of **61** with NaH, followed by treatment with excess *rac*-epichlorohydrin, gave the desired 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **62**.[132]

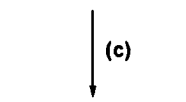
3.2.2 Synthesis of the amine fragments

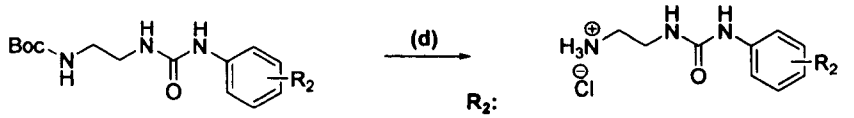
The majority of 1-(2-aminoethyl)-3-phenylureas were obtained from the corresponding substituted phenylisocyanates. Initially, direct reaction of *o*-methylphenylisocyanate with ethylenediamine was attempted (**Scheme 3-2**). This method required drop-wise addition of a solution of the reactive isocyanate to ethylenediamine at 0°C. Although the phenylurea **63** was obtained in moderate yield, formation of the *bis*-urea was also observed, requiring further purification. In comparison, the use of 4-benzyloxyphenylisocyanate (see chapter 2, **Scheme 2-1**) in a similar manner was much more selective, and the desired product **55**

was produced almost quantitatively. The lack of *bis*-urea formation in the latter example may be explained by steric hindrance from the benzyl group, and the associated difficulty of **55** in attacking a second molecule of 4-benzyloxyphenylisocyanate.









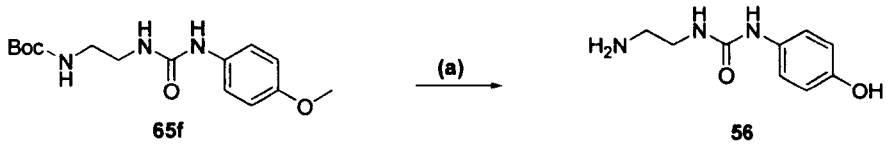
R_2:			R_2:		
65a = H	**65b** = *m*-CH_3	**65c** = *p*-CH_3	**66a** = H	**66b** = *m*-CH_3	**66c** = *p*-CH_3
65d = *o*-OCH_3	**65e** = *m*-OCH_3	**65f** = *p*-OCH_3	**66d** = *o*-OCH_3	**66e** = *m*-OCH_3	**66f** = *p*-OCH_3
65g = *o*-F	**65h** = *m*-F	**65i** = *p*-F	**66g** = *o*-F	**66h** = *m*-F	**66i** = *p*-F
65j = *o*-Cl	**65k** = *m*-Cl	**65l** = *p*-Cl	**66j** = *o*-Cl	**66k** = *m*-Cl	**66l** = *p*-Cl
65m = *o*-Br	**65n** = *m*-Br	**65o** = *p*-Br	**66m** = *o*-Br	**66n** = *m*-Br	**66o** = *p*-Br
65p = *o*-CF_3	**65q** = *m*-CF_3	**65r** = *p*-CF_3	**66p** = *o*-CF_3	**66q** = *m*-CF_3	**66r** = *p*-CF_3

Scheme 3-2: Synthesis of phenyl mono-substituted 1-(2-aminoethyl)-3-(phenyl)ureas derived from ethylenediamine (63, 66a-r).

Reagents and conditions: (a)*o*-tolyl phenylisocyanate, DCM, 0 °C → rt, 64%; (b) di-*tert*-butyl dicarboxylate (Boc_2O), DCM, 87%; (c) phenyl-substituted phenylisocyanate, DCM, 0 °C → rt, 46-95%; (d)MeOH/conc HCl, 80-100%.

Due to the problems encountered in the synthesis of **63**, the mono-Boc protection of ethylenediamine **(54)**[150] was employed to offer improved selectivity when reacting with the phenylisocyanates. As anticipated, stoichiometric addition of a range of substituted phenyl isocyanates to **64**, followed by addition of hexanes to the reaction mixture caused precipitation of the Boc-protected substituted phenylureas **65a-r** (**Scheme 3-2**), which required no further purification. Finally Boc deprotection was achieved cleanly in a mixture of methanol and conc

HCl to give the hydrochloride salts of the mono-substituted 1-(2-aminoethyl)-3-phenylureas **66a-r**.

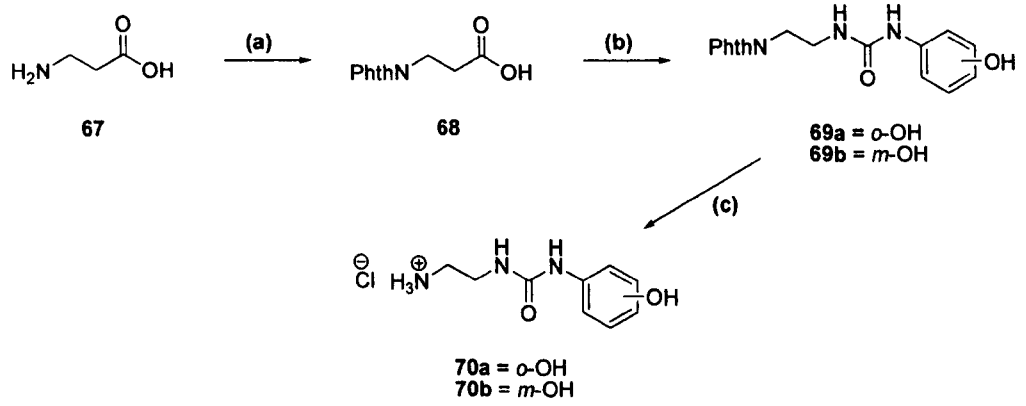


Scheme 3-3: Alternative synthesis of 1-(2-aminoethyl)-3-(4-hydroxyphenyl)urea (56).

Reagents and conditions: (a) BBr_3, DCM, -78°C → rt, 100%.

It was envisaged that **65f** could be used to give a cheaper and more efficient route to **56**. Indeed treatment of **65f** with BBr_3[151], produced **56** in quantitative yield in a single step (**Scheme 3-3**).

Lack of the appropriate isocyanate starting materials, necessitated an alternative strategy for synthesis of the *ortho*- and *meta*-hydroxy substituted derivatives. In these examples, it was thought that the readily available 2- and 3-aminophenols could be used to attack the appropriate aliphatic isocyanate to give the desired urea compounds. With this in mind, synthesis was possible using β-alanine **(67)**, as a starting material (**Scheme 3-4**).



Scheme 3-4: Synthesis of *ortho*- and *meta*-hydroxy substituted 1-(2-aminoethyl)-3-(hydroxyphenyl)urea hydrochlorides (70a-b).

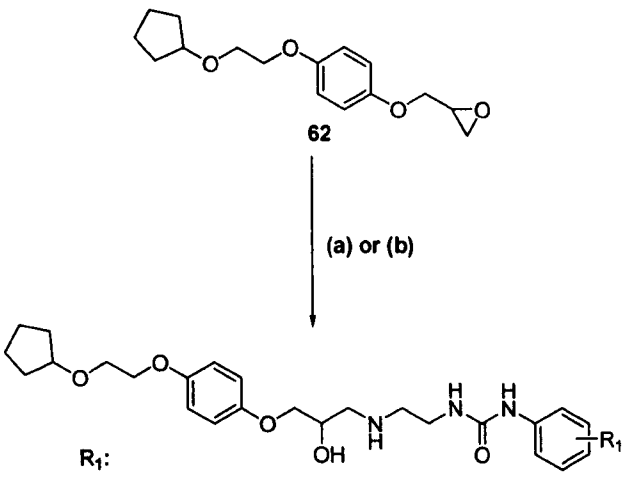
Reagents and conditions: (a) phthalic anhydride, 150 °C, 94%; (b) i. diphenylphosphoryl azide (DPPA), TEA, toluene; ii. Reflux; iii. 2-aminophenol or 3-aminophenol, 75-76%; (c) i. hydrazine monohydrate, EtOH, reflux; ii. acidic workup 51-60%.

The phthalimide protecting group was selected to eliminate the nucleophilic nature of the nitrogen atom, thus preventing potential side reactions during isocyanate formation. As has previously been demonstrated with groups such as Boc, the mono-acylated amine still has a propensity to attack the isocyanate during Curtius rearrangement.[152] Overall, this leads to the undesirable formation of the cyclic Boc-protected urea side-product, and necessitates use of *bis*-protected amine (as is present with phthalimide).[152]

Protection of **67** was achieved with ease through a literature procedure to give **68**.[153] Using DPPA in basic conditions, **68** was converted to the corresponding acyl azide before careful reflux to facilitate Curtius rearrangement to the isocyanate (not isolated).[154] Once evolution of nitrogen had ceased, either 2- or 3-aminophenol was added to produce the corresponding hydroxyphenylureas **69a-b**. The desired amines **70a-b**, were obtained as their hydrochloride salts, by standard hydrazinolysis of the phthalimide group followed by acidic workup (**Scheme 3-4**).

3.2.3 Epoxide opening of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane (62)

Opening of epoxide **62** was effected by refluxing with the appropriate amine in propan-2-ol.[143, 155, 156] Where the amines were present as hydrochloride salts, a slight excess of NaOH was added (**Scheme 3-5**). Yields were typically poor, due to attack on the epoxide by the initial aryloxypropanolamine product. This is not generally a problem in the synthesis of most β-blockers due to isopropylamine or *tert*-butylamine being used to open the epoxide.[59] These low boiling amines are used as the reaction solvent, and are present in such a vast excess, that excessive alkylations are suppressed.



R_1:

71a = H		
71b = *o*-CH_3	**71c** = *m*-CH_3	**71d** = *p*-CH_3
71e = *o*-OCH_3	**71f** = *m*-OCH_3	**71g** = *p*-OCH_3
71h = *o*-F	**71i** = *m*-F	**71j** = *p*-F
71k = *o*-Cl	**71l** = *m*-Cl	**71m** = *p*-Cl
71n = *o*-Br	**71o** = *m*-Br	**71p** = *p*-Br
71q = *o*-CF_3	**71r** = *m*-CF_3	**71s** = *p*-CF_3
71t = *o*-OH	**71u** = *m*-OH	**57** = *p*-OH

Scheme 3-5: Synthesis of phenyl-substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)ureas (71a-u, 57).

Reagents and conditions: (a) **56/63**, propan-2-ol, reflux, 29-31%; (b) **66a-r**, **70a-b**, NaOH, H_2O, propan-2-ol, reflux, 7-29%.

3.2.3.1 Confirmation of epoxide aminolysis by hydroxyphenyl urea compounds

Although relatively less nucleophilic than the primary amine, the phenolic groups present in **56** and **70a-b** have the potential to compete with the amino moiety on the molecules during epoxide opening. Taking **57** as a case study, ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC) and ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC) experiments were carried out to confirm that aminolysis of the epoxide had actually taken place (**Figure 3-1**, **Figure 3-2**).

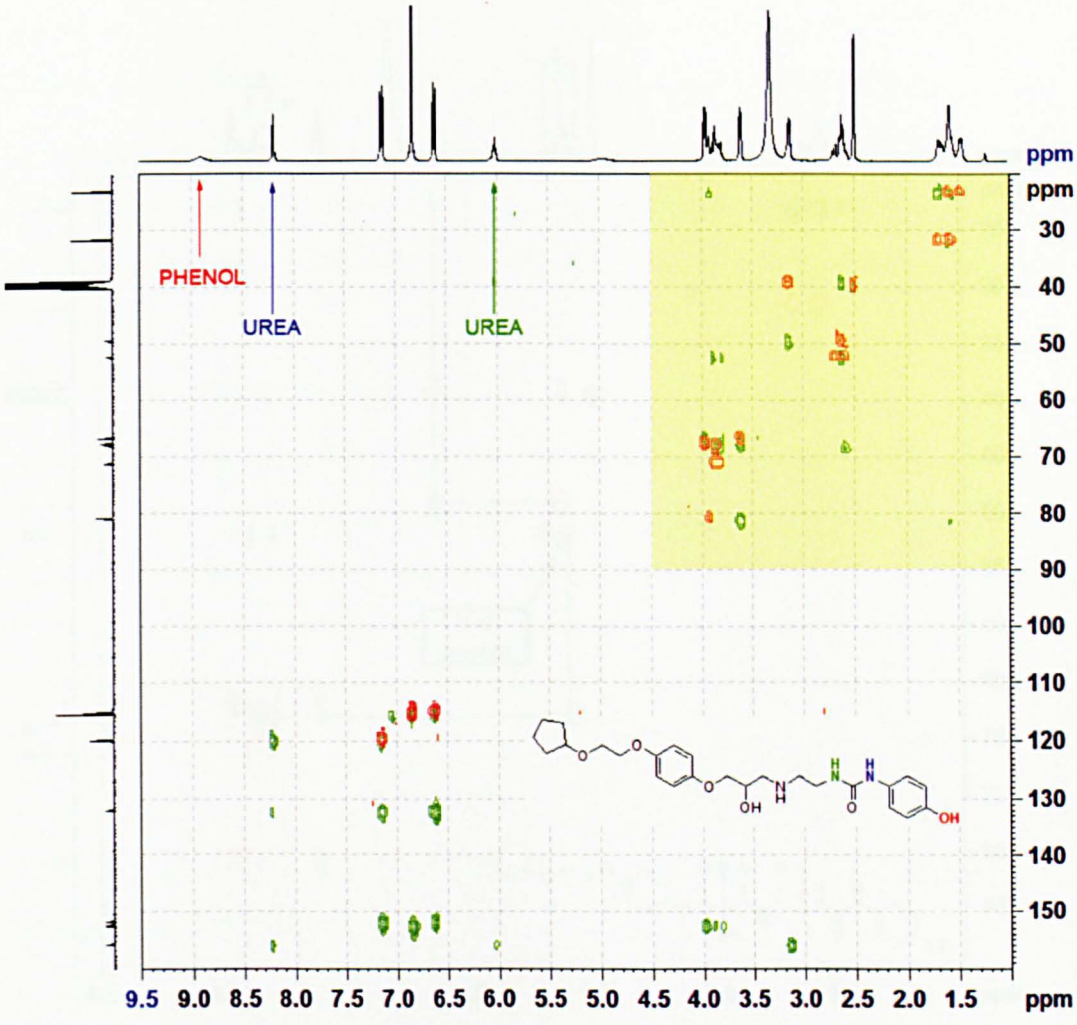


Figure 3-1: Full ^{1}H-^{13}C HSQC/HMBC nuclear magnetic resonance spectroscopy (NMR) experiment overlay of 57 in deuterated dimethyl sulfoxide (DMSO-d_6).

HSQC data is shown in red, HMBC data is overlaid in green. The relevant ^{1}H proton and ^{13}C carbon experiments are displayed on the x- and y-axes respectively. Phenol and urea proton peaks have been highlighted. The yellow section is magnified in Figure 3-2.

The presence of the low-field phenolic proton at 8.91ppm (**Figure 3-1**) and the chemical shift of the diastereotopic 'C' protons (2.56 - 2.71 ppm, **Figure 3-2**) indicate that these protons are adjacent to a nitrogen atom (rather than an oxygen atom), thus aminolysis has occurred. In addition, if the coupling path of the 'E' protons (3.12 – 3.16 ppm, **Figure 3-2)** is followed, there is clear coupling back through the secondary amine to the 'B' proton.

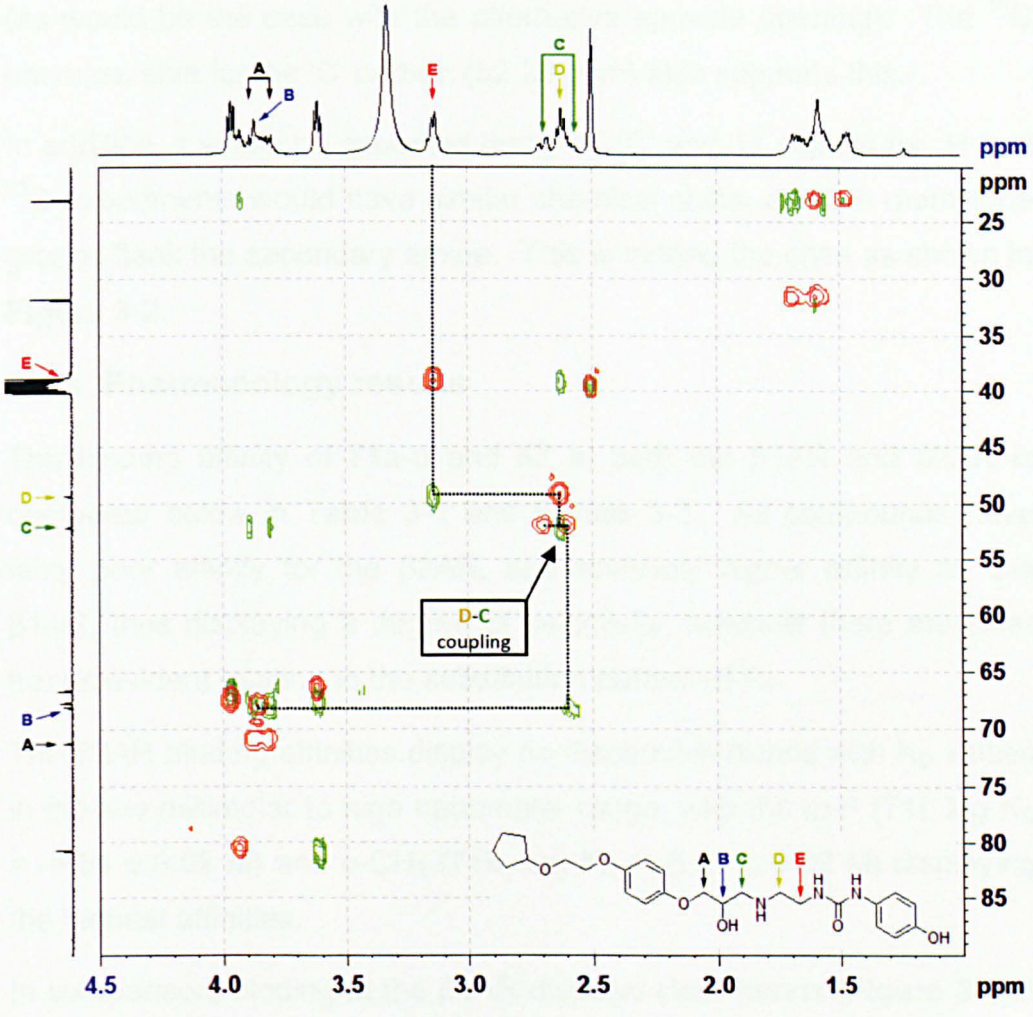


Figure 3-2: ^{1}H-^{13}C HSQC/HMBC NMR experiment overlay of 57 in DMSO-d_6 – magnification of aliphatic region.

Spectral assignment carried out using ^{1}H-^{1}H correlation spectroscopy (COSY) and ^{1}H-^{13}C HSQC experiments. HSQC data is shown in red, HMBC data is overlaid in green. The relevant ^{1}H proton and ^{13}C carbon experiments are displayed on the x- and y-axes respectively. Overlaid spectra clearly display a coupling route from methylene E to methine B; demonstrating aminolysis of the epoxide **62**. The key coupling between 'D' protons and the 'C' carbon is highlighted.

3.2.3.2 Confirmation of core aryloxypropanolamine structure

It is widely accepted that nucleophilic opening of epoxides under neutral or basic conditions proceeds via the least hindered carbon to give the secondary alcohol.[145, 157-159] This configuration of the aryloxypropanolamines was present in all compounds synthesised. Evidence for regioselective opening can be taken from ^{1}H-^{13}C HSQC NMR experiment of **57** (**Figure 3-2**). The 'C' protons at 2.56 – 2.71 ppm display a chemical shift too low to be adjacent to an oxygen atom

(as would be the case with the alternative epoxide opening). The ^{13}C chemical shift for the 'C' carbon (52.20 ppm) also supports this.

In addition, it would be expected that both 'C' and 'D' signals for ^{1}H and ^{13}C experiments would have similar chemical shifts, as both methylene groups flank the secondary amine. This is indeed the case as shown in **Figure 3-2**.

3.2.4 Pharmacology results

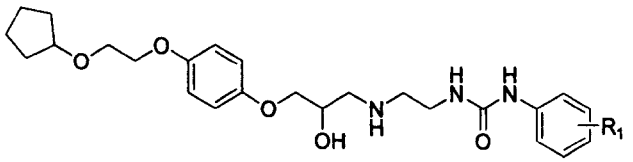
The binding affinity of **71a-u** and **57** at both the β1AR and β2AR is compared below in **Table 3-1** and **Figure 3-3**. All compounds have fairly poor affinity for the β2AR, and relatively higher affinity for the β1AR, thus displaying a degree of selectivity; however there are clear trends evident relating to the substitution pattern of R_1.

The β2AR binding affinities display no discernible trends with K_D values in the low millimolar to high nanomolar range, with the *m*-F (**71i**, log K_D = -6.54 ± 0.02 M) and *o*-CH_3 (**71b**, log K_D = -6.27 ± 0.02 M) displaying the highest affinities.

In comparison, binding at the β1AR displays clear trends **(Figure 3-3)**.If the unsubstituted derivative **(71a)** is taken as a benchmark, it is evident that the *o*-substituted analogues have β1AR affinity that is generally around an order of magnitude lower than **71a**. The exception is the *o*-F compound **71h** which displays comparable affinity to **71a**. The unusually high K_D value for the *o*-F analogue may be because binding at the β1AR is sterically sensitive towards *ortho* substituents. The similarity in atomic radius (van der Waals) between a hydrogen (1.2Å) and fluorine atom (1.35Å)[160] may mean that the *o*-F substituent exerts a similar steric presence to hydrogen. Consequently, larger groups are less well tolerated at the *o*-position. There also appears to be contributing electronic factors, as the steric argument does not hold when comparing the *o*-CH_3 **(71b)** and *o*-CF_3 **(71q)** analogues.

The *m*- and *p*-substituted analogues display much higher β1AR affinity than corresponding *o*-substituted analogues, with *m*-substitution generally affording the highest affinity of all. Overall, the *p*-OH **(57)**, *m*-

F **(71i)**, *m*-Cl **(71l)** and *m*-CH_3 **(71c)** derivatives display the highest affinity at β1AR. Other analogues display either slightly reduced β1AR affinity or, as in the case of *p*-Cl, *m*-OH and *m*-Br compounds, had comparable affinity to when there was no substitution **(71a)**.



	R_1	Binding affinity, log K_D (M)		β_1/β_2 selectivity
		β_1-adrenoceptor	β_2-adrenoceptor	
71a	H	-7.90 ± 0.05	-5.52 ± 0.03	240
71b	*o*-CH_3	-7.33 ± 0.03	-6.27 ± 0.02	11
71c	*m*-CH_3	-8.04 ± 0.04	-6.06 ± 0.03	96
71d	*p*-CH_3	-7.76 ± 0.04	-5.80 ± 0.03	91
71e	*o*-OCH_3	-7.02 ± 0.04	-5.93 ± 0.02	12
71f	*m*-OCH_3	-7.76 ± 0.03	-6.05 ± 0.03	51
71g	*p*-OCH_3	-7.80 ± 0.04	-5.86 ± 0.04	87
71h	*o*-F	-7.82 ± 0.03	-5.94 ± 0.04	76
71i	*m*-F	-8.17 ± 0.03	-6.54 ± 0.02	43
71j	*p*-F	-7.70 ± 0.04	-5.92 ± 0.09	60
71k	*o*-Cl	-7.11 ± 0.03	-5.54 ± 0.03	37
71l	*m*-Cl	-8.14 ± 0.04	-5.59 ± 0.05	355
71m	*p*-Cl	-7.95 ± 0.05	-5.91 ± 0.06	110
71n	*o*-Br	-7.11 ± 0.01	-6.10 ± 0.03	10
71o	*m*-Br	-7.92 ± 0.05	-5.99 ± 0.04	85
71p	*p*-Br	-7.77 ± 0.04	-5.86 ± 0.04	81
71q	*o*-CF_3	-6.94 ± 0.01	-5.84 ± 0.02	13
71r	*m*-CF_3	-7.77 ± 0.06	-5.86 ± 0.06	81
71s	*p*-CF_3	-7.70 ± 0.04	-5.76 ± 0.04	87
71t	*o*-OH	-6.99 ± 0.08	-5.96 ± 0.05	11
71u	*m*-OH	-7.89 ± 0.06	-5.85 ± 0.06	110
57	*p*-OH	-8.16 ± 0.08	-5.45 ± 0.10	513

Table 3-1: Human β_1 and β_2-adrenoceptor binding affinities and receptor selectivity of phenyl-substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)ureas (71a-u, 57).

n ≥ 5 for all assays.

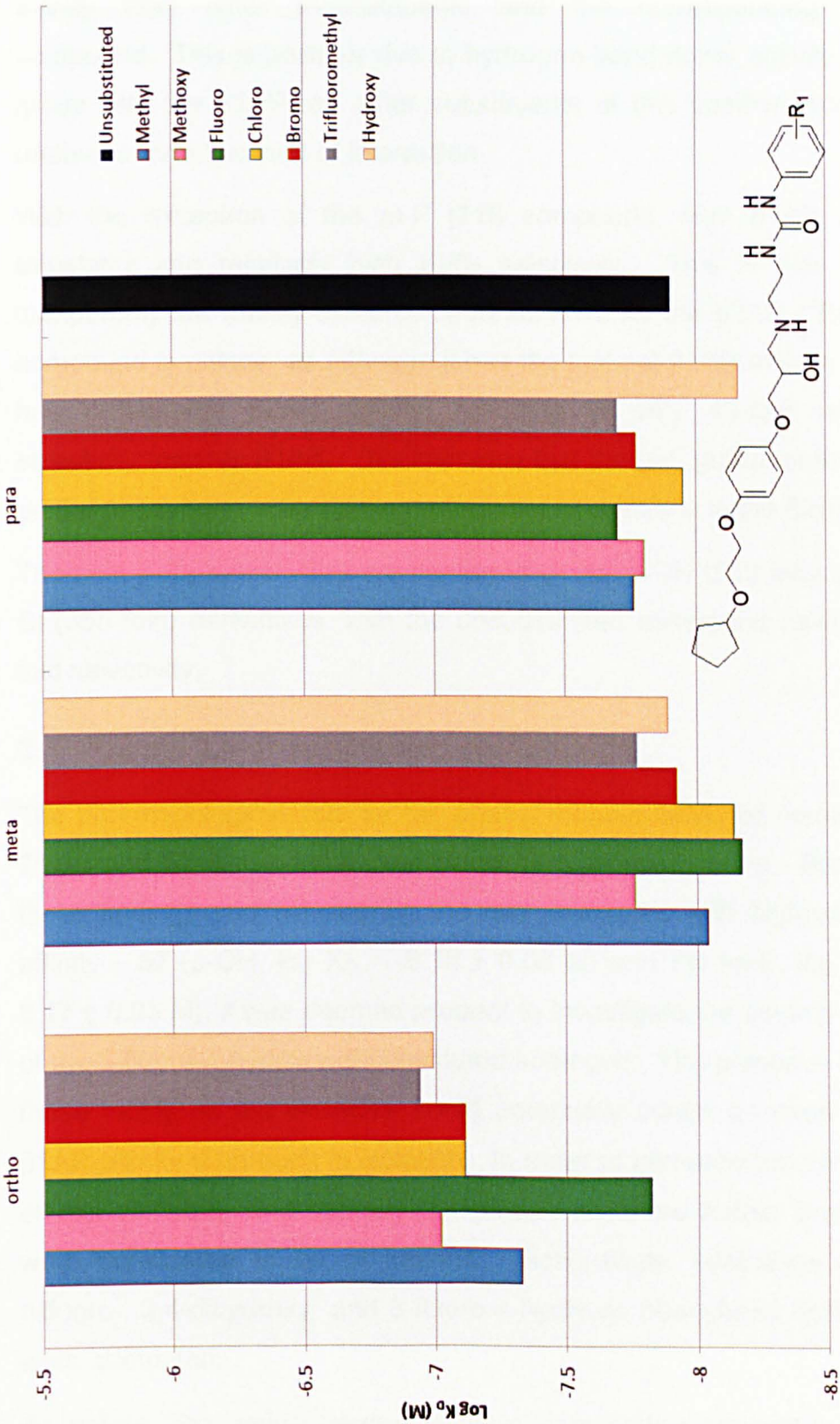


Figure 3-3: Binding affinities phenyl-substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)ureas (71a-u, 57) at the human β_1-adrenoceptor.

The *p*-OH **(57)** analogue is of particular interest, as it has much higher affinity than other *p*-substituents and the corresponding *m*-OH compound. This is possibly due to hydrogen bond-donor activity of this group with the β1AR, as other substituents at this position would be unable to form this kind of interaction.

With the exception of the *m*-F **(71i)** compound, high β1AR affinity translates into relatively high β_1/β_2 selectivity. This is due to the comparably low affinity these compounds have for the β2AR. The *m*-F compound is unique, as although it has the highest β1AR affinity, it also has reasonable β2AR affinity, resulting in only 43-fold receptor selectivity towards β1AR. This indicates that the *m*-F group, or its effect on the phenylurea, may cause preferential interactions in the β2AR.

The best β_1/β_2-selectivities are conferred by the *p*-OH (513 fold) and *m*-Cl (355 fold) derivatives, with the unsubstituted compound having 240 fold selectivity.

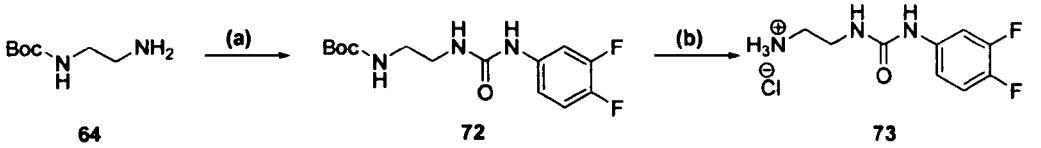
3.3 Phenyl 3,4-di-substituted compounds

The pharmacological data for the phenyl mono-substituted compounds **71a-u** and **57** displayed a clear trend as discussed above. Based on these findings and considering the two analogues with highest β1AR affinity – **57** (*p*-OH, log K_D = -8.16 ± 0.08 M) and **71i** (*m*-F, log K_D = -8.17 ± 0.03 M), it was deemed prudent to investigate the binding affinity of the 3-fluoro-4-hydroxy di-substituted analogue. The presence of both these motifs on the molecule could potentially confer an even higher β1AR affinity than each in isolation. In order to compare the combined effects of fluoro- and hydroxy- functionalities, three further analogues were considered to be of interest. Accordingly, syntheses of 3,4-difluoro-, 3,4-dihydroxy- and 3-fluoro-4-hydroxy- phenylurea derivatives were attempted.

As before, the amine fragments were constructed separately before using each in the aminolysis of epoxide **62**.

3.3.1 Synthesis of the amine fragments

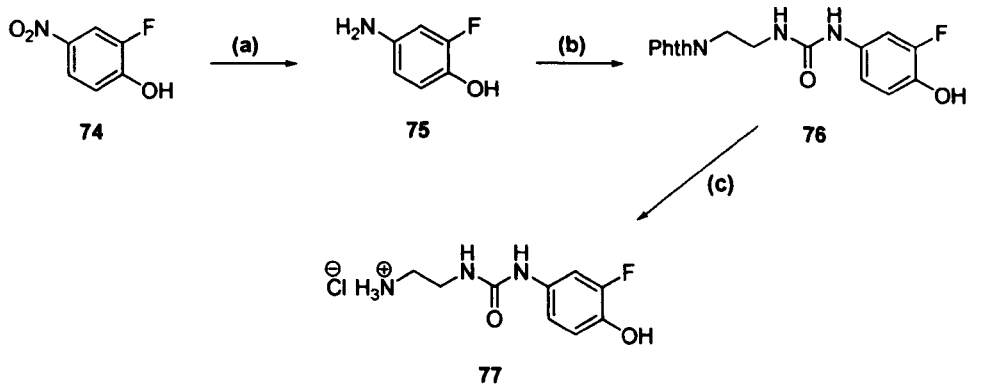
The commercial availability of 3,4-difluorophenyl isocyanate allowed facile synthesis of 1-(2-aminoethyl)-3-(3,4-difluorophenyl)urea **73** as the hydrochloride salt, by reaction with Boc-protected diamine **64** and subsequent acid-mediated Boc removal (**Scheme 3-6**).



Scheme 3-6: Synthesis of 1-(2-aminoethyl)-3-(3,4-difluorophenyl)urea hydrochloride (73).

Reagents and conditions: (a) 3,4-difluorophenylisocyanate, DCM, 75%; (b) MeOH/conc HCl, 100%.

1-(2-aminoethyl)-3-(3-fluoro-4-hydroxyphenyl)urea hydrochloride **77** was constructed in a similar manner to **70a-b** via intermediate Curtius rearrangement of acid **68** to the corresponding isocyanate. Aniline **75** was readily obtained by reduction of 2-fluoro-4-nitrophenol (**74**) by catalytic hydrogenation over Pd(0) (**Scheme 3-7**).



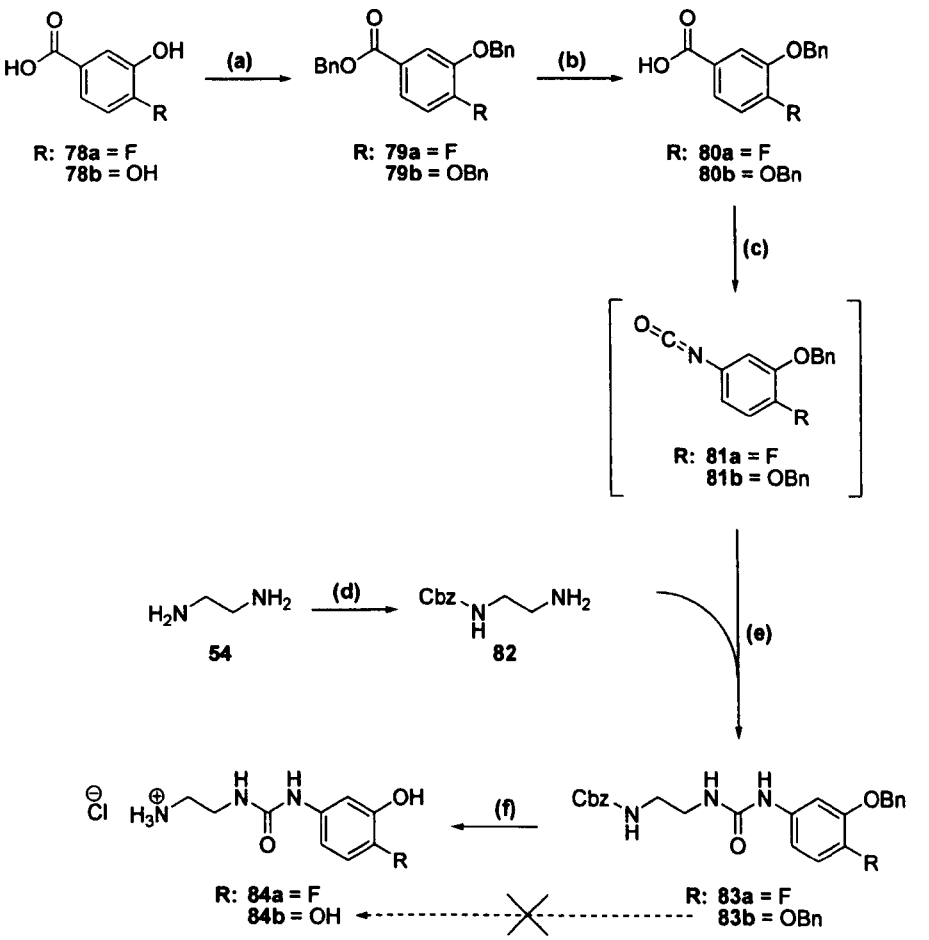
Scheme 3-7: Synthesis of 1-(2-aminoethyl)-3-(3-fluoro-4-hydroxyphenyl)urea hydrochloride (77).

Reagents and conditions: (a) H_2, 10% Pd/C, MeOH, 90%; (b) i. **68**, DPPA, TEA, toluene; ii. Reflux; iii. **75**, 55%; (c) hydrazine monohydrate, EtOH, reflux, 84%

The method employed in **Scheme 3-7** to synthesise **77** could not be used in the case of the 4-fluoro-3-hydroxy- analogue, due to lack of commercial availability of 2-fluoro-5-nitrophenol.

With regards to the 3,4-dihydroxy analogue, attempted synthesis by initial reduction of 4-nitrocatechol was unsuccessful. Although reduction to 4-aminocatechol does occur, the product undergoes spontaneous air oxidation to 2-hydroxy-4-iminoquinone.[161]

Consequently, synthesis of the remaining amines **84a-b** was attempted from the corresponding substituted benzoic acids **78a-b**. Presence of nucleophilic phenol moieties in **78a-b** necessitated protection prior to Curtius rearrangement of the acid group (**Scheme 3-8**). Protection of the phenol resulted in concomitant formation of the benzyl esters **79a-b**, requiring saponification to liberate the free acids **80a-b**.

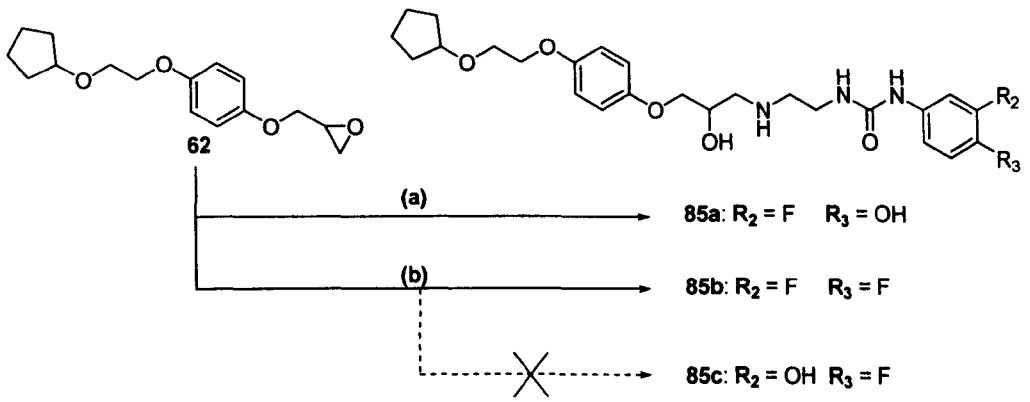


Scheme 3-8: Synthesis of phenyl di-substituted 1-(2-aminoethyl)-3-(phenyl)urea hydrochlorides (84a-b) derived from di-substituted benzoic acids (78a-b).

Reagents and conditions: (a) i. NaH, DMF, 0°C → rt; ii. benzyl bromide (BnBr), 100%; (b) LiOH, H_2O/THF/MeOH, 85-86%; (c) DPPA, TEA, toluene; ii. Reflux; (d) benzyl chloroformate, DCM, 0°C, 36%; (e) Stir, rt; 15-61%; (f) conc HCl, 10% Pd/C, EtOH, 100%.

The benzyl ether protected benzoic acid derivatives **80a-b** then underwent Curtius rearrangement to the corresponding phenylisocyanates **81a-b** (not isolated), before reaction with protected diamine **82**. In this instance, ethylenediamine **(54)** was mono-protected using the carboxybenzyl (Cbz) group, in a manner analogous to mono *tert*-butyloxycarbonyl (Boc) protection (**Scheme 3-2**). This strategy allowed global deprotection of benzyl and Cbz groups by catalytic hydrogenation over Pd(0) in the presence of acid. Although successful for the synthesis of **84a**, the hydrogenation conditions employed did not produce **84b**. The red solid obtained from this reaction was analysed by ^{1}H NMR and high performance liquid chromatography (HPLC). The ^{1}H spectra showed a series of broad peaks uncharacteristic of analogues of **84b**, and relative peak integrations did not correlate with the desired structure. HPLC analysis showed multiple peaks, both broad and sharp with low retention times. It would appear that the attempted deprotection of **83b** resulted in degradation of the molecule, possibly caused by presence of the catechol-type system.

3.3.2 Opening of the epoxide



Scheme 3-9: Synthesis of phenyl di-substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)ureas (85a-c).

Reagents and conditions: (a) **77**, TEA, EtOH, microwave (MW) 80W, 140 °C, 250psi, 13%; (b) **73**, TEA, propan-2-ol, reflux, 28%.

Due to the long reaction times required in previous epoxide openings (compounds **71a-u**, **57**); the conditions for the reaction were varied. In

the case of **85a**, the epoxide **62** was opened using **77** in ethanol under microwave conditions (**Scheme 3-9**). This method has been reported to give complete aminolysis of epoxides in 4 minutes, requiring only 1.5 equivalents of amine relative to the epoxide.[159]

The reaction did not appear to be complete in 4 mins by TLC analysis, so longer reaction times were required. In the cited study, most of the amines used were sterically encumbered, secondary amines and hydrophobic in nature. In comparison, the urea and primary amine moieties present in **77**, make it more polar and poorly soluble in the majority of available solvents. The primary amine group allows further alkylations to occur once the secondary propanolamine is initially generated.

The remaining two amines **73** and **84a** were used to open epoxide **62**, by reflux in propan-2-ol (**Scheme 3-9**). In these reactions TEA was used to neutralise the hydrochloride salts instead of NaOH. It was anticipated this would eliminate potential attack on the epoxides by nucleophilic hydroxide anions.

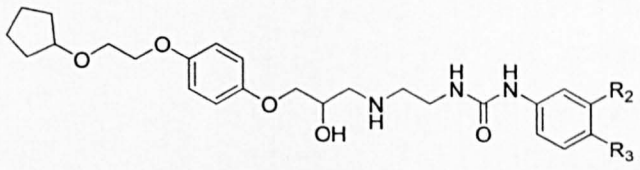
The reaction for target compound **85c** failed. Attempted purification of the crude material by preparative HPLC allowed isolation of the major peak. Although MS analysis of this collected peak displayed the correct mass, ^{1}H NMR analysis indicated the desired product had not formed. The aromatic peaks on the spectrum were found to integrate to areas around twelve times that of the rest of the molecule.

3.3.3 Pharmacology results

The binding affinity of successfully synthesised 3,4-disubstituted analogues **(85a-b)**, is compared to the relevant mono-substituted in **Table 3-2**.

In terms of β1AR affinity, both 3,4-disubstituted compounds **85a** and **85b** have higher affinity than the *p*-F analogue **(71j)**, thus both substitution patterns can be accommodated at the receptor. However, the β1AR affinities are lower than the corresponding *m*-F and *p*-OH compounds, indicating that there is no additive effect, or improvement in

affinity with both groups present. Indeed the slightly lower β1AR affinities for **85a** and **85b**, suggest that favourable interaction of *m*-F or *p*-OH substituents alone is reduced by the presence of a neighbouring fluorine atom.



	R_2	R_3	Binding affinity, log K_D (M)		β_1/β_2 selectivity
			β_1-adrenoceptor	β_2-adrenoceptor	
71a	H	H	-7.90 ± 0.05	-5.52 ± 0.03	240
71i	F	H	-8.17 ± 0.03	-6.54 ± 0.02	43
71j	H	F	-7.70 ± 0.04	-5.92 ± 0.09	60
57	H	OH	-8.16 ± 0.08	-5.45 ± 0.10	513
85a	F	OH	-8.07 ± 0.09	-5.59 ± 0.03	302
85b	F	F	-8.02 ± 0.03	-5.88 ± 0.03	138

Table 3-2: Human β_1 and β_2-adrenoceptor binding affinities and receptor selectivity of phenyl di-substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)ureas (85a-b).

n = 7 for all assays.

Overall, the 3-fluoro-4-hydroxy- compound **(85a)** has lower selectivity than the *p*-OH compound, due to a concomitant slight rise in β2AR affinity. Interestingly, the 3,4-difluoro compound **(85b)** displays better selectivity than either *m*-F or *p*-F analogue. This is due to a reduction in β2AR affinity relative to the *m*-F compound, as well as an increase in β1AR affinity relative to the *p*-F compound – i.e. adoption of different desirable properties of both molecules.

The difficulties thus far encountered synthesising the 3,4-dihydroxy and 4-fluoro-3-hydroxy- compounds, means it is difficult to draw further conclusion regarding 3,4-disubstitution effects.

3.4 Structure-activity relationships of phenyl-substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)ureas

Substitution studies of the phenyl ring adjacent to the urea moiety in **71a**, allow several structure-activity relationships to be inferred.

The unsubstituted ring displays nanomolar range affinity for the β1AR (log K_D = -7.90 ± 0.05 M). The presence of an *o*-substituent relative to the urea reduces β1AR affinity greatly. This occurs in a seemingly sterically-sensitive fashion, as is evident in the relatively high β1AR affinity of the *o*-F analogue. In addition there are likely to be steric and electronic effects on the urea proton adjacent to the ring, due to the proximity of the *o*-substituent. This may account for the differences in binding between the *o*-CH_3 and *o*-CF_3 compounds, as they have opposing electronic effects, but similar atomic radii.

Substituents at the *para* position give β1AR affinity that is comparable or slightly lower than **71a**. The exception to this rule is **57**, containing *p*-OH substitution and much higher β1AR affinity. This suggests a potential hydrogen bond donor interaction with the receptor, as other substituents do not display this activity.

In comparison, *meta* substituents generally improve β1AR affinity relative to **71a** (only *m*-CF_3 and *m*-OCH_3 cause a slight reduction).

It is important to consider that the effects of these substituents will not be localised to their immediate receptor environment. Indeed, the electronic effects on the aromatic ring, and transmitted effects through to the adjacent urea, may alter interactions at the receptor level that are not immediately apparent, and difficult to predict without knowledge of the exact nature of the interactions at these sites.

In terms of the β2AR affinity, there does not appear to be a discernible trend with position or electronic nature of the substituent. All analogues, with the exception of *m*-F gave relatively poor affinity at β2AR.

Overall, the best β_1/β_2-selectivity is achieved with the *m*-Cl and *p*-OH analogues.

Disubstitution with the highest affinity substituents (i.e. 3-fluoro-4-hydroxy-) does not improve affinity at the β1AR and actually causes a slight reduction in affinity and consequently selectivity.

4. MODIFICATION OF THE UREA MOIETY

The concept of isosterism, and later bioisosterism, originated with observations by Irving Langmuir in 1919 on atoms and molecules with a similar arrangement of valence electrons having similar physicochemical properties.[162] More recent definitions of classical bioisosteres relate size, shape, and electron distribution of the group with activity.[160, 163]

The urea moiety may be of importance in imparting high β1AR affinity, and subsequent high β_1/β_2 selectivity. The polar nature of the group, and heteroatom configuration, allows it to function as both a hydrogen bond donator and acceptor. On this basis, isosteric groups with the potential to form similar interactions were considered to be of interest. These would allow evaluation of the sensitivity of the pharmacophore to changes to this group.

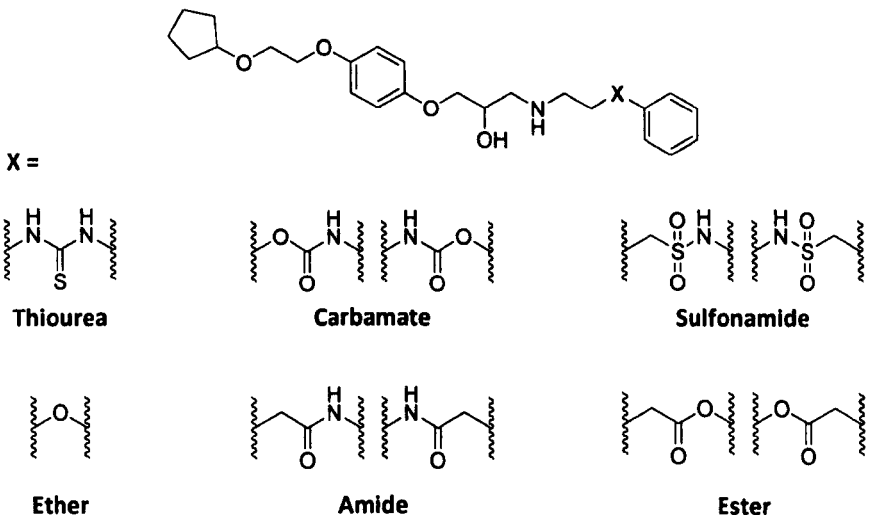


Figure 4-1: Intended target compounds containing replacement functionality of the urea group.

Of particular interest were the thiourea, carbamate, amide, sulfonamide, and ester moieties. In addition, an analogue containing a simple ether

linkage was included in this group. Although it is a non-classical bioisostere,[160] this linker is found in carvedilol and CGP 20712A, which both display high β1AR affinity.[81] With the exception of the thiourea (which can be considered a direct replacement of the urea) and ether, the remaining groups can be installed in two orientations (**Figure 4-1**). Accordingly, synthesis of both variants was attempted.

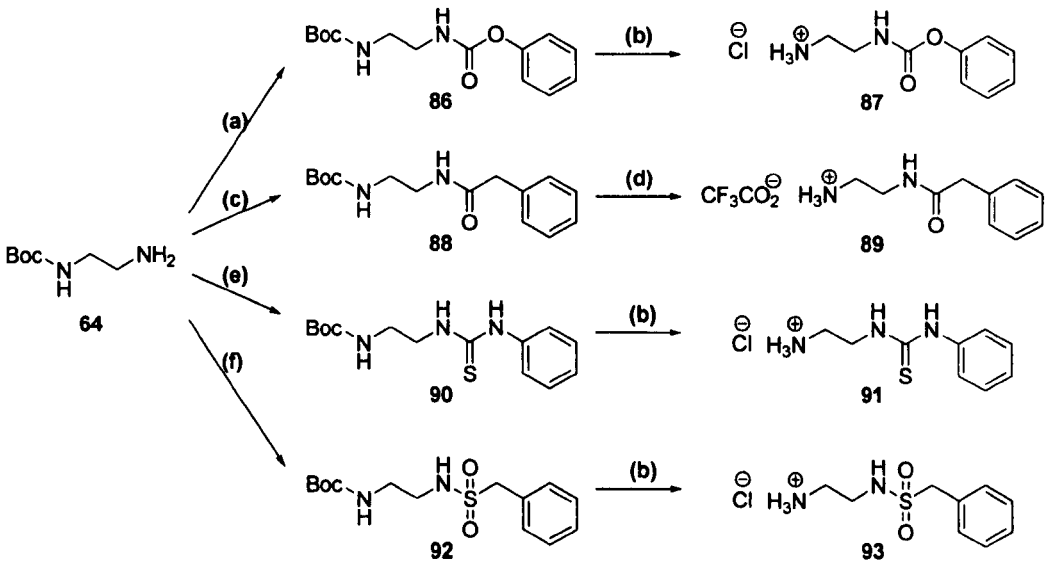
4.1 Principles of synthetic strategy

In a similar fashion to the synthesis of the phenyl-substituted analogues described in **Chapter 3**, the final aryloxypropanolamine compounds were obtained by aminolysis of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** by a series of amine fragments. The synthesis of **62**, is detailed in **Chapter 3**.

To allow comparison of replacement groups directly, the unsubstituted phenyl ring was deemed the best option as a terminal group, even though *p*-OH substitution of this ring offers the best β1AR affinity and β_1/β_2 selectivity (chapter 3). In addition, this strategy avoided the complication of protecting a phenolic functionality on the ring and its subsequent deprotection. Finally, unsubstituted starting materials were found to be more widely available and economically more viable.

4.2 Synthesis of the amine fragments

The synthesis of the amine fragments can be broken down into three major groups defined by the corresponding starting material. The final amine fragments were derived from ethylenediamine (**Scheme 4-1**), GABA (γ-amino butyric acid) (**Scheme 4-2**) or ethanolamine (**Scheme 4-3**).



Scheme 4-1: Ethylenediamine derived amine fragments 87, 89, 91 and 93.

Reagents and conditions: (a) phenyl chloroformate, TEA, DCM, 87%; (b) 4M HCl/dioxane 86-100%; (c) phenylacetyl chloride, TEA, DCM 0 °C → rt, 84%; (d) trifluoroacetic acid (TFA)/DCM 1:1, 100%; (e) phenylisothiocyanate, DCM, 84%; (f) Phenylmethanesulfonyl chloride, TEA, DCM 0 °C → rt, 78%.

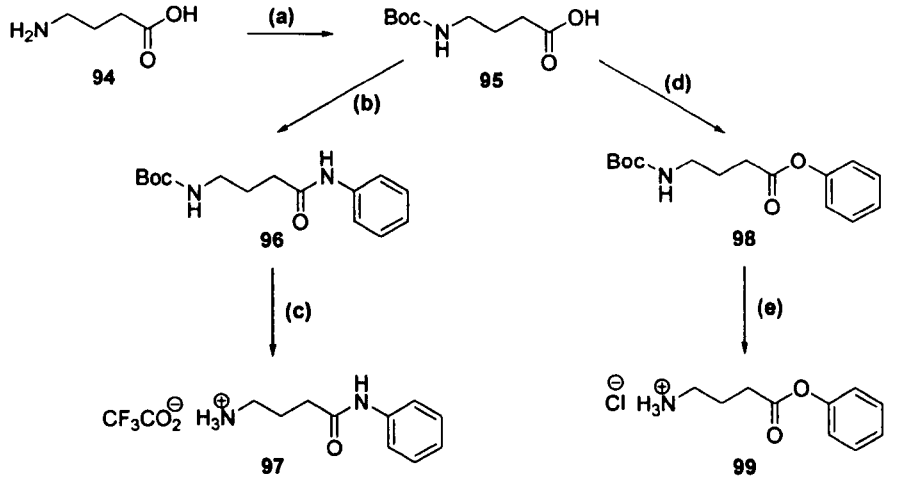
Amines derived from ethylenediamine (**Scheme 4-1**) were synthesised with ease, in high yields. Boc-protected diamine **64** (see **Chapter 3**), was reacted with phenyl chloroformate, phenylacetyl chloride or phenylmethanesulfonyl chloride in the presence of TEA to generate Boc-protected carbamate **(86)**, amide **(88)**, and sulfonamide **(92)** respectively. Reactions with the carbonyl chloride reagents proceeded rapidly, whereas phenylmethanesulfonyl chloride required overnight reaction as expected.

Protected thiourea **(90)**, was produced in an identical fashion to urea **(65a)**, utilising phenylisothiocyanate.

Amines **87**, **89**, **91**, and **93**, were obtained in the acid salt form after acid-mediated removal of the Boc group.

Use of GABA (**94**) (**Scheme 4-2**) allowed facile conversion of the acid terminus to the desired amide or ester. Initial Boc protection of the amine terminus was carried out in water, with THF as a co-solvent, in the presence of $NaHCO_3$ to neutralise the carboxylic acid. Boc-protected GABA **(95)** underwent carboxylate activation with *N,N'*-

dicyclohexylcarbodiimide (DCC), before addition of nucleophile. In the case of aniline, coupling was achieved directly to give amide **96**. The less nucleophilic phenol required a catalytic amount of 4-dimethylaminopyridine (DMAP) as an auxiliary nucleophile to accelerate formation of ester **98**.

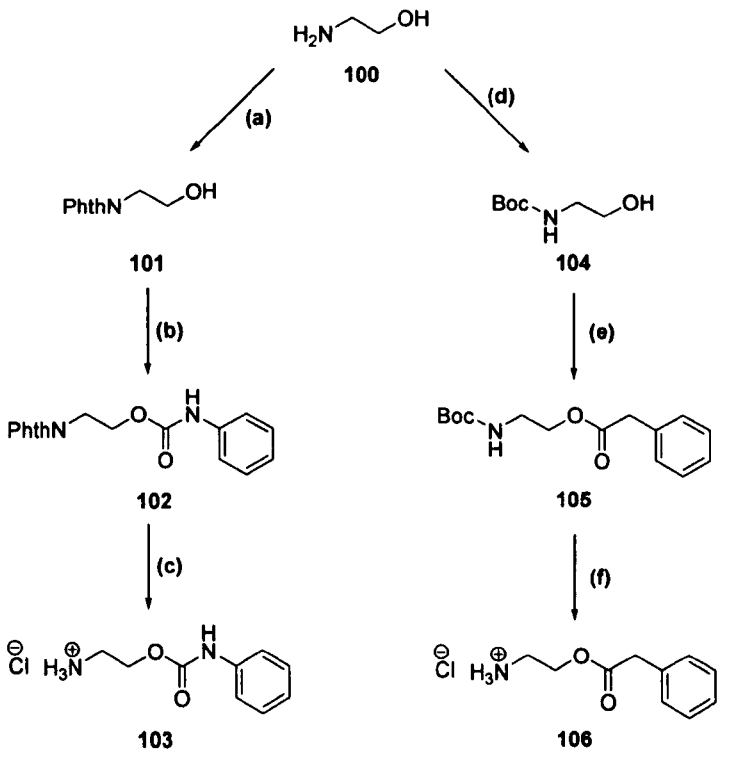


Scheme 4-2: GABA derived amine fragments 97 and 99.

Reagents and conditions: (a) Boc_2O, $NaHCO_3$, water/THF 4:1, 86%; (b) DCC, aniline, DCM, 86%; (c) TFA/DCM 1:1, 100%; (d) DCC, DMAP, phenol, DCM, 69%; (e) 4M HCl/dioxane, 98%.

Amines **97** and **99** were obtained by acid-mediated Boc cleavage.

Finally, ethanolamine **(100)** was used to generate amines **103** and **106**. Once again protection of the amine was necessary, prior to further functionalisation. Phthaloyl protection was achieved in the absence of solvent according to literature procedures[153]. The phthaloyl-protected ethanolamine **(101)**, was reacted with phenylisocyanate directly to give carbamate **102** in poor yield. The poor nucleophilicity of the primary alcohol is likely to be the cause of this, and the reaction may proceed more rapidly if the alcohol is initially deprotonated.



Scheme 4-3: Ethanolamine derived amine fragments 103 and 106.

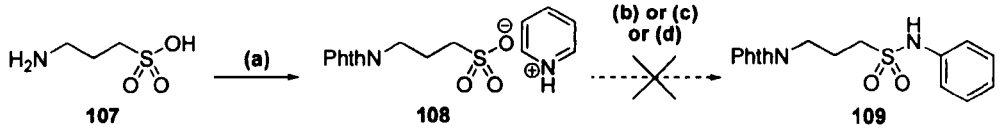
Reagents and conditions: (a) phthalic anhydride, 150 °C, 83%; (b) phenylisocyanate, DCM, 12%; (c) i. hydrazine monohydrate, EtOH, reflux; ii. acidic workup, 74%; (d) Boc_2O, water/THF 3:1, 99%; (e) phenylacetyl chloride, TEA, DCM, 0°C → rt, 94%; (f) 4M HCl/dioxane, 91%.

Hydrazinolysis of the phthaloyl group and subsequent acidic workup afforded the desired amine **(103)** as the hydrochloride salt. Boc-protection of ethanolamine was achieved in much higher yield than the corresponding phthaloyl protection and was carried out according to literature procedures[164]. Subsequent formation of ester **105** was rapidly achieved using phenylacetyl chloride in basic conditions. Acidic Boc cleavage afforded the amine **106** as the hydrochloride salt.

4.2.1 Attempted synthesis of *N*-phenyl-3-aminopropyl sulfonamide

Synthesis of *N*-phenyl-3-aminopropyl sulfonamide was attempted via several routes. Initially, 3-aminopropane sulfonic acid **(107)**, was phthaloyl protected. Protection was initially attempted by heating in the absence of solvent with phthalic anhydride (as for protection of ethanolamine, see above); however only starting material was

recovered from this reaction. Consequently, reflux in pyridine was employed to give the protected sulfonic acid as the pyridinium salt.



Scheme 4-4: Attempted synthesis of *N*-Phenyl-3-aminopropyl sulphonamide (109).

Reagents and conditions: (a) phthalic anhydride, pyridine, reflux, 91%; (b) i. thionyl chloride, reflux; ii. TEA, aniline, DCM; (c) i. triphenylphosphine oxide, triflic anhydride, DCM; ii. TEA, aniline, 0°C → rt; (d) i. trichloroacetonitrile, PPh_3, DCM, reflux; ii. TEA, aniline.

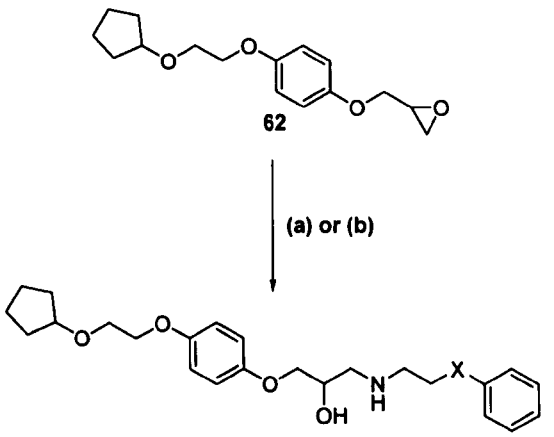
Various attempts were made to activate the sulfonate group on **108** to allow sulfonamide bond formation (**Scheme 4-4**). Firstly reflux of **108** in thionyl chloride was anticipated to generate the sulfonyl chloride. After removal of excess thionyl chloride, the crystalline pyridinium salt had become a yellow waxy solid. This was not isolated, but immediately reacted with aniline in the presence of TEA. Unfortunately TLC analysis failed to show any change after overnight reaction, which would be expected had the sulfonyl chloride formed. It is likely that conversion from the sulfonate to the sulfonyl chloride did not proceed by this method.

Activation of sulfonate salts using triphenylphosphine oxide/triflic anhydride prior to exposure of amine has been shown to give sulfonamides in high yields.[165, 166] When attempted, this method did not give the desired sulfonamide; as confirmed by ^{1}H NMR and MS analysis.

Finally, activation was attempted using trichloroacetonitrile and triphenylphosphine.[167] Although formation of triphenylphosphine oxide was noted (indicating displacement of a sulfonate oxygen), formation of the sulfonamide was not evident by ^{1}H NMR and MS analysis.

4.3 Opening of the epoxides

Commercially available 2-phenoxyethylamine was used to open epoxide **62** to generate the desired ether derivative **110a** (**Scheme 4-5**). As the free amine was available, no base was required, and ring opening was carried out in neutral conditions, by reflux in propan-2-ol with an excess of the amine. Remaining analogues **110b-f**, were obtained by opening epoxide **62** with amines **89**, **91**, **93**, **97** and **103** in the presence of TEA as described previously in **Chapter 3**.



Scheme 4-5: Synthesis of analogues of 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)urea modified at the urea moiety 110a-e.

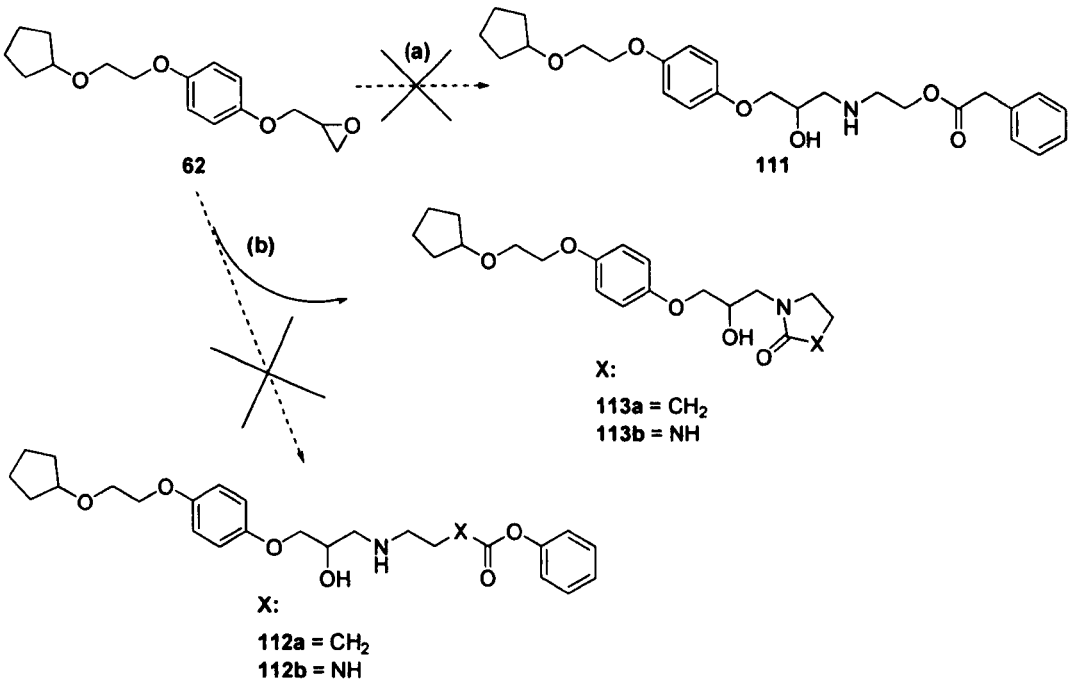
Reagents and conditions: (a) 2-phenoxyethylamine, propan-2-ol, reflux, 68%; (b) **89**, **91**, **93**, **97** and **103**, TEA, propan-2-ol, reflux, 16-45%.

4.3.1 Problematic epoxide openings

Attempted opening of epoxide **62** with **87**, and **99** and **106** did not give the desired ester (**111**, **112a**) and carbamate (**112b**) derivatives (**Scheme 4-6**). In the case of the phenyl acetate ester (**111**), analysis by HPLC indicated a single peak. When the ^{1}H NMR spectrum was examined, the areas of the spectrum where the propanolamine backbone is normally visible, displayed a multitude of indistinguishable peaks. Although overall integration of these peaks was correct, some

of the individual peaks represented half the expected area. Initially, the observed spectrum was attributed to rotational isomerism brought about by the ester group. However raising the temperature of the ^{1}H NMR from 25°C to 50°C resulted in no change in the spectrum. If individual rotamers were indeed visible initially, then the raise in experimental temperature would have allowed free rotation about the C-O bond and aligned the individual rotamer signals.

Attempts to synthesise phenolic ester **112a** and carbamate **112b**, resulted in intramolecular cyclisation reactions to the corresponding lactam **113a** and oxazolidinone **113b** (**Scheme 4-6**).



Scheme 4-6: Attempted synthesis of ester and phenolic carbamate analogues 111 and 112a-b.

Reagents and conditions: (a) **106**, TEA, EtOH; (b) **87/99**, TEA, EtOH, 80W, 140 °C, 250psi, 6-7%.

The ability of phenol to act as a leaving group, especially under the thermodynamic conditions employed for epoxide opening, promoted intramolecular attack of the secondary amine on the carbonyl group in these reactions.

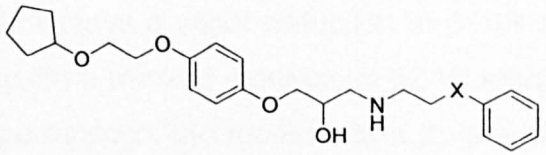
The acidic nature of phenol means its conjugate base, the phenoxide anion portrays leaving group ability. As such, any future attempted syntheses are likely to require installation of the phenol as a terminal step. Such a reaction could be carried out after epoxide opening of **62** with either GABA or 3-aminopropanoic acid. The GABA derivative could be carboxylate-activated and converted to the phenyl ester **112a**. In order to generate the corresponding carbamate **112b**, conversion of the 3-aminopropanoic acid derivative to the corresponding alkyl isocyanate, prior to reaction with phenol, may be a possible route. In both cases, the appropriate amine/alcohol protection strategy would have to be employed to prevent intramolecular cyclisation reactions.

Previous investigations into the nature of the nitrogen atom on the aryloxypropanolamines, have shown tertiary nitrogen structures to have considerably lower affinity for the β1AR. As such, these side-products were found not to be useful.[124, 126, 168] Indeed, the secondary amine motif is required to form a salt-bridge interaction with an aspartate residue in the receptor (see **Chapter 1**).[16, 25, 169, 170]

4.4 Pharmacology results

A comparison of parent urea compound **71a** pharmacology and that of the analogues is made in **Table 4-1**.

With the exception of amide compound **110e**, the remaining analogues display at least a 0.5 log unit increase in affinity for the β2AR over the urea compound **(71a)**. Affinity for the β1AR is generally reduced, although still reasonable with all K_D values remaining below 100 nM. The exception to this is phenyl acetamide **110f**, which actually displays a higher affinity, with a β1AR K_D value of around 4 nM.



	X	Binding affinity, log K_D (M)		β_1/β_2 selectivity
		β_1-adrenoceptor	β_2-adrenoceptor	
71a	NH(C=O)NH	-7.90 ± 0.05	-5.52 ± 0.10	240
110a	O	-7.46 ± 0.04	-6.04 ± 0.02	26
110b	NH(C=S)NH	-7.09 ± 0.06	-6.16 ± 0.04	9
110c	$NH(SO_2)CH_2$	-7.59 ± 0.03	-6.03 ± 0.04	36
110d	O(C=O)NH	-7.53 ± 0.07	-6.33 ± 0.03	16
110e	$CH_2(C=O)NH$	-7.15 ± 0.03	-5.83± 0.02	21
110f	$NH(C=O)CH_2$	-8.42 ± 0.03	-7.27 ± 0.02	14

Table 4-1: Human β_1 and β_2-adrenoceptor binding affinities and receptor selectivity of 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)urea analogues modified at the urea moiety (110a-f).

$n \geq 6$ for all assays.

Remarkably, replacement of the urea moiety with a simple ether linkage retains reasonable β1AR activity. In comparison to the other replacement groups, the ether **110a** has no hydrogen bond donor activity, yet is still comparable to the rest of the analogues in terms of β1AR activity and overall selectivity.

The two amide analogues **110e-f**, display quite striking differences in their activity profile. Broadly speaking, each amide can be thought to represent the individual nitrogen groups present on the urea compound, in isolation. Butanamide **110e** retains low β2AR affinity, but also displays a reduction in β1AR affinity relative to urea **71a**. Conversely, phenyl acetamide **110f** has a higher β1AR affinity than **110e**, but also exhibits a marked increase in affinity for the β2AR. It appears that N-H group adjacent to the ring is important in suppressing β2AR affinity, whereas the more distant N-H group (alkyl chain side of the urea) is important for high β1AR activity. In addition, the presence of the same N-H group promotes β2AR binding. The urea group incorporates both of these individual features, offering good β1AR affinity whilst suppressing β2AR affinity.

Carbamate **110d** displays a slight reduction in β1AR affinity compared to the urea, alongside a marked increase in β2AR affinity. Although this illustrates the importance of the more distant (to the phenyl) N-H group in promoting β1AR binding, the presence of the more electronegative oxygen atom does not appear particularly deleterious in comparison to butanamide **110e** (where the corresponding group is CH_2). Additionally, the ether compound **110a** contains an oxygen atom in the same relative position, and retains a similar level of β1AR affinity. This indicates that an electronegative atom (possibly a hydrogen bond acceptor), at or near this position, is important for β1AR activity. However the additional presence of hydrogen bond donor activity (as in N-H) appears to offer even greater interaction with the receptor. The marked rise in β2AR affinity of the carbamate, with respect to the urea, indicates that the presence of an electronegative atom is also important for interaction with this receptor. Again, replacement of the oxygen with CH_2, as in amide **110e**, shows further reduction in β2AR affinity.

The sulfonamide **110c**, displays a similar profile to other analogues containing the distant N-H (such as **110f**). It retains β1AR affinity with a slight increase in β2AR affinity.

Finally, the thiourea **110b** exhibits diminished affinity for β1AR and a raised β2AR affinity. It might be expected that the urea and thiourea would have similar properties, on account of the above observations regarding the two N-H groups. The difference observed may be attributable to the diverse nature of the urea and thiourea groups. There is a stark difference in the polarity across the C=O bond in urea and the C=S in thiourea (C and S have similar electronegativity, whereas O is much more electronegative).[160] The urea group is able to function as both an efficient donor and acceptor of hydrogen-bonds (due to the basicity of the oxygen atom).[171] In comparison, the larger size of the sulfur atom (van der Waals radii: O = 1.4Å, S = 1.85Å) and lower charge density means it is less basic and consequently less effective as a hydrogen-bond acceptor.[171] However, the thiourea is

more acidic, and thus a better hydrogen-bond donor than the urea group.[171]

Overall, the lower β1AR affinity of the thiourea may be a consequence of reduced hydrogen-bond acceptor functionality. Indeed the higher β1AR affinity of the ether compound may further point towards the importance of a good hydrogen-bond acceptor in this area.

Additionally, there may also be a steric effect to consider as larger groups, such as the thiourea and sulfonamide appear to have reduced β1AR affinity.

Unfortunately, due to difficulties synthesising the phenolic carbamate **112b**, esters **111** and **112a** and propyl sulfonamide derivatives, a full comparison is not possible, especially with regard to determining the full effects of the N-H group adjacent to the phenyl.

Overall, the best selectivity was retained with the parent urea compound.

4.5 Structure-activity relationships of 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(phenyl)urea analogues modified at the urea moiety

Modification of the urea moiety has allowed several structure-activity relationships to be determined.

The best β_1/β_2 selectivity was retained by the parent urea compound **71a**. Of all the functional groups investigated as direct bioisosteres, or indirect replacements (i.e. ether), good affinity for β1AR, and a varying degree of selectivity (9 – 36 fold) was maintained. As such, the urea group is not essential for receptor binding in either sub-types, but does seem to offer a role in differential selectivity between the receptors.

The presence of an electronegative atom, at a distance of two carbon units from the propanolamine nitrogen, seems important for affinity at both receptors, although further studies would be required to determine the optimal length of the carbon linker. It appears this electronegative

atom may be important as a hydrogen bond acceptor. If the electronegative atom also has hydrogen bond donator capability (e.g. N-H), this appears to offer greater β_1/β_2 selectivity by increasing affinity for the β1AR.

Initial observations indicate the N-H group adjacent to the ring may be important in reducing β2AR binding. Full analyses cannot be conducted due to difficulties in synthesising the alternate carbamate and sulfonamide compounds, as well as the ester derivatives.

Hydrogen bond acceptor functionality may be of importance as the thiourea shows lower affinity for the β1AR, compared to the parent urea.

As well as the electronic and hydrogen bond effects, the presence of larger groups at this position appears to be less well tolerated at the β1AR.

The urea group offers the best receptor selectivity, although the best β1AR affinity is imparted by phenyl acetamide **110f**.

Overall, the ability to modify the urea moiety without hugely deleterious effects on β1AR affinity and maintaining some degree of $\beta_1/\ \beta_1$ selectivity indicates this part of the ligand is amenable to more extensive modification that could offer an enhanced selectivity profile.

5. VARIANTS OF THE ARYLOXY GROUP AND ASSOCIATED LINKERS

There are several studies in the literature that investigate substitution of the aryloxypropanolamine ring,[74, 92, 95, 102, 126] as well as the nature of the substituent *para*- to the propanolamine[91, 98, 99, 103, 106, 107, 172] side chain (see **Chapter 1**).[105]

Incorporating known SAR into the design, with the aims of improving selectivity for the β1AR over the β2AR, and improving affinity for the β1AR; the synthesis of a third series of analogues was initiated. Based upon previous findings (*cf.* **Chapters 3 and 4**), analogues were synthesised incorporating the urea moiety, and either *m*-Cl or *p*-OH substitution of the phenylurea.

5.1 Modification of the terminus attached to the ethylene glycol linker

Investigations into this region of similar molecules have been extensive, with a variety of motifs and alkyl chain length being compared[105, 106]. Whilst retaining the *p*-OH substitution on the phenylurea (as in **57**), modifications were made to the terminus of the ethylene glycol motif in the parent compound. The ethylene glycol unit itself has been shown to impart good affinity for the β1AR as well as good β1AR/β2AR selectivity in a variety of analogues, [105, 106] thus modifications were limited to the group attached to this linker. These analogues included the 4-fluorophenethyl-, cyclopropylmethyl- and ethyl- derivatives, previously shown to have good β1AR affinity.[105, 106]

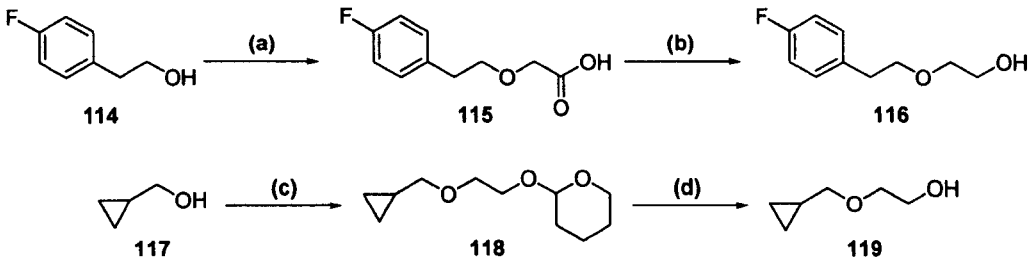
5.1.1 Principles behind synthetic strategy

Synthesis was adopted in a similar fashion to that described for **57** (**Chapter 3**). Once again the epoxide and amine fragments were combined in a convergent step, under basic or neutral conditions to

generate the desired aryloxypropanolamine pharmacophore. Each fragment was synthesised in isolation with 1-(2-aminoethyl)-3-(4-hydroxyphenyl)urea **(56)**, being employed from previous synthesis.

5.1.2 General synthesis

Initially, synthesis of the 2-(alkyloxy)ethanol fragments was required, where there was lack of commercial availability. 2-(4-fluorophenethyloxy)ethanol **(116)** was constructed according to literature methods (**Scheme 5-1**).[98] This involved condensation of 2-(4-fluorophenyl)ethanol **(114)** with chloroacetic acid after deprotonation of the alcohol with NaH. Reduction of the product, 2-(4-fluorophenethyloxy)acetic acid **(115)** to the alcohol **116**, was achieved using $LiAlH_4$.



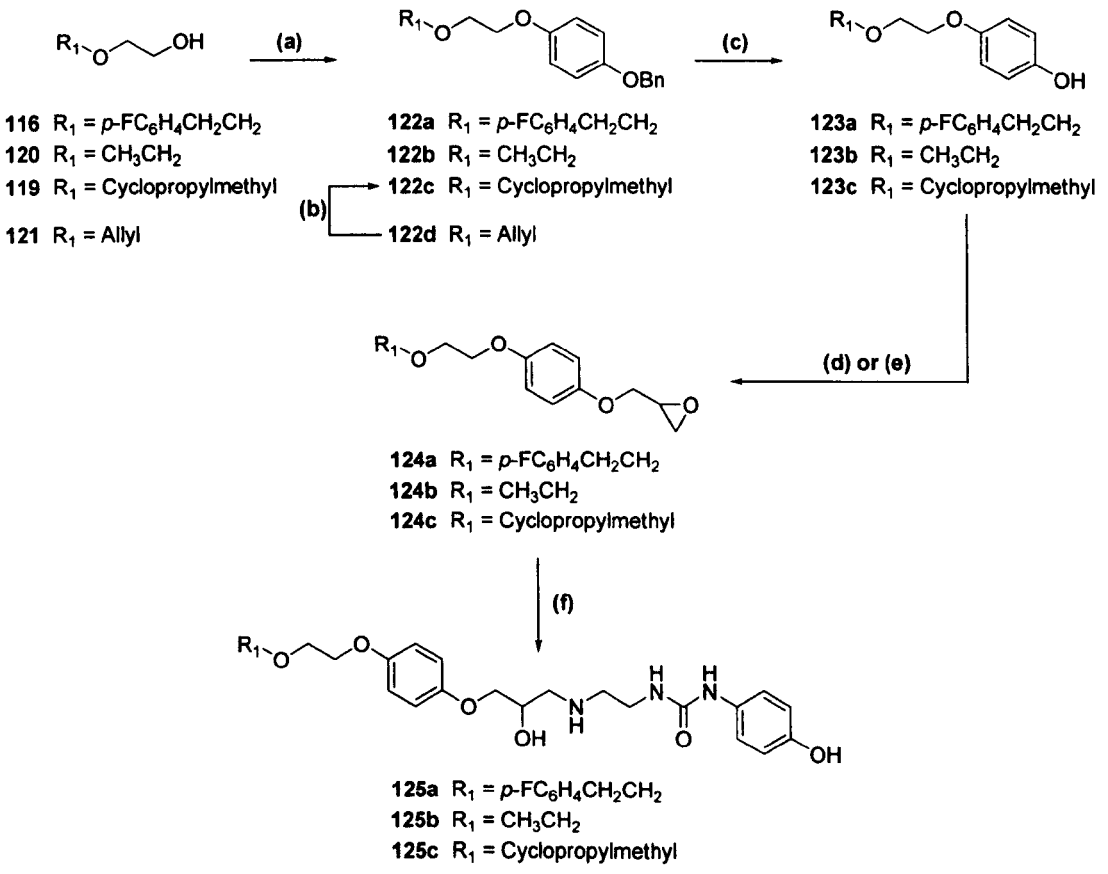
Scheme 5-1: Synthesis of 2-(alkyloxy)ethanol fragments (116 and 119).

Reagents and conditions: (a) i. NaH, DMF 60 °C; ii. chloroacetic acetic acid, 60 °C, 50%; (b) $LiAlH_4$, THF, 0 °C, 67%; (c) NaH, THF; ii. 2-chloroethoxytetrahydro-*2H*-pyran, 21 % (d) pyridinium *para*-toluenesulfonate (PPTS), EtOH, 55 °C, 79%.

2-(cyclopropylmethoxy)ethanol **(119)** synthesis initially required condensation of cyclopropylmethanol **(117)** with 2-chloroethoxytetrahydro-*2H*-pyran. The reaction proceeded, though in low yield, which is likely due to a combination of factors. Firstly, **117** may not be stable to formal deprotonation by NaH due to allylic rearrangement. Secondly, the ether product **118** may be volatile such that loss through rotary evaporation may have been encountered.

Deprotection of the THP-protected alcohol was carried out in acidic conditions using PPTS, according to literature procedures.[173]

The remainder of epoxide syntheses was carried out largely in the manner described for the cyclopentyl derivative **62** in **Chapter 3**. Initially, the 2-(alkyloxy)ethanols **116**, **119**, and **120** were condensed with 4-(benzyloxy)phenol using Mitsunobu chemistry (**Scheme 5-2**). Use of different azodicarboxylates was attempted, however diethyl azodicarboxylate (DEAD) and DIAD were found to offer the best results. Although DBAD is designed for easy removal on acidic workup[147, 148], the removal of the hydrazine product was found not to be problematic.



Scheme 5-2: Synthesis of 1-(2-(3-(4-(2-(alkyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-((4-hydroxy)phenyl)urea analogues varied at the ethylene glycol linker terminus (125a-c).

Reagents and conditions: (a)PPh_3, 3-(benzyloxy)phenol, DEAD or DBAD or DIAD, DCM, 35 – 85%; (b) diethyl zinc, diiodomethane, toluene, 0 °C →rt, 97%; (c) H_2, 10% Pd/C, EtOH, 68 – 100%; (d) i. 2M $NaOH_{(aq)}$; ii. *rac*-epichlorohydrin, 60 °C, 62 – 84%; (e) i. NaH, DMF; ii. *rac*-epichlorohydrin, 71 – 84%; (f) **56**, propan-2-ol, reflux, 21 – 43%.

Benzyl deprotection of ethers **122a-c** was achieved *via* catalytic hydrogenation using 10% Pd/C, as previously described to afford

phenols **123a-c**. Finally, alkylation of phenols **123a-c** was carried out in one of two conditions. In the case of **123c**, aqueous NaOH was employed as a base[143], followed by reflux in *rac*-epichlorohydrin. This approach was selected, as NaH was deemed too strong a base in the presence of the cyclopropyl group. The remaining phenols were deprotonated using NaH as the base, in DMF, before stirring in an excess of *rac*-epichlorohydrin at room temperature, based on literature precedent[132].

The poor yields obtained in the initial synthetic steps for the cyclopropylmethoxy- analogue, prompted an alternative route to be investigated. A more direct route was envisaged by employing Simmons-Smith chemistry[139]. Coupling of the readily-available 2-(allyloxy)ethanol **(121)**, with 4-(benzyloxy)phenol under Mitsunobu conditions, allowed formation of the ether **122d**. Direct conversion of **122d** to **122c**, in near-quantitative yield, was achieved using diethyl zinc and diiodomethane as Simmons-Smith reagents.[139] This route eliminates several problems of the initial synthesis. Firstly, the reaction can be monitored with ease by visualisation under UV light at 254 nm, due to the presence of the aryl groups. Consequently, this allows easier purification by column chromatography. Secondly the non-volatile nature of the ether product **122d**, eradicates any loss through evaporation during solvent removal. Finally, the late installation of the cyclopropyl group means there is no need to use cyclopropylmethanol **(117)**, as an initial starting material – thus removing any loss through incompatibilities with NaH. Overall, ether **122c** is obtained over 2 high-yielding steps rather than three steps, two of which are low to moderate in yield.

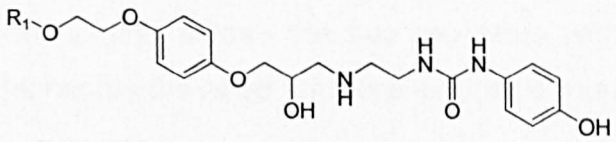
Aryloxypropanolamines **125a-c** were obtained by overnight reflux of epoxides **124a-c**, in propan-2-ol, with an excess of amine **56** present.

5.1.3 Pharmacology results

5.1.3.1 Whole cell binding

The β1AR and β2AR binding affinities, and subsequent selectivity of the newly synthesised analogues, are compared in **Table 5-1**.

As can be seen, all four compounds are highly selective towards the β1AR. The bulkier and more extensive 4-fluorophenethyl compound **125a** displayed the highest β1AR affinity of all, but also a raised affinity for the β2AR and consequently the lowest degree of relative receptor selectivity.



	R_1	Binding affinity, log K_D (M)		β_1/β_2 selectivity
		β_1-adrenoceptor	β_2-adrenoceptor	
57	cyclopentyl	-8.16 ± 0.08	-5.45 ± 0.10	513
125a	4-fluorophenethyl	-8.50 ± 0.07	-5.99 ± 0.04	326
125b	ethyl	-7.08 ± 0.04	log IC_{50} > -4*	HIGH
125c	cyclopropylmethyl	-7.52 ± 0.03	log IC_{50} > -4*	HIGH

Table 5-1: Human β_1 and β_2-adrenoceptor binding affinities and receptor selectivity of 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-((4-hydroxy)phenyl)urea analogues varied at the ethylene glycol linker terminus (125a-c).

n ≥ 5 for all assays. * maximum concentration of compound applied within limits of experiment, with no binding activity detected.

Although the smaller ethyl and cyclopropylmethyl $\mathbf{R_1}$ groups caused a lowering of the β1AR affinity relative to the parent cyclopentyl compound **57**, they also displayed no detectable binding at the β2AR when concentrations of 100 μM were applied. The concentration of compound that can be applied is limited by the amount of DMSO required to dissolve it, and therefore by the amount of DMSO that can be applied in the assay. In the case of **125b** and **125c**, a maximum concentration of 100 μM of compound was tolerated. Even at this concentration, neither compound showed complete displacement of the radioligand. Consequently, although it is possible to report a log IC_{50}

value of > -4 M for both **125b** and **125c**, an accurate value for K_D cannot be extrapolated. It is entirely possible that neither **125b** nor **125c** bind to the β2AR, however it is not possible to determine this from the assay employed. It is evident that both **125b** and **125c** are highly selective towards the β1AR, with **125c** being the more selective of the two compounds on account of its higher β1AR affinity.

Overall, the larger groups such as cyclopentyl and 4-fluorophenethyl impart higher levels of binding for the β1AR, but also raise affinity for the β2AR. It may be that both receptors share similar bulk-preferring pockets at this part of the binding site for these ligands.

The variability in binding across the two receptors with these simple modifications, highlights the need for more extensive modification to this part of the core structure.

When the cyclopropylmethyl compound **125c** is compared to LK 204-545 **(44)** (see **Chapter 1**), it is evident that although the presence of the nitrile group increases affinity for the β1AR, the selectivity conferred by **125c** may be higher due to lack of β2AR binding within the limits of the assay. Bearing in mind the difficulty encountered in synthesising **44**, the lower β1AR affinity (but higher selectivity) offered by lack of the nitrile group on **125c** seems acceptable.

5.1.3.2 *In-vivo* activity of 1-(2-(3-(4-(2-(cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea (125c)

The anticipated high β_1/β_2-selectivity of 1-(2-(3-(4-(2-(cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea **(125c)**, prompted an investigation into whether high selectivity would be maintained *in vivo*.

A small study (n = 4) was carried out by Professor Sheila Gardiner (School of Biomedical Sciences, QMC, University of Nottingham), monitoring regional hemodynamics in the conscious rat, [174, 175] according to the following procedure. All drugs were administered by

intra-venous injection through the tail. After a baseline period of at least 30 minutes, all animals were given atropine (1 mg/kg) as a bolus dose, with maintenance dosing of 1 mg/kg/h throughout the experiment to block vagal effects on reflex tachycardia. Starting 45 minutes after initial atropine administration, animals were given 3 minute infusions of either salbutamol (1.8 µg/kg/min) or isoprenaline (360 ng/kg/min). After 20 minutes, those animals that had been administered salbutamol were given isoprenaline, and those that had been administered isoprenaline were given salbutamol (doses as above). After a further 20 minutes, all animals were given a single bolus dose of **125c** (10 mg/kg), and 30 minutes thereafter, β-agonist administration was repeated as above.

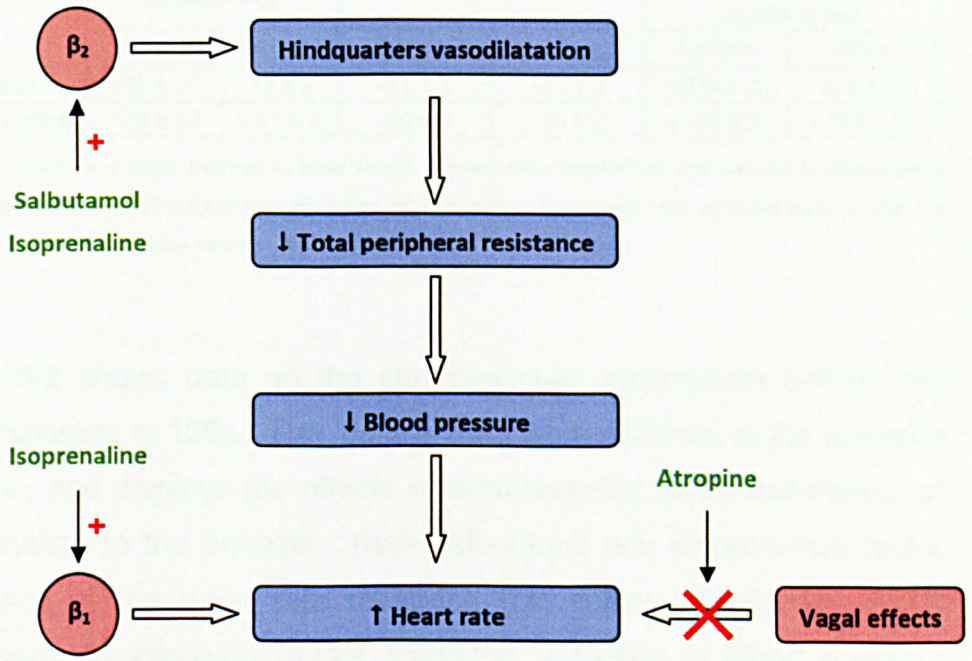


Figure 5-1: Pictorial representation of homeostatic response to peripheral vasodilatation elicited by β_2-adrenoceptor activation in the *in-vivo* rat model.

As can be seen in **Figure 5-1**, administration of salbutamol, a selective β2AR agonist causes β_2-mediated hindquarters vasodilatation. This in turn causes a fall in total peripheral resistance and subsequent drop in blood pressure evoking a reflex tachycardia response. The tachycardia is comprised of both sympathetic and vagal components. The effects of the vagal reflex can be removed by pre-treatment with the muscarinic

antagonist, atropine. As such, any observed reflex tachycardia is sympathetic in this model, and mediated by the β1AR in the heart.

When isoprenaline (a non-selective β1AR and β2AR agonist) is applied in the place of salbutamol, the same reflex tachycardia is observed downstream of β2AR activation. In addition, direct, non-reflex activation of the β1AR occurs, increasing tachycardia further.

This model allows parameters such as tachycardia, hypotension and vascular conductance to be recorded from the freely moving animal.

	Heart rate response (beats/min)		Hypotension (mm Hg)		% Increase in hindquarters vascular conductance	
	Before	After	Before	After	Before	After
Salbutamol	+55 ± 14	+7 ± 4	-11 ± 1	-11 ± 3	+106 ± 20	+98 ± 15
Isoprenaline	+74 ± 12	+11 ± 4	-19 ± 3	-18 ± 2	+126 ±20	+129 ± 19

Table 5-2: Effect on changes (relative to basal levels) in heart rate, hypotension and increase in hindquarters vascular conductance elicited by either salbutamol or isoprenaline, before and after administration of 125c (10 mg/kg) in the conscious, freely-moving rodent model (n = 4).

Table 5-2 shows data on the cardiovascular parameters before and after exposure to **125c**. The 'before' data were obtained in the absence of **125c**, and displays the effects of administering either salbutamol or isoprenaline to the animals. Both salbutamol and isoprenaline cause increases in the heart rate response (i.e. reflex tachycardia, β1AR mediated), hypotension (β2AR mediated reduction in blood pressure through vasodilatation), and an increase in vascular conductance (β2AR mediated). The tachycardia in the isoprenaline experiments is clearly higher – a reflection of direct β1AR mediated cardiac stimulation.

The 'after' data was obtained by administering **125c** before repeated agonist challenge by either isoprenaline or salbutamol. The β1AR-mediated tachycardia elicited directly by isoprenaline, and indirectly by salbutamol, is considerably reduced by **125c** in both cases to little over basal levels. Within the realms of experimental error, the β2AR related parameters are unaffected. Although quantitative values for receptor selectivity cannot be obtained, this preliminary study has demonstrated

the ability of **125c** to block agonist responses at the β1AR in-vivo, without affecting β2AR related parameters measured in the hindquarters vascular bed. This demonstrates, in principle, the role of **125c** as a potentially potent and specific β1AR antagonist.

5.1.3.3 Reporter gene assays

An assessment of the functional profile of **125c** was carried out in a high expression reporter gene assay by Dr. Jillian Baker.

In cells expressing the human β1AR, ligands with agonist activity are able to activate the receptor and initiate the secondary messenger signalling system (G-protein activation of AC, and production of cAMP – see **Chapter 1**). The downstream expression of the reporter gene (after phosphorylation of CREB by PKA and subsequent binding the CRE promoter) produces secreted placental alkaline phosphatase (SPAP), which is secreted by the cell and can then be quantified.[27, 176]

Application of **125c** to the assay cells was found to cause SPAP production, indicating **125c** possesses agonist activity at the human β1AR. However, the physiological relevance of this activity is unclear for a number of reasons. Firstly, the assay comprises of cells with very high surface receptor expression which is not comparable to physiological levels. Secondly the effect of signal amplification inherent to the secondary messenger system means a very weak agonist stimulus can result in considerable levels of SPAP production downstream.

An accurate evaluation of whether **125c** is an efficacious agonist would require an assay measuring activation of AC, thus minimising signal amplification. Additionally, physiological relevance could only be assessed in an assay employing physiological levels of surface receptor concentration.

5.2 Modification of the aryloxy group adjacent to the propanolamine

To gain further insight into the effect on β1AR and β2AR affinity from modification of the central aryloxypropanol ring, synthesis of a series of analogues was initiated based upon known ligands with high affinity for β1AR;[81] propranolol **(7)**, bupranolol **(22)** and ICI 89406 **(41)** (**Table 5-3**); as well as commercial availability of precursors.

Bupranolol and propranolol are actually more selective towards the β2AR (**Table 5-3**). Despite this, their high affinity for the β1AR makes them suitable candidates for investigation.

The results from **Chapter 3** indicate that additional selectivity for the β1AR is conferred by the presence of *m*-Cl or *p*-OH substituents on the phenylurea ring. Combining these fragments with the aryloxypropanolamine motifs of the high β1AR affinity compounds was postulated to give compounds with both high β1AR affinity and high selectivity.

	Compound	Binding affinity, log K_D (M)		β_1/β_2 selectivity
		β_1-adrenoceptor	β_2-adrenoceptor	
7	Propranolol	-8.16 ± 0.08	-9.08± 0.06	-8*
22	Bupranolol	-8.51 ± 0.04	-9.85± 0.05	-22*
41	ICI 89406	-8.91 ± 0.09	-7.07± 0.06	69

Table 5-3: Human β_1 and β_2-adrenoceptor binding affinities and receptor selectivity of propranolol, bupranolol and ICI 89406.

Data taken from selectivity study carried out by Dr. Jillian Baker using the same assay techniques.[81] * These compounds are actually more selective towards the β2AR.

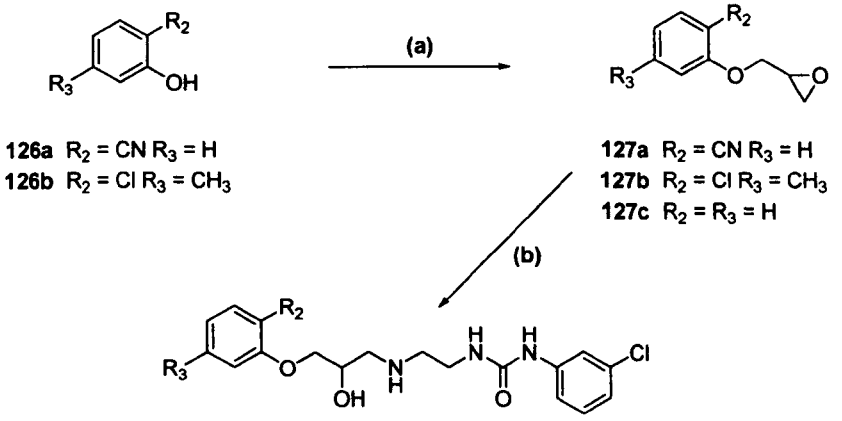
In addition, the corresponding *m*-Cl analogues of **125b** and **125c** were made. The phenyl derivative was also synthesised as there are no substituents on the ring of the aryloxypropanolamine component. The remaining *p*-OH analogues are yet to be synthesised.

5.2.1 Principles of synthetic strategy

Once again, synthesis of the appropriate epoxide was required before opening with 1-(2-aminoethyl)-3-(3-chlorophenyl)urea **(66k)**. Phenyl glycidyl ether **(127c)** was commercially available and epoxides **124b** and **124c** had been previously synthesised (see above).

5.2.2 General synthesis

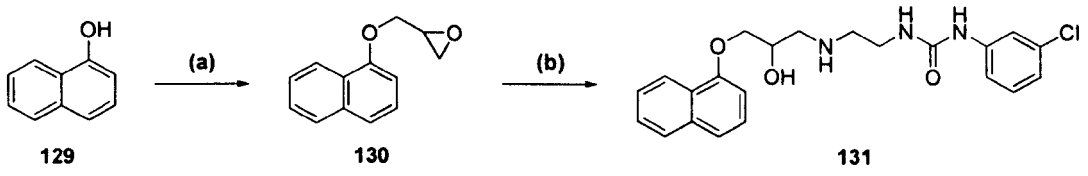
Synthesis of aromatic glycidyl ether compounds **127a**, **127b** and **130** was carried out in a similar fashion to previous phenol alkylation reactions, with *rac*-epichlorohydrin (see above and **Chapter 3**). In each case, NaH was used as the base to generate the sodium phenolate salt, before overnight reaction with excess *rac*-epichlorohydrin (**Scheme 5-3** and **Scheme 5-4**).



Scheme 5-3: Synthesis of 1-(2-(3-(aryloxy)-2-hydroxypropylamino)ethyl)-3-(3-chloro)phenyl)urea analogues with modified substituents on the aryl group adjacent to propanolamine (128a-c)

Reagents and conditions: (a) i. NaH, DMF; ii. *rac*-epichlorohydrin, 21 – 50%; (b) **66k**, TEA, propan-2-ol, reflux, 11 – 14%.

The alkylation of phenols **126a-b** provided the desired compounds **127a-b**, though in much lower yield than the corresponding reaction involving 1-naphthol **(129)**.

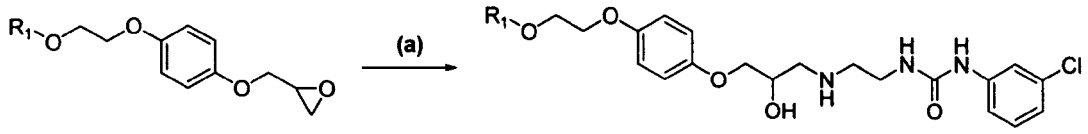


129 130 131

Scheme 5-4: Synthesis of 1-(2-(2-hydroxy-3-(naphthalen-1-yloxy)propylamino)ethyl)-3-(3-chlorophenyl)urea (131).

Reagents and conditions: (a) i. NaH, DMF; ii. *rac*-epichlorohydrin, 100%; (b) **66k**, TEA, propan-2-ol, reflux, 16%;

Opening of epoxide compounds **127a-c**, **130**, and **124b-c** was carried out under previously described conditions, by the amine **66k** in propan-2-ol, using TEA to neutralise the hydrochloride salt (**Scheme 5-3**, **Scheme 5-4** and **Scheme 5-5**).



124b $R_1 = CH_3CH_2$
124c R_1 = Cyclopropylmethyl

132a $R_1 = CH_3CH_2$
132b R_1 = Cyclopropylmethyl

Scheme 5-5: Synthesis of 1-(2-(3-(4-(2-(alkyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-((3-chloro)phenyl)urea analogues varied at the ethylene glycol linker terminus.

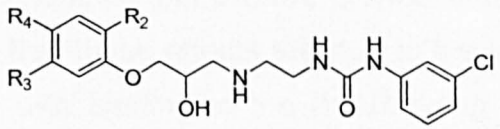
Reagents and conditions: (a) **66k**, TEA, propan-2-ol, reflux, 9 – 11%.

As before, all desired aryloxypropanolamine compounds were obtained in relatively low yield.

5.2.3 Pharmacology results

Pharmacology data for the *m*-Cl analogues **128a-c, 131** and **132a-b** is particularly interesting (**Table 5-4**). Firstly, if *m*-Cl compounds **71l**, **132a** and **132b** are compared with the corresponding *p*-OH compounds **57, 125b** and **125c** (**Table 5-1**), trends between different alkyloxy substituents are preserved. The cyclopentyloxy- compounds **57** and **71l** have higher β1AR affinity than their corresponding ethoxy- and cyclopropylmethoxy- counterparts.

Ethoxy- compound **132a** displays a higher β1AR affinity than the corresponding compound containing the *p*-OH substituent **(125b)**, which would not be expected. In fact, the binding profiles between *m*-Cl compounds **132a** and **132b** is essentially identical, whereas in the case of the corresponding *p*-OH compounds, the cyclopropylmethoxy- motif offers a higher β1AR affinity.



	R_2	R_3	R_4	Binding affinity, log K_D (M)		β_1/β_2 selectivity
				β_1-adrenoceptor	β_2-adrenoceptor	
71l	H	H	2-(cyclopentyloxy)ethoxy	-8.14 ± 0.04	-5.59 ± 0.05	355
128a	CN	H	H	-9.23 ± 0.05	-7.15 ± 0.06	120
128b	Cl	CH_3	H	-8.88 ± 0.04	-7.62 ± 0.11	18
128c	H	H	H	-8.52 ± 0.04	-7.00 ± 0.07	33
131	naphthyl ring			-8.39 ± 0.08	-7.52 ± 0.03	7
132a			2-(ethoxy)ethoxy	-7.50 ± 0.06	-5.20 ± 0.04	200
132b			2-(cyclopropylmethoxy)ethoxy	-7.53 ± 0.06	-5.24 ± 0.03	195

Table 5-4: Human β_1 and β_2-adrenoceptor binding affinities and receptor selectivity of 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-((3-chloro)phenyl)urea analogues varied at the ethylene glycol linker terminus / aromatic ring adjacent to the propanolamine (128-c, 131, 132a-b).

n ≥ 5 for all assays.

The difference between the *p*-OH and *m*-Cl substituents appears to arise from the change in affinity for the β2AR. The difference between the cyclopentyl compounds **57** and **71l** is not so great. However with the ethoxy- and cyclopropylmethoxy- compounds, changing the substitution pattern of the phenylurea from *p*-OH to *m*-Cl causes an increase in affinity for the β2AR, although binding is still poor. Overall, this means the pattern of *p*-OH compounds offering better β_1/β_2 selectivity than the corresponding *m*-Cl remains.

The remaining compounds from **Table 5-4** posses no alkyl substituent at the R_4 position. Remarkably, very minor changes to the nature of the aromatic component of the aryloxypropanolamine motif, confer quite large changes to the binding profiles of each compound to the two

receptors. Interestingly, all these aryl motifs (compounds **128a-c** and **131**) have very high β1AR affinity as well high β2AR affinity.

The presence of an *o*-CN group on **128a** augments the affinity for the β1AR to sub-nanomolar levels, whilst only marginally increasing affinity for the β2AR relative to the unsubstituted compound **128c**. In comparison, compound **128b** (where R_2 = Cl and R_3 = CH_3) exhibits a slightly raised β1AR affinity, but a more pronounced increase in β2AR affinity. It may be that these effects are due to the electronic nature of the aromatic ring, with binding to the β1AR being favoured by more electron deficient rings such as that on **128a**. In comparison, the compounds with an alkyloxy-substituent at the R_4 position display a much reduced affinity for the β2AR, as well as a reduction in β1AR affinity. The 1,4-dialkaryl ether system means the central aromatic ring is more electron proficient due to mesomeric donation by each of the oxygen atoms.

In addition to apparent electronic effects, the effect due to the steric presence of the R_4 group is apparent. Both the 2-(cyclopropylmethoxy)ethoxy- and 2-(ethoxy)ethoxy- compounds have lower β1AR affinities, whereas the bulkier 2-(cyclopentyloxy)ethoxy- compound has a higher β1AR affinity. So although R_4 substituents appear to diminish affinity for the β1AR and β2AR considerably, the termination of this substituent in a bulkier group allows the β1AR affinity to be regained at the relative expense of β2AR affinity – i.e. selectivity improves. This indicates that although both receptors tolerate *para*-substituents on the aromatic ring less well, relative to no substitution at this position, the β1AR appears to have a preference for bulkier groups attached to this substituent, but further away from the aryloxypropanolamine motif.

The importance of the 1-(2-aminoethyl)-3-(3-chlorophenyl)urea group in imparting β_1/β_2-selectivity is further demonstrated by **128b** and **131**. The β2AR selective compounds propranolol and bupranolol (**Table 5-3**), have been converted to β1AR selective compounds by replacement of the *N*-alkyl group with this motif.

In order for the full steric and electronic effects of ring substituents to be compared, a much more comprehensive study is necessary to elucidate structure activity relationships. Quite evidently both the β1AR and β2AR are sensitive to minor changes at the aromatic region of the aryloxypropanolamine motif.

In addition, the full ranges of corresponding analogues bearing the p-OH substituent on the phenylurea ring are yet to be synthesised. Earlier results (**Table 5-1**) would indicate that these would confer much better selectivity over the *m*-Cl analogues, through a reduction in affinity for the β2AR.

5.3 Structure-activity relationships

The modifications to the core pharmacophore introduced in this chapter are in some cases quite subtle, though produce quite drastic changes in the binding profiles of the relevant compounds.

In terms of *p*-substituents on the aromatic ring of the aryloxypropanolamine motif, it is evident that these cause a reduction in affinity for both the β1AR and β2AR, relative to no substituent on the ring. The reduction in affinity is more drastic for the β2AR. The affinity reduction for the β1AR imparted by this modification can be reversed if the ethylene glycol linker is terminated with a more bulky cyclopentyl or extended 4-fluorophenethyl group. This indicates the presence of a bulk-preferring pocket in the β1AR, some distance away from the core aryloxypropanolamine motif, which is not present in the β2AR.

The electron withdrawing CN group placed *ortho* to the oxypropanolamine chain confers very high β1AR affinity without great increases in β2AR binding. However the overall effect when several substituents are present on this ring is more complicated.

In the case of LK 204-545 **(44)**, the combination of an *o*-CN and *p*-alkyloxy group produces gains in terms of β1AR affinity but, also a potential loss in selectivity due to an associated rise in β2AR affinity, in comparison to **125c**.

This complicated multi-substituent effect is again evident when a 2-chloro-5-methyl- system is present as both β1AR and β2AR affinities increase considerably relative the unsubstituted compound.

Simple aryl groups (not extensively substituted) are tolerated with high affinity at both receptors. The relative sensitivity of the receptors to modifications to the ring on the aryloxypropanolamine motif is apparent, as relatively minor changes bring about changes in selectivity.

The ability of both receptors to tolerate a larger aromatic system is evident from the high affinity imparted by the naphthyl group on both receptors.

The aryloxypropanolamine is known to be essential for binding at both receptors[170], and as would be expected, subtle change on or near to the motif produce distinct differences in the binding profile of subsequent ligands. In order to get a clearer picture of the effect of changes to this region of the core pharmacophore, a more extensive library of molecules needs to be synthesised.

6. CONCLUSIONS AND FUTURE WORK

6.1 General conclusions

Using LK 204-545 **(44)** as an initial lead compound, an extensive library of aryloxypropanolamine analogues were synthesised, characterised and underwent *in-vitro* testing for binding at human β_1 and β_2 adrenoceptors (carried out by Dr Jillian Baker).

A novel and safer route for the synthesis of **44** was devised, though was found to be protracted and low yielding. This route could be improved by selection of a more appropriate protecting group to replace PMB. The affinity of **44** for the β1AR was found to be lower than the literature value[131], though this is likely to be due to differences in assays used.

On the basis of synthetic ease, a new lead was selected omitting the nitrile motif of **44**. The cyclopropylmethyl group was replaced with a cyclopentyl group with the aim of increasing β1AR affinity[106] whilst retaining a facile synthetic route.

Modification of various moieties on the new lead compound **57**, allowed the generation of new SAR and ultimately afforded the highly β1AR selective compound **125c**. This compound was selected for a pilot *in-vivo* study in the regional hemodynamic rat model[174, 175] and displayed essentially selective β1AR antagonist activity (carried out by Professor Sheila Gardiner).

A summary of the SAR generated is given below.

6.1.1 Substitution of the aromatic ring adjacent to the urea

Substitution of the aromatic ring adjacent to the urea was investigated, including *ortho*-, *meta*-, and *para*- substitution with a variety of electron donating and withdrawing groups (trifluoromethyl, fluoro, chloro, bromo, methyl, hydroxy, and methoxy). The unsubstituted analogue **71a** was also synthesised.

With the exception of the *m*-F derivative **71i**, all variants had poor β2AR affinity (K_D = 537 nM – 3548 nM). Relative to the unsubstituted variant **71a**, all *ortho*-substituents were found to reduce β1AR affinity in a sterically sensitive fashion (*o*-F retained reasonable β1AR affinity). The *meta*-substituents generally increased β1AR affinity, whilst *para*-substituents generally caused a small reduction in β1AR affinity, with the exception of the *p*-OH variant which displayed extremely high β1AR affinity (K_D = 7 nM).

Overall, the highest β1AR affinity was displayed by the *m*-F, *m*-Cl and *p*-OH analogues, whilst best β_1/β_2 selectivity was afforded by *p*-OH, *m*-Cl and unsubstituted analogues.

Attempts to combine substituents conferring the highest affinity (*m*-F and *p*-OH) to produce a 3,4-disubstituted compound resulted in lower β1AR affinity overall.

6.1.2 Modification of the urea moiety

Isosteres of the urea moiety were investigated, including thiourea, sulfonamide, amide, and carbamate. Additionally, the ether compound **110a** was synthesised.

Overall, the parent urea compound **71a** retained the best β_1/β_2 selectivity. Generally, replacement groups resulted in lower affinity for the β1AR and raised β2AR affinity. Although the urea is not essential for binding at the β1AR receptor (the ether compound **110a** retains relatively high affinity), its ability to act as both hydrogen bond donor and acceptor seems key to selectivity.

The two amide compounds **110e** and **110f** indicate that the two N-H groups present on the urea have very specific roles affecting receptor binding. The N-H group adjacent to the ring seems to be important in suppressing β2AR affinity, whereas the more distant N-H group (alkyl chain side of the urea) is important for high β1AR activity. Indeed phenylacetamide **110f** displays higher β1AR affinity than the parent urea compound.

The thiourea **110b** displays lower β1AR affinity than the parent urea compound **71a**, which may suggest the importance of a good hydrogen-bond acceptor motif.

Larger groups (sulfonamide, thiourea) appear to be less well tolerated than smaller groups in this area.

A complete investigation of analogues was not possible due to difficulties synthesising the ester, phenolic carbamate, and *N*-phenyl sulfonamide compounds.

6.1.3 Variants of the aryloxy group and associated linkers

The aromatic ring of the aryloxypropanolamine is particularly sensitive to substituent changes.

Simple aromatic derivatives bearing either phenyl or naphthyl rings possess high affinity at both receptor sub-types, and thus poor selectivity. The N-alkyl substituent is likely to be responsible for the degree of selectivity exhibited by compounds **128c** and **131**.

Substitution *para*- to the oxypropanolamine group results in a reduction in affinity for both receptors, relative to the unsubstituted compound **128c**. However, the reduction in affinity is more pronounced for the β2AR. Termination of the ethylene glycol linker with a bulkier group allows affinity for the β1AR to return. Thus cyclopentyl **(57)** and 4-fluorophenethyl **(125a)** compounds were found to have higher β1AR affinities than the corresponding ethyl **(125b)** and cyclopropylmethyl **(125c)** variants. These groups have a small effect on raising the β2AR affinities; hence selectivity was lower in the bulkier compounds.

The electronic nature of the aryloxy group may be key in determining the binding profile of these compounds. Installation of an electron withdrawing *o*-CN group alone proffers extremely high β1AR affinity and good selectivity, whereas the 2-chloro-5-methyl analogue has raised affinity at both receptor subtypes. Overall, it is likely that a balance of steric and electronic factors is important in determining the best selectivity and affinity for the β1AR.

The interplay between different ring modifications is best displayed when comparing LK 204-545 **(44)** with **125c**. LK 204-545 has high β1AR affinity and selectivity. Although removal of the nitrile group causes a small reduction in β1AR affinity, there is also no detectable β2AR affinity within experimental limits for **125c**, thus high selectivity is retained.

Overall, a more extensive investigation into ring substituents is necessary as well as completion of synthesis of the corresponding analogues with *p*-OH substitution in place of the *m*-Cl group.

6.2 Future work

Work carried out to date has provided several avenues for further investigation, these are detailed below.

6.2.1 Computational studies

6.2.1.1 QSAR

The structural similarity of the analogues, yet diversity of binding profiles of the compounds synthesised to date warrants an in-depth QSAR study to be undertaken. Although QSAR algorithms such as CoMFA assume a single binding conformation in the receptor of all ligands included, such a study could generate a wealth of valuable information based upon fixed parameters and attributes of functional groups.

The information generated, would be used to design and synthesise a new library of molecules. This kind of iterative process allows a more rational approach to drug design.

6.2.1.2 Molecular modelling

The recent publication of coordinates from crystal structures for the β1AR and β2AR may prove timely for this project.[31, 36, 38, 41] In particular, the β1AR has been co-crystallised with the antagonist cyanopindolol.[41] Taking the conformation of cyanopindolol as a general template for antagonist binding at the β1AR, the structures of

compounds synthesised in this project could be aligned and visualised. Similar modelling experiments on the β2AR, and subsequent comparison, may reveal key favourable or unfavourable interactions responsible for the high selectivity of compounds such as **57** and **125a-c**.

Identification of these key residues, or even residues near the binding site which could be accessed, would form the basis of structural modifications designed to access them.

6.2.2 Further modification of the phenylurea

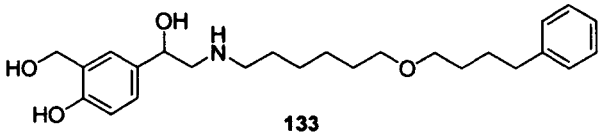
The differences in β1AR affinity displayed by the installation of simple substituents on the aromatic ring adjacent to the urea group, provide a basis for much more extensive modification.

Firstly the aromatic ring could be substituted for non-aromatic rings, and alkyl groups of differing size, as well as investigating substituent effects. This would evaluate the ability of the receptor to accommodate bulkier and more flexible structures, in comparison to the planar aromatic groups. Previous studies have shown some alkyl-substituted ureas (such as ethyl- and *n*-butyl) to have better antagonist potency and cardioselectivity than their substituted phenylurea analogues.[126]

Replacement of the phenyl ring with a variety of heterocycles may prove useful. Heteroatoms such as nitrogen in pyridine could potentially form polar interactions with acidic residues, whilst still maintaining π-stacking interactions. Also, larger aromatic structures may be tolerated differentially between the β1AR and β2AR.

6.2.3 Amine-urea linker study

A more in-depth study into the nature of the carbon linker between the amino and urea motifs could be of use. It is known that extremely lengthy, flexible structures such as salmeterol **(133)** can be accommodated by the β2AR[177], however similar studies with the β1AR do not appear to have been conducted.



133

Figure 6-1: Salmeterol – a long acting selective β_2 agonist

The branching of this linker could also be investigated. Branching would offer the potential to tether on additional functional groups to this part of the molecule. Previously, branching on the amino-substituent has been shown to improve antagonist potency in molecules (the *tert*-butyl substituent often imparts higher antagonist potency than corresponding *iso*-propyl derivatives[94, 96], but can have lower cardioselectivity[93]).

6.2.4 Aryloxy group modification

There is much scope for investigation of the aryloxy group. The nature of the aryl group itself had been shown to be relatively open to modification, with heteroatom containing aromatics (e.g. pindolol **25**), five membered rings (e.g. timolol **26**) and bi/tricyclic structures (e.g. propranolol **7** and carazolol **24**) being tolerated.

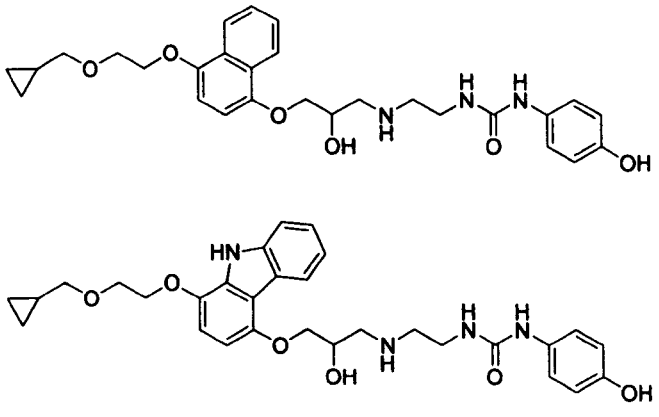


Figure 6-2: Analogues of 125c bearing the aromatic core of propranolol (top) and carazolol (bottom)

Replacement of the aromatic group, whilst retaining *para*-substitution could potentially result in compounds with higher affinity for the β1AR, whilst retaining the high cardioselectivity. **Figure 6-2** shows possible

analogues of **125c** bearing the naphthyl and carbazolyl groups of propranolol **(7)** and carazolol **(24)** respectively. The parent compounds **7** and **24** possess relatively high affinity at the β1AR, but poor selectivity.

So far, the variety of *para*-substituents investigated in this project has been limited to 2-(ethoxy)ethoxy-, 2-(cyclopropylmethoxy)ethoxy, 2-(cyclopentyloxy)ethoxy, and 2-(4-fluorophenethyloxy)ethoxy)- (see **Chapter 5**). There is much scope to alter this substituent, including a study into length of the linker between the aryloxy group and the terminal alkyl/aryl substituent, the effects of branching this linker and the nature of the terminal group.

6.2.5 Removal of ISA

Initial reporter gene-based assays of **125c** indicate it is able to activate the β1AR. These assays were carried by Dr. Jillian G Baker (Institute of Cell Signalling, Queens Medical Centre, Nottingham) using established methodology[27, 81, 141] based on CHO-K1 cells expressing human β_1-adrenoceptors and transfected with the SPAP reporter gene.[176]

The extent and physiological relevance of activation cannot be quantified without more extensive study using AC based assays in cells with physiological levels of surface receptor expression. The presence of ISA is unsurprising when the structure of **125c** is considered with respect to existing SAR. The presence of the alkyloxy group *para*- to the oxypropanolamine motif would be expected to impart a degree of agonist activity on the molecule. Such activity may possibly be eliminated using previous techniques (see **Chapter 1**) of removal of the oxygen atom linker on the *para*-substituent, or reducing its basicity (e.g. by installation of an electron withdrawing group on the adjacent aromatic ring).

It is possible to derive empirically that the agonist effects of **125c** are likely to be quite low, based upon the *in-vivo* studies carried out. In these experiments, **125c** was clearly acting as a selective antagonist of the β1AR.

6.2.6 Optimisation of synthetic route

Although attempts to improve the synthetic route have been successful throughout this work, the extent to which it is amenable to parallel synthesis is limited.

Improvement of the Mitsunobu reactions can be envisaged by use of resin-bound PPh_3, allowing ease of removal of triphenylphosphine oxide. Reagents such as DBAD can be eliminated by acidic workup. Overall, this may eliminate the need for silica-based purification from these reactions.

The lowest yielding reaction in the pathway is the terminal epoxide opening. An alternative route could be employed where the epoxide is opened with either *N*-benzyl-2-nitroethylamine or *N*-benzyl-2-azidoethylamine present in stoichiometric quantities. Opening of the epoxide with a secondary amine should eliminate the expected side products, as well as the need to use a large excess of amine. Subsequent reduction of the nitro/azide group would then liberate a reactive primary amine, which could be further functionalised as the appropriate urea. The desired secondary amine could then be released after hydrogenolysis of the N-benzyl group. Such a route may improve the overall yield obtained. In addition, purification of the final compound is likely to be relatively straightforward as it would not require separation of mixtures of amines.

7. EXPERIMENTAL

7.1 General chemistry

Chemicals and solvents were purchased from standard suppliers and used without further purification. Merck Kieselgel 60, 230-400 mesh, for flash column chromatography (FCC) was supplied by Merck KgaA (Darmstadt, Germany) and deuterated solvents were purchased from Goss International Limited (England) and Sigma-Aldrich Company Ltd (England).

Unless otherwise stated, reactions were carried out at ambient temperature. Reactions were monitored by thin layer chromatography on commercially available precoated aluminium backed plates (Merck Kieselgel 60 F_{254}). Visualisation was by examination under UV light (254 and 366 nm). General staining carried out with Ninhydrin (solution in ethanol), $KMnO_4$ or phosphomolybdic acid (PMA). All organic extracts after aqueous work-up procedures were dried over $MgSO_4$ or Na_2SO_4 before gravity filtering and evaporation to dryness. Organic solvents were evaporated *in vacuo* at ≤ 40°C (water bath temperature). Purification using preparative layer chromatography (PLC) was carried out using Fluka silica gel 60 PF254 containing gypsum (200 mm x 200 mm x 1 mm). Flash chromatography was performed using Merck Kieselgel 60 (0.040-0.063 mm).

Melting points (Mp) were recorded on a Reichert 7905 apparatus or Perkin Elmer Pyris 1 differential scanning calorimeter and were uncorrected. Fourier transform – infrared (FT-IR) spectra were recorded as thin films or KBr discs in the range of 4000 – 500 cm^{-1} using and Avatar 360 Nicolet FT-IR spectrophotometer. Optical rotation was measured on a Bellingham-Stanley ADP220 polarimeter.

High resolution mass spectra (HRMS) – time of flight, electrospray (TOF ES +/-) were recorded on a Waters 2795 separation module/micromass LCT platform.

^{1}H NMR spectra were recorded on a Bruker-AV 400 at 400.13 MHz. ^{13}C NMR spectra were recorded at 101.62 MHz. Chemical shifts (δ) are recorded in parts per million (ppm) with reference to the chemical shift of the deuterated solvent/an internal tetramethylsilane (TMS) standard. Coupling constants (J) and carbon-fluorine coupling constants (J_{CF}) are recorded in Hz and the significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m), doublet of doublets (dd), doublet of triplets (dt). Spectra were assigned using appropriate COSY, distortionless enhanced polarisation transfer (DEPT), HSQC and HMBC sequences. Unless otherwise stated all spectra were recorded in $CDCl_3$.

Analytical HPLC was performed using system 1a/1b and either system 2 or system 3 to confirm purity. All retention times (R_t) are quoted in minutes. System 1: Phenomenex Onyx Monolithic reverse phase C_{18} column (100 x 4.6 mm), a flow rate of 5.00 mL/min (system 1a) or 3.00 mL/min (system 1b) and UV detection at 287 nm. Linear gradient 5% - 95% solvent B over 10 minutes. Solvent A: 0.1% formic acid (FA) in water; solvent B: 0.1% FA in MeCN.

System 2: Vydac reverse phase C_8 column (150 x 4.6 mm), a flow rate of 1.00 mL/min and UV detection at 287 nm. Linear gradient 5% - 95% solvent B over 24 minutes. Solvent A: 0.06% TFA in water; solvent B: 0.06% TFA in MeCN.

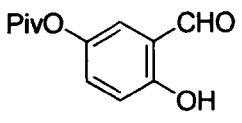
System 3: Waters symmetry reverse phase C_{18} column (75 x 4.6 mm), a flow rate of 1.00 mL/min and UV detection at 287 nm. Linear gradient 5% - 95% solvent B over 20 minutes. Solvent A: 0.1% FA in water; solvent B: 0.1% FA in MeOH.

Preparative HPLC was performed using a Phenomenex Onyx Monolithic reverse phase C_{18} column (100 x 10 mm), a flow rate of 14.10 mL/min and UV detection at 287 nm. Samples were run in 5% - 95% solvent B over 10 minutes. Solvent A: 0.1% FA in water; solvent B: 0.1% FA in MeCN.

1-(2-(3-(2-Cyano-4-(2-(cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea hydroformate (44)

The experimental procedure for this compound is listed after the entry for 1-(2-aminoethyl)-3-(4-hydroxyphenyl)urea **(56)**.

3-Formyl-4-hydroxyphenyl pivalate (46)



2,5-Dihydroxybenzaldehyde **(45)** (5.237 g, 37.92 mmol) was dissolved in dry DMF (40 mL) at 0 °C, under a nitrogen atmosphere. TEA (4.200 g, 5.813 mL, 41.71 mmol, 1.1 molar equivalents (eq)) followed by dropwise addition of pivaloyl chloride (4.801, 4.599 mL, 39.81 mmol, 1.05 eq). The mixture was then stirred at room temperature for 4 hours. After quenching with MeOH, all solvent was removed *in vacuo*. The resulting slurry was dispersed in water (100 mL) and extracted with ethyl acetate (EtOAc) (3 x 50 mL). The combined organic layers were washed with brine (50 mL) before concentration. Purification via FCC (eluent EtOAc/hexanes 20:80) afforded 6.100 g of viscous yellow oil

Yield: 72%.

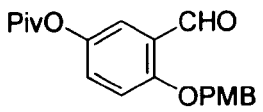
IR: 3411 (br, O-H, stretch (str)), 2976 (alkyl C-H. str), 2937, 2874 (aldehyde C-H, str), 1754 (ester C=O, str), 1690 (aldehyde C=O, str), 1608, 1586, 1494 (aryl, str), 1398 ($C(CH_3)_3$, str), 1293, 1188 (ester C-O, str), 1109 (C-OH, str).

^{1}H NMR: δ 9.98 (s, 1H, CHO), 7.18 (d, *J* = 2.8 Hz, 1H, aryl 2-H), 7.00 (dd, *J* = 8.9/2.8 Hz, 1H, aryl 6-H), 6.94 (d, *J* = 8.9 Hz, 1H, aryl 5-H), 6.60 (br s, 1H, OH), 1.40 (s, 9H, $(CH_3)_3$).

^{13}C NMR: δ 188.71 (CHO), 178.10 (ester C=O), 154.52 (aryl 4-C), 145.54 (aryl 1-C), 128.53 (aryl 3-C), 124.25 (aryl 6-C), 122.89 (aryl 2-C), 115.51 (aryl 5-C), 39.35 ($C(CH_3)$), 27.10 ($(CH_3)_3$).

m/z: HRMS (TOF ES$^-$) $C_{12}H_{13}O_4$ $[M-H]^-$ calcd 221.0819; found 221.0818.

4-(4-Methoxybenzyloxy)-3-formylphenyl pivalate (47)



NaH 60% suspension in mineral oil (495 mg, equivalent to 297 mg of NaH, 12.37 mmol, 1.1 eq) was suspended in dry DMF (10 mL) at 0 °C, under a nitrogen atmosphere. To this was added 3-formyl-4-hydroxyphenyl pivalate **(46)** (2.500 g, 11.25 mmol) in dry DMF (5 mL) and *p*-methoxybenzyl bromide (2.488 g / 1.783 mL, 12.37 mmol, 1.1 eq) in dry DMF (5 mL). After overnight stirring at room temperature and removal of DMF *in vacuo*, the resulting slurry was dispersed in water (30 mL) and extracted with diethyl ether (Et_2O) (4 x 25 mL). The combined organic fractions were washed with aqueous 2 M NaOH (20 mL) and brine (20 mL). After concentration the crude material was purified by FCC (eluent EtOAc/petroleum ether 40-60 (PE) 5:95 for 2 column volumes, 10:90 for 6 column volumes then 30:70) to give 1.267 g of white crystalline solid.

Yield: 33%.

Mp: 69 – 71 °C.

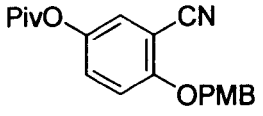
IR: 2972 (alkyl C-H, str), 2941, 2867 (aldehyde C-H, str), 1749 (ester C=O, str), 1684 (aldehyde C=O, str), 1612, 1587, 1517 (aryl, str), 1394 ($C(CH_3)_3$, str), 1268, 1255 (ester C-O, str), 1127, 1093 (C-O-C, str), 869 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 10.46 (s, 1H, CHO), 7.51 (d, *J* = 3.2 Hz, 1H, aryl 2-H), 7.34 (d, *J* = 9.0 Hz, 2H, PMB C-H *meta* to methoxy group), 7.23 (dd, *J* = 8.6/2.9 Hz, 1H, aryl 6-H), 7.06 (d, *J* = 9.0 Hz, 1H, aryl 5-H), 6.92 (d, *J* = 8.6 Hz, 2H, PMB C-H *ortho* to methoxy group), 5.10 (s, 2H, PMB CH_2), 3.81 (s, 3H, PMB CH_3), 1.34 (s, 9H, $(CH_3)_3$).

^{13}C NMR: δ 188.94 (CHO), 177.17 (ester C=O), 159.80 (PMB $COCH_3$), 158.74 (aryl 4-C), 144.81 (aryl 1-C), 129.23 (PMB C-H *meta* to methoxy group), 129.01 (PMB quaternary (4°) C *para* to methoxy group), 127.87 (aryl 6-C), 125.69 (aryl 3-C), 120.79 (aryl 2-C), 114.26 (aryl 5-C), 114.21 (PMB C-H *ortho* to methoxy group), 70.97 (PMB CH_2), 53.34 (PMB CH_3), 39.09 ($C(CH_3)_3$), 27.15 ($(CH_3)_3$).

m/z: HRMS (TOF ES$^+$) $C_{20}H_{23}O_5$ $[MH]^+$ calcd 365.1359; found 365.1341.

4-(4-Methoxybenzyloxy)-3-cyanophenyl pivalate (48)



4-(4-Methoxybenzyloxy)-3-formylphenyl pivalate **(47)** (1.250 g, 3.65 mmol) was dissolved in THF (5 mL) and aqueous 37% NH_3 solution (20 mL) with stirring. To this was added iodine (1.019 g, 4.02 mmol, 1.1 eq) and the mixture stirred for 2 hours at room temperature. After quenching with excess aqueous 10% sodium thiosulphate solution, the entire mixture was extracted with DCM (4 x 25 mL) and concentrated to afford 1.210 g of brown crystalline solid requiring no further purification.

Yield: 98%.

Mp: 95 – 97 °C.

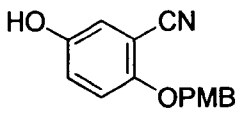
IR: 2970, 2936, 2877 (alkyl C-H, str), 2231 (CN, str), 1749 (ester C=O, str), 1612, 1586, 1516 (aryl, str), 1392 ($C(CH_3)_3$, str), 1273, 1256 (ester C-O, str), 1119, 1098 (C-O-C, str), 825 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 7.36 (d, *J* = 8.5 Hz, 2H, PMB C-H *meta* to methoxy group), 7.28 (d, *J* = 3.1 Hz, 1H, aryl 2-H), 7.19 (dd, *J* = 9.2/2.6 Hz, 1H, aryl 6-H), 6.98 (d, *J* = 9.2 Hz, 1H, aryl 5-H), 6.91 (d, *J* = 8.8 Hz, 2H, PMB C-H *ortho* to methoxy group), 5.13 (s, 2H, PMB CH_2), 3.81 (s, 3H, PMB CH_3), 1.34 (s, 9H, $(CH_3)_3$).

^{13}C NMR: δ 177.04 (ester C=O), 159.81 (PMB $COCH_3$), 158.15 (aryl 4-C), 144.25 (aryl 1-C), 128.97 (PMB C-H *meta* to methoxy group), 127.73 (aryl 6-C), 127.58 (PMB 4° C *para* to methoxy group), 126.59 (aryl 2-C), 115.65 (CN), 114.31 (PMB C-H *ortho* to methoxy group), 113.98 (aryl 5-C), 103.09 (aryl 3-C), 71.21 (PMB CH_2), 55.46 (PMB CH_3), 39.22 ($C(CH_3)_3$), 27.18 ($(CH_3)_3$).

m/z: HRMS (TOF ES$^+$) $C_{20}H_{21}NNaO_4$ $[MNa]^+$ calcd 362.1363; found 362.1388.

2-(4-Methoxybenzyloxy)-5-hydroxybenzonitrile (49)



4-(4-Methoxybenzyloxy)-3-cyanophenyl pivalate **(48)** (1.200 g, 3.54 mmol) and sodium *tert*-butoxide (850 mg, 8.84 mmol, 2.5 eq) were dissolved in MeOH (30 mL) and stirred for 2 hours. After removal of MeOH *in vacuo*, the crude product was dispersed in water (40 mL) and washed with DCM (1 x 30 mL). This formed an inseparable emulsion and was passed through a celite filter (suction) to effect layer separation. The aqueous layer was then acidified to pH 4 with careful addition of conc HCl, before extraction with DCM (4 25 mL). Concentration of the combined organic extracts gave 311 mg of an orange solid, requiring no further purification.

Yield: 34%.

Mp: 165 – 167 °C.

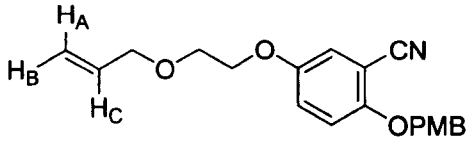
IR: 3299 (br, O-H, str), 3055 (aryl C-H, str), 2930, 2835 (alkyl C-H, str), 2243 (CN, str), 1614, 1587, 1499 (aryl, str), 822 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 7.36 (d, *J* = 8.5 Hz, 2H, PMB C-H *meta* to methoxy group), 7.03 (d, *J* = 2.9 Hz, 1H, aryl 6-H), 6.98 (dd, *J* = 8.6/2.9 Hz, 1H, aryl 4-H), 6.91 (d, *J* = 8.6 Hz, 2H, PMB C-H *ortho* to methoxy group), 6.88 (d, *J* = 9.2 Hz, 1H, aryl 3-H), 5.21 (br, s, OH), 5.07 (s, 2H, PMB CH_2), 3.81 (s, 3H, PMB CH_3).

^{13}C NMR: δ 159.71 (PMB $COCH_3$), 154.97 (aryl 2-C), 149.59 (aryl 5-C), 129.03 (PMB C-H *meta* to methoxy group), 128.10 (PMB 4° C *para* to methoxy group), 121.79 (aryl 4-C), 119.78 (aryl 6-C), 116.31 (CN), 115.18 (aryl 3-C), 114.23 (PMB C-H *ortho* to methoxy group), 103.09 (aryl 1-C), 71.47 (PMB CH_2), 55.45 (PMB CH_3).

m/z: HRMS (TOF ES^-) $C_{15}H_{12}NO_3$ $[M-H]^-$ calcd 254.0823; found 254.0839.

5-(2-(Allyloxy)ethoxy)-2-(4-methoxybenzyloxy)benzonitrile (50)



2-Allyloxyethanol (174 mg, 182 µL, 1.70 mmol, 1.5 eq), triphenylphosphine (449 mg, 1.70 mmol, 1.5 eq), and 2-(4-methoxybenzyloxy)-5-hydroxybenzonitrile **(49)** (290 mg, 1.14 mmol) were dissolved in DCM (10 mL). DIAD (346 mg, 337 µL, 1.70 mmol, 1.5 eq) was added dropwise to the reaction mixture and allowed to stir overnight. The reaction mixture was then transferred to a separating funnel and washed with aqueous 2 M NaOH (1 x 10 mL), and brine (1 x 10 mL). After concentration of the organic layer, purification was achieved via FCC (eluent EtOAc/PE 10:90 for 3 column volumes then 30:70) to give the desired product as clear colourless oil in quantitative yield.

Yield: 100%.

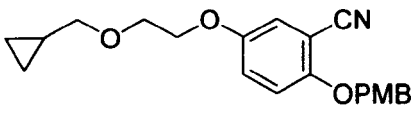
IR: 3019 (alkene C-H, str), 2936 (alkyl C-H, str), 2231 (CN, str), 1499 (aryl, str), 1107 (C-O-C, str), 930 (alkene C-H, deformation).

^{1}H NMR: δ 7.35 (d, *J* = 8.7 Hz, 2H, PMB C-H *meta* to methoxy group), 7.08 (s, 1H, aryl 6-H), 7.07 (dd, *J* = 8.6/3.1 Hz, 1H, aryl 4-H), 6.92 (d, *J* = 9.4 Hz, 1H, aryl 3-H), 6.90 (d, *J* = 8.6 Hz, 2H, PMB C-H *ortho* to methoxy group), 5.82 – 5.98 (m, 1H, H_C), 5.30 (ddt, *J* = 17.3/1.5/1.5 Hz, 1H, H_A), 5.21 (ddt, *J* = 10.1/1.5/1.5 Hz, 1H, H_B), 5.08 (s, 2H, PMB CH_2), 4.05 – 4.09 (m, 4H, CH_2=CHCH_2, CH_2OAr), 3.81 (s, 3H, PMB CH_3), 3.76 (t, *J* = 4.8 Hz, 2H, CH_2CH_2OAr).

^{13}C NMR: δ 159.70 (PMB $COCH_3$), 155.06 (aryl 2-C), 152.78 (aryl 5-C), 134.51 (C-H_C), 129.00 (PMB C-H *meta* to methoxy group), 128.09 (PMB 4° C *para* to methoxy group), 121.69 (aryl 4-C), 118.68 (aryl 6-C), 117.64 (CH_2=CH), 116.45 (CN), 115.01 (aryl 3-C), 114.21 (PMB C-H *ortho* to methoxy group), 103.97 (aryl 1-C), 72.53 (CH_2=CHCH_2), 71.39 (PMB CH_2), 68.55, 68.46 (OCH_2CH_2O), 55.42 (PMB CH_3).

m/z: HRMS (TOF ES^+) $C_{20}H_{22}NO_4$ $[MH]^+$ calcd 340.1543; found 340.1508.

5-(2-(Cyclopropylmethoxy)ethoxy)-2-(4-methoxybenzyloxy)benzonitrile (51)



5-(2-(Allyloxy)ethoxy)-2-(4-methoxy benzyloxy)benzonitrile **(50)** (408 mg, 1.20 mmol) was dissolved in dry toluene (5 mL) under a nitrogen atmosphere and cooled to 0 °C with stirring. Diethyl zinc (2.40 mL as 1 M/hexanes, 2.40 mmol, 2 eq) was added, followed by dropwise addition of diiodomethane (644 mg / 194 µL, 2.40 mmol, 2 eq). After stirring for 15 minutes, the vessel was allowed to reach room temperature, and then stirred overnight. The reaction was quenched with aqueous saturated ammonium chloride solution (20 mL), before extraction with DCM (3 x 20 mL). After removal of solvent *in vacuo*, the residue was purified by FCC (eluent MeOH/DCM 1:199). The title compound was the first of two bands to be eluted from the column as 121 mg of dark yellow oil.

Yield: 29%.

IR: 3050 (cyclopropyl (^{c}Pr) C-H, str), 2935, 2872 (alkyl C-H, str), 2228 (CN), 1614, 1586, 1500 (aryl, str), 1109 (C-O-C, str), 824 (aryl C-H, bend, *para*-disubstituted ring).

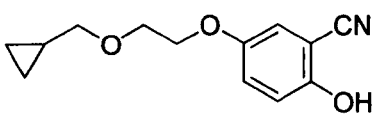
^{1}H NMR: δ 7.33 (d, *J* = 8.7 Hz, 2H, PMB C-H *meta* to methoxy group), 7.06 (s, 1H, aryl 6-H), 7.05 (dd, *J* = 7.4/3.2 Hz, 1H, aryl 4-H), 6.90 (d, *J* = 9.4 Hz, 1H, aryl 3-H), 6.87 (d, *J* = 8.6 Hz, 2H, PMB C-H *ortho* to methoxy group), 5.04 (s, 2H, PMB CH_2), 4.04 (t, *J* = 4.6 Hz, 2H, CH_2OAr), 3.77 (s, 3H, PMB CH_3), 3.76 (t, *J* = 4.8 Hz, 2H, CH_2CH_2OAr), 3.34 (d, *J* = 6.9 Hz, 2H, ^{c}PrCH_2O), 1.00 – 1.12 (m, 1H, ^{c}Pr CH), 0.47 – 0.58 (m, 2H, ^{c}Pr CH_2)*, 0.15 – 0.26 (^{c}Pr CH_2)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR: δ 159.52 (PMB $COCH_3$), 154.84 (aryl 2-C), 152.64 (aryl 5-C), 128.87 (PMB C-H *meta* to methoxy group), 127.95 (PMB 4° C *para* to methoxy group), 121.52 (aryl 4-C), 118.52 (aryl 6-C), 116.37 (CN), 114.80 (aryl 3-C), 114.03 (PMB C-H *ortho* to methoxy group), 102.67

(aryl 1-C), 76.24 (^{c}PrCH$_2$O), 71.16 (PMB CH$_2$), 68.69, 68.40 (OCH$_2$CH$_2$O), 55.27 (PMB CH$_3$), 10.51 (^{c}Pr CH), 3.07 (^{c}Pr CH$_2$).

m/z: HRMS (TOF ES$^+$) $C_{21}H_{24}NO_4$ [MH]$^+$ calcd 354.1700; found 354.1698.

5-(2-(Cyclopropylmethoxy)ethoxy)-2-hydroxybenzonitrile (52)



The title compound was found to be the second band eluted from the flash column during synthesis of 5-(2-(cyclopropylmethoxy)ethoxy)-2-(4-methoxybenzyloxy)benzonitrile **(51)** as 71 mg of clear, colourless oil.

Yield: 25%.

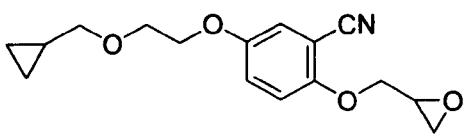
IR: 3280 (broad, O-H, str), 3083 (^{c}Pr C-H, str), 3006 (aryl C-H, str), 2934, 2876 (alkyl C-H, str), 2228 (CN, str), 1510 (aryl, str), 1370 (O-H, bend), 1093 (C-O-C, str).

^{1}H NMR: δ 7.66 (br s, 1H, OH), 6.94 (dd, *J* = 9.3/3.1 Hz, 1H, aryl 4-H), 6.87 (d, *J* = 3.0 Hz, 1H, aryl 6-H), 6.86 (d, *J* = 9.1 Hz, 1H, aryl 3-H), 4.04 (t, *J* = 4.6 Hz, 2H, CH$_2$OAr), 3.81 (t, *J* = 4.8 Hz, 2H, *CH$_2$*CH$_2$OAr), 3.40 (d, *J* = 6.9 Hz, 2H, ^{c}PrC*H$_2$*O), 1.02 – 1.14 (m, 1H, ^{c}Pr CH), 0.46 – 0.59 (m, 2H, ^{c}Pr CH$_2$)*, 0.15 – 0.27 (^{c}Pr CH$_2$)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR: δ 153.75 (aryl 2-C), 151.96 (aryl 5-C), 122.76 (aryl 4-C), 117.89 (aryl 6-C), 116.75 (aryl 3-C), 116.63 (CN), 99.45 (aryl 1-C), 76.52 (^{c}PrCH$_2$O), 68.87, 68.19 (OCH$_2$CH$_2$O), 10.44 (^{c}Pr CH), 3.26 (^{c}Pr CH$_2$).

m/z: HRMS (TOF ES$^-$) $C_{13}H_{14}NO_3$ [M-H]$^-$ calcd 232.0979; found 232.0981.

2-((Oxiran-2-yl)methoxy)-5-(2-(cyclopropylmethoxy)ethoxy)benzonitrile (53)



5-(2-(Cyclopropylmethoxy)ethoxy)-2-hydroxybenzonitrile **(52)** (70 mg, 0.30

mmol) and TEA (33 mg, 46 µL, 0.33 mmol, 1.1 eq) were dissolved in *rac*-epichlorohydrin (5 mL) and heated under reflux at 80 °C for 2 hours. Excess *rac*-epichlorohydrin was removed *in vacuo* before dissolving the crude residue in EtOAc (30 mL). After washing with aqueous 1 M HCl (10 mL), aqueous 1 M NaOH (10 mL) and brine (10 mL), the organic layer was concentrated to afford the desired epoxide in quantitative yield as clear colourless oil.

Yield: 100%.

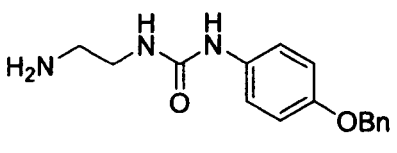
IR: 2962, 2919 (alkyl C-H, str), 2227 (CN, str), 1501 (aryl, str), 1261, 800 (epoxide C-O, str), 1096 (C-O-C, str).

^{1}H NMR: δ 7.09 (dd, *J* = 9.0/3.1 Hz, 1H, aryl 4-H), 7.06 (d, *J* = 2.8 Hz, 1H, aryl 6-H), 6.92 (d, *J* = 9.0 Hz, 1H, aryl 3-H), 4.30 (dd, *J* = 11.3/2.8 Hz, 1H, $ArOCH_2$) 4.06 (t, *J* = 4.6 Hz, 2H, CH_2OAr), 4.01 (dd, *J* = 11.3/5.4 Hz, 1H, $ArOCH_2$), 3.77 (t, *J* = 4.6 Hz, 2H, CH_2CH_2OAr), 3.34 (d, *J* = 7.2 Hz, 2H, $^cPrCH_2O$), 3.31 – 3.38 (m, 1H, epoxide CH), 2.89 (dd, *J* = 4.7/4.7 Hz, 1H, epoxide CH_2), 2.79 (dd, *J* = 4.9/2.7 Hz, 1H, epoxide CH_2), 1.00 – 1.13 (m, 1H, ^{c}Pr CH), 0.46 – 0.59 (m, 2H, ^{c}Pr CH_2)*, 0.15 – 0.27 (^{c}Pr CH_2)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR: δ 154.70 (aryl 2-C), 153.00 (aryl 5-C), 121.67 (aryl 4-C), 118.65 (aryl 6-C), 116.16 (CN), 114.52 (aryl 3-C), 102.55 (aryl 1-C), 76.29 ($^cPrCH_2O$), 70.16 ($ArOCH_2$), 68.72, 68.52 (OCH_2CH_2O), 50.03 (epoxide CH), 45.54 (epoxide CH_2), 10.53 (^{c}Pr CH), 3.08 (^{c}Pr CH_2).

m/z: HRMS (TOF ES$^+$) $C_{16}H_{20}NO_4$ $[MH]^+$ calcd 290.1387; found 290.1411.

1-(2-Aminoethyl)-3-(4-(benzyloxy)phenyl)urea (55)



A solution of 4-(benzyloxy) phenylisocyanate (3.739g 16.61 mmol) in anhydrous DCM (30 mL) was dripped into a flask containing vigorously stirred 1,2-ethanediamine **(54)** (6 mL, 89.80 mmol, 5.4 eq) under nitrogen. Instant precipitation of a white solid was noted and the reaction was allowed to stir for a further 3

hours. After removal of all volatiles, the crude solid was washed with Et_2O, before drying to give 4.472 g of white solid.

Yield: 94%.

Mp: 147 – 149 °C.

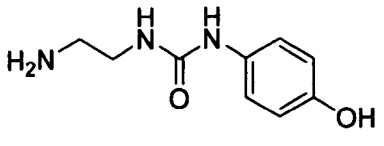
IR: 3300 (primary (1°) amine N-H, str), 2932, 2864 (alkyl C-H, str), 1642 (urea C=O, str), 1604 (1° amine N-H, bend), 1111 (C-O, str), 830 (aryl C-H, bend, *para*-disubstituted ring), 741, 697 (aryl C-H, bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 8.48 (s, 1H, NHAr), 7.31 – 7.44 (m, 5H, aromatic benzyl CH), 7.28, 6.88 (d, *J* = 9.0 Hz, 2 x 2H, *para*-disubstituted ring), 6.24 (t, *J* = 5.2 Hz, 1H, N*H*CONHAr), 5.02 (s, 2H, PhCH_2O), 4.27 (br s, 2H, NH_2), 3.10 – 3.17 (m, 2H, CH_2NH), 2.67 (t, *J* = 6 Hz, CH_2NH_2).

^{13}C NMR (DMSO-d_6): δ 155.72 (C=O), 152.88, 137.37, 133.97 (4° C), 128.37, 127.71, 127.63 (benzyl CH), 119.29, 114.87 (aryl CH), 69.37 (benzyl CH_2), 40.87 (*C*H_2NH_2), 40.38 (*C*HNH).

m/z: HRMS (TOF ES$^+$) $C_{16}H_{20}N_3O_2$ $[MH]^+$ calcd 286.1550; found 286.1547.

1-(2-Aminoethyl)-3-(4-hydroxyphenyl)urea (56)



Method A

1-(2-Aminoethyl)-3-(4-(benzyloxy)phenyl) urea **(55)** (113 mg, 0.40 mmol) was stirred overnight in a solution of concentrated HCl (10 mL). All solvent was removed *in vacuo* and the residue redissolved in water (10 mL) before neutralisation with 0.5 M aqueous NaOH. After removal of water *in vacuo*, the residue was dissolved in the minimum amount of MeOH and filtered (gravity) before purification by PLC (eluent 37% aq NH_3/MeOH/DCM 2:25:73). This gave 56 mg of brown semi-solid.

Yield: 73%.

Method B

tert-Butyl 2-(3-(4-methoxyphenyl)ureido)ethylcarbamate **(65f)** (3.00 g, 9.70 mmol was dissolved in DCM (20 mL) under a nitrogen atmosphere and cooled to -78 °C. BBr_3 (1.0 M in DCM, 50 mL, 50 mmol, 5.15 eq) was added before stirring at room temperature for 2 hours. The reaction mixture was quenched by addition of MeOH over an ice bath before removal of all volatiles *in vacuo*. The crude solid was purified over a silica plug (initial eluent DCM, followed by 37% aq NH_3/MeOH/DCM 2:25:73) to give the desired brown semi-solid in quantitative yield.

Yield: 100%.

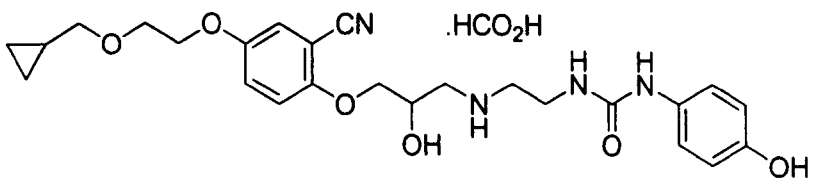
IR: 3339 (1° amine N-H, str), 3118 (br, O-H, str), 1643 (urea C=O, str), 1615 (1° amine N-H, bend), 1570 (aryl, str), 836 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR (MeOD-d_4): δ 7.17 (d, *J* = 8.7 Hz, 2H, aryl 3-H and 5-H), 6.73 (d, *J* = 8.7 Hz, 2H, aryl 2-H and 6-H), 3.45 (t, *J* = 5.6 Hz, 2H, CH_2NH(C=O)NH), 3.05 (t, *J* = 6.0 Hz, 2H, C$H_2$$NH_2$).

^{13}C NMR (MeOD-d_4): δ 159.38 (4° aryl 4-C), 154.70 (C=O), 132.02 (4° aryl 1-C), 123.61, 116.32 (aryl CH), 41.73 (CH_2NH_2), 38.79 (CH_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{14}N_3O_2$ $[MH]^+$ calcd 196.1081; found 196.1081.

1-(2-(3-(2-Cyano-4-(2-(cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea hydroformate (44)



2-((Oxiran-2-yl)methoxy)-5-(2-(cyclopropylmethoxy)ethoxy)benzonitrile **(53)** (52 mg, 0.18 mmol) and 1-(2-aminoethyl)-3-(4-hydroxyphenyl)urea **(56)** (70 mg, 0.36 mmol, 2 eq) were dissolved in propan-2-ol (5 mL) and

heated under reflux at 90 °C overnight. After removal of all volatiles *in vacuo*, the crude product was purified by PLC (eluent 37% aq NH_3/MeOH/DCM 1:10:89) and preparative HPLC to give 2 mg of hygroscopic white solid.

Yield: 2%.

^{1}H NMR (DMSO-d_6): δ 8.39 (br s, 1H, OH), 8.19 (s, 1H, NH(C=O)N*H*Ar), 7.31 (d, *J* = 3.0 Hz, 1H, cyanophenyl ring 3-H), 7.23 (dd, *J* = 9.4/3.0 Hz, 1H, cyanophenyl ring 5-H), 7.18 (d, *J* = 9.1 Hz, 1H, cyanophenyl ring 6-H), 7.13 (d, *J* = 8.7 Hz, 2H, hydroxyphenyl ring 2-H and 6-H), 6.61 (d, *J* = 8.7 Hz, 2H, hydroxyphenyl ring 3-H and 5-H), 6.02 (t, *J* = 5.1 Hz, 1H, N*H*(C=O)NHAr), 3.97 – 4.10 (m, 4H, CH_2OAr, ArOCH_2), 3.84 – 3.93 (m, 1H, CH(OH)), 3.68 (t, *J* = 4.7 Hz, 2H, CH_2CH$_2$OAr), 3.28 (d, *J* = 6.7 Hz, 2H, ^{c}PrCH_2O), 3.13 (dt, *J* = 5.7/5.4 Hz, 2H, NHCH$_2$CH_2), 2.56 – 2.77 (m, 4H, CH(OH)CH_2NH, NHCH_2CH$_2$), 0.93 – 1.07 (m, 1H, ^{c}Pr CH), 0.40 – 0.52 (m, 2H, ^{c}Pr CH$_2$)*, 0.13 – 0.20 (^{c}Pr CH$_2$)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR (MeOD-d_4): δ 159.66 (hydroxyphenyl ring 4-C), 156.18 (C=O), 154.78 (cyanophenyl ring 1-C), 154.53 (cyanophenyl ring 4-C), 132.07 (hydroxyphenyl ring 1-C), 123.59 (hydroxyphenyl ring 2-C and 6-C), 123.07 (cyanophenyl ring 5-C), 119.49 (cyanophenyl ring 3-C), 117.35 (CN), 116.36 (hydroxyphenyl ring 3-C and 5-C), 115.63 (cyanophenyl ring 6-C), 103.11 (cyanophenyl ring 2-C), 77.09 (^{c}PrCH$_2$O), 72.81 (ArOCH$_2$), 70.01 (CH$_2$CH$_2$OAr), 69.61 (CH$_2$OAr), 67.55 (CH(OH)), 51.97, 50.79 (CH(OH)CH$_2$NH, NHCH$_2$CH$_2$), 38.85 (NHCH$_2$CH$_2$), 11.36 (^{c}Pr CH), 3.44 (^{c}Pr CH$_2$).

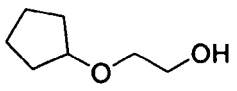
m/z: HRMS (TOF ES$^+$) $C_{25}H_{33}N_4O_6$ $[MH]^+$ calcd 485.2395; found 485.2408.

HPLC R_t: 3.40 (System 1b), 9.32 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea (57)

The experimental procedure for this compound is listed after the entry for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropyl amino)ethyl)-3-(3-hydroxyphenyl)urea **(71u)**.

2-(Cyclopentyloxy)ethanol (59)



Zirconium chloride (10.021 g, 43 mmol, 1.1 eq) was dissolved in dry THF (100 mL) under a nitrogen atmosphere. To this was added sodium borohydride (6.507 g, 172 mmol, 4.4 eq) in portions at room temperature with stirring, resulting in hydrogen gas evolution and formation of a cream suspension. A solution of cyclopentanone ethylene ketal **(58)** (5.000 g, 4.85 mL, 39 mmol) in dry THF (50 mL) was added slowly whilst maintaining the vessel temperature between 0 – 5 °C. After stirring at room temperature for 4 hours, the mixture was quenched with cautious addition of aqueous 2 M HCl over an ice bath. All organic solvent was removed *in vacuo* and the remaining aqueous slurry extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (1 x 30 mL) before concentration to a crude oil. This was purified by FCC (eluent EtOAc/hexanes 50:50) to give 4.114 g of clear colourless oil.

Yield: 81%.

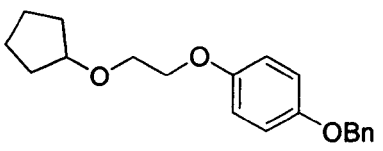
IR: 2943, 2865 (alkyl C-H, str), 1109 (C-O-C, str)

^{1}H NMR: δ 3.84 – 3.89 (m, 1H, CH), 3.60 – 3.63 (m, CH_2OH), 3.41 (t, *J* = 4.9 Hz, 2H, ^{c}PeOCH_2), 2.84 (t, *J* = 5.6 Hz, 1H, OH), 1.51 – 1.72 (m, 6H, cyclopentyl (^{c}Pe) CH_2), 1.38 – 1.50 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR: δ 81.80 (CH), 69.94 (^{c}PeOCH_2), 61.80 (CH_2OH), 32.17 (2-C and 5-C ^{c}Pe ring), 23.46 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^-$) $C_8H_{15}O_4$ $[M+HCO_2]^-$ calcd 175.0976; found 175.0995.

1-(2-(Cyclopentyloxy)ethoxy)-4-(benzyloxy)benzene (60)



2-(Cyclopentyloxy)ethanol **(59)** (3.751 g, 28.81 mmol), triphenylphosphine (9.448 g, 36.02 mmol, 1.25 eq), and 4-(benzyloxy)phenol (5.769 g, 28.81 mmol, 1 eq) were dissolved in DCM (70 mL). DBAD (8.294 g, 36.02 mmol, 1.25 eq) in DCM (20 mL) was added dropwise to the reaction mixture and allowed to stir overnight. After removal of approximately half of the solvent from the reaction mixture, the resulting slurry was diluted with hexanes (100 mL) and washed with aqueous 1 M HCl (2 x 50 mL), aqueous 1 M NaOH (2 x 50 mL), water (2 x 50 mL) and brine (1 x 50 mL). The organic layer was concentrated and redissolved in DCM (30 mL). On addition of hexanes a precipitate of triphenylphosphine oxide began to form. The flask was left in the freezer for 1 hour before filtration of the precipitate and washing with hexanes and Et_2O. After concentration of the filtrate, purification was achieved via FCC (eluent Et_2O/hexanes 10:90) to give 6.75 g of clear colourless oil.

Yield: 75%.

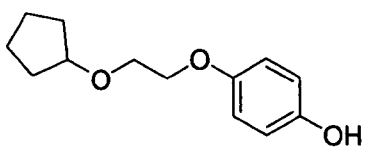
IR: 2870, 2954 (alkyl C-H, str), 1507 (aryl, str), 1109 (C-O-C, str), 824 (aryl C-H, bend, *para*-disubstituted ring), 738, 696 (aryl C-H bend, phenyl ring).

^{1}H NMR: δ 7.34 – 7.48 (m, 5H, aromatic benzyl CH), 6.91, 6.96 (d, J = 9.2 Hz, 2 x 2H, aryl-dioxy ring), 5.04 (s, 2H, PhCH_2O), 4.09 (t, J = 4.9 Hz, 2H, CH_2OArOBn), 4.02 – 4.06 (m, 1H, ^{c}Pe CH), 3.76 (t, J = 5.3 Hz, 2H, CH_2CH$_2$OArOBn), 1.73 – 1.84 (m, 6H, ^{c}Pe CH_2), 1.56 – 1.63 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR: δ 153.02, 153.26 (4° C, aryl-dioxy ring), 137.29 (4° benzyl C), 127.44, 127.83, 128.50 (benzyl CH), 115.62, 115.68 (CH aryl-dioxy ring), 81.89 (^{c}Pe CH), 70.55 (benzyl CH_2), 68.16 (*C*H$_2$OArOBn), 67.28 (*C*H$_2$CH$_2$OArOBn), 32.27 (2-C and 5-C ^{c}Pe ring), 23.55 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES^+) $C_{20}H_{25}O_3$ $[MH]^+$ calcd 313.1798; found 313.1766.

4-(2-(Cyclopentyloxy)ethoxy)phenol (61)



1-(2-(Cyclopentyloxy)ethoxy)-4-(benzyloxy) benzene **(60)** (6.326 g, 20.25 mmol) was dissolved in EtOH (120 mL) before hydrogenating over 10% Pd/C (633 mg) at room temperature and atmospheric pressure overnight. After addition of powdered charcoal and filtration over celite, no workup was required and the desired compound isolated in quantitative yield as clear oil.

Yield: 100%.

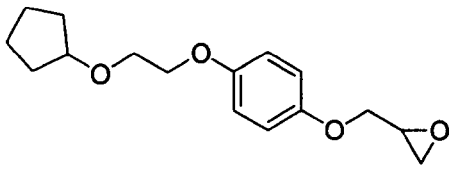
IR: 3381 (br, O-H, str), 2960, 2871 (alkyl C-H, str), 1510 (aryl, str), 1104 (C-O-C, str), 827 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 7.60 (br s, 1H, OH), 6.69, 6.73 (d, J = 9.2 Hz, 2 x 2H, *para*-disubstituted phenol), 3.96 – 3.99 (m, 3H, CH, CH_2OAr), 3.70 (t, J = 5.0 Hz, 2H, ^{c}PeOCH_2), 1.62 – 1.78 (m, 6H, ^{c}Pe CH_2), 1.45 – 1.53 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR: δ 150.05, 152.01 (4° C), 115.53, 115.88 (CH phenolic ring), 82.05 (^{c}Pe CH), 67.82 (CH_2OAr), 67.11 (CH_2CH_2OAr), 32.87 (2-C and 5-C ^{c}Pe ring), 23.20 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES^-) $C_{13}H_{17}O_3$ $[M-H]^-$ calcd 221.1183; found 221.1191.

2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane (62)



NaH 60% suspension in mineral oil (863 mg, equivalent to 518 mg of NaH, 21.58 mmol, 1.1 eq) was suspended in dry DMF (20 mL) with stirring under a nitrogen atmosphere. After 5 minutes 4-(2-(cyclopentyloxy)ethoxy)phenol **(61)** (4.360 g, 19.61 mmol) in dry DMF (20 mL) was added dropwise with the vessel cooled over an ice bath. This was then allowed to stir at room temperature for 20 minutes before

addition of *rac*-epichlorohydrin (15.34 mL, 196.10 mmol, 10 eq). The mixture was stirred for 7 hours then quenched cautiously with MeOH. After removal of all volatiles, the crude residue was partitioned between water (30 mL) and Et_2O (30 mL) and the aqueous layer washed again with Et_2O (3 x 30 mL). The combined organic extracts were concentrated before purification over a silica plug (initial wash with hexanes, followed by EtOH/DCM 5:95) to give 4.558 g of clear yellow oil.

Yield: 84%.

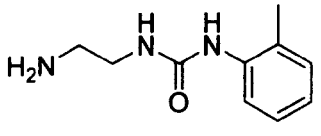
IR: 3052 (epoxide C-H, str, weak), 2961, 2870 (alkyl C-H, str), 1508 (aryl, str), 1110 (C-O-C, str), 828 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 6.80 (s, 4H, aryl C-H), 4.11 (dd, *J* = 11.1/3.1 Hz, 1H, ArOC*H*$_2$CH), 3.99 (t, *J* = 4.9 Hz, 2H, C*H*$_2$OAr), 3.92 – 3.96 (m, 1H, ^{c}Pe CH), 3.82 (dd, *J* = 11.1/5.7, 1H, ArOC*H*$_2$CH), 3.67 (t, *J* = 5.3 Hz, 2H, ^{c}PeOC*H*$_2$), 3.25 – 3.29 (m, 1H, epoxide C*H*), 2.82 (d, *J* = 4.9/4.9 Hz, 1H, epoxide C*H*$_2$), 2.67 (dd, *J* = 5.0/2.7 Hz, 1H, epoxide C*H*$_2$), 1.58 – 1.77 (m, 6H, ^{c}Pe C*H*$_2$), 1.41 – 1.56 (m, 2H, ^{c}Pe C*H*$_2$).

^{13}C NMR: δ 152.65, 153.36 (4° C), 115.46, 115.53 (aryl CH), 69.35 (ArOCH$_2$CH), 68.08 (*C*H$_2$OAr), 67.18 (*C*H$_2$CH$_2$OAr), 50.13 (epoxide CH), 44.49 (epoxide CH$_2$), 32.17 (2-C and 5-C ^{c}Pe ring), 23.45 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{16}H_{22}NaO_4$ $[MNa]^+$ calcd 301.1410; found 301.1414.

1-(2-Aminoethyl)-3-(2-methylphenyl)urea (63)



1,2-Ethanediamine **(54)** (11.3 g, 12.5 mL, 188 mmol, 50 eq) was diluted with dry DCM (15 mL) under a nitrogen atmosphere and cooled to 0 °C. To this, a solution of *o*-tolylphenyl isocyanate (500 mg, 466 µL, 3.76 mmol) in dry DCM (7.5 mL) was added dropwise. After 30 minutes of stirring at room temperature, all solvent was removed and the crude

slurry purified by FCC (eluent 37% aq NH_3/MeOH/DCM 2:30:68) to give 466 mg of beige solid.

Yield: 64%.

Mp: 179 – 181 °C.

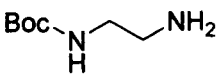
IR: 3325 (br, 1° amine N-H, str), 2926, 2864 (alkyl C-H, str), 1634 (urea C=O, str), 1587 (aryl, str), 753 (aryl C-H, bend, *ortho*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 7.81 (d, *J* = 8.1 Hz, 1H, aryl 6-H), 7.70 (br s, 1H, NH(C=O)N*H*Ar), 7.05 – 7.11 (m, 2H, aromatic C-H), 6.85 (dd, *J* = 8.1/8.1 Hz, 1H, aromatic C-H), 6.64 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 3.07 (dt, *J* = 6.0/6.0 Hz, 2H, CH_2NH), 2.61 (t, *J* = 6.2 Hz, 2H, CH_2NH$_2$), 2.17 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6): δ 155.52 (C=O), 138.35, 126.56 (4° C), 130.02, 126.02, 121.68, 120.35 (Aryl C-H), 42.43 (*C*H_2NH), 41.81 (*C*H_2NH$_2$), 17.95 (*C*H_3).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{16}N_3O$ $[MH]^+$ calcd 194.1288; found 194.1308.

***tert*-Butyl 2-aminoethylcarbamate (64)**



1,2-Ethanediamine **(54)** (50 mL, 927 mmol, 8.75 eq) was diluted in DCM (200mL) with vigorous stirring. Di-*tert*-butyl dicarbonate (23.2 g, 106 mmol) was dissolved in DCM (1.3 L) and then added dropwise to the solution of 1,2-ethanediamine over 24 hours. After removal of all volatiles, the remaining residue was partitioned between water (250 mL) and DCM (250 mL). The aqueous layer was washed again with DCM (250 mL) before combining the organic solvents and concentrating. The residue was dissolved in aqueous 0.5 M $KHSO_4$ (250 mL) and washed with DCM (2 x 100 mL). The aqueous layer was then basified with aqueous 2 M NaOH before final extraction with DCM (4 x 100 mL). The combined organic extracts were dried and concentrated to give 12.98g of viscous translucent oil.

Yield: 87%.

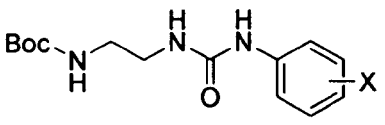
IR: 3354 (1° amine N-H, str), 2977, 2933 (alkyl C-H, str), 1694 (carbamate C=O, str), 1524 (carbamate – amide II* N-H bend), 1392, 1366 ($C(CH_3)_3$, str), 1252, 1174 (C-O, str). *nomenclature derived from hydrogen-bonded and non-hydrogen bonded bands shown with amides.

^{1}H NMR: δ 5.16 (br s, 1H, CONH), 3.06 – 3.10 (m, 2H, CH_2NH), 2.71 (t, J = 6 Hz, CH_2NH_2), 1.36 (s, 9H, $C(CH_3)_3$), 1.24 (br s, 2H, NH_2).

^{13}C NMR: δ 156.26 (C=O), 79.25 ($C(CH_3)_3$), 42.85, 41.70 (CH_2), 28.42 ($C(CH_3)_3$).

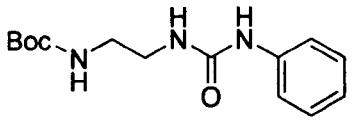
m/z: HRMS (TOF ES$^+$) $C_7H_{17}N_2O_2$ $[MH]^+$ calcd 161.1285; found 161.1300.

General procedure for synthesis of phenyl substituted *tert*-butyl 2-(3-phenylureido)ethylcarbamates



tert-Butyl 2-aminoethylcarbamate **(64)** (1 eq) was dissolved in dry DCM (10 mL) and cooled to 0 °C with stirring under a nitrogen atmosphere. To this was added dropwise, a solution of the desired substituted phenylisocyanate (500 mg) in dry DCM (5 mL). The mixture was stirred overnight at room temperature, before addition of hexanes until precipitation occurred. The solid mass was collected by filtration (vacuum) and washed with hexanes before drying *in vacuo*.

***tert*-Butyl 2-(3-phenylureido)ethylcarbamate (65a)**



Yield: 46%.

Mp: 153 – 155 °C.

IR: 3323 (br, carbamate N-H, str), 2980, 2936 (alkyl C-H, str), 1682 (carbamate C=O, str), 1646 (urea C=O, str), 1547 (aryl, str), 755, 694 (aryl C-H bend phenyl ring).

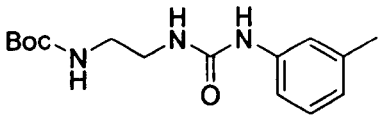
^{1}H NMR (DMSO-d_6): δ 8.53 (s, 1H, NH(C=O)N*H*Ph), 7.37 (d, J = 7.7 Hz, 2H, 2-H and 6-H phenyl ring), 7.20 (dd, J = 7.5/7.5 Hz, 2H, 3-H and 5-H phenyl ring), 6.86 – 6.89 (m, 2H, 4-H phenyl ring, N*H*(C=O)NHPh), 6.16 (t, J = 5.5 Hz, 1H, O(C=O)N*H*), 3.11 (dt, J = 6.1/6.1 Hz, 2H,

C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.38 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d$_6$): δ 155.73, 155.29 (C=O), 140.54 (4° C), 128.63 (phenyl 3-C and 5-C), 120.98 (phenyl 4-C), 117.63 (phenyl 2-C and 6-C), 77.64 (Boc 4° C), 40.46, 39.01 (CH$_2$), 28.26 (CH$_3$).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{22}N_3O_3$ [MH]$^+$ calcd 280.1656; found 280.1668.

***tert*-Butyl 2-(3-*m*-tolylureido)ethylcarbamate (65b)**



Yield: 59%.

Mp: 135 – 137 °C.

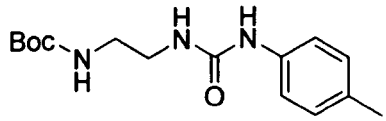
IR: 3354 (br, carbamate N-H, str), 2978, 2940 (alkyl C-H, str), 1687 (carbamate C=O, str), 1658 (urea C=O, str), 1530 (aryl, str), 786 (aryl C-H bend *meta*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d$_6$): δ 8.43 (s, 1H, NH(C=O)N*H*Ar), 7.21 (s, 1H, aryl 2-H), 7.15 (d, *J* = 8.4 Hz, 1H, aryl 6-H), 7.08 (dd, *J* = 7.6/7.6 Hz, 1H, aryl 5-H), 6.86 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 6.69 (d, *J* = 7.3 Hz, 1H, aryl 4-H), 6.12 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.11 (dt, *J* = 6.4/6.4 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 2.23 (s, 3H, CH$_3$), 1.37 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d$_6$): δ 155.71, 155.26 (C=O), 140.43, 137.69 (4° C), 128.46 (aryl 5-C), 121.74 (aryl 4-C), 118.18 (aryl 2-C), 114.84 (aryl 6-C), 77.63 (Boc 4° C), 40.46, 39.02 (CH$_2$), 28.25 (Boc CH$_3$), 21.25 (CH$_3$).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{24}N_3O_3$ [MH]$^+$ calcd 294.1812; found 294.1838.

***tert*-Butyl 2-(3-*p*-tolylureido)ethylcarbamate (65c)**



Yield: 86%.

Mp: 145 – 147 °C.

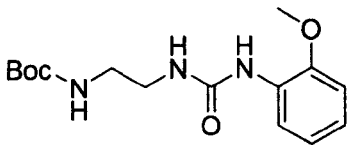
IR: 3345 (br, carbamate N-H, str), 2982, 2928 (alkyl C-H, str), 1680 (carbamate C=O, str), 1655 (urea C=O, str), 1520 (aryl, str), 823 (aryl C-H bend *para*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.39 (s, 1H, NH(C=O)N*H*Ar), 7.25 (d, *J* = 8.4 Hz, 2H, aryl 2-H and 6-H) 7.01 (d, *J* = 8.3 Hz, 2H, aryl 3-H and 5-H), 6.85 (t, *J* = 5.1 Hz, 1H, N*H*(C=O)NHAr), 6.09 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.10 (dt, *J* = 6.0/6.0 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.98 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 2.20 (s, 3H, CH_3), 1.37 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6): δ 155.72, 155.35 (C=O), 137.96, 129.68 (4° C), 117.76, 129.03 (aryl CH), 77.63 (Boc 4° C), 40.50, 39.00 (CH_2), 28.26 (Boc CH_3), 20.31 (CH_3).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{24}N_3O_3$ $[MH]^+$ calcd 294.1812; found 294.1839.

***tert*-Butyl 2-(3-(2-methoxyphenyl)ureido)ethylcarbamate (65d)**



Yield: 76%.

Mp: 155 – 157 °C.

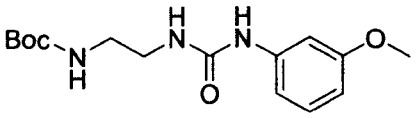
IR: 3328 (br, carbamate N-H, str), 2978, 2927 (alkyl C-H, str), 1689 (carbamate C=O, str), 1650 (urea C=O, str), 1540 (aryl, str), 751 (aryl C-H bend *ortho*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.06 (dd, *J* = 7.5/2.0 Hz,1H, aryl 6-H), 7.91 (s, 1H, NH(C=O)N*H*Ar), 6.90 – 6.96 (m, 2H, aromatic C-H, N*H*(C=O)NHAr), 6.80 – 6.88 (m, 3H, aromatic C-H, O(C=O)N*H*), 3.82 (s, 3H, OC*H*$_3$), 3.10 (dt, *J* = 6.4/6.4 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.98 (dt, *J* = 5.9/5.9 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6): δ 155.66, 155.24 (C=O), 147.27, 129.46 (4° C), 120.93, 120.45, 110.54 (aromatic C-H), 117.94 (aryl 6-C), 77.63 (Boc 4° C), 55.64 (CH_3), 40.48, 38.70 (CH_2), 28.26 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{24}N_3O_4$ $[MH]^+$ calcd 310.1761; found 310.1763.

tert-Butyl 2-(3-(3-methoxyphenyl)ureido)ethylcarbamate (65e)



Yield: 93%.

Mp: 159 – 161 °C.

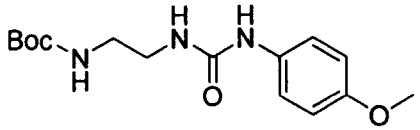
IR: 3326 (br, carbamate N-H, str), 2978, 2926 (alkyl C-H, str), 2837 (O-CH_3, str, weak), 1678 (carbamate C=O, str), 1647 (urea C=O, str), 1513 (aryl, str), 787 (aryl C-H bend *meta*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.53 (s, 1H, NH(C=O)N*H*Ar), 7.13 (dd, *J* = 2.2/2.2 Hz, 1H, aryl 2-H), 7.10 (dd, *J* = 8.1/8.1 Hz, 1H, aryl 5-H), 6.83 – 6.87 (m, 2H, aryl 6-H, N*H*(C=O)NHAr), 6.46 (dd, *J* = 8.1/2.0 Hz, 1H, aryl 4-H), 6.14 (t, *J* = 5.6 Hz, 1H, O(C=O)N*H*), 3.70 (s, 3H, OC*H*$_3$), 3.11 (dt, *J* = 6.4/6.4 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH_3)$_3$).

^{13}C NMR (DMSO-d_6): δ 159.63, 141.76 (4° C), 155.72, 155.19 (C=O), 129.35 (aryl 5-C), 110.00 (aryl 6-C), 106.42 (aryl 4-C), 103.41 (aryl 2-C), 77.65 (Boc 4° C), 54.83 (CH_3), 40.42, 38.99 (CH_2), 28.26 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{24}N_3O_4$ $[MH]^+$ calcd 310.1761; found 310.1744.

tert-Butyl 2-(3-(4-methoxyphenyl)ureido)ethylcarbamate (65f)



Yield: 72%.

Mp: 102 – 104 °C.

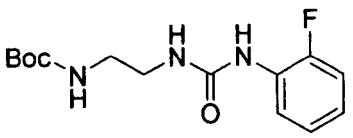
IR: 3350 (br, carbamate N-H, str), 2971, 2939 (alkyl C-H, str), 2836 (O-CH_3, str, weak), 1684 (carbamate C=O, str), 1643 (urea C=O, str), 1511 (aryl, str), 829 (aryl C-H bend *para*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.31 (s, 1H, NH(C=O)N*H*Ar), 7.27 (d, *J* = 9.0 Hz, 2H, aryl 2-H and 6-H), 6.85 (t, *J* = 4.6 Hz, 1H, N*H*(C=O)NHAr), 6.80 (d, *J* = 9.0 Hz, 2H, aryl 3-H and 5-H), 6.04 (t, *J* = 5.6 Hz, 1H, O(C=O)N*H*) 3.68 (s, 3H, OC*H*$_3$), 3.10 (dt, *J* = 6.4/6.4 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.98 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH_3)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.72, 155.51 (C=O), 153.90, 133.65 (4° C), 119.42 (aryl 2-C and 6-C), 113.85 (aryl 3-C and 5-C), 77.63 (Boc 4° C), 55.12 (CH_3), 40.54, 38.81 (CH_2), 28.26 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{24}N_3O_4$ $[MH]^+$ calcd 310.1761; found 310.1769.

***tert*-Butyl 2-(3-(2-fluorophenyl)ureido)ethylcarbamate (65g)**



Yield: 83%.

Mp: 181 – 183 °C.

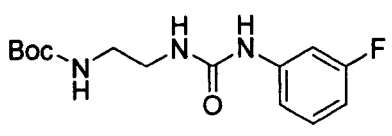
IR: 3368, 3331 (carbamate/urea N-H, str), 2981, 2935 (alkyl C-H, str), 1673 (carbamate C=O, str), 1661 (urea C=O, str), 1551 (aryl, str), 1256 (C-F, str), 752 (aryl C-H bend *ortho*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.32 (s, 1H, NH(C=O)N*H*Ar), 8.11 (dd, *J* = 7.2 Hz, 1H, aryl 5-H), 7.16 (ddd, *J* = 11.8/8.2/1.4 Hz, 1H, aryl 3-H), 7.06 (dd, *J* = 7.9/7.9 Hz, 1H, aryl 4-H), 6.85 – 6.93 (m, 2H, N*H*(C=O)NHAr, aryl 6-H), 6.66 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.12 (dt, *J* = 6.3/6.3 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6): δ 155.71, 154.96 (C=O), 151.59 (J_{CF} = 240.6 Hz, CF), 128.33 (J_{CF} = 10.3 Hz, aryl 1-C), 124.35 (J_{CF} = 3.3 Hz, aryl 4-C), 121.48 (J_{CF} = 7.3 Hz, aryl 6-C), 120.16 (aryl 5-C), 114.77 (J_{CF} = 19.0 Hz, aryl 3-C), 77.65 (Boc 4° C), 40.35, 38.77 (CH_2), 28.25 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}FN_3O_3$ $[MH]^+$ calcd 298.1561; found 298.1550.

***tert*-Butyl 2-(3-(3-fluorophenyl)ureido)ethylcarbamate (65h)**



Yield: 53%.

Mp: 114 – 116 °C.

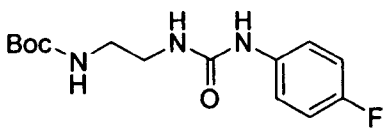
IR: 3353 (carbamate N-H, str), 2982, 2944 (alkyl C-H, str), 1679 (carbamate C=O, str), 1654 (urea C=O, str), 1515 (aryl, str), 1277 (C-F, str), 773 (aryl C-H bend *meta*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.78 (s, 1H, NH(C=O)N*H*Ar), 7.45 (ddd, *J* = 12.3/2.2/2.2 Hz, 1H, aryl 2-H), 7.22 (ddd, *J* = 8.2/8.2/8.2 Hz, 1H, aryl 5-H), 7.01 (dd, *J* = 8.2/1.1 Hz, 1H, aryl 6-H), 6.86 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 6.68 (dd, *J* = 8.7/2.5 Hz, 1H, aryl 4-H), 6.22 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.12 (dt, *J* = 6.4/6.44 Hz, 2H, C*H*$_2$NH(C=O)NH), 3.00 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d_6): δ 162.43 (J_{CF} = 240.0 Hz, CF), 155.73, 155.05 (C=O), 142.46 (J_{CF} = 11.5 Hz, aryl 1-C), 130.10 (J_{CF} = 9.8 Hz, aryl 5-C), 113.27 (aryl 6-C), 107.23 (J_{CF} = 21.3 Hz, aryl 4-C), 104.24 (J_{CF} = 26.5 Hz, aryl 2-C), 77.65 (Boc 4° C), 40.31, 39.04 (CH$_2$), 28.25 (Boc CH$_3$).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}FN_3O_3$ [MH]$^+$ calcd 298.1561; found 298.1569.

tert-Butyl 2-(3-(4-fluorophenyl)ureido)ethylcarbamate (65i)



Yield: 85%.

Mp: 152 – 154 °C.

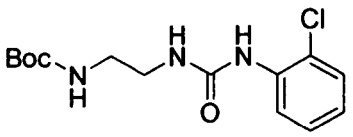
IR: 3340 (carbamate N-H, str), 2979, 2940 (alkyl C-H, str), 1686 (carbamate C=O, str), 1639 (urea C=O, str), 1509 (aryl, str), 1286 (C-F, str), 834 (aryl C-H bend *para*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.56 (s, 1H, NH(C=O)N*H*Ar), 7.38 (dd, *J* = 7.0/5.0 Hz, 2H, aryl 2-H and 6-H), 7.04 (dd, *J* = 8.9/8.9 Hz, 2H, aryl 3-H and 5-H), 6.86 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 6.13 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.11 (dt, *J* = 6.4/6.44 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d_6): δ 156.86 (J_{CF} = 237.0 Hz, CF), 155.73, 155.33 (C=O), 136.91 (aryl 1-C), 119.22 (J_{CF} = 7.5 Hz, aryl 2-C and 6-C), 115.08 (J_{CF} = 22.0 Hz, aryl 3-C and 5-C), 77.64 (Boc 4° C), 40.43, 38.86 (CH$_2$), 28.26 (Boc CH$_3$).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}FN_3O_3$ [MH]$^+$ calcd 298.1561; found 298.1550.

tert-Butyl 2-(3-(2-chlorophenyl)ureido)ethylcarbamate (65j)



Yield: 52%.

Mp: 167 – 169 °C.

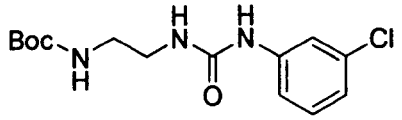
IR: 3332 (carbamate N-H, str), 2972, 2933 (alkyl C-H, str), 1673 (carbamate C=O, str), 1665 (urea C=O, str), 1528 (aryl, str), 751 (aryl C-H bend *ortho*-disubstituted aromatic ring), 656 (C-Cl, bend).

^{1}H NMR (DMSO-d$_6$): δ 8.14 (d, *J* = 8.2 Hz, 1H, aryl 6-H), 8.03 (s, 1H, NH(C=O)N*H*Ar), 7.38 (dd, *J* = 8.0/1.4 Hz, 1H, aryl 3-H), 7.23 (ddd, *J* = 7.8/7.8/1.4 Hz, 1H, aryl C-H), 7.07 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 6.94 (dd, *J* = 7.8/1.5 Hz, 1H, aryl C-H), 6.87 (t, *J* = 5.2 Hz, 1H, O(C=O)N*H*), 3.13 (dt, *J* = 6.3/6.3 Hz, 2H, C*H*$_2$NH(C=O)NH), 3.00 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.38 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d$_6$): δ 155.68, 154.85 (C=O), 136.73, 121.10 (4° C), 129.05 (aryl 3-C), 127.42, 122.38 (aryl C-H), 120.73 (aryl 6-C), 77.64 (Boc 4° C), 40.28, 38.96 (CH_2), 28.24 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}ClN_3O_3$ $[MH]^+$ calcd 314.1266; found 314.1274.

tert-Butyl 2-(3-(3-chlorophenyl)ureido)ethylcarbamate (65k)



Yield: 60%.

Mp: 117 – 119 °C.

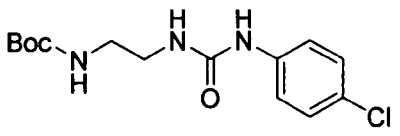
IR: 3341 (carbamate N-H, str), 2979, 2942 (alkyl C-H, str), 1685 (carbamate C=O, str), 1642 (urea C=O, str), 1528 (aryl, str), 780 (aryl C-H bend *meta*-disubstituted aromatic ring), 639 (C-Cl, bend).

^{1}H NMR (DMSO-d$_6$): δ 8.76 (s, 1H, NH(C=O)N*H*Ar), 7.66 (s, 1H, aryl 2-H), 7.21 (dd, *J* = 7.9/7.9 Hz, 1H, aryl 5-H), 7.17 (d, *J* = 8.2 Hz, 1H, aryl C-H), 6.92 (d, *J* = 7.7 Hz, 1H, aryl C-H), 6.87 (t, *J* = 5.1 Hz, 1H, N*H*(C=O)NHAr), 6.23 (t, *J* = 5.3 Hz, 1H, O(C=O)N*H*), 3.11 (dt, *J* = 6.1/6.1 Hz, 2H, C*H*$_2$NH(C=O)NH), 3.00 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.73, 155.01 (C=O), 142.09, 133.07 (4° C), 130.22 (aryl 5-C), 120.57 (aryl C-H), 116.96 (aryl 2-C), 115.97 (aryl C-H), 77.65 (Boc 4° C), 40.28, 38.92 (CH_2), 28.25 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}ClN_3O_3$ $[MH]^+$ calcd 314.1266; found 314.1276.

***tert*-Butyl 2-(3-(4-chlorophenyl)ureido)ethylcarbamate (65l)**



Yield: 82%.

Mp: 165 – 167 °C.

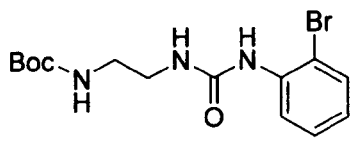
IR: 3339 (carbamate N-H, str), 2979, 2941 (alkyl C-H, str), 1685 (carbamate C=O, str), 1641 (urea C=O, str), 1528 (aryl, str), 829 (aryl C-H bend *para*-disubstituted aromatic ring), 644 (C-Cl, bend).

^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H, NH(C=O)N*H*Ar), 7.41 (d, *J* = 8.9 Hz, 2H, aryl C-H), 7.25 (d, *J* = 8.9 Hz, 2H, aryl C-H), 6.86 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 6.20 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.11 (dt, *J* = 6.4/6.4 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH_3)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.73, 155.12 (C=O), 139.56, 124.42 (4° C), 128.45, 119.09 (aryl C-H), 77.65 (Boc 4° C), 40.35, 38.78 (CH_2), 28.25 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}ClN_3O_3$ $[MH]^+$ calcd 314.1266; found 314.1261.

***tert*-Butyl 2-(3-(2-bromophenyl)ureido)ethylcarbamate (65m)**



Yield: 90%.

Mp: 136 – 138 °C.

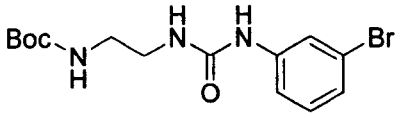
IR: 3345, 3312 (carbamate/urea N-H, str), 2976, 2926 (alkyl C-H, str), 1686 (carbamate C=O, str), 1657 (urea C=O, str), 1539 (aryl, str), 745 (aryl C-H bend *ortho*-disubstituted aromatic ring), 663 (C-Br, bend).

^{1}H NMR (DMSO-d_6): δ 8.06 (d, *J* = 8.2 Hz, 1H, aryl 6-H), 7.85 (s, 1H, NH(C=O)N*H*Ar), 7.54 (dd, *J* = 8.0/1.4 Hz, 1H, aryl 3-H), 7.27 (ddd, *J* = 7.8/7.8/1.4 Hz, 1H, aryl C-H), 7.14 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 6.85 – 6.90 (m, 2H, aryl C-H, O(C=O)N*H*), 3.12 (dt, *J* = 6.4/6.4 Hz, 2H, CH_2NH(C=O)NH), 3.01 (dt, *J* = 5.8/5.8 Hz, 2H, CH_2NH(C=O)O), 1.38 (s, 9H, C(CH_3)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.67, 154.87 (C=O), 137.83, 112.15 (4° C), 132.33 (aryl 3-C), 127.91, 123.13 (aryl C-H), 121.53 (aryl 6-C), 77.63 (Boc 4° C), 40.29, 39.00 (CH_2), 28.24 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}BrN_3O_3$ [MH]$^+$ calcd 358.0761; found 358.0760.

***tert*-Butyl 2-(3-(3-bromophenyl)ureido)ethylcarbamate (65n)**



Yield: 85%.

Mp: 122 - 124°C.

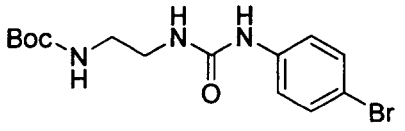
IR: 3338 (carbamate N-H, str), 2979, 2940 (alkyl C-H, str), 1685 (carbamate C=O, str), 1645 (urea C=O, str), 1539 (aryl, str), 778 (aryl C-H bend *meta*-disubstituted aromatic ring), 682 (C-Br, bend).

^{1}H NMR (DMSO-d_6): δ 8.76 (s, 1H, NH(C=O)N*H*Ar), 7.81 (dd, *J* = 1.8/1.8 Hz, 1H, aryl 2-H), 7.21 (ddd, *J* = 8.5/1.4/1.4 Hz, 1H, aryl C-H), 7.16 (dd, *J* = 7.8/7.8 Hz, 1H, aryl 5-H), 7.05 (ddd, *J* = 7.7/1.2/1.2 Hz, 1H, aryl C-H), 6.86 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 6.24 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.11 (dt, *J* = 6.3/6.3 Hz, 2H, CH_2NH(C=O)NH), 3.00 (dt, *J* = 5.8/5.8 Hz, 2H, CH_2NH(C=O)O), 1.37 (s, 9H, C(CH_3)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.73, 155.00 (C=O), 142.25, 121.66 (4° C), 130.55 (aryl 5-C), 123.47 (aryl C-H), 119.83 (aryl 2-C), 116.36 (aryl C-H), 77.65 (Boc 4° C), 40.28, 38.89 (CH_2), 28.25 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}BrN_3O_3$ [MH]$^+$ calcd 358.0761; found 358.0750.

tert-Butyl 2-(3-(4-bromophenyl)ureido)ethylcarbamate (65o)



Yield: 86%.

Mp: 192 – 194 °C.

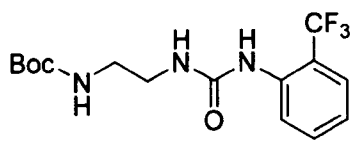
IR: 3337 (carbamate N-H, str), 2978, 2938 (alkyl C-H, str), 1686 (carbamate C=O, str), 1643 (urea C=O, str), 1528 (aryl, str), 826 (aryl C-H bend *para*-disubstituted aromatic ring), 641 (C-Br, bend).

^{1}H NMR (DMSO-d$_6$): δ 8.70 (s, 1H, NH(C=O)N*H*Ar), 7.37 (s, 4H, aryl C-H), 6.86 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 6.20 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.11 (dt, *J* = 6.3/6.3 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d$_6$): δ 155.71, 155.06 (C=O), 139.96, 112.23 (4° C), 131.32, 119.51 (aryl C-H), 77.63 (Boc 4° C), 40.33, 38.85 (CH$_2$), 28.24 (Boc CH$_3$).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}BrN_3O_3$ $[MH]^+$ calcd 358.0761; found 358.0767.

tert-Butyl 2-(3-(2-(trifluoromethyl)phenyl)ureido)ethylcarbamate (65p)



Yield: 95%.

Mp: 149 – 151 °C.

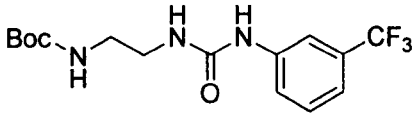
IR: 3331 (carbamate N-H, str), 2979, 2931 (alkyl C-H, str), 1688 (carbamate C=O, str), 1654 (urea C=O, str), 1541 (aryl, str), 1322 (C-F, str), 766 (aryl C-H bend *ortho*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d$_6$): δ 7.96 (d, *J* = 8.2 Hz, 1H, aryl C-H), 7.80 (s, 1H, NH(C=O)N*H*Ar), 7.60 (d, *J* = 7.9 Hz, 1H, aryl C-H), 7.56 (dd, *J* = 8.1/8.1 Hz, 1H, aryl C-H), 7.17 (dd, *J* = 7.4/7.4 Hz, 1H, aryl C-H), 7.03 – 7.10 (m, 1H, N*H*(C=O)NHAr), 6.82 – 6.90 (m, 1H, O(C=O)N*H*), 3.08 – 3.18 (m, 2H, C*H*$_2$NH(C=O)NH), 2.95 - 3.06 (m, 2H, C*H*$_2$NH(C=O)O), 1.38 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.70, 155.07 (C=O), 137.36 (4° C), 132.74, 124.64, 122.59 (aryl C-H), 125.77 (J_{CF} = 5.3 Hz, aryl C-H), 124.10 (J_{CF} = 273.0 Hz, CF_3), 77.65 (Boc 4° C), 40.27, 38.90 (CH_2), 28.24 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{21}F_3N_3O_3$ $[MH]^+$ calcd 348.1530; found 348.1529.

***tert*-Butyl 2-(3-(3-(trifluoromethyl)phenyl)ureido)ethylcarbamate (65q)**



Yield: 90%.

Mp: 102 – 103 °C.

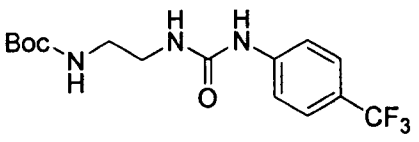
IR: 3338 (carbamate N-H, str), 2980, 2937 (alkyl C-H, str), 1686 (carbamate C=O, str), 1654 (urea C=O, str), 1569 (aryl, str), 1339 (C-F, str), 795 (aryl C-H bend *meta*-disubstituted aromatic ring), 700 (C-F, bend).

^{1}H NMR (DMSO-d_6): δ 8.93 (s, 1H, NH(C=O)N*H*Ar), 7.97 (s, 1H, aryl 2-H), 7.49 (d, *J* = 8.3 Hz, 1H, aryl 6-H), 7.43 (dd, *J* = 7.6/7.6 Hz, 1H, aryl 5-H), 7.21 (d, *J* = 7.4 Hz, 1H, aryl 4-H), 6.87 (t, *J* = 5.1 Hz, 1H, N*H*(C=O)NHAr), 6.28 (t, *J* = 5.2 Hz, 1H, O(C=O)N*H*), 3.13 (dt, *J* = 6.3/6.3 Hz, 2H, C*H*$_2$NH(C=O)NH), 3.01 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH_3)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.75, 155.12 (C=O), 141.40, (4° C), 129.73 (aryl 5-C), 129.41 (J_{CF} = 30.9 Hz, aryl 3-C), 124.31 (J_{CF} = 272.3 Hz, CF_3), 121.11 (aryl 6-C), 117.19 (J_{CF} = 4.2 Hz, aryl 4-C), 113.52 (J_{CF} = 4.1 Hz, aryl 2-C), 77.66 (Boc 4° C), 40.27, 38.94 (CH_2), 28.25 (Boc CH_3).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{21}F_3N_3O_3$ $[MH]^+$ calcd 348.1530; found 348.1540.

***tert*-Butyl 2-(3-(4-(trifluoromethyl)phenyl)ureido)ethylcarbamate (65r)**



Yield: 63%.

Mp: 194 – 196 °C.

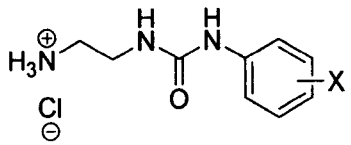
IR: 3391, 3336 (urea/carbamate N-H, str), 2981, 2935 (alkyl C-H, str), 1699 (carbamate C=O, str), 1666 (urea C=O, str), 1553 (aryl, str), 1329 (C-F, str), 838 (aryl C-H bend *para*-disubstituted aromatic ring), 657 (C-F, bend).

^{1}H NMR (DMSO-d_6): δ 8.99 (s, 1H, NH(C=O)N*H*Ar), 7.59 (d, *J* = 9.1 Hz, 2H, aryl C-H), 7.55 (d, *J* = 9.1 Hz, 2H, aryl C-H), 6.88 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 6.31 (t, *J* = 5.5 Hz, 1H, O(C=O)N*H*), 3.13 (dt, *J* = 6.3/6.3 Hz, 2H, C*H*$_2$NH(C=O)NH), 3.01 (dt, *J* = 5.8/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH_3)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.74, 154.91 (C=O), 144.26, 121.07 (4° C), 125.96 (J_{CF} = 3.7 Hz, aryl 3-C and 5-C), 122.04 (J_{CF} = 226.7 Hz, CF_3), 117.19 (aryl 2-C and 6-C), 77.66 (Boc 4° C), 40.24, 38.89 (CH_2), 28.24 (Boc CH_3).

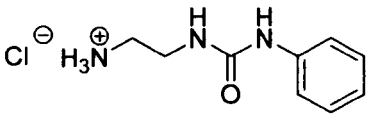
m/z: HRMS (TOF ES$^+$) $C_{15}H_{21}F_3N_3O_3$ $[MH]^+$ calcd 348.1530; found 348.1516.

General procedure for synthesis of phenyl substituted 1-(2-aminoethyl)-3-(phenyl)urea hydrochlorides



The desired phenyl substituted Boc-protected phenylurea (compounds **65a-65r**) was dissolved in MeOH (6 mL) with the aid of sonication and heat if necessary. This was then added to vigorously stirred concentrated aqueous HCl (5 mL) and stirred for 3 hours. All solvents were removed *in vacuo* and the resulting hydrochloride salts of the desired compounds were freeze-dried.

1-(2-Aminoethyl)-3-phenylurea hydrochloride (66a)



Yield: 92%.

Mp: 186 – 188 °C.

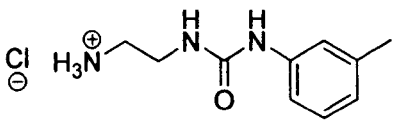
IR: 3329, 3234 (urea N-H, str), 2958 (NH_3^+, str), 2761, 2681 (alkyl C-H, str), 1660 (urea C=O, str), 1597, 1499 (NH_3^+, bend), 1563 (aryl, str), 764, 693 (aryl C-H bend phenyl ring).

^{1}H NMR (DMSO-d$_6$): δ 8.93 (s, 1H, NH(C=O)N*H*Ph), 7.89 (br s, 3H, NH_3^+), 7.41 (d, *J* = 7.6 Hz, 2H, 2-H and 6-H phenyl ring), 7.22 (dd, *J* = 7.5/7.5 Hz, 2H, 3-H and 5-H phenyl ring), 6.90 (dd, *J* = 7.3/7.3 Hz, 1H, 4-H phenyl ring), 6.34 (t, *J* = 5.7 Hz, N*H*(C=O)NHPh), 3.30 – 3.34 (m, 2H, C*H*$_2$NH(C=O)NH), 2.83 – 2.93 (m, 2H, C*H*$_2$NH$_3^+$).

^{13}C NMR (DMSO-d$_6$): δ 155.76 (C=O), 140.33 (4° C), 128.64 (phenyl 3-C and 5-C), 121.23 (phenyl 4-C), 117.80 (phenyl 2-C and 6-C), 39.12, 37.17 (*C*H$_2$).

m/z: HRMS (TOF ES$^+$) $C_9H_{14}N_3O$ $[MH]^+$ calcd 180.1131; found 180.1140.

1-(2-Aminoethyl)-3-(3-methylphenyl)urea hydrochloride (66b)



Yield: 100%.

Mp: 185 – 187 °C.

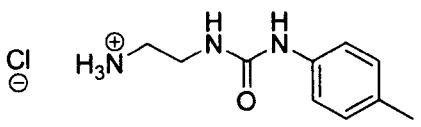
IR: 3323 (urea N-H, str), 3009 (NH_3^+, str), 2753 (alkyl C-H, str), 1677 (urea C=O, str), 1612, 1489 (NH_3^+, bend), 1567 (aryl, str), 775 (aryl C-H bend *meta*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d$_6$): δ 9.01 (s, 1H, NH(C=O)N*H*Ar), 8.02 (br s, 3H, NH_3^+), 7.18 – 7.25 (m, 2H, aryl 2-H, aryl C-H), 7.09 (dd, *J* = 7.7/7.7 Hz, 1H, aryl 5-H), 6.67 – 6.75 (m, 2H, aryl C-H, N*H*(C=O)NHAr), 3.31 (dt, *J* = 6.2/6.2 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.87 (t, *J* = 6.2 Hz, C*H*$_2$NH$_3^+$), 2.23 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6): δ 155.82 (C=O), 140.33, 137.67 (4° C), 128.46 (aryl 5-C), 121.90, 118.25, 114.94 (aryl C-H), 39.04 ($CH_2NH_3^+$), 37.14 ($CH_2NH(C=O)NH$), 21.26 (CH_3)

m/z: HRMS (TOF ES$^+$) $C_{10}H_{16}N_3O$ $[MH]^+$ calcd 194.1288; found 194.1289.

1-(2-Aminoethyl)-3-(4-methylphenyl)urea hydrochloride (66c)



Yield: 96%.

Mp: 214 – 216 °C (literature (lit). 213 °C)[143].

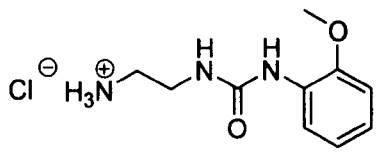
IR: 3312 (urea N-H, str), 3005 (NH_3^+, str), 1670 (urea C=O, str), 1603, 1512 (NH_3^+, bend), 1558 (aryl, str), 810 (aryl C-H bend *para*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.95 (s, 1H, NH(C=O)N*H*Ar), 8.01 (br s, 3H, NH_3^+), 7.29 (d, *J* = 8.4 Hz, 2H, aryl 2-H and 6-H), 7.02 (d, *J* = 8.4 Hz, 2H, aryl 3-H and 5-H), 6.63 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.31 (dt, *J* = 6.2/6.2 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.86 (t, *J* = 6.2 Hz, 2H, C*H*$_2$NH$_3^+$), 2.20 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6): δ 155.85 (C=O), 137.82, 129.84 (4° C), 129.01 (aryl 3-C and 5-C), 117.84 (aryl 2-C and 6-C), 39.07 ($CH_2NH_3^+$), 37.13 ($CH_2NH(C=O)NH$), 20.32 (CH_3).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{16}N_3O$ $[MH]^+$ calcd 194.1288; found 194.1283.

1-(2-Aminoethyl)-3-(2-methoxyphenyl)urea hydrochloride (66d)



Yield: 85%.

Mp: 156 - 158°C.

IR: 3373, 3282 (urea N-H, str), 2998 (NH_3^+, str), 1647 (urea C=O, str), 1576, 1499 (NH_3^+, bend), 1533 (aryl, str), 755 (aryl C-H bend *ortho*-disubstituted aromatic ring).

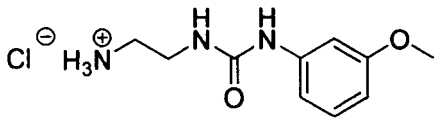
^{1}H NMR (DMSO-d_6): δ 8.05 (dd, *J* = 7.8/1.8 Hz, 1H, aryl 6-H), 8.03 (s, 1H, NH(C=O)N*H*Ar), 8.00 (br s, 3H, NH_3^+), 7.19 – 7.26 (m, 1H,

N*H*(C=O)NHAr), 6.96 (dd, *J* = 7.9/1.6 Hz, 1H, aryl 3-H), 6.88 (ddd, *J* = 7.4/7.4/1.8 Hz, 1H, aryl C-H), 6.84 (ddd, *J* = 7.8/7.8/1.7 Hz, 1H, aryl C-H), 3.82 (s, 3H, CH_3), 3.31 (dt, *J* = 6.1/6.1 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.87 (tq, *J* = 5.8/5.8 Hz, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d$_6$): δ 156.06 (C=O), 147.90, 129.61 (4° C), 121.75, 120.89 (aryl C-H), 118.62 (aryl 6-H), 111.10 (aryl 3-H), 56.13 (CH_3), 39.63 (*C*$H_2NH_3^+$), 37.50 (*C*H_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{16}N_3O_2$ $[MH]^+$ calcd 210.1237; found 210.1234.

1-(2-Aminoethyl)-3-(3-methoxyphenyl)urea hydrochloride (66e)



Yield: 99%.

Mp: 183 – 188 °C.

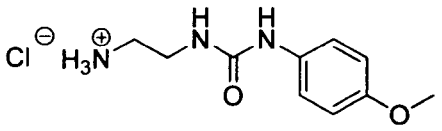
IR: 3389, 3301 (urea N-H, str), 2883 (NH_3^+, str), 1658 (urea C=O, str), 1576, 1497 (NH_3^+, bend), 1522 (aryl, str), 785 (aryl C-H bend *meta*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d$_6$): δ 9.03 (s, 1H, NH(C=O)N*H*Ar), 7.95 (br s, 3H, NH_3^+), 7.16 (dd, *J* = 2.2/2.2 Hz, 1H, aryl 2-H), 7.11 (dd, *J* = 8.1/8.1 Hz, 1H, aryl 5-H), 6.89 (dd, *J* = 8.1/1.7 Hz, 1H, aryl 6-H), 6.61(t, *J* = 5.7 Hz, 1H, N*H*(C=O)NHAr), 6.48 (dd, *J* = 8.1/2.4 Hz, 1H, aryl 4-H), 3.69 (s, 3H, CH_3), 3.32 (dt, *J* = 6.2/6.2 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.87 (tq, *J* = 5.9/5.9 Hz, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d$_6$): δ 159.60, 141.60 (4° C), 155.69 (C=O), 129.36 (aryl 5-C), 110.15 (aryl 6-C), 106.53 (aryl 4-C), 103.60 (aryl 2-C), 54.86 (CH_3), 39.06 ($CH_2NH_3^+$), 37.13 (*C*H_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{16}N_3O_2$ $[MH]^+$ calcd 210.1237; found 210.1216.

1-(2-Aminoethyl)-3-(4-methoxyphenyl)urea hydrochloride (66f)



Yield: 88%.

Mp: 180 – 182 °C.

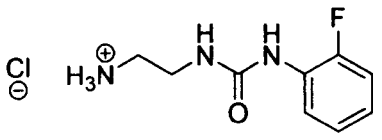
IR: 3335 (urea N-H, str), 3007 (NH_3^+, str), 1629 (urea C=O, str), 1508 (NH_3^+, bend), 1570 (aryl, str), 827 (aryl C-H bend *para*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 8.75 (s, 1H, NH(C=O)N*H*Ar), 7.93 (br s, 3H, NH_3^+), 7.30 (d, *J* = 9.1 Hz, 2H, aryl 2-H and 6-H), 6.81 (d, *J* = 9.1 Hz, 2H, aryl 3-H and 5-H), 6.47 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.69 (s, 3H, CH_3), 3.33 (dt, *J* = 6.1/6.1 Hz, 2H, CH_2NH(C=O)NH), 2.86 (tq, *J* = 5.8/5.8 Hz, C$H_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 156.44, 154.49, 133.88 (4° C), 120.05 (aryl 2-C and 6-C), 114.30 (aryl 3-C and 5-C), 55.59 (CH_3), 39.90 ($CH_2NH_3^+$), 37.67 (CH_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{16}N_3O_2$ $[MH]^+$ calcd 210.1237; found 210.1237.

1-(2-Aminoethyl)-3-(2-fluorophenyl)urea hydrochloride (66g)



Yield: 97%.

Mp: 208 – 210 °C.

IR: 3320 (urea N-H, str), 3011 (NH_3^+, str), 2755 (alkyl C-H, str), 1678 (urea C=O, str), 1605, 1490 (NH_3^+, bend), 1561 (aryl, str), 1258 (C-F, str), 745 (aryl C-H bend *ortho*-disubstituted aromatic ring).

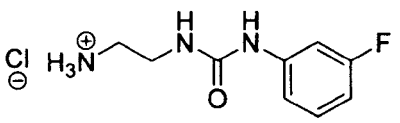
^{1}H NMR (DMSO-d_6): δ 8.57 (d, *J* = 2.1 Hz, 1H, NH(C=O)N*H*Ar), 8.05 - 8.10 (m, 4H, aryl C-H, NH_3^+), 7.11 – 7.21 (m, 2H, aryl 3-H, N*H*(C=O)NHAr), 7.08 (dd, *J* = 7.8/7.8 Hz, 1H, aryl C-H), 6.90 – 6.96 (m, 1H, aryl C-H), 3.34 (dt, *J* = 6.0/6.0 Hz, 2H, CH_2NH(C=O)NH), 2.88 (t, *J* = 6.2 Hz, 2H, C$H_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.44 (C=O), 151.85 (J_{CF} = 241.2 Hz, C-F), 128.65 (J_{CF} = 10.3 Hz, aryl 1-C), 124.34 (J_{CF} = 3.6 Hz, aryl C-H), 121.93

(J_{CF} = 7.4 Hz, aryl C-H), 120.64 (aryl C-H), 114.90 (J_{CF} = 19.1 Hz, aryl 3-C), 38.82 ($CH_2NH_3^+$), 37.11 (CH_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}FN_3O$ $[MH]^+$ calcd 198.1037; found 198.1034.

1-(2-Aminoethyl)-3-(3-fluorophenyl)urea hydrochloride (66h)



Yield: 91%.

Mp: 184 – 186 °C.

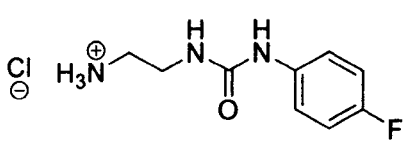
IR: 3310 (urea N-H, str), 3005 (NH_3^+, str), 2686 (alkyl C-H, str), 1678 (urea C=O, str), 1610, 1494 (NH_3^+, bend), 1558 (aryl, str), 1271 (C-F, str), 785 (aryl C-H bend *meta*-disubstituted aromatic ring), 682 (C-F, bend).

^{1}H NMR (DMSO-d_6): δ 9.42 (s, 1H, NH(C=O)N*H*Ar), 8.00 (br s, 3H, NH_3^+), 7.46 (ddd, *J* = 12.3/2.2/2.2 Hz, 1H, aryl 2-H), 7.23 (ddd, *J* = 8.2/8.2/7.0 Hz, 1H, aryl 5-H), 7.06 (dd, *J* = 8.2/1.8 Hz, 1H, aryl 6-H), 6.76 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 6.69 (ddd, *J* = 8.7/8.7/2.5 Hz, 1H, aryl 4-H), 3.33 (dt, *J* = 6.2/6.2 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.87 (tq, *J* = 5.4/5.4 Hz, 2H, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 162.40 (J_{CF} = 240.1 Hz, C-F), 155.58 (C=O), 142.37 (J_{CF} = 11.6 Hz, aryl 1-C), 130.13 (J_{CF} = 9.7 Hz, aryl 5-C), 113.38 (J_{CF} = 2.2 Hz, aryl 6-C), 107.39 (J_{CF} = 21.2 Hz, aryl 4-C), 104.31 (J_{CF} = 26.5 Hz, aryl 2-C), 39.19 ($CH_2NH_3^+$), 37.09 (CH_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}FN_3O$ $[MH]^+$ calcd 198.1037; found 198.1027.

1-(2-Aminoethyl)-3-(4-fluorophenyl)urea hydrochloride (66i)



Yield: 100%.

Mp: 203 – 205 °C.

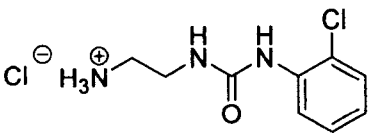
IR: 3336 (urea N-H, str), 3012 (NH_3^+, str), 1647 (urea C=O, str), 1615, 1509 (NH_3^+, bend), 1565 (aryl, str), 1225 (C-F, str), 827 (aryl C-H bend *para*-disubstituted aromatic ring), 756 (C-F, bend).

^{1}H NMR (DMSO-d_6): δ 9.17 (s, 1H, NH(C=O)N*H*Ar), 8.02 (br s, 3H, NH_3^+), 7.42 (dd, *J* = 9.2/5.0 Hz, 2H, aryl 2-H and 6-H), 7.05 (dd, *J* = 8.9/8.9 Hz, 2H, aryl 3-H and 5-H), 6.62 – 6.73 (m, 1H, N*H*(C=O)NHAr), 3.27 – 3.38 (m, 2H, C*H*$_2$NH(C=O)NH), 2.86 (tq, *J* = 5.7/5.7 Hz, 2H, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 156.93 (J_{CF} = 237.2 Hz, C-F), 155.83 (C=O), 136.81 (J_{CF} = 2.3 Hz, 1-C), 119.31 (J_{CF} = 7.5 Hz, aryl 2-C and 6-C), 115.09 (J_{CF} = 22.0 Hz, aryl 3-C and 5-C), 39.07 (*C*$H_2NH_3^+$), 37.15 (*C*H$_2$NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}FN_3O$ $[MH]^+$ calcd 198.1037; found 198.1021.

1-(2-Aminoethyl)-3-(2-chlorophenyl)urea hydrochloride (66j)



Yield: 80%.

Mp: 205 – 207 °C.

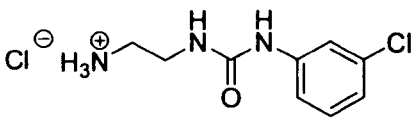
IR: 3327 (urea N-H, str), 2978 (NH_3^+, str), 2748 (alkyl C-H, str), 1675 (urea C=O, str), 1589, 1528 (NH_3^+, bend), 1567 (aryl, str), 748 (aryl C-H bend *ortho*-disubstituted aromatic ring), 627 (C-Cl, bend).

^{1}H NMR (DMSO-d_6): δ 8.44 (br s, 1H, NH(C=O)N*H*Ar), 8.12 (dd, *J* = 8.3/1.5 Hz, 1H, aryl C-H), 8.07 (br s, 3H, NH_3^+), 7.52 (t, *J* = 5.6 Hz, 1H, N*H*(C=O)NHAr), 7.40 (dd, *J* = 8.0/1.5 Hz, 1H, aryl C-H), 7.24 (dd, *J* = 7.8/1.4 Hz, 1H, aryl C-H), 6.96 (dd, *J* = 7.8/1.5 Hz, 1H, aryl C-H), 3.31 – 3.39 (m, 2H, C*H*$_2$NH(C=O)NH), 2.89 (br s, 2H, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.78 (C=O), 136.97, 121.98 (4° C), 129.58, 127.87, 123.19, 121.51 (aryl C-H), 38.93 (*C*$H_2NH_3^+$), 37.54 (*C*H$_2$NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}ClN_3O$ $[MH]^+$ calcd 214.0742; found 214.0724.

1-(2-Aminoethyl)-3-(3-chlorophenyl)urea hydrochloride (66k)



Yield: 92%.

Mp: 193 – 195 °C.

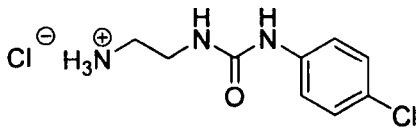
IR: 3319 (urea N-H, str), 3009 (NH_3^+, str), 1681 (urea C=O, str), 1598, 1506 (NH_3^+, bend), 1558 (aryl, str), 774 (aryl C-H bend *meta*-disubstituted aromatic ring), 680 (C-Cl, bend).

^{1}H NMR (DMSO-d_6): δ 9.25 (s, 1H, NH(C=O)N*H*Ar), 7.86 (br s, 3H, NH_3^+), 7.66 – 7.70 (m, 1H, aryl 2-H), 7.20 – 7.27 (m, 2H, aryl C-H), 6.94 (ddd, *J* = 6.7/2.2/2.2 Hz, 1H, aryl C-H), 6.62 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.32 (dt, *J* = 6.2/6.2 Hz, 2H, CH_2NH(C=O)NH), 2.88 (tq, *J* = 5.8/5.8 Hz, 2H, C$H_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.53 (C=O), 141.95, 133.06 (4° C), 130.27, 120.79, 116.10 (aryl C-H), 117.11 (aryl 2-C), 39.70 (*C*$H_2NH_3^+$), 37.16 (*C*H_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}ClN_3O$ $[MH]^+$ calcd 214.0742; found 214.0737.

1-(2-Aminoethyl)-3-(4-chlorophenyl)urea hydrochloride (66l)



Yield: 94%.

Mp: 223 – 225 °C.

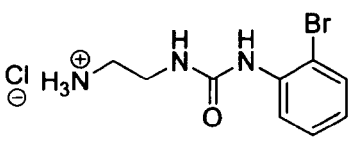
IR: 3380, 3307 (urea N-H, str), 2879 (NH_3^+, str), 1647 (urea C=O, str), 1593, 1491 (NH_3^+, bend), 1518 (aryl, str), 836 (aryl C-H bend *para*-disubstituted aromatic ring), 647 (C-Cl, bend).

^{1}H NMR (DMSO-d_6): δ 9.25 (s, 1H, NH(C=O)N*H*Ar), 7.95 (br s, 3H, NH_3^+), 7.45, 7.27 (d, *J* = 8.9 Hz, 2 x 2H, aryl C-H), 6.67 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.32 (dt, *J* = 6.2/6.2 Hz, 2H, CH_2NH(C=O)NH), 2.87 (tq, *J* = 5.7/5.7 Hz, 2H, C$H_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.61 (C=O), 139.43, 124.59 (4° C), 128.47, 119.19 (aryl C-H), 39.24 (*C*$H_2NH_3^+$), 37.13 (*C*H_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}ClN_3O$ $[MH]^+$ calcd 214.0742; found 214.0732.

1-(2-Aminoethyl)-3-(2-bromophenyl)urea hydrochloride (66m)



Yield: 89%.

Mp: 195 – 197 °C.

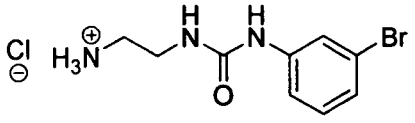
IR: 3329 (urea N-H, str), 3014 (NH_3^+, str), 2743 (alkyl C-H, str), 1676 (urea C=O, str), 1582, 1527 (NH_3^+, bend), 1558 (aryl, str), 749 (aryl C-H bend *ortho*-disubstituted aromatic ring), 668 (C-Br, bend).

^{1}H NMR (DMSO-d_6): 8.09 (br s, 3H, NH_3^+), 7.99 – 8.06 (m, 2H, NH(C=O)N*H*Ar, aryl C-H), 7.54 – 7.60 (m, 2H, N*H*(C=O)NHAr, aryl C-H), 7.28 (dd, *J* = 7.8/1.4 Hz, 1H, aryl C-H), 6.91 (dd, *J* = 8.0/1.6 Hz, 1H, aryl C-H), 3.29 – 3.38 (m, 2H, CH_2NH(C=O)NH), 2.89 (tq, *J* = 5.6/5.6 Hz, 2H, C$H_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.34 (C=O), 137.63, 112.60 (4° C), 132.41, 127.94, 123.50, 121.87 (aryl C-H), 38.79 (*C*$H_2NH_3^+$), 37.12 (*C*H_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}BrN_3O$ $[MH]^+$ calcd 258.0237; found 258.0211.

1-(2-Aminoethyl)-3-(3-bromophenyl)urea hydrochloride (66n)



Yield: 98%.

Mp: 196 – 198 °C.

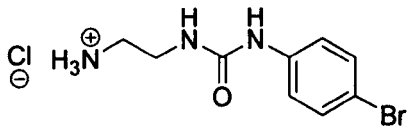
IR: 3324 (urea N-H, str), 3009 (NH_3^+, str), 1680 (urea C=O, str), 1589, 1495 (NH_3^+, bend), 1556 (aryl, str), 772 (aryl C-H bend *meta*-disubstituted aromatic ring), 680 (C-Br, bend).

^{1}H NMR (DMSO-d_6): δ 9.41 (s, 1H, NH(C=O)N*H*Ar), 7.98 (br s, 3H, NH_3^+), 7.82 (dd, *J* = 1.9/1.9 Hz, 1H, aryl 2-H), 7.28 (ddd, *J* = 8.2/1.9/0.9 Hz, 1H, aryl C-H), 7.17 (dd, *J* = 8.0/8.0 Hz, 1H, aryl 5-H), 7.06 (ddd, *J* = 7.9/1.9/1.0 Hz, 1H, aryl C-H), 6.76 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.32 (dt, *J* = 6.3/6.3 Hz, 2H, CH_2NH(C=O)NH), 2.87 (t, *J* = 5.8 Hz, 2H, C$H_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.52 (C=O), 142.14, 121.59 (4° C), 130.27 (aryl 5-C), 123.58, 116.39 (aryl C-H), 119.85 (aryl 2-C), 39.19 ($CH_2NH_3^+$), 37.10 (CH_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}BrN_3O$ $[MH]^+$ calcd 258.0237; found 258.0213.

1-(2-Aminoethyl)-3-(4-bromophenyl)urea hydrochloride (66o)



Yield: 99%.

Mp: 229 – 231 °C (lit. 234 °C)[143].

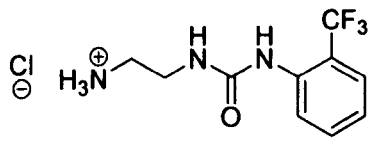
IR: 3292 (urea N-H, str), 3010 (NH_3^+, str), 1675 (urea C=O, str), 1590, 1487 (NH_3^+, bend), 1553 (aryl, str), 820 (aryl C-H bend *para*-disubstituted aromatic ring), 621 (C-Br, bend).

^{1}H NMR (DMSO-d_6): δ 9.30 (s, 1H, NH(C=O)N*H*Ar), 7.98 (br s, 3H, NH_3^+), 7.37 - 7.43 (m, 4H, aryl C-H), 6.71 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.32 (dt, *J* = 6.3/6.3 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.87 (t, *J* = 5.6 Hz, 2H, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.59 (C=O), 139.88, 112.42 (4° C), 131.35, 119.61 (aryl C-H), 39.21 ($CH_2NH_3^+$), 37.12 (CH_2NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}BrN_3O$ $[MH]^+$ calcd 258.0237; found 258.0211.

1-(2-Aminoethyl)-3-(2-trifluoromethylphenyl)urea hydrochloride (66p)



Yield: 97%.

Mp: 153 – 155 °C.

IR: 3317 (urea N-H, str), 2998 (NH_3^+, str), 1636 (urea C=O, str), 1573 (aryl, str), 1322 (C-F, str), 763 (aryl C-H bend *ortho*-disubstituted aromatic ring).

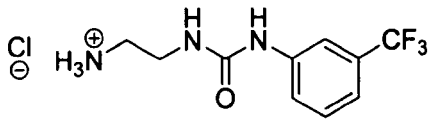
^{1}H NMR (DMSO-d_6): 8.09 (br s, 3H, NH_3^+), 8.01 (s, 1H, NH(C=O)N*H*Ar), 7.94 (d, *J* = 8.3 Hz, 1H, aryl 6-H), 7.62 (d, *J* = 7.9 Hz, 1H, aryl 3-H), 7.58 (dd, *J* = 8.1/8.1 Hz, 1H, aryl C-H), 7.46 (t, *J* = 5.5 Hz,

1H, N*H*(C=O)NHAr), 7.21 (dd, *J* = 7.6/7.6 Hz, 1H, aryl C-H), 3.32 – 3.39 (m, 2H, CH_2NH(C=O)NH), 2.89 (t, *J* = 6.3 Hz, 2H, C$H_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.63 (C=O), 137.09 (aryl 1-C), 132.78, 123.07 (aryl C-H), 125.85 (J_{CF} = 5.3 Hz, aryl 3-C), 125.16 (aryl 6-C), 124.03 (J_{CF} = 273.0 Hz, aryl CF_3), 119.32 (J_{CF} = 29.0 Hz, aryl 2-C), 38.81 (*C*$H_2$$NH_3^+$), 37.22 (*C*$H_2$NH(C=O)NH).

m/z: HRMS (TOF ES^+) $C_{10}H_{13}F_3N_3O$ $[MH]^+$ calcd 248.1005; found 248.0988.

1-(2-Aminoethyl)-3-(3-trifluoromethylphenyl)urea hydrochloride (66q)



Yield: 94%.

Mp: 175 – 177 °C.

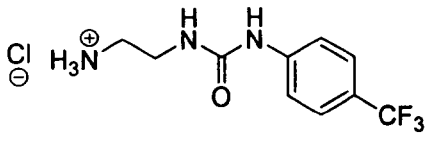
IR: 3302 (urea N-H, str), 3000 (NH_3^+, str), 1682 (urea C=O, str), 1604 (NH_3^+, bend), 1575 (aryl, str), 1342 (C-F, str), 799 (aryl C-H bend *meta*-disubstituted aromatic ring), 700 (C-F, bend).

^{1}H NMR (DMSO-d_6): 9.59 (s, 1H, NH(C=O)N*H*Ar), 7.92 – 8.04 (m, 4H, aryl 2-H, NH_3^+), 7.55 (d, *J* = 8.5 Hz, 1H, aryl 6-H), 7.45 (dd, *J* = 7.8/7.8 Hz, 1H, aryl 5-H), 7.22 (d, *J* = 7.6 Hz, 1H, aryl 4-H), 6.80 (t, *J* = 5.7 Hz, 1H, N*H*(C=O)NHAr), 3.30 – 3.40 (m, 2H, CH_2NH(C=O)NH), 2.89 (t, *J* = 6.1 Hz, 2H, C$H_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 156.10 (C=O), 141.77 (aryl 1-C), 130.19 (aryl 5-C), 129.83 (J_{CF} = 31.3 Hz, aryl 3-C), 124.75 (J_{CF} = 272.4, aryl 3-C), 121.61 (aryl 6-C), 117.76 (J_{CF} = 3.9 Hz, aryl 4-C), 114.03 (J_{CF} = 4.0 Hz, aryl 2-C), 39.22 (*C*$H_2$$NH_3^+$), 37.59 (*C*$H_2$NH(C=O)NH)

m/z: HRMS (TOF ES^+) $C_{10}H_{13}F_3N_3O$ $[MH]^+$ calcd 248.1005; found 248.1019.

1-(2-Aminoethyl)-3-(4-trifluoromethylphenyl)urea hydrochloride (66r)



Yield: 96%.

Mp: 203 – 206 °C.

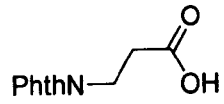
IR: 3311 (urea N-H, str), 3007 (NH_3^+, str), 1681 (urea C=O, str), 1607 (NH_3^+, bend), 1573 (aryl, str), 1330 (C-F, str), 839 (aryl C-H bend *para*-disubstituted aromatic ring).

^{1}H NMR (DMSO-d_6): δ 9.66 (s, 1H, NH(C=O)N*H*Ar), 8.02 (br s, 3H, NH_3^+), 7.62 (d, *J* = 8.7 Hz, 2H, aryl 2-H and 6-H), 7.57 (d, *J* = 8.9 Hz, 2H, aryl 3-H and 5-H), 6.87 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.35 (dt, *J* = 6.1/6.1 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.89 (t, *J* = 6.2 Hz, 2H, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.46 (C=O), 144.19 (aryl 1-C), 125.96 (J_{CF} = 3.7 Hz, aryl 3-C and 5-C), 124.67 (J_{CF} = 270.7 Hz, CF_3), 121.03 (J_{CF} = 32.0 Hz, aryl 4-C), 117.26 (aryl 2-C and 6-C), 38.84 (*C*$H_2$$NH_3^+$), 37.11 (*C*$H_2$NH(C=O)NH).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{13}F_3N_3O$ $[MH]^+$ calcd 248.1005; found 248.1026.

3-Phthalimidopropanoic acid (68)



Phthalic anhydride (14.8 g, 0.1 mol) and β-alanine **(67)** (8.9 g, 0.1 mol, 1 eq) were heated at 150 °C with stirring under a condenser for 2 hours. After cooling to room temperature, the crude solid was dispersed in water (150 mL) and collected by filtration (suction) before drying to give 20.7 g of white crystalline solid requiring no further purification.

Yield: 94 %.

Mp: 151 - 153 °C

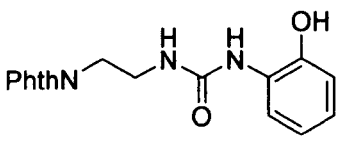
IR: 3210 (br, O-H, str), 1770 (phth C=O, str), 1701 (br, C=O, str), 1610 (aryl, str), 1377 (O-H, bend), 729 (aryl C-H, bend, *ortho*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 12.39 (s, 1H, COOH), 7.81-7.89 (m, 4H, aryl C-H), 3.79 (t, *J* = 7.3 Hz, 2H, phth-NC*H*$_2$), 2.60 (t, *J* = 7.4 Hz, 2H, C*H*$_2$COOH).

^{13}C NMR (DMSO-d_6): δ 172.14 (acid C=O), 167.63 (phth C=O), 134.42, 131.65, 123.05 (phth CH, 4 °C), 33.60 (phth-NCH$_2$) 32.37 (*C*H$_2$COOH).

m/z: HRMS (TOF ES$^+$) $C_{11}H_{10}NO_4$ $[MH]^+$ calcd 220.0604; found 220.0615.

1-(2-Hydroxyphenyl)-3-(2-phthalimidoethyl)urea (69a)



A solution of 3-phthalimidopropanoic acid **(68)** (2.000 g, 9.12 mmol), DPPA (1.966 mL, 9.12 mmol, 1 eq) and TEA (2.543 ml, 2 eq, 18.25 mmol) in dry toluene (60 mL) was stirred at room temperature, under a nitrogen atmosphere. After disappearance of starting materials by TLC (approximately 1 hour), the mixture was refluxed to promote conversion to the isocyanate. After evolution of nitrogen gas had ceased, the reaction mixture was split into half (by volume). 2-aminophenol (747 mg, 1.5 eq, 6.84 mmol) was added to one half of the isocyanate solution and stirred under reflux for 16 hours. On cooling to room temperature a yellow precipitate formed, which was collected by filtration (suction) and washed with EtOAc. On drying, 867 mg of pale yellow solid was obtained requiring no further purification.

Yield: 75 %.

Mp: 219 – 220 °C.

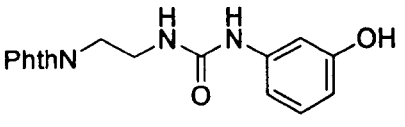
IR: 3368 (O-H, str), 3342 (urea N-H, str), 1770, 1709 (phth C=O, str), 1674 (urea C=O, str), 1575 (aryl, str), 740, 712 (aryl C-H, bend, *ortho*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 9.76 (s, 1H, OH), 7.81 - 7.90 (m, 4H, phth CH), 7.77 (dd, *J* = 7.8/1.5 Hz, 1H, hydroxyphenyl 6-H), 7.76 (s, 1H, NH(C=O)N*H*Ar), 6.95 (t, *J* = 6.2, 1H, N*H*(C=O)NHAr), 6.62 – 6.78 (m, 3H, hydroxyphenyl 3-H, 4-H, 5-H), 3.66 (t, *J* = 5.3Hz, 2H, phth-NC*H*$_2$), 3.35 (dt, *J* = 6.0/5.8 Hz, 2H, C*H*$_2$-NH).

^{13}C NMR (DMSO-d_6): δ 167.98 (phth C=O), 155.49 (urea C=O), 145.34 (hydroxyphenyl 2-C), 134.33 (phth C-H), 131.77 (phth 4° C), 128.34 (hydroxyphenyl 1-C), 123.02, (phth C-H), 121.24, 119.05, 114.58 (hydroxyphenyl 3-C, 4-C, 5-C), 118.45 (hydroxyphenyl 6-C) , 38.24 (phth-NCH_2) , 37.27 (CH_2-NH).

m/z: HRMS (TOF ES^+) $C_{17}H_{16}N_3O_4$ $[MH]^+$ calcd 326.1135; found 326.1165.

1-(3-Hydroxyphenyl)-3-(2-phthalimidoethyl)urea (69b)



Isocyanate solution was prepared as described for 1-(2-hydroxyphenyl)-3-(2-phthalimidoethyl) urea **(69a)**. To the remaining half portion was added 3-aminophenol (747 mg, 1.5 eq, 6.84 mmol) and stirred under reflux for 16 hours.

After cooling and removal of solvent, the crude residue was dispersed in EtOAc (50 mL) and washed with aqueous 2M HCl (2 x 30 mL). Concentration of the organic layer gave 1.134 g of pale yellow solid.

Yield: 76 %.

Mp: 225 – 228 °C.

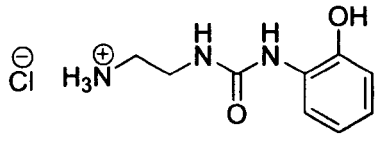
IR: 3393 (O-H, str), 3343 (urea N-H, str), 1769, (phth C=O, str), 1694 (urea C=O, str), 1563 (aryl, str), 727 (aryl C-H, bend, *ortho*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 9.15 (br s, 1H, OH), 8.31 (s, 1H, NH(C=O)N*H*Ar), 7.81 – 7.88 (m, 4H, phth CH), 6.91 – 6.95 (m, 2H, aryl 2-H, 5-H), 6.63 (dd, *J* = 8.0/1.0 1H, aryl 6-H), 6.26 (dd, *J* = 7.6/1.7 Hz, 1H, aryl 4-H), 6.20 (t, *J* = 5.8 Hz, 1H, N*H*(C=O)NHAr), 3.68 (t, *J* = 5.7 Hz, 2H, phth-NCH_2), 3.34 (dt, *J* = 5.7/5.7 Hz, 2H, CH_2-NH).

^{13}C NMR (DMSO-d_6): δ 167.97 (phth C=O), 157.58 (hydroxyphenyl 3-C), 155.14 (urea C=O), 141.44 (hydroxyphenyl 1-C), 134.27 (phth C-H), 131.76 (phth 4° C), 129.11 (hydroxyphenyl 5-C), 122.96 (phth C-H), 108.58 (hydroxyphenyl 6-C), 108.21 (hydroxyphenyl 4-C), 104.91 (hydroxyphenyl 3-C), 38.13 (phth-NCH_2), 37.51 (CH_2-NH).

m/z: HRMS (TOF ES$^+$) $C_{17}H_{16}N_3O_4$ $[MH]^+$ calcd 326.1135; found 326.1169.

1-(2-Aminoethyl)-3-(2-hydroxyphenyl)urea hydrochloride (70a)



A solution of 1-(2-hydroxyphenyl)-3-(2-phthalimidoethyl)urea **(69a)** (700mg, 2.13 mmol) and hydrazine monohydrate (232 µl, 4.5 mmol, 2.1 eq) in EtOH (20 mL) was stirred under reflux for 2 hours. After cooling to room temperature, solvent was removed *in vacuo*. The crude residue was dispersed in EtOAc (30 mL) and washed with aqueous 2M HCl (2 x 30 mL). The combined aqueous layers were concentrated *in vacuo* to give 296 mg of yellow solid requiring no further purification.

Yield: 60 %.

Mp: 160 – 165 °C.

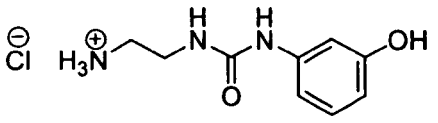
IR: 3346 (O-H, str), 3267 (urea N-H, str), 3137, 3023 (br, NH_3^+, str), 1645 (urea C=O, str), 1568 (aryl, str), 751 (aryl C-H, bend, *ortho*-disubstituted ring)

^{1}H NMR (DMSO-d$_6$): δ 9.92 (br s, 1H, OH), 8.11 (s,1H, NH(C=O)N*H*Ar), 7.82 (dd, *J* = 7.9/1.7 Hz, 1H, aryl 6-H), 7.27 (t, *J* = 5.6 Hz, 1H, N*H*(C=O)N*H*Ar), 6.84 (dd, *J* = 7.7/1.5 Hz, 1H, aryl 3-H), 6.74 (ddd *J* = 7.4/7.4/1.7 Hz, 1H, aryl 4-H or 5-H), 6.67 (ddd, *J* = 7.8/7.8/1.5 Hz, 1H, aryl 4-H or 5-H), 3.31 (dt, *J* = 6.2/5.9 Hz, 2H, C*H*$_2$NH), 2.86 (t, *J* = 6.3 Hz, 2H, C*H*$_2$NH$_3^+$).

^{13}C NMR (DMSO-d$_6$): δ 156.05 (C=O), 145.86 (aryl 2-C), 128.12 (aryl 1-C), 121.64 (aryl 4-C), 118.96 (aryl 6-C), 118.87 (aryl 5-C), 114.92 (aryl 3-C), 39.15 ($CH_2NH_3^+$), 37.14 (CH_2NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{14}N_3O_2$ $[MH]^+$ calcd 196.1081; found 196.1071.

1-(2-Aminoethyl)-3-(2-hydroxyphenyl)urea hydrochloride (70b)



Phthalimide deprotection of 1-(3-hydroxyphenyl)-3-(2-phthalimidoethyl) urea **(69b)** (700 mg, 2.13 mmol) was carried out as described for 1-(2-hydroxyphenyl)-3-(2-phthalimidoethyl)urea **(69a)** to give 252 mg of yellow solid requiring no further purification.

Yield: 51 %.

Mp: 106 – 109 °C.

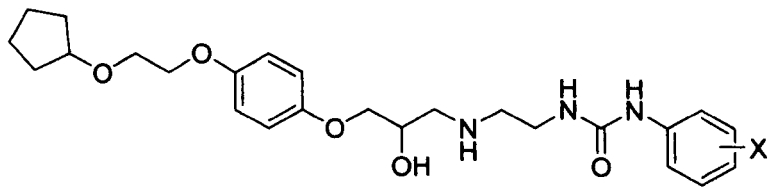
IR: 3406 (br, O-H, str), 3104 (br, NH_3^+, str), 2899, 2595 (alkyl C-H, str), 1682 (urea C=O, str), 1557 (aryl, str), 763 (aryl C-H, bend, *meta*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 8.94 (s, 1H, NH(C=O)N*H*Ar), 8.05 (br s, 3H, NH_3^+), 6.96 – 6.98, (m, 2H, aryl 2H, 5-H), 6.78 (m, 1H, aryl 6-H), 6.30 (dd, *J* = 8.0/2.3/0.7 Hz, 1H aryl 4-H), 3.31 (t, *J* = 6.4 Hz, 2H, C*H*$_2$ NH_3^+), 2.83 (dt, *J* = 6.0/6.0 Hz, 1H, C*H*$_2$NH).

^{13}C NMR (DMSO-d_6): δ 157.71 (aryl 3-C), 155.78 (C=O), 141.47 (aryl 1-C), 129.18 (aryl 5-C), 108.54 (aryl 6-C), 108.41 (aryl 4-C), 104.89 (aryl 2-C), 39.18 ($CH_2NH_3^+$), 37.14 (CH_2NH).

m/z: HRMS (TOF ES$^+$) $C_9H_{14}N_3O_2$ $[MH]^+$ calcd 196.1081; found 196.1065.

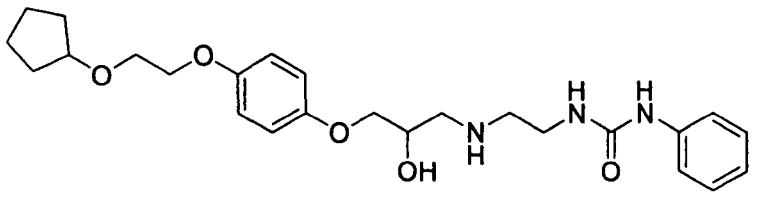
General procedure for synthesis of substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(aryl)ureas



Substituted 1-(2-aminoethyl)-3-(aryl)urea (1 eq, compounds **56, 63**, or **66a-r**) and 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (50 mg, 0.18 mmol) were suspended in propan-2-ol (3 mL). In the case

where 1-(2-aminoethyl)-3-(aryl)ureas were hydrochloride salts, NaOH (1.1 eq as 10 M aqueous solution) was also added. The mixture was heated under reflux overnight, after which all solvent was removed *in vacuo*. The crude residue was purified via PLC (eluent 37% aq NH_3/MeOH/DCM 2:10:88). Analogues with substitution *meta* to the urea group were purified using a weaker eluent (NH_3/MeOH/DCM 2:5:93). The final aryloxypropanolamines were freeze-dried to give white solids. Where further purification was necessary by preparative HPLC, the final compounds were isolated as the hydroformate salt.

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-phenylurea (71a)



Yield: 22%.

Mp: 109 – 115 °C.

IR: 3310 (br, O-H, str), 2930, 2868 (alkyl C-H, str), 1634 (urea C=O, str), 1509, 1567 (aryl, str), 1110 (C-O-C, str), 764, 693 (aryl C-H, bend, *phenyl* ring).

^{1}H NMR (DMSO-d$_6$): δ 8.61 (s, 1H, NH(C=O)N*H*Ar), 7.38 (dd, *J* = 8.7/1.1 Hz, 2H, 2-H and 6-H phenyl ring), 7.20 (dd, *J* = 7.4/7.4 Hz, 2H, 3-H and 5-H phenyl ring), 6.81 – 6.89 (m, 5H, 4-H phenyl ring, aryl-dioxy ring), 6.22 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.07 (br s, 1H, NH) 3.79 – 3.97 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.19 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH$_2$CH_2), 2.75 (dd, *J* = 12.0/4.0 Hz, 1H, CH(OH)CH_2NH), 2.68 (t, *J* = 6.0 Hz, 2H, NHCH_2CH$_2$), 2.63 (dd, *J* = 12.2/6.8 Hz, 1H, CH(OH)CH_2NH), 1.52 – 1.74 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.52 (m, 2H, ^{c}Pe CH_2).

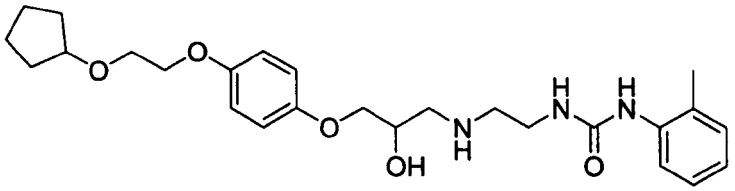
^{13}C NMR (DMSO-d$_6$): δ 155.37 (C=O), 152.70, 152.53, 140.59 (aryl 4° C), 128.64 (phenyl 3-C and 5-C), 120.2943 (phenyl 4-C), 117.57 (phenyl 2-C and 6-C), 115.34 (*C*H aryl-dioxy ring), 80.85 (^{c}Pe *C*H),

71.14 (ArOCH_2), 67.79 (CH(OH)), 67.71 (CH_2OAr), 66.73 (^{c}PeOCH_2), 51.98 (CH(OH)CH_2NH), 49.14 (NHCH_2CH_2), 38.78 (NHCH_2CH_2), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{36}N_3O_5$ $[MH]^+$ calcd 458.2649; found 458.2611.

HPLC R_t: 3.62 (System 1b), 12.67 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-methylphenyl)lurea (71b)



Yield: 31%.

Mp: 114 – 115 °C.

IR: 3329 (br, O-H, str), 2928, 2871 (alkyl C-H, str), 1636 (urea C=O, str), 1509, 1567 (aryl, str), 1109 (C-O-C, str), 827 (aryl C-H, bend, *para*-disubstituted ring), 764 (aryl C-H, bend, *ortho* -disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 7.80 (d, J = 7.6 Hz, 1H, aryl 6-H), 7.70 (s, 1H, NH(C=O)NHAr), 7.05 – 7.11 (m, 2H, C-H tolyl ring), 6.80 – 6.87 (m, 5H, C-H aryl-dioxy ring, C-H tolyl ring), 6.60 (t, J = 5.4 Hz, 1H, NH(C=O)NHAr), 4.95 (br s, 1H, NH), 3.77 – 4.01 (m, 6H, CH_2OAr, ^{c}Pe CH, CH(OH), ArOCH_2), 3.61 (t, J = 4.9 Hz, 2H, ^{c}PeOCH_2), 3.17 (dt, J = 5.9/5.9 Hz, 2H,), 2.70 (dd, J = 11.8/4.2 Hz, 1H, CH(OH)CH_2NH), 2.64 (t, J = 6.0 Hz, 3H, NHCH_2CH$_2$), 2.59 (dd, J = 11.7/6.3 Hz, CH(OH)CH_2NH), 2.17 (s, 3H, CH_3), 1.52 – 1.76 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.52 (m, 2H, ^{c}Pe CH_2).

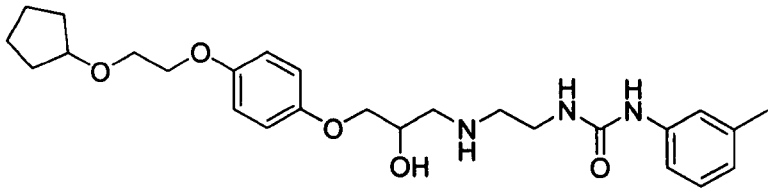
^{13}C NMR (DMSO-d_6): δ 155.48 (C=O), 152.75, 152.49, 138.31 (aryl 4° C), 130.03, 126.02, 121.72 (aryl C-H), 120.42 (aryl 6-C), 115.32 (*C*H aryl-dioxy ring), 80.85 (^{c}Pe *C*H), 71.25 (ArOCH_2), 68.17 (*C*H(OH)), 67.70 (CH_2OAr), 66.73 (^{c}PeOCH_2), 52.34 (CH(OH)CH_2NH), 49.39

($NHCH_2CH_2$), 39.10 ($NHCH_2CH_2$), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring), 17.97 (CH_3).

m/z: HRMS (TOF ES^+) $C_{26}H_{38}N_3O_5$ $[MH]^+$ calcd 472.2806; found 472.2764.

HPLC R_t: 4.07 (System 1b), 7.60 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-methylphenyl)urea (71c)



Yield: 9%.

Mp: 112 – 114 °C.

IR: 3408 (urea N-H, str), 3316 (br, O-H, str), 2930, 2870 (alkyl C-H, str), 1633 (urea C=O, str), 1509, 1569 (aryl, str), 1111 (C-O-C, str), 819 (aryl C-H, bend, *para*-disubstituted ring), 760 (aryl C-H, bend, *meta*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 8.51 (s, 1H, NH(C=O)N*H*Ar), 7.21 (s, 1H, aryl 2-H), 7.10 (d, *J* = 8.4 Hz, 1H, aryl 6-H), 7.08 (dd, *J* = 7.6/7.6 Hz, 1H, aryl 5-H), 6.85, 6.83 (d, *J* = 9.2 Hz, 2 x 2H, C-H aryl-dioxy ring), 6.46 (d, *J* = 7.4 Hz, 1H aryl 4-H), 6.20 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.09 (br s, 1H, NH), 3.78 – 4.00 (m, 6H, C*H*$_2$OAr, ^{c}Pe C*H*, C*H*(OH), ArOC*H*$_2$), 3.61 (t, *J* = 4.9 Hz, 2H, ^{c}PeOC*H*$_2$), 3.19 (dt, *J* = 5.9/5.9 Hz, 2H, $NHCH_2CH_2$), 2.77 (dd, *J* = 11.8/3.6 Hz, 1H, CH(OH)C*H*$_2$NH), 2.61 – 2.73 (m, 3H, CH(OH)C*H*$_2$NH, $NHCH_2CH_2$), 2.23 (s, 3H, CH_3), 1.52 – 1.75 (m, 6H, ^{c}Pe C*H*$_2$), 1.41 – 1.52 (m, 2H, ^{c}Pe C*H*$_2$).

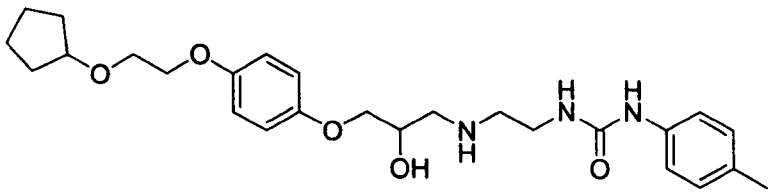
^{13}C NMR (DMSO-d_6): δ 155.38 (C=O), 152.66, 152.53, 140.47, 121.72 (aryl 4° C), 137.70 (aryl 4-C), 128.48 (aryl 5-C), 118.12 (aryl 2-C), 115.32 (*C*H aryl-dioxy ring), 114.80 (aryl 6-C), 80.84 (^{c}Pe *C*H), 71.08 (ArO*C*H$_2$), 69.77 (*C*H(OH)), 67.69 (*C*H$_2$OAr), 66.72 (^{c}PeO*C*H$_2$), 51.82

($CH(OH)CH_2NH$), 49.05 ($NHCH_2CH_2$), 38.56 ($NHCH_2CH_2$), 31.79 (2-C and 5-C ^cPe ring), 23.13 (3-C and 4-C ^cPe ring),21.26 (CH_3).

m/z: HRMS (TOF ES^+) $C_{26}H_{38}N_3O_5$ $[MH]^+$ calcd 472.2806; found 472.2825.

HPLC R_t: 4.25 (System 1b), 8.28 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-methylphenyl)urea (71d)



Yield: 19%.

Mp: 142 – 144 °C.

IR: 3319 (br, O-H, str), 2926, 2868 (alkyl C-H, str), 1634 (urea C=O, str), 1509, 1566 (aryl, str), 1110 (C-O-C, str), 821 (aryl C-H, bend, *para*-disubstituted ring).

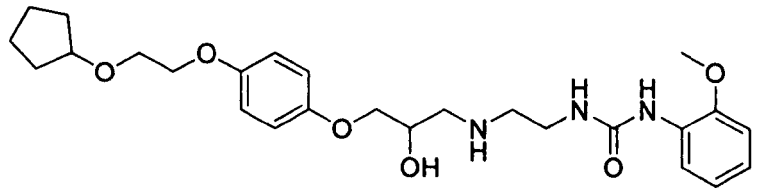
^{1}H NMR (DMSO-d_6): δ 8.46 (s, 1H, NH(C=O)N*H*Ar), 7.26 (d, *J* = 8.4 Hz, 2H, aryl 2-H and 6-H), 7.01 (d, *J* = 8.3 Hz, 2H, aryl 3-H and 5-H), 6.82, 6.85 (d, *J* = 9.2 Hz, 2 x 2H, C-H aryl-dioxy ring), 6.14 (t, *J* = 5.5 Hz, 1H, N*H*(C=O)NHAr), 5.04 (br s, 1H, NH), 3.79 – 3.97 (m, 6H, C*H*$_2$OAr, ^{c}Pe C*H*, C*H*(OH), ArOC*H*$_2$), 3.61 (t, *J* = 4.9 Hz, 2H, ^{c}PeOC*H*$_2$), 3.18 (dt, *J* = 5.8/5.8 Hz, 2H, $NHCH_2CH_2$), 2.74 (dd, *J* = 11.9/3.6 Hz, 1H, $CH(OH)CH_2NH$), 2.59 – 2.70 (m, 3H, $CH(OH)CH_2NH$, $NHCH_2CH_2$), 2.21 (s, 3H, CH_3), 1.52 – 1.76 (m, 6H, ^{c}Pe C*H*$_2$), 1.41 – 1.52 (m, 2H, ^{c}Pe C*H*$_2$).

^{13}C NMR (DMSO-d_6): δ 155.42 (C=O), 152.78, 152.51, 138.02, 129.61 (aryl 4° C), 129.02 (2-C and 6-C tolyl ring), 117.67 (3-C and 5-C tolyl ring), 115.31 (*C*H aryl-dioxy ring), 80.84 (^{c}Pe *C*H), 71.12 ($ArOCH_2$), 67.81(*C*H(OH)), 67.68 (CH_2OAr), 66.71 ($^cPeOCH_2$), 51.98 ($CH(OH)CH_2NH$), 49.16 ($NHCH_2CH_2$), 38.48 ($NHCH_2CH_2$), 31.79 (2-C and 5-C ^{c}Pe ring), 23.13 (3-C and 4-C ^{c}Pe ring), 20.30 (CH_3).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{38}N_3O_5$ $[MH]^+$ calcd 472.2806; found 472.2834.

HPLC R_t: 4.18 (System 1b), 13.52 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-methoxyphenyl)urea (71e)



Yield: 27%.

Mp: 78 – 83 °C.

IR: 3332 (br, O-H, str), 2929, 2864 (alkyl C-H, str), 1651 (urea C=O, str), 1508, 1549 (aryl, str), 1109 (C-O-C, str), 823 (aryl C-H, bend, *para*-disubstituted ring), 748 (aryl C-H, bend, *ortho* -disubstituted ring).

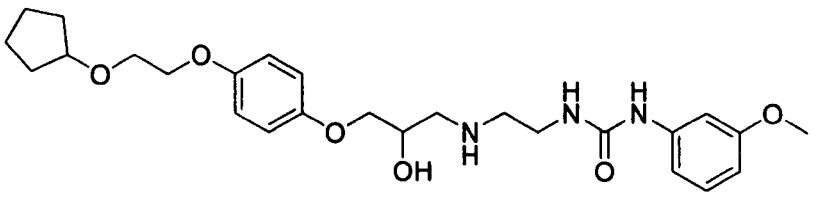
^{1}H NMR (DMSO-d_6): δ 8.07 (dd, *J* = 7.6/2.0 Hz, 1H, aryl 6-H), 7.97 (s, 1H, NH(C=O)N*H*Ar), 6.92 – 6.99 (m, 2H, C-H methoxyphenyl ring, N*H*(C=O)NHAr), 6.79 – 6.89 (m, 6H, C-H aryl-dioxy ring, C-H methoxyphenyl ring), 5.07 (br s, 1H, NH), 3.76 – 4.00 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.82 (s, 3H, CH_3), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.18 (dt, *J* = 5.9/5.9 Hz, 2H, NHCH_2CH_2), 2.74 (dd, *J* = 11.9/3.9 Hz, 1H, CH(OH)CH_2NH), 2.59 – 2.70 (m, 3H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.32 (C=O), 152.70, 152.52, 147.30, 129.51 (aryl 4° C), 120.90, 120.45, 110.54 (aryl C-H), 117.98 (aryl 6-C), 115.32 (*C*H aryl-dioxy ring), 80.85 (^{c}Pe *C*H), 71.14 (ArOCH$_2$), 67.80 (*C*H(OH)), 67.69 (*C*H$_2$OAr), 66.73 (^{c}PeOCH$_2$), 55.63 (CH_3), 52.03 (CH(OH)*C*H$_2$NH), 49.23 (NHCH$_2$CH$_2$), 38.93 (NHCH$_2$CH$_2$), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{38}N_3O_6$ $[MH]^+$ calcd 488.2755; found 488.2777.

HPLC R_t: 3.77 (System 1b), 12.87 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-methoxyphenyl)urea (71f)



Yield: 16%.

Mp: 103 – 110 °C.

IR: 3316 (br, O-H, str), 2928, 2869 (alkyl C-H, str), 1630 (urea C=O, str), 1509, 1570 (aryl, str), 1110 (C-O-C, str), 824 (aryl C-H, bend, *para*-disubstituted ring), 765 (aryl C-H, bend, *meta*-disubstituted ring).

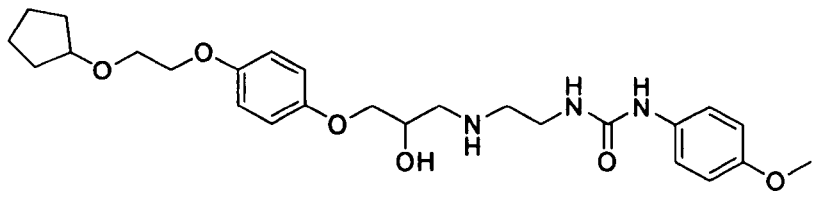
^{1}H NMR (DMSO-d_6): δ 8.63 (s, 1H, NH(C=O)N*H*Ar), 7.14 (dd, *J* = 2.2/2.2 Hz, 1H, aryl 2-H), 7.10 (dd, *J* = 8.1/8.1 Hz, 1H, aryl 5-H), 6.79 – 6.89 (m, 5H, C-H aryl-dioxy ring, aryl 6-H), 6.46 (ddd, *J* = 8.2/2.4/0.6 Hz, 1H aryl 4-H), 6.22 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.05 (br s, 1H, NH), 3.79 – 3.97 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.69 (s, 3H, CH_3), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.18 (dt, *J* = 5.9/5.9 Hz, 2H, NHCH_2CH_2), 2.73 (dd, *J* = 11.9/3.9 Hz, 1H, CH(OH)CH_2NH), 2.50 – 2.70 (m, 3H, CH(OH)CH_2NH, NHCH_2CH_2), 1.52 – 1.74 (m, 6H, ^{c}Pe CH_2), 1.41 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.28 (C=O), 159.64, 152.71, 152.52, 141.84 (aryl 4° C), 129.36 (aryl 5-C), 115.34 (*C*H aryl-dioxy ring), 109.94 (aryl 6-C), 106.33 (aryl 4-C), 103.35 (aryl 2-C), 80.85 (^{c}Pe *C*H), 71.16 (ArO*C*H_2), 67.87 (*C*H(OH)), 67.70 (*C*H_2OAr), 66.73 (^{c}PeO*C*H_2), 54.82 (CH_3), 52.02 (CH(OH)*C*H_2NH), 49.13 (NH*C*H_2CH_2), 38.77 (NHCH_2*C*H_2), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{38}N_3O_6$ $[MH]^+$ calcd 488.2755; found 488.2786.

HPLC R_t: 3.40 (System 1b), 12.42 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-methoxyphenyl)urea (71g)



Yield: 7%.

Mp: 121 – 125 °C.

IR: 3327 (br, O-H, str), 2927, 2867 (alkyl C-H, str), 1635 (urea C=O, str), 1509, 1565 (aryl, str), 1110 (C-O-C, str), 828 (aryl C-H, bend, *para*-disubstituted ring).

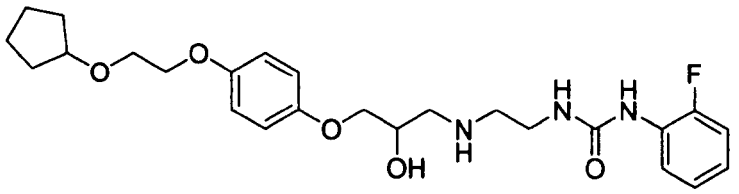
^{1}H NMR (DMSO-d_6): δ 8.37 (s, 1H, NH(C=O)N*H*Ar), 7.10 (d, *J* = 9.0 Hz, 2H, aryl 2-H and 6-H), 6.82 – 6.87 (m, 4H, C-H aryl-dioxy ring), 6.80 (d, *J* = 9.0 Hz, 2H, aryl 3-H and 5-H), 6.07 (t, *J* = 5.5 Hz, 1H, N*H*(C=O)NHAr), 5.00 (br s, 1H, NH), 3.75 – 4.00 (m, 6H, C*H*$_2$OAr, ^{c}Pe C*H*, C*H*(OH), ArOC*H*$_2$), 3.68 (s, 3H, CH_3), 3.61 (t, *J* = 4.7 Hz, 2H, ^{c}PeOC*H*$_2$), 3.15 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH$_2$C*H*$_2$), 2.55 – 2.76 (m, 4H, CH(OH)C*H*$_2$NH, NHC*H*$_2$CH$_2$), 1.52 – 1.74 (m, 6H, ^{c}Pe C*H*$_2$), 1.41 – 1.52 (m, 2H, ^{c}Pe C*H*$_2$).

^{13}C NMR (DMSO-d_6): δ 155.55 (C=O), 153.82, 152.73, 152.49, 133.75 (aryl 4° C), 119.28 (2-C and 6-C methoxyphenyl ring), 115.32 (*C*H aryl-dioxy ring), 113.86 (3-C and 5-C methoxyphenyl ring), 80.85 (^{c}Pe *C*H), 71.20 (ArO*C*H$_2$), 68.03 (*C*H(OH)), 67.69 (*C*H$_2$OAr), 66.73 (^{c}PeO*C*H$_2$), 55.12 (CH_3), 52.17 (CH(OH)*C*H$_2$NH), 49.33 (NH*C*H$_2$CH$_2$), 38.27 (NHCH$_2$*C*H$_2$), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{38}N_3O_6$ $[MH]^+$ calcd 488.2755; found 488.2717.

HPLC R_t: 3.37 (System 1b), 12.02 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-fluorophenyl)urea (71h)



Yield: 11%.

Mp: 115 – 117 °C.

IR: 3322 (br, O-H, str), 2929, 2871 (alkyl C-H, str), 1640 (urea C=O, str), 1509, 1568 (aryl, str), 1241 (C-F, str), 1110 (C-O-C, str), 827 (aryl C-H, bend, *para*-disubstituted ring), 764 (aryl C-H, bend, *ortho* - disubstituted ring), 744 (C-F, bend).

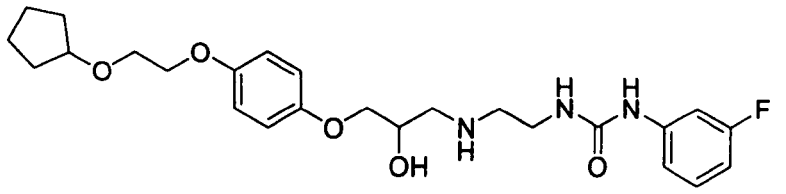
^{1}H NMR (DMSO-d_6): δ 8.38 (s, 1H, NH(C=O)N*H*Ar), 8.12 (dd, *J* = 8.3/1.5 Hz, 1H, aryl 6-H), 7.16 (ddd, *J* = 11.8/8.2/1.4 Hz, 1H, aryl 3-H), 7.06 (dd, *J* = 8.2/8.2 Hz, 1H, aryl 5-H), 6.87 – 6.94 (m, 1H, aryl 4-H), 6.85, 6.82 (d, *J* = 9.3 Hz, 2 x 2H, C-H aryl-dioxy ring), 6.70 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr) 4.99 (br s, 1H, NH), 3.77 – 4.01 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.18 (dt, *J* = 6.0/6.0 Hz, 2H, NHCH$_2$CH_2), 2.71 (dd, *J* = 11.8/3.9 Hz, 1H, CH(OH)CH_2NH), 2.56 – 2.67 (m, 3H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.51 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.41 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.16 (C=O), 152.75, 152.50, 136.85 (aryl 4° C), 125.40 (aryl 6-C), 124.35 (aryl 5-C), 121.43 (aryl 4-C), 114.67 (aryl 3-C), 115.31 (*C*H aryl-dioxy ring), 80.84 (^{c}Pe *C*H), 71.22 (ArOCH$_2$), 68.06 (*C*H(OH)), 67.69 (CH$_2$OAr), 66.72 (^{c}PeOCH$_2$), 51.18 (CH(OH)*C*H$_2$NH), 48.89 (NHCH$_2$CH$_2$), 38.99 (NHCH$_2$CH$_2$), 31.79 (2-C and 5-C ^{c}Pe ring), 23.13 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}FN_3O_5$ [MH]$^+$ calcd 476.2555; found 476.2596.

HPLC R_t: 4.05 (System 1b), 12.35 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-fluorophenyl)urea (71i)



Yield: 27%.

Mp: 108 – 110 °C.

IR: 3317 (br, O-H, str), 2926, 2869 (alkyl C-H, str), 1636 (urea C=O, str), 1509, 1569 (aryl, str), 1118 (C-O-C, str), 1238 (C-F, str), 827 (aryl C-H, bend, *para*-disubstituted ring), 765 (C-F, bend).

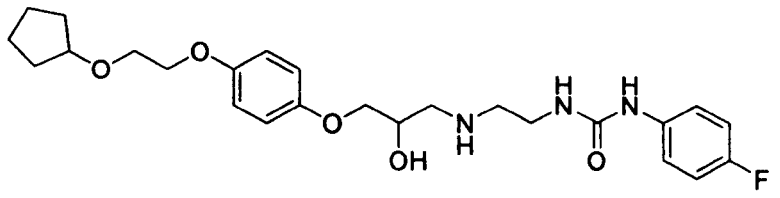
^{1}H NMR (DMSO-d_6): δ 8.86 (s, 1H, NH(C=O)N*H*Ar), 7.45 (ddd, *J* = 12.3/2.2/2.2 Hz, 1H, aryl 2-H), 7.22 (ddt, *J* = 8.2/8.2.2/8.2 Hz, 1H, aryl 5-H), 7.00 (ddd, *J* = 8.2/1.2 Hz, 1H, aryl 6-H), 6.79 – 6.89 (m, 4H, C-H aryl-dioxy ring), 6.68 (ddd, *J* = 8.2/8.2/2.1 Hz, 1H, aryl 4-H), 6.28 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.04 (br s, 1H, NH), 3.77 – 4.01 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.18 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH_2CH_2), 2.57 – 2.79 (m, 4H, CH(OH)CH_2NH, NHCH_2CH_2), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 162.44 (J_{CF} = 240.8 Hz, C-F), 155.11 (C=O), 152.72, 152.51 (aryl 4° C), 142.53 (J_{CF} = 11.8 Hz, aryl 1-C), 130.11 (J_{CF} = 10.9 Hz, aryl 5-C), 115.33 (*C*H aryl-dioxy ring), 113.22 (J_{CF} = 2.7 Hz, aryl 6-C), 107.16 (J_{CF} = 20.5 Hz, aryl 4-C), 104.16 (J_{CF} = 26.2 Hz, aryl 2-C), 80.85 (^{c}Pe *C*H), 71.17 (ArO*C*H$_2$), 67.93 (*C*H(OH)), 67.70 (*C*H$_2$OAr), 66.73 (^{c}PeO*C*H$_2$), 52.05 (CH(OH)*C*H$_2$NH), 49.06 (NH*C*H$_2$CH$_2$), 38.66 (NHCH$_2$*C*H$_2$), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}FN_3O_5$ [MH]$^+$ calcd 476.2555; found 476.2514.

HPLC R_t: 4.02 (System 1b), 13.32 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-fluorophenyl)urea (71j)



Yield: 19%.

Mp: 130 – 133 °C.

IR: 3318 (br, O-H, str), 2927, 2868 (alkyl C-H, str), 1630 (urea C=O, str), 1509, 1572 (aryl, str), 1111 (C-O-C, str), 1232 (C-F, str), 827 (aryl C-H, bend, *para*-disubstituted ring), 764 (C-F, bend).

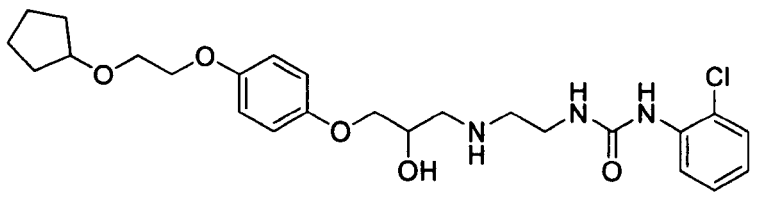
^{1}H NMR (DMSO-d_6): δ 8.69 (s, 1H, NH(C=O)N*H*Ar), 7.39 (dd, *J* = 9.1/5.0 Hz, 2H, 2-H and 6-H fluorophenyl ring), 7.04 (dd, *J* = 8.9/8.9 Hz, 2H, 3-H and 5-H fluorophenyl ring), 6.80 – 6.88 (m, 4H, aryl-dioxy ring), 6.23 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.13 (br s, 1H, NH), 3.78 – 4.00 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.15 – 3.24 (m, 2H, NHCH_2CH_2), 2.78 (dd, *J* = 11.9/3.7 Hz, 1H, CH(OH)CH_2NH), 2.61 – 2.74 (m, 3H, CH(OH)CH_2NH, NHCH_2CH_2), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.45 (C=O), 152.66, 152.55 (aryl 4° C), 136.94 (J_{CF} = 1.8 Hz, 1-C), 119.15 (J_{CF} = 8.0 Hz, 2-C and 6-C fluorophenyl ring), 115.33 (*C*H aryl-dioxy ring), 115.08 (J_{CF} = 21.6 Hz, 3-C and 5-C fluorophenyl ring), 80.85 (^{c}Pe *C*H), 71.07 (ArO*C*H_2), 67.70 (*C*H_2OAr), 67.49 (*C*H(OH)), 66.73 (^{c}PeO*C*H_2), 51.80 (CH(OH)*C*H_2NH), 49.02 (NH*C*H_2CH_2), 38.43 (NHCH_2*C*H_2), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}FN_3O_5$ $[MH]^+$ calcd 476.2555; found 476.2529.

HPLC R_t: 4.12 (System 1b), 13.20 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-chlorophenyl)urea (71k)



Yield: 9%.

Mp: 99 - 103°C.

IR: 3317 (br, O-H, str), 2929, 2868 (alkyl C-H, str), 1638 (urea C=O, str), 1508, 1559 (aryl, str), 1112 (C-O-C, str), 820 (aryl C-H, bend, *para*-disubstituted ring), 750 (aryl C-H, bend, *ortho* -disubstituted ring), 730 (C-Cl, bend).

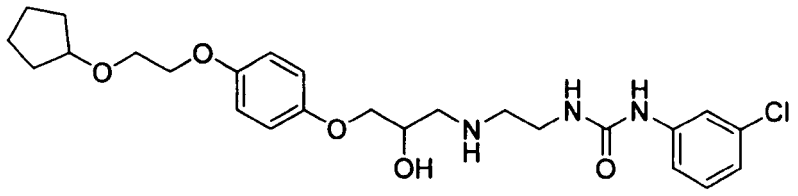
^{1}H NMR (DMSO-d_6): δ 8.14 (dd, *J* = 8.3/1.3 Hz, 1H, aryl 6-H), 8.09 (s, 1H, NH(C=O)N*H*Ar), 7.38 (dd, *J* = 8.0/1.4 Hz, 1H, aryl 3-H), 7.22 (ddd, *J* = 7.8/7.8/1.4 Hz, 1H, aryl 5-H), 7.07 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 6.93 (ddd, *J* = 7.8/7.8/1.5 Hz, 1H, aryl 4-H), 6.79 – 6.86 (m, 4H, C-H aryl-dioxy ring), 4.98 (br s, 1H, NH), 3.73 – 4.01 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.18 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH$_2$CH_2), 2.57 – 2.72 (m, 4H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.51 – 1.74 (m, 6H, ^{c}Pe CH_2), 1.41 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 154.89 (C=O), 152.76, 152.50, 136.83 (aryl 4° C), 129.07 (aryl 3-C), 127.42 (aryl 5-C), 122.34 (aryl 4-C), 120.81 (aryl 6-C), 115.33 (*C*H aryl-dioxy ring), 80.86 (^{c}Pe *C*H), 71.24 (ArOCH$_2$), 68.15 (*C*H(OH)), 67.71 (*C*H$_2$OAr), 66.73 (^{c}PeOCH$_2$), 52.30 (CH(OH)*C*H$_2$NH), 49.22 (NHCH$_2$*C*H$_2$), 39.19 (NHCH$_2$*C*H$_2$), 31.81 (2-C and 5-C ^{c}Pe ring), 23.15 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}ClN_3O_5$ [MH]$^+$ calcd 492.2260; found 492.2254.

HPLC R_t: 3.50 (System 1b), 13.09 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-chlorophenyl)urea (71l)



Yield: 17%.

Mp: 102 – 109 °C.

IR: 3316 (br, O-H, str), 2930, 2869 (alkyl C-H, str), 1639 (urea C=O, str), 1509, 1570 (aryl, str), 1119 (C-O-C, str), 819 (aryl C-H, bend, *para*-disubstituted ring), 765 (C-Cl, bend).

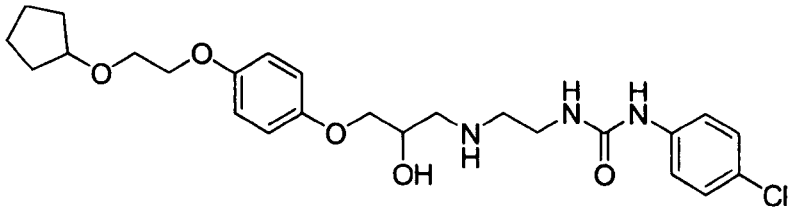
^{1}H NMR (DMSO-d_6): δ 8.85 (s, 1H, NH(C=O)N*H*Ar), 7.67 (dd, *J* = 2.0/2.0 Hz, 1H, aryl 2-H), 7.22 (dd, *J* = 8.1/8.1 Hz, 1H, aryl 5-H), 7.16 (ddd, *J* = 8.2/1.8/1.2 Hz, 1H, aryl 6-H), 6.92 (ddd, *J* = 7.7/2.1/1.2 Hz, 1H, aryl 4-H), 6.85 (d, *J* = 9.3 Hz, 2H, C-H aryl-dioxy ring), 6.82 (d, *J* = 9.3 Hz, 2H, C-H aryl-dioxy ring), 6.29 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.03 (br s, 1H, NH), 3.77 – 4.00 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.18 (dt, *J* = 5.9/5.9 Hz, 2H, NHCH$_2$CH_2), 2.57 – 2.77 (m, 4H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.51 – 1.74 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.08 (C=O), 152.71, 152.51, 146.03, 133.10 (aryl 4° C), 130.24 (aryl 5-C), 120.51 (aryl 4-C), 116.87 (aryl 2-C), 115.90 (aryl 6-C), 115.32 (*C*H aryl-dioxy ring), 80.85 (^{c}Pe *C*H), 71.17 (ArO*C*H$_2$), 67.92 (*C*H(OH)), 67.70 (*C*H$_2$OAr), 66.72 (^{c}PeO*C*H$_2$), 52.05 (CH(OH)*C*H$_2$NH), 49.04 (NH*C*H$_2$CH$_2$), 38.90 (NHCH$_2$*C*H$_2$), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}ClN_3O_5$ $[MH]^+$ calcd 492.2260; found 492.2250.

HPLC R_t: 3.88 (System 1b), 13.87 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-chlorophenyl)urea (71m)



Yield: 18%.

Mp: 133 – 140 °C.

IR: 3321 (br, O-H, str), 2926, 2867 (alkyl C-H, str), 1633 (urea C=O, str), 1510, 1565 (aryl, str), 1110 (C-O-C, str), 827 (aryl C-H, bend, *para*-disubstituted ring), 764 (C-Cl, bend).

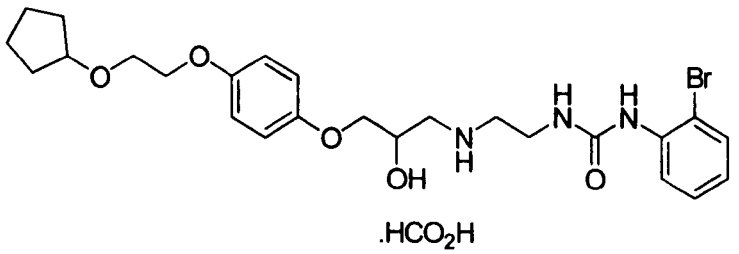
^{1}H NMR (DMSO-d_6): δ 8.76 (s, 1H, NH(C=O)N*H*Ar), 7.41, 7.25 (d, *J* = 8.9 Hz, 2 x 2H, C-H of chlorophenyl ring), 6.81 – 6.86 (m, 4H, C-H aryl-dioxy ring), 7.23 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.00 (br s, 1H, NH), 3.76 – 3.99 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.17 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH_2CH_2), 2.57 – 2.72 (m, 4H, CH(OH)CH_2NH, NHCH_2CH_2), 1.52 – 1.74 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.15 (C=O), 152.72, 152.50, 139.62, 124.32 (aryl 4° C), 128.45, 118.99 (CH chlorophenyl ring), 115.31 (*C*H aryl-dioxy ring), 80.84 (^{c}Pe *C*H), 71.19 (ArO*C*H_2), 68.01 (*C*H(OH)), 67.69 (*C*H_2OAr), 66.72 (^{c}PeO*C*H_2), 52.12 (CH(OH)*C*H_2NH), 49.14 (NH*C*H_2CH_2), 38.95 (NHCH_2*C*H_2), 31.79 (2-C and 5-C ^{c}Pe ring), 23.13 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}ClN_3O_5$ $[MH]^+$ calcd 492.2260; found 492.2284.

HPLC R_t: 3.80 (System 1b), 13.73 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-bromophenyl)urea hydroformate (71n)



.HCO_2H

Yield: 9%.

Mp: semi-solid.

IR: 3360 (br, O-H, str), 2950, 2870 (alkyl C-H, str), 1678 (urea C=O, str), 1508, 1581 (aryl, str), 1130 (C-O-C, str), 826 (aryl C-H, bend, *para*-disubstituted ring), 753 (aryl C-H, bend, *ortho* -disubstituted ring), 722 (C-Br, bend).

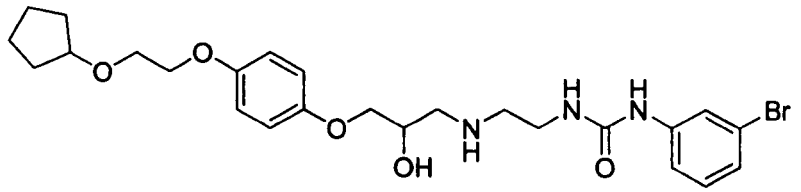
^{1}H NMR (DMSO-d_6): δ 8.04 (dd, *J* = 8.3/1.5 Hz, 1H, aryl 6-H), 7.98 (s, 1H, NH(C=O)N*H*Ar), 7.56 (dd, *J* = 8.0/1.4 Hz, 1H, aryl 3-H), 7.22 – 7.32 (m, 2H, aryl 5-H, N*H*(C=O)NHAr), 6.79 – 6.94 (m, 5H, aryl 4-H, aryl-dioxy ring), 3.76 – 4.15 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.18 – 3.56 (m, 10H, H_2O, NHCH$_2$CH_2), 2.55 – 3.13 (m, 4H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.30 (C=O), 152.70, 152.42, 137.62 112.54 (aryl 4° C), 132.62 (aryl 3-C), 127.95 (aryl 5-C), 123.49 (aryl 4-C), 121.89 (aryl 6-C), 115.39, 115.35 (*C*H aryl-dioxy ring), 80.85 (^{c}Pe *C*H), 70.63 (ArOCH$_2$), 67.71 (*C*H$_2$OAr), 66.71 (^{c}PeOCH$_2$), 65.09 (*C*H(OH)), 50.58 (CH(OH)CH$_2$NH), 48.21 (NHCH$_2$CH$_2$), 37.21 (NHCH$_2$CH$_2$), 31.79 (2-C and 5-C ^{c}Pe ring), 23.13 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}BrN_3O_5$ $[MH]^+$ calcd 536.1755; found 536.1800.

HPLC R_t: 4.09 (System 1b), 13.34 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-bromophenyl)urea (71o)



Yield: 12%.

Mp: 120 – 122 °C.

IR: 3323 (br, O-H, str), 2926, 2868 (alkyl C-H, str), 1630 (urea C=O, str), 1509, 1565 (aryl, str), 1111 (C-O-C, str), 825 (aryl C-H, bend, *para*-disubstituted ring), 683 (C-Br, bend).

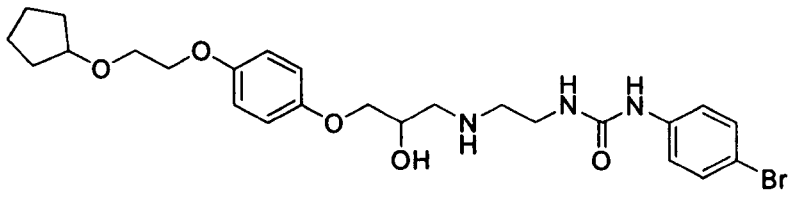
^{1}H NMR (DMSO-d_6): δ 8.83 (s, 1H, NH(C=O)N*H*Ar), 7.81 (dd, *J* = 1.9/1.9 Hz, 1H, aryl 2-H), 7.21 (ddd, *J* = 8.2/1.7/1.7 Hz, 1H, aryl 6-H), 7.16 (dd, *J* = 7.7/7.7 Hz, 1H, aryl 5-H), 7.05 (ddd, *J* = 7.7/1.2/1.2 Hz, 1H, aryl 4-H), 6.85 (d, *J* = 9.3 Hz, 2H, C-H aryl-dioxy ring), 6.82 (d, *J* = 9.3 Hz, 2H, C-H aryl-dioxy ring), 6.29 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 5.02 (br s, 1H, NH), 3.76 – 4.00 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.13 – 3.21 (m, 2H, NHCH$_2$CH_2), 2.71 (dd, *J* = 11.7/3.7 Hz, 1H, CH(OH)CH_2NH), 2.67 (dd, *J* = 6.1/6.1 Hz, 2H, NHCH_2CH$_2$), 2.60 (dd, *J* 11.9/6.7 Hz, 1H, CH(OH)CH_2NH), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.05 (C=O), 152.72, 152.49, 142.33, 121.69 (aryl 4° C), 130.57 (aryl 5-C), 123.41 (aryl 4-C), 121.69 (aryl 2-C), 116.29 (aryl 6-C), 115.33 (*C*H aryl-dioxy ring), 80.85 (^{c}Pe *C*H), 71.89 (ArO*C*H$_2$), 67.97 (*C*H(OH)), 67.70 (*C*H$_2$OAr), 66.73 (^{c}PeO*C*H$_2$), 52.08 (CH(OH)*C*H$_2$NH), 49.06 (NH*C*H$_2$CH$_2$), 38.70 (NHCH$_2$*C*H$_2$), 31.81 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}BrN_3O_5$ $[MH]^+$ calcd 536.1755; found 536.1735.

HPLC R_t: 4.47 (System 1b), 14.42 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-bromophenyl)urea (71p)



Yield: 23%.

Mp: 148 – 150 °C.

IR: 3326 (br, O-H, str), 2926, 2869 (alkyl C-H, str), 1633 (urea C=O, str), 1509, 1560 (aryl, str), 1110 (C-O-C, str), 822 (aryl C-H, bend, *para*-disubstituted ring).

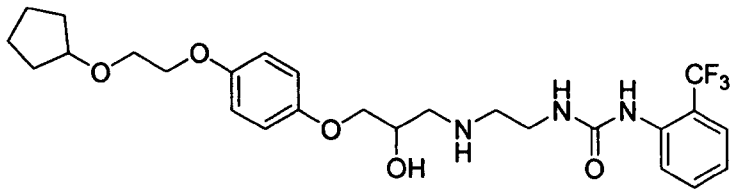
^{1}H NMR (DMSO-d_6): δ 8.77 (s, 1H, NH(C=O)N*H*Ar), 7.37 (s, 4H, C-H of bromophenyl ring), 6.84 (d, *J* = 9.3 Hz, 2H, C-H aryl-dioxy ring), 6.82 (d, *J* = 9.2 Hz, 2H, C-H aryl-dioxy ring), 6.25 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.03 (br s, 1H, NH), 3.78 – 3.97 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.17 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH$_2$CH_2), 2.71 (dd, *J* = 11.8/3.9 Hz, 1H, CH(OH)CH_2NH), 2.57 – 2.68 (m, 3H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.51 – 1.74 (m, 6H, ^{c}Pe CH_2), 1.40 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.12 (C=O), 152.69, 152.49, 140.04, 112.14 (aryl 4° C), 131.34, 119.43 (CH bromophenyl ring), 115.30 (CH aryl-dioxy ring), 80.84 (^{c}Pe CH), 71.15 (ArOCH$_2$), 67.92 (CH(OH)), 67.68 (CH$_2$OAr), 66.72 (^{c}PeOCH$_2$), 52.05 (CH(OH)CH$_2$NH), 49.08 (NHCH$_2$CH$_2$), 38.85 (NHCH$_2$CH$_2$), 31.79 (2-C and 5-C ^{c}Pe ring), 23.13 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}BrN_3O_5$ $[MH]^+$ calcd 536.1755; found 536.1773.

HPLC R_t: 4.47 (System 1b), 14.45 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-(trifluoromethyl)phenyl)urea (71q)



Yield: 12%.

Mp: 128 – 130 °C.

IR: 3330 (br, O-H, str), 2923, 2866 (alkyl C-H, str), 1645 (urea C=O, str), 1510, 1571 (aryl, str), 1121 (C-O-C, str), 1323 (C-F, str), 820 (aryl C-H, bend, *para*-disubstituted ring), 768 (aryl C-H, bend, *ortho*-disubstituted ring).

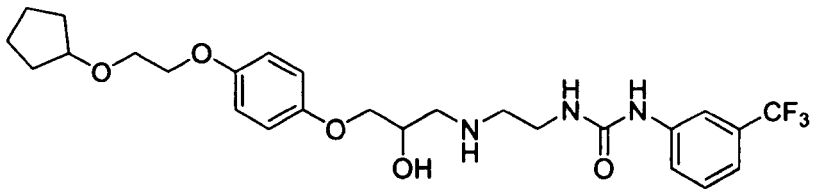
^{1}H NMR (DMSO-d_6): δ 7.95 (d, *J* = 8.4 Hz, 1H, aryl 6-H), 7.86 (s, 1H, NH(C=O)N*H*Ar), 7.60 (d, *J* = 7.9 Hz, 1H, aryl 3-H), 7.55 (dd, *J* = 7.9/7.9 Hz, 1H, aryl 5-H), 7.17 (dd, *J* = 7.6/7.6 Hz, 1H, aryl 4-H), 7.08 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 6.84 (s, 4H, aryl-dioxy ring), 5.04 (br s, 1H, NH), 3.77 – 4.02 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.9 Hz, 2H, ^{c}PeOCH_2), 3.21 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH_2CH_2), 2.62 – 2.77 (m, 4H, CH(OH)CH_2NH, NHCH_2CH_2), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.42 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.12 (C=O), 152.69, 152.52, 137.36 (aryl 4° C), 132.72 (aryl 5-C), 126.82 (J_{CF} = 274.6 Hz, CF_3), 125.79 (aryl 3-C), 124.77 (aryl 6-C), 122.59 (aryl 4-C), 115.32 (*C*H aryl-dioxy ring), 80.84 (^{c}Pe *C*H), 71.13 (ArO*C*H$_2$), 67.80 (*C*H(OH)), 67.69 (*C*H$_2$OAr), 66.71 (^{c}PeO*C*H$_2$), 52.03 (CH(OH)*C*H$_2$NH), 49.03 (NH*C*H$_2$CH$_2$), 39.02 (NHCH$_2$*C*H$_2$), 31.78 (2-C and 5-C ^{c}Pe ring), 23.13 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{35}F_3N_3O_5$ $[MH]^+$ calcd 526.2523; found 526.2538.

HPLC R_t: 4.27 (System 1b), 13.55 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-(trifluoromethyl)phenyl)urea (71r)



Yield: 7%.

Mp: 96 – 98 °C.

IR: 3331 (br, O-H, str), 2927, 2869 (alkyl C-H, str), 1633 (urea C=O, str), 1509, 1566 (aryl, str), 1125 (C-O-C, str), 1344 (C-F, str), 821 (aryl C-H, bend, *para*-disubstituted ring), 764 (aryl C-H, bend, *meta*-disubstituted ring), 702 (C-F, bend).

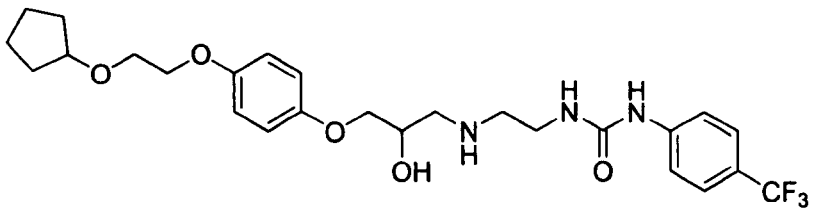
^{1}H NMR (DMSO-d_6): δ 9.03 (s, 1H, NH(C=O)N*H*Ar), 7.97 (s, 1H, aryl 2-H), 7.50 (d, *J* = 8.5 Hz, 1H, aryl 6-H), 7.44 (dd, *J* = 7.7/7.7 Hz, 1H, aryl 5-H), 7.21 (d, *J* = 7.5, 1H, aryl 4-H), 6.85 (d, *J* = 9.2 Hz, 2H, C-H aryl-dioxy ring), 6.82 (d, *J* = 9.3 Hz, 2H, C-H aryl-dioxy ring), 6.36 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 5.13 (br s, 1H, NH), 3.81 – 3.97 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.9 Hz, 2H, ^{c}PeOCH_2), 3.22 (dt, *J* = 5.7/5.7 Hz, 2H, NHCH$_2$CH_2), 2.63 – 2.83 (m, 4H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.51 – 1.74 (m, 6H, ^{c}Pe CH_2), 1.41 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.22 (C=O), 152.65, 152.55, 141.43, (aryl 4° C), 129.74 (aryl 5-C), 124.31 (J_{CF} = 270.6 Hz, CF_3), 121.06 (aryl 6-C), 117.12 (aryl 4-C), 115.32 (*C*H aryl-dioxy ring), 113.44 (aryl 2-C), 80.85 (^{c}Pe *C*H), 71.05 (ArOCH$_2$), 67.70 (*C*H$_2$OAr), 67.51 (*C*H(OH)), 66.72 (^{c}PeOCH$_2$), 51.74 (CH(OH)CH$_2$NH), 48.83 (NHCH$_2$CH$_2$), 38.60 (NHCH$_2$*C*H$_2$), 31.80 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{35}F_3N_3O_5$ $[MH]^+$ calcd 526.2523; found 526.2474.

HPLC R_t: 4.64 (System 1b), 14.77 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-(trifluoromethyl)phenyl)urea (71s)



Yield: 26%.

Mp: 134 – 136 °C.

IR: 3293 (br, O-H, str), 2932, 2871 (alkyl C-H, str), 1694 (urea C=O, str), 1509, 1568 (aryl, str), 1122 (C-O-C, str), 1328 (C-F, str), 821, 841 (aryl C-H, bend, *para*-disubstituted ring), 703 (C-F, bend).

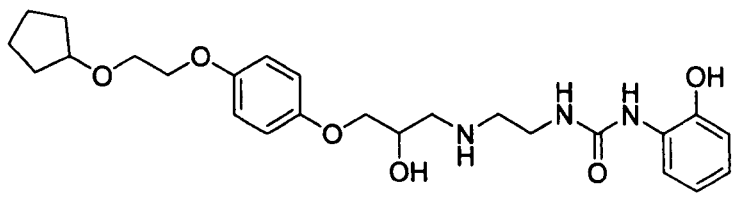
^{1}H NMR (DMSO-d_6): δ 9.08 (s, 1H, NH(C=O)N*H*Ar), 7.59 (d, *J* = 9.0 Hz, 2H, 2-H and 6-H of trifluoromethylphenyl ring), 7.55 (d, *J* = 9.1 Hz, 2H, 3-H and 5-H of trifluoromethylphenyl ring), 6.85 (d, *J* = 9.2 Hz, 2H, C-H aryl-dioxy ring), 6.82 (d, *J* = 9.3 Hz, 2H, C-H aryl-dioxy ring), 6.38 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 5.03 (br s, 1H, NH), 3.77 – 4.01 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.9 Hz, 2H, ^{c}PeOCH_2), 3.21 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH$_2$CH_2), 2.74 (dd, *J* = 11.8/3.8 Hz, 1H, CH(OH)CH_2NH), 2.69 (t, *J* = 5.9 Hz, 2H, NHCH_2CH$_2$), 2.63 (dd, *J* = 12.0/6.6 Hz, 1H, CH(OH)CH_2NH), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.38 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 154.97 (C=O), 152.69, 152.51, 144.32, (aryl 4° C), 125.93 (J_{CF} = 3.4 Hz, 3-C and 5-C of trifluoromethylphenyl ring), 124.68 (J_{CF} = 269.6 Hz, CF_3), 120.82 (J_{CF} = 34.7 Hz, 4-C of trifluoromethylphenyl ring), 117.11 (2-C and 6-C of trifluoromethylphenyl ring), 115.31 (*C*H aryl-dioxy ring), 80.84 (^{c}Pe *C*H), 71.13 (ArO*C*H$_2$), 67.83 (*C*H(OH)), 67.68 (*C*H$_2$OAr), 66.71 (^{c}PeO*C*H$_2$), 51.96 (CH(OH)*C*H$_2$NH), 48.94 (NH*C*H$_2$CH$_2$), 38.64 (NHCH$_2$*C*H$_2$), 31.78 (2-C and 5-C ^{c}Pe ring), 23.12 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{35}F_3N_3O_5$ $[MH]^+$ calcd 526.2523; found 526.2480.

HPLC R_t: 4.67 (System 1b), 14.90 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea (71t)



To 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (51 mg, 0.18 mmol) was added TEA (75 µl, 0.53 mmol, 3 eq), 1-(2-aminoethyl)-3-(2-hydroxyphenyl)urea hydrochloride **(70a)** (83 mg, 0.36 mmol, 2 eq) and propan-2-ol (5 mL). The mixture was stirred under reflux for 16 hours. After removal of all volatiles *in vacuo*, the crude residue was purified by PLC (eluent 37% aq NH_3/MeOH/DCM 2:5:93) to give 18 mg of beige semi-solid.

Yield: 21 %.

IR: 3319 (br, O-H, str), 2920, 2900 (alkyl, C-H, str), 1653 (urea C=O, str), 1558, 1506 (aryl, str), 1109 (C-O-C, str), 823 (aryl, C-H, bend, *para*-disubstituted ring), 750 (aryl, C-H, bend, *ortho*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 8.01 (s, 1H, NH(C=O)N*H*Ar), 7.83 (dd, *J* = 7.8/1.6 Hz, 1H, aryl 6-H), 6.91 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 6.85 (d, *J* = 9.7 Hz, 2H, aryl-dioxy ring), 6.82 (d, *J* = 9.30 Hz, 2H, aryl-dioxy ring), 6.78 (dd, *J* = 7.8/1.7 Hz, 1H, aryl 3-H), 6.73, (ddd, *J* = 7.7/7.3/1.2 Hz, 1H, aryl 4-H), 6.68, (ddd, *J* = 7.8/7.3/1.7 Hz, 1H, aryl 5-H), 6.09 (br s, 1H, phenol), 3.80 – 4.00 (m, 6H C*H*$_2$OAr, ^{c}Pe C*H*, C*H*(OH), ArOC*H*$_2$), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOC*H*$_2$), 3.19 (dt, *J* = 5.8/5.8 Hz, 2H, NHCH$_2$C*H*$_2$), 2.75 (dd, *J* = 12.1/3.9 Hz, 1H, CH(OH)C*H*$_2$NH), 2.61 – 2.69 (m, 3H, NHC*H*$_2$CH$_2$, CH(OH)C*H*$_2$NH), 1.40-1.72 (m, 8H, ^{c}Pe C*H*$_2$).

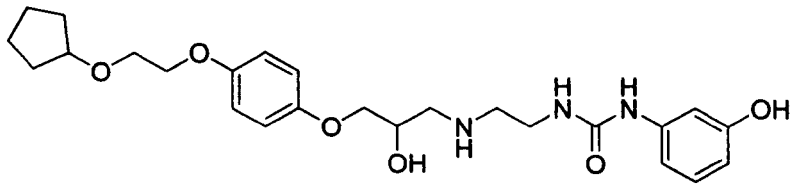
^{13}C NMR (DMSO-d_6): δ 155.81 (C=O), 152.70, 152.52, 145.63, 128.43 (aryl 4° C), 121.34 (aryl 4-C), 119.04 (aryl 5-C), 118.76 (aryl 6-C), 115.34 (CH aryl-dioxy ring), 114.82 (aryl 3-C), 80.84 (^{c}Pe CH), 71.14 (ArOCH$_2$), 67.75 (CH(OH)), 67.72 (CH$_2$OAr), 66.72 (^{c}PeOCH$_2$), 51.95

(CH(OH)CH_2NH), 49.18 (NHCH_2CH_2), 40.11(NHCH_2CH_2), 31.79 (2-C and 5-C ^{c}Pe ring), 23.12 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{36}N_3O_6$ $[MH]^+$ calcd 474.2599; found 474.2600.

HPLC R_t: 4.10 (System 1b), 11.84 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-hydroxyphenyl)urea (71u)



Epoxide opening of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy) methyl)oxirane **(62)** with 1-(2-aminoethyl)-3-(3-hydroxyphenyl)urea hydrochloride **(70b)**, was carried out as described in the procedure for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification was achieved via PLC (eluent 37% aq NH_3/MeOH/DCM 2:10:88) to give 21 mg of beige semi-solid.

Yield: 25 %.

IR: 3350 (br, O-H, str), 2932, 2868 (alkyl, C-H, str), 1675 (urea C=O, str), 1559, 1507 (aryl, str), 1108 (C-O-C, str), 826 (aryl, C-H, bend, *para*-disubstituted ring), 765 (aryl, C-H, bend, *meta*-disubstituted ring).

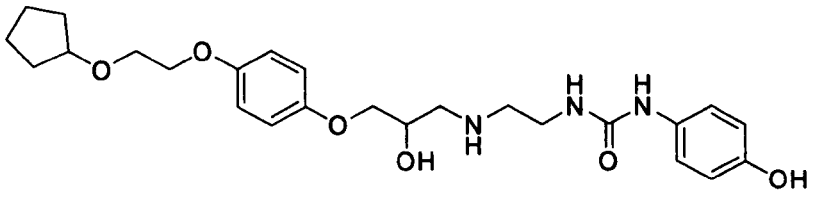
^{1}H NMR (DMSO-d_6): δ 9.18 (br s, 1H, phenol), 8.47 (s, 1H, NH(C=O)N*H*Ar), 6.98 (s, 1H, aryl 2-H), 6.96 (dd, *J* = 8.1 Hz, 1H, aryl 5-H), 6.77 – 6.90 (m, 4H, aryl-dioxy ring), 6.71 (d, *J* = 7.8 Hz, 1H, aryl 6-H) , 6.28 (dd, *J* = 7.8/1.8 Hz, 1H, aryl 4-H), 6.16 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 3.75 - 4.02 (m, 6H, CH_2OAr, ^{c}PeC*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.15 (dt, *J* = 5.7/5.7 Hz, 2H, NHCH_2CH_2), 2.70 (dd, *J* = 12.0/3.7 Hz, 1H, CH(OH)CH_2NH), 2.54 – 2.67 (m, 3H, CH(OH)CH_2NH, NHCH_2CH_2),1.38 – 1.74 (m, 8H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.24 (C=O), 157.65, 152.73, 152.51, 141.66, (aryl 4° C), 129.20 (aryl 5-C), 115.34 (CH aryl-dioxy ring), 108.38 (aryl 6-C), 108.10 (aryl 4-C), 104.68 (aryl 2-C), 80.84 (^{c}Pe CH), 71.22 ($ArOCH_2$), 68.01 (CH(OH)), 67.72 (CH_2OAr), 66.72 ($^{c}PeOCH_2$), 52.14 ($CH(OH)CH_2NH$), 49.23 ($NHCH_2CH_2$), 38.99 ($NHCH_2CH_2$), 31.79 (2-C and 5-C ^{c}Pe ring), 23.12 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{36}N_3O_6$ $[MH]^+$ calcd 474.2599; found 474.2642.

HPLC R_t: 3.90 (System 1b), 11.02 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea (57)



Refer to general procedure for synthesis of aromatically substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(aryl)ureas.

Yield: 29%.

Mp: 135 - 138°C.

IR: 3311 (br, O-H, str), 2929, 2869 (alkyl C-H, str), 1636 (urea C=O, str), 1509, 1568 (aryl, str), 1111 (C-O-C, str), 820 (aryl C-H, bend, *para*-disubstituted ring).

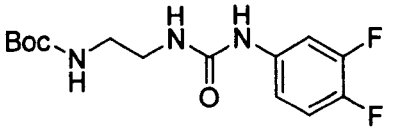
^{1}H NMR (DMSO-d_6): δ 8.91 (br s, 1H, phenol), 8.20 (s, 1H, NH(C=O)N*H*Ar), 7.13 (d, *J* = 8.8 Hz, 2H, aryl C-H *ortho* to urea), 6.84 (s, 4H, aryl-dioxy ring), 6.62 (d, *J* = 8.8 Hz, 2H, aryl C-H *ortho* to phenol), 6.02 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 4.96 (br s, 1H, NH), 3.79 – 3.97 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), $ArOCH_2$), 3.61 (t, *J* = 4.8 Hz, 2H, $^{c}PeOCH_2$), 3.12 – 3.16 (m, 2H, $NHCH_2CH_2$), 2.56 – 2.71 (m, 4H, $CH(OH)CH_2NH$, $NHCH_2CH_2$), 1.54 – 1.69 (m, 6H, ^{c}Pe CH_2), 1.42 – 1.51 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 152.74, 152.49, 151.85, 132.14 (aryl 4° C), 155.65 (C=O), 119.72 (aryl C-H *ortho* to urea), 115.33 (*C*H aryl-dioxy ring), 115.04 (aryl C-H *ortho* to phenol), 80.84 (^{c}Pe *C*H), 71.23 (ArO*C*H$_2$), 68.08 (*C*H(OH)), 67.71 (*C*H$_2$OAr), 66.72 (^{c}PeO*C*H$_2$), 52.20 (CH(OH)*C*H$_2$NH), 49.38 (NH*C*H$_2$CH$_2$), 39.15 (NHCH$_2$*C*H$_2$), 31.78 (2-C and 5-C ^{c}Pe ring), 23.12 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{36}N_3O_6$ $[MH]^+$ calcd 474.2599; found 474.2578.

HPLC R_t: 3.17 (System 1b), 10.30 (System 3).

***tert*-Butyl 2-(3-(3,4-difluorophenyl)ureido)ethylcarbamate (72)**



Refer to general procedure for synthesis of phenyl substituted *tert*-butyl 2-(3-phenylureido)ethylcarbamates. Product isolated as brown oil.

Yield: 75%.

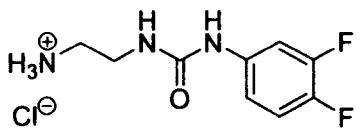
IR: 3336 (carbamate N-H, str), 2980, 2942 (alkyl C-H, str), 1683 (carbamate C=O, str), 1641 (urea C=O, str), 1514 (aryl, str), 1287 (C-F, str).

^{1}H NMR (DMSO-d_6): δ 8.81 (s, 1H, NH(C=O)N*H*Ar), 7.62 (ddd, *J* = 13.7/7.5/2.6 Hz, 1H, aryl 2-H), 7.26 (ddd, *J* = 10.6/9.5/9.2 Hz, 1H, aryl 5-H), 6.98 - 7.05 (m, 1H, aryl 6-H), 6.84 (t, *J* = 4.6 Hz, 1H, N*H*(C=O)NHAr), 6.24 (t, *J* = 5.8 Hz, 1H, O(C=O)N*H*), 3.11 (dt, *J* = 6.2/5.8 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.99 (dt, *J* = 6.2/5.8 Hz, 2H, C*H*$_2$NH(C=O)O), 1.37 (s, 9H, C(CH$_3$)$_3$).

^{13}C NMR (DMSO-d_6): δ 155.71, 155.09 (C=O), 117.17 (J_{CF} = 19.1 Hz, aryl 5-C), 113.53 (J_{CF} = 8.1 Hz, aryl 6-C), 106.39 (J_{CF} = 22.1 Hz, aryl 2-C), 77.63 (Boc 4° C), 45.99 (*C*H$_2$NH(C=O)NH), 40.28 (BocNH*C*H$_2$), 28.22 (Boc CH$_3$).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{20}F_2N_3O_3$ $[MH]^+$ calcd 316.1467; found 316.1460.

1-(2-Aminoethyl)-3-(3,4-difluorophenyl)urea hydrochloride (73)



tert-Butyl 2-(3-(3,4-difluorophenyl)ureido) ethylcarbamate **(72)** (1.966 g, 6.23 mmol) was dissolved in MeOH (10 mL) with vigorous stirring. To this was added 4 M HCl/dioxane (40 mL) and the solution stirred for 4 hours. After removal of all solvents *in vacuo*, the crude residue was triturated with toluene and dried to give a beige solid in quantitative yield.

Yield: 100%.

Mp: 191 – 193 °C.

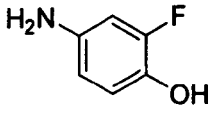
IR: 3311 (urea N-H, str), 3011 (NH_3^+, str), 1676 (urea C=O, str), 1573 (aryl, str), 1516 (NH_3^+, bend), 1242 (C-F, str).

^{1}H NMR (DMSO-d_6): δ 9.51 (s, 1H, NH(C=O)N*H*Ar), 8.05 (br s, 3H, NH_3^+), 7.64 (ddd, *J* = 14.0/7.8/2.6 Hz, 1H, aryl 2-H), 7.27 (ddd, *J* = 10.4/9.2/9.2 Hz, 1H, aryl 5-H), 7.02 – 7.10 (m, 1H, aryl 6-H), 6.78 (br s, 1H, N*H*(C=O)NHAr), 3.33 (t, *J* = 6.2/Hz, 2H, CH_2NH(C=O)NH), 2.86 (tq, *J* = 5.8/5.8 Hz, 2H, C$H_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 155.61 (C=O), 137.73 (J_{CF} = 11.3 Hz, aryl 1-C), 117.20 (J_{CF} = 17.8 Hz, aryl 5-C), 113.59 (J_{CF} = 8.8 Hz, aryl 6-C), 106.41 (J_{CF} = 22.1 Hz, aryl 2-C), 46.00 (CH_2NH(C=O)NH), 37.09 ($CH_2$$NH_3^+$).

m/z: HRMS (TOF ES^+) $C_9H_{12}F_2N_3O$ $[MH]^+$ calcd 216.0943; found 216.0937.

4-Amino-2-fluorophenol (75)



2-Fluoro-4-nitrophenol **(74)** (1.19g, 7.57mmol) was dissolved in methanol (40mL) and hydrogenated over 10% Pd/C (125 mg), at room temperature and atmospheric pressure. The suspension was filtered over celite and washed with excess MeOH. Removal of excess solvent *in vacuo* afforded 867 mg of light brown solid.

Yield: 90 %.

Mp: 169 – 172 °C.

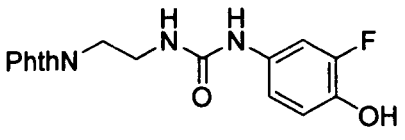
IR: 3354 (OH, str), 3292 (NH, str), 1608, 1525 (aryl, str), 1401 (O-H, bend), 1206 (C-F, str), 1110 (C-OH, str), 728 (C-F, str).

^{1}H NMR (DMSO-d_6): δ 8.57 (s, 1H, OH), 6.62 (dd, *J* = 10.1/8.5 Hz, 1H, 6-H), 6.34 (dd, *J* = 10.7/2.8 Hz, 1H, 3-H), 6.20 (ddd, *J* = 8.3/2.8/1.1 Hz, 1H, 5-H), 4.68 (s, 2H, NH_2).

^{13}C NMR (DMSO-d_6): δ 151.53 (J_{CF} = 238.3 Hz, 2-C), 141.94 (J_{CF} = 8.7 Hz, 4-C), 134.68 (J_{CF} = 13.1 Hz, 1-C), 118.43 (J_{CF} = 4.8Hz, 6-C), 109.97 (J_{CF} = 2.4 Hz, 5-C), 102.19 (J_{CF} = 21.4 Hz, 3-C).

m/z: HRMS (TOF ES$^+$) C_6H_7FNO $[MH]^+$ calcd 128.0506; found 128.0504.

1-(3-Fluoro-4-hydroxyphenyl)-3-(2-phthalimidoethyl)urea (76)



Isocyanate solution was prepared as described for 1-(2-hydroxyphenyl)-3-(2-phthalimidoethyl) urea **(69a)** via curtius reaction starting with 3-phthalimidopropanoic acid **(28)** (1.000 g, 4.56 mmol). To this was added 4-amino-2-fluorophenol **(75)** (830 mg, 6.53 mmol, 1.4 eq) and stirred with heating under reflux overnight. After cooling, the formed precipitate was collected by filtration (suction) and washed with EtOAc, which on drying gave 866 mg of solid.

Yield: 55 %.

Mp: 226 – 227 °C.

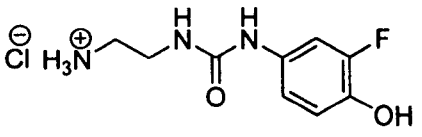
IR: 3373 (OH, str), 3227 (urea NH, str), 1772, 1709 (phth C=O, str), 1653 (urea C=O, str), 1608 (aryl, str), 1404 (O-H, bend), 1227, 724 (C-F, str).

^{1}H NMR (DMSO-d_6): δ 9.26 (br s, 1H, OH), 8.21 (s, 1H, NH(C=O)N*H*Ar), 7.77 – 7.92 (m, 4H, phth C-H), 7.16 – 7.25 (m, 1H, aryl C-H), 6.69 – 6.79 (m, 2H, aryl C-H), 6.20 (t, *J* = 5.97 Hz, 1H, N*H*(C=O)NHAr), 3.67 (t, *J* = 5.3 Hz, 2H, NCH_2), 3.30 – 3.35 (m, 2H, CH_2NH).

^{13}C NMR (DMSO-d_6): δ 168.03 (phth C=O), 155.38 (urea C=O), 150.45 (J_{CF} = 238.7 Hz, 3-C phenylurea), 134.27, 122.96 (phth C-H), 132.56 (J_{CF} = 16.3 Hz, 4-C phenylurea), 131.82 (phth 4° C), 128.85 (1-C phenylurea), 117.44, 114.22 (J_{CF} = 3.9 Hz, 5-C and 6-C phenylurea), 106.85 (J_{CF} = 21.8 Hz, 2-C phenylurea), 38.19 (NCH_2), 37.58 (CH_2NH).

m/z: HRMS (TOF ES$^+$) $C_{17}H_{15}FN_3O_4$ $[MH]^+$ calcd 344.1041; found 344.1014.

1-(2-Aminoethyl)-3-(3-fluoro-4-hydroxyphenyl)urea hydrochloride (77)



Phthalimide deprotection of 1-(3-fluoro-4-hydroxyphenyl)-3-(2-phthalimidoethyl) urea **(76)** (800 mg, 2.33 mmol) was carried out as described in the synthesis of 1-(2-aminoethyl)-3-(2-hydroxyphenyl)urea hydrochloride **(70a)** to give 487 mg of white solid requiring no further purification.

Yield: 84 %.

Mp: 190 - 195 °C.

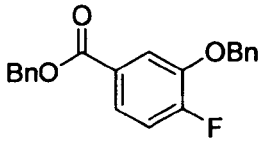
IR: 3372 (OH, str), 3128 (br, urea NH, str), 3042 (br, $NH3^+$, str), 1674 (urea C=O, str), 1612 (aryl, str), 1284 (O-H, bend), 1213 (C-F, str).

^{1}H NMR (DMSO-d_6): δ 9.35 (br s, 1H, OH), 8.93 (s, 1H, NH(C=O)N*H*Ar), 7.98 (br s, 3H, NH_3^+), 7.33 – 7.42 (m, 1H, aryl C-H), 6.78 – 6.88 (m, 2H, aryl C-H), 6.55 (t, *J* = 5.1 Hz, 1H, N*H*(C=O)NHAr), 3.30 (dt, *J* = 6.1/5.6 Hz, 2H, C*H*$_2$NH), 2.79 – 2.91 (m, 2H, $NH_3^+CH_2$).

^{13}C NMR (DMSO-d_6): δ155.86 (C=O), 150.49 (J_{CF} = 232.0 Hz, aryl 3-C), 139.01 (J_{CF} = 11.0 Hz, aryl 4-C), 132.57 (J_{CF} = 8.2 Hz, aryl 1-C), 117.61, 114.08 (J_{CF} = 3.3 Hz, aryl 5-C and 6-C), 106.70 (J_{CF} = 22.5 Hz, aryl 2-C), 39.10 (CH_2NH), 37.17 ($NH_3^+CH_2$).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}FN_3O_2$ $[MH]^+$ calcd 214.0986; found 214.1002.

Benzyl 3-(benzyloxy)-4-fluorobenzoate (79a)



NaH 60% suspension in mineral oil (546 mg, equivalent to 338 mg of NaH, 14 .09 mmol, 2.2 eq) was weighed into a flame-dried flask and suspended in dry DMF (30 mL), under a nitrogen atmosphere over an ice bath. To this was added 4-fluoro-3-hydroxybenzoic acid **(78a)** (1.000 g, 6.41 mmol) and the mixture was stirred at room temperature for 30 minutes. Benzyl bromide (2.410 g, 1.676 mL, 14.09 mmol, 2.2 eq) was added to the flask, and the mixture stirred for a further 48 hours at room temperature. After removal of solvent *in vacuo*, the crude slurry was dissolved in water (30 mL) before extraction with Et_2O (3 x 20 mL). The combined organic layers were washed with aqueous 2 M NaOH (1 x 30 mL). Removal of solvent afforded the desired compound as colourless translucent oil in quantitative yield with no further purification required.

Yield: 100%.

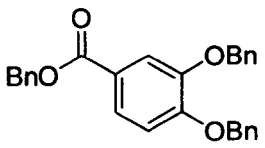
IR: 3066 (aryl C-H, str), 2933 (alkyl C-H, str), 1718 (ester C=O, str), 1287 (C-F, str), 1202 (ester C-O, str), 1099 (C-O-C, str), 761 (C-F, bend), 697 (aryl C-H bend, phenyl ring).

^{1}H NMR: δ 7.82 (dd, *J* = 7.9/1.8 Hz, 1H, aryl 2-H), 7.73 (ddd, *J* = 8.4/4.4/2.1 Hz, 1H, aryl 6-H), 7.34 – 7.53 (m, 10H, aromatic benzyl C-H), 7.15 (dd, *J* = 10.5/8.2 Hz, 1H, aryl 5-H), 5.39 (s, 2H, benzyl ester CH_2), 5.19 (s, 2H, benzyl ether CH_2).

^{13}C NMR: δ 165.44 (C=O), 155.98 (J_{CF} = 254.8 Hz, aryl 4-C), 146.69 (J_{CF} = 10.2 Hz, aryl 3-C), 136.00, 135.93 (benzyl 4° C), 128.66, 128.65, 128.34, 128.28, 128.20, 127.59 (aromatic benzyl C-H), 126.54 (J_{CF} =3.4 Hz, aryl 1-C), 123.65 (J_{CF} =8.5 Hz, aryl 6-C), 116.64 (J_{CF} =2.8 Hz, aryl 2-C), 116.17(J_{CF} =19.2 Hz, aryl 5-C), 71.25 (benzyl ether CH_2), 66.90 (benzyl ester CH_2).

m/z: HRMS (TOF ES$^+$) $C_{21}H_{18}FO_3$ $[MH]^+$ calcd 337.1234; found 337.1208.

Benzyl 3,4-bis(benzyloxy)benzoate (79b)



Refer to the procedure for benzyl 3-(benzyloxy)-4-fluorobenzoate **(79a)**. 3,4-dihydroxybenzoic acid **(78b)** was benzyl-protected in a similar manner to 4-fluoro-3-hydroxybenzoic acid **(78a)**, using 3.3 eq of benzyl bromide to afford a waxy yellow solid in quantitative yield requiring no further purification.

Yield: 100%.

Mp: 69.5 – 71.5 °C (lit. 66 – 67 °C)[178].

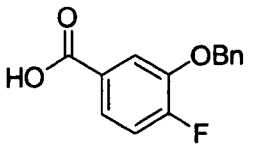
IR: 3030 (aryl C-H, str), 2923 (alkyl C-H, str), 1693 (ester C=O, str), 1599, 1588, 1516 (aryl, str), 1275 (ester C-O, str), 741, 696 (aryl C-H bend, phenyl ring).

^{1}H NMR: δ 7.65 – 7.72 (m, 2H, aryl 2-H and 6-H), 7.28 – 7.52 (m, 15H, aromatic benzyl C-H), 6.93 (d, *J* = 8.7 Hz, 1H, aryl 5-H), 5.33 (s, 2H, benzyl ester CH_2), 5.23, 5.20 (s, 2 x 2H, 2 x benzyl ether CH_2).

^{13}C NMR: δ 166.17 (C=O), 153.10 (aryl 4-C), 148.43 (aryl 3-C), 136.94, 136.64, 136.35 (benzyl 4° C), 128.70, 128.67, 128.63, 128.26, 128.18, 128.10, 128.03, 12.51, 127.23 (benzyl C-H), 124.26 (aryl 1-C), 123.11 (aryl 6-C), 115.75 (aryl 2-C), 113.35 (aryl 5-C), 71.34, 70.93 (benzyl ether CH_2), 66.60 (benzyl ester CH_2).

m/z: HRMS (TOF ES$^+$) $C_{28}H_{25}O_4$ $[MH]^+$ calcd 425.1747; found 425.1706.

3-(Benzyloxy)-4-fluorobenzoic acid (80a)



Benzyl 3-(benzyloxy)-4-fluorobenzoate **(79a)** (2.067g, 6.15 mmol and lithium hydroxide (736 mg, 30.74 mmol, 5 eq) were dissolved in water/THF/MeOH 1:1:1 (45 mL) in a flask flushed with nitrogen. After overnight stirring, THF and MeOH were removed *in vacuo* and the remaining aqueous slurry diluted to 30 mL with more water. After washing with Et_2O (2 x 30 mL), the aqueous layer was acidified with conc HCl to approximately pH 4. The acidified aqueous layer was

extracted with DCM (2 x 30 mL). Removal of organic solvent *in vacuo*, gave 1.299 g of the desired free acid as a white solid requiring no further purification.

Yield: 86%.

Mp: 207 – 209 °C.

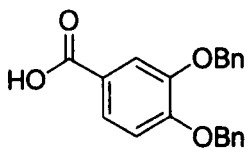
IR: 3036 (aryl C-H, str), 2954, 2881, 2843, 2624 (CO_2H O-H, str), 1678 (CO_2H C=O, str), 1610, 1520 (aryl, str), 1302 (C-F, str), 740 (C-F, bend), 765, 695 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 13.06 (br s, 1H, CO_2H), 7.74 (dd, J = 8.3/2.7 Hz, 1H, aryl 2-H), 7.57 (ddd, J = 8.3/4.5/1.9 Hz, 1H, aryl 6-H), 7.47 (d, J = 8.3 Hz, 2H, benzyl 2-H and 6-H), 7.40 (dd, J = 7.5/7.5 Hz, 2H, benzyl 3-H and 5-H), 7.28 – 7.37 (m, 2H, aryl 5-H, benzyl 4-H), 5.24 (s, 2H, benzyl CH_2).

^{13}C NMR (DMSO-d_6): δ 166.37 (C=O), 154.65 (J_{CF} = 245.7 Hz, aryl 4-C), 146.06 (J_{CF} = 11.3 Hz, aryl 3-C), 139.16 (benzyl 4° C), 128.46, 128.07, 127.74 (benzyl C-H), 127.60 (J_{CF} = 3.0 Hz, aryl 1-C), 123.01 (J_{CF}= 7.9 Hz, aryl 6-C), 116.09 (J_{CF} = 19.7 Hz, aryl 5-C), 115.9 (J_{CF} = 3.0 Hz, aryl 2-C), 70.24 (benzyl CH_2).

m/z: HRMS (TOF ES$^-$) $C_{14}H_{10}FO_3$ $[M-H]^-$ calcd 245.0619; found 245.0617.

3,4-*bis*(Benzyloxy)benzoic acid (80b)



Benzyl 3,4-bis(benzyloxy)benzoate **(79b)** was hydrolysed as described in the procedure for 3-(benzyloxy)-4-fluorobenzoic acid **(80a)**. The desired free acid was collected as white solid requiring no further purification.

Yield: 85%.

Mp: 189 – 191 °C (lit. 187 – 188 °C)[178].

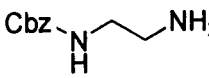
IR: 3033 (aryl C-H, str), 2906, 2864, 2547 (CO_2H O-H, str), 1679 (CO_2H C=O, str), 1601, 1521 (aryl, str), 761, 694 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 12.56 (br s, 1H, CO_2H), 7.52 – 7.59 (m, 2H, aryl 2-H and 6-H), 7.41 – 7.49 (m, 4H, 2 x benzyl 2-H and 6-H), 7.26 – 7.41 (m, 6H, 2 x benzyl 3-H, 4-H and 5-H), 7.11 (d, J = 8.2 Hz, 1H, aryl 5-H), 5.20, 5.15 (s, 2 x 2H, 2 x benzyl CH_2).

^{13}C NMR (DMSO-d_6): δ 166.88 (C=O), 152.01 (aryl 4-C), 147.57 (aryl 3-C), 136.86, 136.57 (benzyl 4° C), 128.25, 128.20, 127.71, 127.61, 127.32, 127.27 (benzyl C-H), 123.44, 123.40 (aryl 1-C, aryl 6-C), 114.70 (aryl 2-C), 112.96 (aryl 5-C), 70.09, 69.88 (benzyl CH_2).

m/z: HRMS (TOF ES^-) $C_{21}H_{17}O_4$ $[M-H]^-$ calcd 333.1132; found 333.1109.

Benzyl 2-aminoethylcarbamate (82)



Ethylenediamine **(54)** (16.71 mL, 250 mmol, 5 eq) was dissolved in dry DCM (50 mL) and cooled to 0 °C with vigorous stirring. A solution of benzylchloroformate (7.14 mL, 50 mmol) in dry DCM (250 mL) was dripped slowly into the solution of ethylenediamine over 10 minutes. The mixture was allowed to stir overnight at room temperature before washing with aqueous 2 M NaOH (1 x 100 mL) and water (2 x 100 mL). The organic layer was then extracted with aqueous 1 M HCl (3 x 100 mL). The combined acidic aqueous layers were basified with aqueous 2 M NaOH before finally extracting with DCM (3 x 80 mL). Concentration of the organic fractions gave poor yield, and the desired product was found to be trapped in the basified aqueous layer. This aqueous layer was concentrated *in vacuo* before re-extraction with DCM (3 x 50 mL). After concentration of the combined organic layers, eth crude product was purified by FCC (eluent 37% aq NH_3/MeOH/DCM 1:5:94) to give 3.51 g of colourless semi-solid.

Yield: 36%.

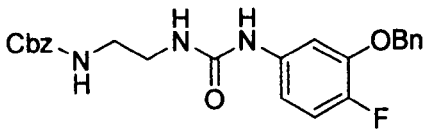
IR: 3321 (NH_2, str), 3032 (aryl C-H, str), 2948 (alkyl C-H, str), 1690 (carbamate C=O, str), 1541 (aryl, str), 746, 697 (aryl C-H bend, phenyl ring).

^{1}H NMR: δ 7.19 – 7.42 (m, 5H, aromatic benzyl C-H), 5.53 (br s, 1H, carbamate NH), 5.06 (s, 2H, benzyl CH_2), 3.19 (dt, *J* = 5.9/5.9 Hz, 2H, NHCH_2), 2.76 (t, *J* = 5.9 Hz, 2H, CH_2NH$_2$), 1.32 (br s, 1H, NH_2).

^{13}C NMR: δ 156.79 (C=O), 136.60 (benzyl 4° C), 128.51, 128.08 (aromatic benzyl C-H, 66.62 (benzyl CH_2), 43.74 ($NHCH_2$), 41.67 (CH_2NH_2).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{15}N_2O_2$ $[MH]^+$ calcd 195.1128; found 195.1128.

Benzyl 2-(3-(3-(benzyloxy)-4-fluorophenyl)ureido)ethylcarbamate (83a)



A solution of 3-(benzyloxy)-4-fluorobenzoic acid **(80a)** (1.000 g, 4.06 mmol), DPPA (918 µL, 4.26 mmol, 1.05 eq) and TEA (1.132 mL, 8.12 mmol, 2 eq) in dry toluene (20 mL) was stirred at room temperature, under a nitrogen atmosphere. After disappearance of starting materials by TLC (approximately 1 hour), the mixture was refluxed to promote conversion to the isocyanate. After evolution of nitrogen gas had ceased, benzyl 2-aminoethylcarbamate **(82)** (868 mg, 4.47 mmol, 1.1 eq) was added and the reaction cooled to room temperature. The formed precipitate was collected by filtration (suction) and washed with MeOH, before drying to afford 1.090 g of white solid.

Yield: 61%.

Mp: 209 – 211 °C.

IR: 3303 (N-H, str), 2949 (alkyl C-H, str), 1685 (carbamate C=O, str), 1637 (urea C=O, str), 1615, 183, 1523 (aryl, str), 1282 (C-F, str), 746, 695 (aryl C-H, bend, phenyl ring).

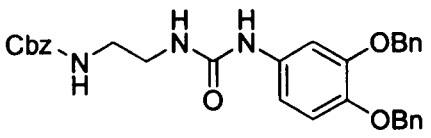
^{1}H NMR (DMSO-d$_6$): δ 8.56 (s, 1H, NH(C=O)N*H*Ar), 7.23 – 7.52 (m, 12H, aromatic benzyl C-H, aryl 6-H, carbamate NH), 7.06 (dd, *J* = 11.1/9.0 Hz, 1H, aryl 5-H), 6.81 – 6.89 (m, 1H, aryl 2-H), 6.18 (t, *J* = 5.2

Hz, N*H*(C=O)NHAr), 5.14 (s, 2H, benzyl carbamate CH_2), 5.02 (s, 2H, benzyl ether CH_2), 3.14, 3.09 (t, *J* = 5.5 Hz, 2 x 2H, NHCH_2CH_2NH).

^{13}C NMR (DMSO-d_6): δ 156.28, 155.26 (C=O), 145.82 (J_{CF} = 11.6 Hz, aryl 3-C), 137.28 (J_{CF} = 3.5 Hz, aryl 1-C), 137.15, 136.53 (benzyl 4° C), 128.27, 128.36, 128.04, 127.78 (benzyl C-H), 115.68 (J_{CF} = 16.5 Hz, aryl 5-C), 109.74 (J_{CF} = 5.2 Hz, aryl 6-C), 105.28 (aryl 2-C), 70.07 (benzyl ether CH_2), 65.27 (benzyl carbamate CH_2), 45.99, 40.75 (NHCH_2CH_2NH).

m/z: HRMS (TOF ES$^+$) $C_{24}H_{25}FN_3O_4$ $[MH]^+$ calcd 438.1824; found 438.1792.

Benzyl 2-(3-(3,4-*bis*(benzyloxy)phenyl)ureido)ethylcarbamate (83b)



3,4-*bis*(Benzyloxy)benzoic acid **(80b)** was reacted in a similar fashion to 3-(benzyloxy)-4-fluorobenzoic acid **(80a)** as described in the procedure for benzyl 2-(3-(3-(benzyloxy)-4-fluorophenyl)ureido)ethylcarbamate **(83a)**. In this case the precipitate formed was not the desired product and the remaining crude mixture was purified by FCC (eluent EtOAc/PE 30:70 to 50:50 over 20 column volumes, then 50:50 to 100:0 over 10 column volumes), to give 479 mg of white solid.

Yield: 15%.

Mp: 171 – 179 °C.

IR: 3424 (N-H, str), 3034 (aryl C-H, str), 2934 (alkyl C-H, str), 1684 (carbamate C=O, str), 1636 (urea C=O, str), 1601, 1576, 1513 (aryl, str), 750, 696 (aryl C-H bend, phenyl ring).

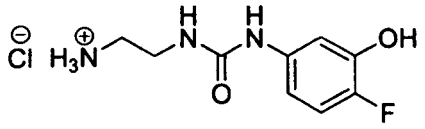
^{1}H NMR (DMSO-d_6): δ 8.42 (s, 1H, NH(C=O)N*H*Ar), 7.25 – 7.48 (m, 12H, aromatic benzyl C-H, aryl 2-H), 7.21 (dd, *J* = 7.8/7.8 Hz, 2H, benzyl 3-H and 5-H), 7.14 (d, *J* = 7.8 Hz, benzyl 2-H and 6-H), 6.95 (t, *J* = 7.5Hz, 1H, carbamate NH), 6.90 (d, *J* = 8.9 Hz, 1H, aryl 5-H), 6.81 (dd, *J* = 8.4/2.3 Hz, 1H, aryl 6-H), 6.16 (t, *J* = 5.3 Hz, 1H,

N*H*(C=O)NHAr), 5.05 (s, 2H, benzyl carbamate CH_2), 5.03, 5.02 (s, 2 x 2H, benzyl ether CH_2), 3.12, 3.08 (t, *J* = 5.5 Hz, 2 x 2H, NHCH_2CH_2NH).

^{13}C NMR (DMSO-d$_6$): δ 155.38, 148.51 (C=O), 142.65 (aryl 3-C), 139.15 (aryl 4-C), 137.60, 137.22, 137.15 (benzyl 4° C), 128.79, 128.33, 128.27, 127.73, 127.63, 127.55, 127.51 (benzyl C-H), 121.85 (aryl 1-C), 119.89, 119.84 (aryl 5-C, aryl 6-C), 105.61 (aryl 2-C), 70.90, 70.02 (benzyl ether CH_2), 65.22 (benzyl carbamate CH_2), 45.97, 40.84 (NHCH_2CH_2NH).

m/z: HRMS (TOF ES$^+$) $C_{31}H_{32}N_3O_5$ $[MH]^+$ calcd 526.2336; found 526.2322.

1-(2-Aminoethyl)-3-(4-fluoro-3-hydroxyphenyl)urea hydrochloride (84a)



Benzyl 2-(3-(3-(benzyloxy)-4-fluoro phenyl)ureido)ethylcarbamate **(83a)** was hydrogenated as described in the procedure for 4-(2-(cyclopentyloxy) ethoxy)phenol **(61)**, with the addition of a few drops of conc HCl. The reaction mixture was filtered over celite with additional MeOH washings, before concentration of the filtrate. The hydrochloride salt was collected in quantitative yield.

Yield: 100%.

Mp: 186 – 188 °C.

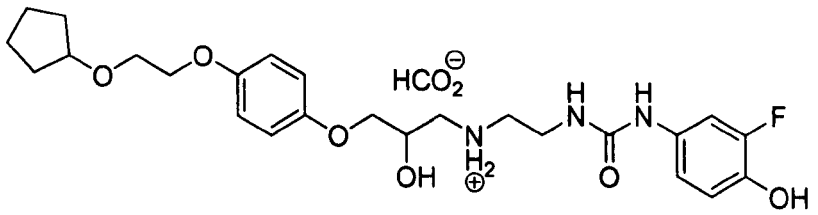
IR: 3350 (O-H, str), 3266 (urea N-H, str), 3142 (NH_3^+, str), 2948 (alkyl C-H, str), 1670 (urea C=O, str), 1619, 1563, 1516 (aryl, str), 1182 (C-F, str), 1125 (C-O, str), 779 (C-F, bend).

^{1}H NMR (DMSO-d$_6$): δ 9.74 (br s, 1H, OH), 9.00 (s, 1H, NH(C=O)N*H*Ar), 8.02 (br s, 3H, NH_3^+), 7.17 (dd, *J* = 6.0/2.4 Hz, 1H, aryl 2-H), 6.94 (dd, *J* = 11.2/8.8 Hz, 1H, aryl 5-C), 6.75 (ddd, *J* = 8.8/3.2/2.8 Hz, 1H, aryl 6-C), 6.57 – 6.66 (br m, 1H, N*H*(C=O)NHAr), 3.25 – 3.35 (br m, 2H, C$H_2$$NH_3^+$), 2.85 (dt, *J* = 6.0/5.6 Hz, 2H, CH_2NH).

^{13}C NMR (DMSO-d_6): δ 155.73 (C=O), 146.05 (J_{CF} = 235.2 Hz, aryl 4-C), 144.56 (J_{CF} = 11.9 Hz, aryl 3-C), 136.80 (J_{CF} = 3.0 Hz, aryl 1-C), 115.51 (J_{CF} = 19.2 Hz, aryl 5-C), 108.30 (J_{CF} = 5.6 Hz, aryl 6-C), 107.47 (J_{CF} = 1.6 Hz, aryl 2-C), 46.01 (CH_2NH), 37.10 ($CH_2NH_3^+$).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}FN_3O_2$ $[MH]^+$ calcd 214.0986; found 214.0973.

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-fluoro-4-hydroxyphenyl)urea hydroformate (85a)



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (50 mg, 0.18 mmol), TEA (50 µL, 0.36 mmol, 2 eq) and 1-(2-aminoethyl)-3-(3-fluoro-4-hydroxyphenyl)urea hydrochloride **(77)** (67 mg, 0.27 mmol, 1.5 eq) were dissolved in EtOH (2 mL) before exposing to MW conditions (140 °C, 80W, 250 psi) for 8 minutes. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:5:94) and preparative HPLC afforded 13 mg of white semi-solid.

Yield: 13 %.

IR: 3377 (br, OH, str), 2912, 2868 (alkyl C-H, str), 1647 (urea C=O, str), 1614, 1568, 1508 (aryl, str), 1236 (C-F, str), 822 (aryl C-H, bend, *para*-disubstituted ring).

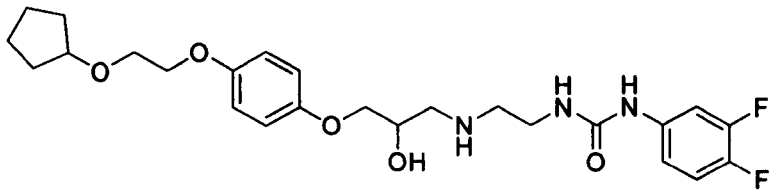
^{1}H NMR (DMSO-d_6): δ 9.30 (br s, 1H, phenolic OH), 8.60 (s, 1H, NH(C=O)N*H*Ar), 7.32 – 7.42 (m, 1H, phenylurea C-H), 6.75 – 6.92 (m, 6H, C-H aryl-dioxy ring, phenylurea C-H), 6.26 (t, *J* = 5.6 Hz, 1H, N*H*(C=O)NHAr), 3.75 – 4.04 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.61 (t, *J* = 4.8 Hz, 2H, ^{c}PeOCH_2), 3.20 – 3.27 (m, 2H, NHCH$_2$CH_2), 2.72 – 2.94 (m, 4H, CH(OH)CH_2NH, NHCH_2CH$_2$), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.41 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 155.74 (C=O), 152.79, 152.54 (aryl-dioxy ring 4° C), 148.93 (aryl 4-C), 102.53 (J_{CF} = 20.6 Hz, aryl 2-C), 115.43, 115.39 (aryl-dioxy ring C-H), 80.88 (^{c}Pe *C*H), 70.92 (ArO*C*H_2), 69.04 (*C*H(OH)), 67.76 (*C*H_2OAr), 66.73 (^{c}PeO*C*H_2), 50.89 (CH(OH)*C*H_2NH), 48.73 (NH*C*H_2CH_2), 37.43 (NHCH_2*C*H_2), 31.81 (2-C and 5-C ^{c}Pe ring), 23.14 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}FN_3O_6$ $[MH]^+$ calcd 492.2504; found 492.2503.

HPLC R_t: 3.82 (System 1b), 10.65 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3,4-difluorophenyl)urea (85b)



Epoxide opening of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy) methyl)oxirane **(62)** with 1-(2-aminoethyl)-3-(3,4-difluorophenyl)urea hydrochloride **(73)**, was carried out as described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification was achieved via PLC (eluent 37% aq NH_3/MeOH/DCM 2:10:88) to give 50 mg of yellow solid.

Yield: 28%.

Mp: 113 – 115 °C.

IR: 3323 (br, OH, str), 2929, 2871 (alkyl C-H, str), 1645 (urea C=O, str), 1581 (N-H, bend), 1510 (aryl, str), 1234 (C-F, str), 759 (C-F, bend).

^{1}H NMR (DMSO-d_6): δ 8.90 (s, 1H, NH(C=O)N*H*Ar), 7.63 (ddd, *J* = 13.8/7.9/2.6 Hz, 1H, aryl 2-H), 7.26 (ddd, *J* = 10.4/9.2/9.2 Hz, 1H, aryl 5-H), 6.96 – 7.06 (m, 1H, aryl 6-H), 6.84 (s, 4H, C-H aryl-dioxy ring), 6.32 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 5.11 (br s, 1H, OH), 3.76 – 4.03 (m, 6H, C*H*$_2$OAr, ^{c}Pe C*H*, C*H*(OH), ArOC*H*$_2$), 3.61 (t, *J* = 4.6 Hz, 2H, ^{c}PeOC*H*$_2$), 3.14 – 3.24 (m, 2H, NHCH$_2$C*H*$_2$), 2.59 – 2.81 (m, 4H,

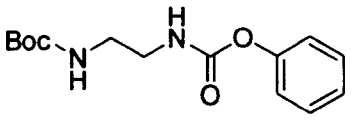
CH(OH)CH_2NH, NHCH_2CH_2), 1.52 – 1.75 (m, 6H, ^{c}Pe CH_2), 1.41 – 1.52 (m, 2H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d$_6$): δ 155.19 (C=O), 153.83 (J_{CF} = 233.5 Hz, aryl 3-C), 151.24 (J_{CF} = 257.3 Hz, aryl 4-C), 127.26 (aryl 1-C), 117.2 (J_{CF} = 16.1 Hz, aryl 5-C) 115.32 (*C*H aryl-dioxy ring), 113.5 (J_{CF} = 10.5 Hz, aryl 6-C), 106.30, (J_{CF} = 24.1Hz, aryl 2-C), 80.84 (^{c}Pe *C*H), 71.08 (ArO*C*H$_2$), 68.64 (*C*H(OH)), 67.68 (*C*H$_2$OAr) 66.71 (^{c}PeO*C*H$_2$), 51.82 (CH(OH)*C*H$_2$NH), 48.93 (NH*C*H$_2$CH_2), 38.67 (NHCH_2*C*H$_2$), 31.79 (2-C and 5-C ^{c}Pe ring), 23.13 (3-C and 4-C ^{c}Pe ring)

m/z: HRMS (TOF ES$^-$) $C_{25}H_{32}F_2N_3O_5$ [M-H]$^-$ calcd 492.2316; found 492.2361.

HPLC R$_t$: 4.65 (System 1b), 13.70 (System 3).

Phenyl-2-(*tert*-butyloxycarbonyl)aminoethylcarbamate (86)



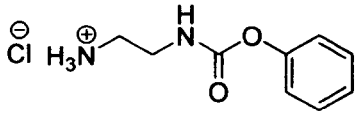
tert-Butyl 2-aminoethylcarbamate **(64)** (1.000 g, 6.25 mmol) and TEA (759 mg, 1.045 mL, 7.5 mmol, 1.2 eq) were dissolved in dry DCM (20 mL) under a nitrogen atmosphere. Phenylchloroformate (1.076 g, 862 µL, 6.87 mmol, 1.1 eq) was added and the mixture stirred for 1 hour. After confirmation of total amine consumption by TLC, the mixture was diluted to 50 mL with DCM before washing with aqueous 1 M NaOH (1 x 50 mL), and water (1 x 50 mL). Removal of all volatiles gave 1.521 g of cream solid which was used without any further purification.

Yield: 87%.

IR: 3333 (carbamate N-H, str), 2977 (alkyl, C-H, str), 1716, 1689 (carbamate C=O, str), 1526 (aryl, str), 1366 (C(CH$_3$)$_3$, str), 769, 699 (aryl C-H bend, phenyl ring).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{21}N_2O_4$ [MH]$^+$ calcd 281.1496; found 281.1516.

Phenyl 2-aminoethylcarbamate hydrochloride (87)



Phenyl-2-(*tert*-butyloxycarbonyl)aminoethyl carbamate **(86)** was dissolved in Et_2O (15 mL) and 4 M HCl / dioxane (15 mL). After 10 minutes the formed precipitate was filtered (suction) and washed with Et_2O to give a white solid in quantitative yield.

Yield: 100 %.

Mp: 166 – 168 °C.

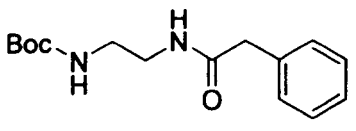
IR: 3336 (carbamate N-H, str), 2891 (NH_3^+, str), 1715 (carbamate C=O, str), 1536 (aryl, str), 761, 708 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 8.23 (br s, 3H, NH_3^+), 7.97 (t, *J* = 5.3 Hz, 1H, carbamate NH), 7.38 (dd, *J* = 7.7/7.7 Hz, 2H, aryl 3-H and 5-H), 7.20 (dd, *J* = 7.7/7.1 Hz, 1H, aryl 4-H), 7.13 (d, *J* = 7.7 Hz, 2H, aryl 2-H and 6-H), 3.35 (dt, *J* = 6.1/6.1 Hz, 2H, CH_2NH), 2.86 – 3.01 (m, 2H, $CH_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 154.49 (C=O), 150.95 (aryl 1-C), 129.21 (aryl 3-C and 5-C), 125.03 (aryl 4-C), 121.77 (aryl 2-C and 6-C), 38.41, 38.20 (CH_2).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}N_2O_2$ $[MH]^+$ calcd 181.0972; found 181.0965.

***tert*-Butyl 2-(2-phenylacetamido)ethylcarbamate (88)**



tert-Butyl 2-aminoethylcarbamate **(64)** (1 g, 6.25 mmol) and TEA (958 µL, 6.88 mmol, 1.1 eq) were dissolved in dry DCM (20 mL) and cooled to 0 °C under a nitrogen atmosphere. Phenyl acetyl chloride (826 µL, 6.25 mmol, 1 eq) was added and the mixture stirred at room temperature for 2 hours. The TEA.HCl salt was filtered before concentration of the filtrate *in vacuo*. The crude residue was dissolved in EtOAc (50 mL) and washed with acidified water (25 mL, pH 4 adjusted using aqueous 1M $KHSO_4$), aqueous 2M NaOH (25 mL) and

water (25 mL). The organic layer was concentrated *in vacuo* to give 1.460 g of white solid requiring no further purification.

Yield: 84 %.

Mp: 133 – 135 °C.

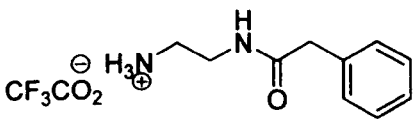
IR: 3370 (amide N-H, str), 3332 (carbamate N-H, str), 2983, 2940 (alkyl C-H, str), 1687 (carbamate C=O, str), 1649 (amide I, C=O str), 1530 (amide II, N-H bend), 1497 (aryl, str), 1365 ($C(CH_3)$, str), 698 (aryl C-H bend, phenyl ring).

^{1}H NMR ($CDCl_3$): δ 7.25 – 7.39 (m, 5H, phenyl CH), 6.05 (br s, 1H, amide NH), 4.87 (br s, 1H, carbamate NH), 3.55 (s, 2H, $CH_2C=O$), 3.29 – 3.33 (m, 2H, CH_2NH), 3.18 – 3.22 (m, 2H, CH_2NH), 1.42 (s, 9H, $(CH_3)_3$).

^{13}C NMR ($CDCl_3$): δ 171.85 (amide C=O), 156.83 (carbamate C=O), 134.94 (phenyl 1-C), 129.53, 129.12 (phenyl 2-C, 3-C, 5-C, 6-C), 127.45 (phenyl 4-C), 79.76 (Boc 4 °C), 43.92 ($CH_2C=O$), 40.80, 40.42 ($NHCH_2CH_2NH$), 28.50 ($(CH_3)_3$).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{23}N_2O_3$ $[MH]^+$ calcd 279.1703; found 279.1686.

2-(2-Phenylacetamido)ethylammmonium trifluoroacetate (89)



tert-Butyl 2-(2-phenylacetamido)ethyl carbamate **(88)** (1.310 g, 4.71 mmol) was dissolved in TFA/DCM (20 mL 1:1) and stirred for 2 hours at room temperature. Removal of volatiles *in vacuo* gave 1.518 g of semi-solid requiring no further purification.

Yield: 100%.

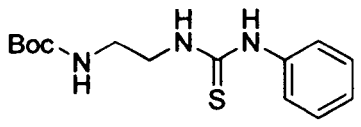
IR: 3054 (br, NH_3^+, str), 1653 (amide I, C=O str), 1538 (amide II, N-H bend), 715 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 8.30 (t, J = 5.4 Hz, 1H, amide NH), 7.90 (br s, 3H, NH_3^+), 7.20 – 7.31 (m, 5H, phenyl CH), 3.43 (s, 2H, $CH_2C=O$), 3.28 (dt, J = 6.0/6.4 Hz, 2H, CH_2NH), 2.84 – 2.90 (m, 2H, $CH_2NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 171.03 (amide C=O), 158.46 (J_{CF} = 33.6, formate C=O), 136.05 (phenyl 1-C), 129.16, 128.23 (phenyl 2-C, 3-C, 5-C, 6-C), 126.44 (phenyl 4-C), 42.29 (CH_2C=O), 38.60 ($CH_2NH_3^+$), 36.63 ($CH_2CH_2CH_2$).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{15}N_2O$ $[MH]^+$ calcd 179.1179; found 179.1196.

tert-Butyl 2-(3-phenylthioureido)ethylcarbamate (90)



tert-Butyl 2-aminoethylcarbamate **(64)** (593 mg, 3.70 mmol) and phenylisothiocyanate (442 µL, 3.70 mmol, 1 eq) were reacted in a manner analogous to that described in the general procedure for synthesis of phenyl substituted 1-(2-aminoethyl)-3-(phenyl)urea hydrochlorides **(65a – 65r)** to give 920 mg of white solid.

Yield: 84%.

Mp: 138 – 140 °C.

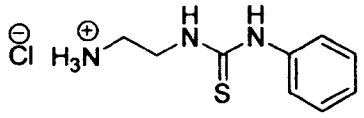
IR: 3327 (br, carbamate N-H, str), 1688 (carbamate C=O, str), 1588 (aryl, str), 1277 (thiourea, str), 1180 (C=S, str), 693 (aryl C-H bend phenyl ring).

^{1}H NMR ($CDCl_3$): δ 7.85 (br s, 1H, NH(C=S)N*H*Ph), 7.42 (dd, *J* = 7.7/7.7 Hz, 2H, 3-H and 5-H phenyl ring), 7.29 (dd, *J* = 7.3/7.3 Hz, 1H, 4-H phenyl ring), 7.22 (d, *J* = 7.7 Hz, 2H, 2-H and 6-H phenyl ring), 6.85 (br s, 1H, O(C=O)N*H*), 4.90 (br s, 1H, N*H*(C=S)NHPh), 3.74 (dt, *J* = 5.9/5.2 Hz, 2H, C*H*$_2$NH(C=S)NH), 3.32 (dt, *J* = 6.3/5.6 Hz, 2H, C*H*$_2$NH(C=O)O), 1.34 (s, 9H, C(CH_3)$_3$).

^{13}C NMR ($CDCl_3$): δ 181.17 (C=S), 157.02 (C=O), 136.05 (4° C), 130.20 (phenyl 3-C and 5-C), 127.39 (phenyl 4-C), 125.46 (phenyl 2-C and 6-C), 79.99 (Boc 4° C), 47.00, 39.83 (CH_2), 28.38 (CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{22}N_3O_2S$ $[MH]^+$ calcd 296.1427; found 296.1409.

1-(2-Aminoethyl)-3-phenylthiourea hydrochloride (91)



tert-Butyl 2-(3-phenylthioureido)ethyl carbamate **(90)** (882 mg, 2.99 mmol) was stirred overnight in 4 M HCl in dioxane (20 mL). Removal of all volatiles *in vacuo* gave 592 mg of cream crystalline solid requiring no further purification.

Yield: 86%.

Mp: 183 – 185 °C.

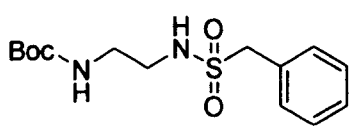
IR: 3355, 3166 (thiourea N-H, str), 2949 (NH_3^+, str), 2804, 2629 (alkyl C-H, str), 1595 (aryl, str), 1320 (thiourea, str), 1095 (C=S, str), 733, 693 (aryl C-H bend phenyl ring).

^{1}H NMR (DMSO-d_6): δ 10.10 (s, 1H, NH(C=S)N*H*Ph), 8.22 (t, *J* = 5.5 Hz, N*H*(C=S)NHPh), 8.08 (br s, 3H, NH_3^+), 7.46 (dd, *J* = 8.6/1.1Hz, 2H, 2-H and 6-H phenyl ring), 7.32 (dd, *J* = 7.4/7.4 Hz, 2H, 3-H and 5-H phenyl ring), 7.11 (dd, *J* = 7.4/7.4 Hz, 1H, 4-H phenyl ring), 3.74 (dt, *J* = 6.4/5.9 Hz, 2H, C*H*$_2$NH(C=O)NH), 2.95 – 3.06 (m, 2H, C*H*$_2$$NH_3^+$).

^{13}C NMR (DMSO-d_6): δ 180.99 (C=S), 139.08 (4° C), 128.08, 124.26, 123.11 (Phenyl C-H), 41.23, 37.92 (*C*H$_2$).

m/z: HRMS (TOF ES$^+$) $C_9H_{14}N_3S$ $[MH]^+$ calcd 196.0903; found 196.0889.

***tert*-Butyl 2-(benzylsulfonamido)ethylcarbamate (92)**



tert-Butyl 2-aminoethylcarbamate **(64)** (500 mg, 3.12 mmol) and TEA (478 µL, 3.43 mmol, 1.1 eq) were dissolved in dry DCM (10 mL) under a nitrogen atmosphere. Phenylmethanesulfonyl chloride (595 mg, 3.12 mmol, 1 eq) in DCM (5 mL) was added dropwise whilst cooling the mixture over an ice bath. After stirring at room temperature overnight, the crude mixture was diluted to 30 mL with DCM before washing with aqueous 1 M $KHSO_4$ (20 mL), aqueous 1 M NaOH (20 mL) and water (20 mL). Removal of all volatiles *in vacuo* gave 761 mg of white solid requiring no further purification.

Yield: 78 %.

Mp: 110 – 114 °C.

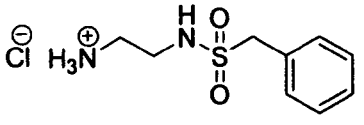
IR: 3366 (carbamate N-H, str), 3293 (sulfonamide N-H, str), 2983, 2939 (alkyl C-H, str), 1691 (carbamate C=O, str), 1532 (aryl, str), 1367, 1171 (sulfonamide, str), 698 (aryl C-H bend phenyl ring).

^{1}H NMR ($CDCl_3$): δ 7.34 – 7.42 (m, 5H, phenyl C-H), 4.78 – 4.93 (m, 2H, 2 x N-H), 4.25 (s, 2H, SO_2CH_2), 3.11 – 3.23 (m, 2H, C=ONHCH_2), 2.96 – 3.08 (m, 2H, CH_2NHSO$_2$), 1.43 (s, 9H, $C(CH_3)_3$).

^{13}C NMR ($CDCl_3$): δ 156.52 (C=O), 130.76, 129.0, 128.92 (Phenyl C-H), 129.39 (4° C), 80.00 (Boc 4° C), 59.07 (SO_2CH_2), 44.05 (CH_2NHSO$_2$), 41.04 (C=ONHCH_2), 28.48 (CH_3).

m/z: HRMS (TOF ES$^+$) $C_{14}H_{23}N_2O_4S$ $[MH]^+$ calcd 315.1373; found 315.1397.

_N_-(Aminoethyl)benzylsulfonamide hydrochloride (93)



Boc-deprotection of *tert*-butyl 2-(benzylsulfonamido)ethylcarbamate **(92)** (704 mg, 2.24 mmol) was achieved as described for *tert*-butyl 2-(3-phenylthioureido)ethylcarbamate **(90)**, giving 552 mg of cream solid requiring no further purification.

Yield: 98 %.

Mp: 191.5 – 193.5 °C.

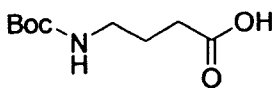
IR: 3338 (sulfonamide N-H, str), 2999 (NH_3^+, str), 1589, 1502 (aryl, str), 697 (aryl C-H bend phenyl ring).

^{1}H NMR ($CDCl_3$): δ 8.08 (br s, 3H, NH_3^+), 7.45 (t, J = 5.9 Hz, 1H, $NHSO_2$), 7.33 – 7.42 (m, 5H, phenyl C-H), 4.40 (s, 2H, SO_2CH_2), 3.16 (dt, J = 6.8/5.9 Hz, 2H, CH_2NHSO$_2$), 2.77 – 2.90 (m, 2H, $NH_3^+CH_2$).

^{13}C NMR ($CDCl_3$): δ 130.81, 128.37, 128.09 (Phenyl C-H), 130.05 (4° C), 57.22 (SO_2CH_2), 39.17 (CH_2).

m/z: HRMS (TOF ES$^+$) $C_9H_{15}N_2O_2S$ $[MH]^+$ calcd 215.0849; found 215.0845.

tert-Butyl 3-carboxypropylcarbamate (95)



γ-Aminobutyric acid **(94)** (3.946 g, 38.27 mmol) and $NaHCO_3$ (7.07 g, 84.19 mmol, 2.2 eq) were dissolved in water/THF (4:1, 100 mL). Boc_2O (9.186 g, 42.09 mmol, 1.1 eq) was added and the mixture stirred at room temperature for 48 hours. THF was removed *in vacuo* before washing the remaining aqueous mixture with DCM (2 x 50 mL). The aqueous layer was then acidified using 2M aqueous HCl to pH 4 before extraction with DCM (4 x 30 mL). The organic layers were combined and concentrated to give 6.711 g of clear colourless oil requiring no further purification.

Yield: 86%.

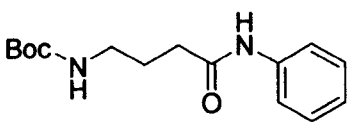
IR: 2984 (O-H, str), 1717 (acid C=O, str), 1700 (carbamate C=O, str), 1369 ($C(CH_3)$, str), 1264, 1165 (C-O, str).

^{1}H NMR ($CDCl_3$): δ 10.13 (br s, 1H, CO_2H), 5.05 – 5.15 (br m, 1H, NH), 2.90 – 3.13 (br m, 2H, $NHCH_2$), 2.24 (t, J = 7.3 Hz, 2H, CH_2CO_2H), 1.68 (tt, J = 7.0/7.0 Hz, 2H, $CH_2CH_2CH_2$), 1.23 – 1.38 (m, 9H, $(CH_3)_3$)

^{13}C NMR ($CDCl_3$): δ 177.58 (CO_2H), 156.28 (carbamate C=O), 79.17 (Boc 4° C), 39.65 (CH_2NH), 30.65 (CH_2CO_2H), 28.21 (($CH_3)_3$)), 24.94 ($CH_2CH_2CH_2$)

m/z: HRMS (TOF ES^+) $C_9H_{18}NO_4$ $[MH]^+$ calcd 204.1230; found 204.1250.

tert-Butyl 3-(phenylcarbamoyl)propylcarbamate (96)



tert-Butyl 3-carboxypropylcarbamate **(95)** (921 mg, 4.53 mmol) and DCC (1.028 g, 4.98 mmol, 1.1 eq) were dissolved in DCM (25 mL) and stirred for 30 minutes. Aniline (454 µL, 4.98 mmol, 1.1 eq) was added and the mixture stirred at room temperature for 48 hours. The reaction mixture was diluted to 50 ml with DCM before washing with acidified water (30 ml, pH 4 adjusted using aqueous 1M $KHSO_4$), saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was concentrated *in vacuo* and the crude residue purified via FCC

(eluent EtOAc/hexanes 10:90 to 80:20 over 10 column volumes). The product was recrystallised from MeCN to give 700 mg of crystalline white solid.

Yield: 86 %.

Mp: 141 – 144 °C.

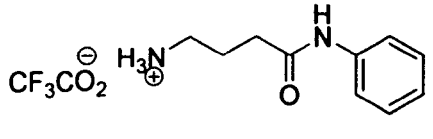
IR: 3336 (amide N-H, str), 3329 (carbamate N-H, str), 2932, 2851 (alkyl C-H, str), 1691 (carbamate C=O, str), 1653 (amide I, C=O str), 1573 (aryl, str), 1531 (amide II, N-H bend), 1365 ($C(CH_3)$, str).

^{1}H NMR ($CDCl_3$): δ 8.71 (br s, 1H, amide NH), 7.60 (d, *J* = 7.8 Hz, 2H, 2-H and 6-H phenyl ring), 7.31 (dd, *J* = 7.6/7.6 Hz, 2H, 3-H and 5-H phenyl ring), 7.08 (dd, *J* = 7.4/7.4 Hz, 1H, 4-H phenyl ring), 4.81 (br s, 1H, carbamate NH), 3.25 (dt, *J* = 6.0/6.0 Hz, 2H, $NHCH_2$), 2.38 (t, *J* = 6.5 Hz, 2H, CH_2CONH), 1.88 (tt, *J* = 6.4 Hz, 2H ($CH_2CH_2CH_2$), 1.46 (s, 9H, $(CH_3)_3$).

^{13}C NMR ($CDCl_3$): δ 171.31 (amide C=O), 157.36 (carbamate C=O), 138.57 (phenyl 1-C), 129.01 (phenyl 3-C and 5-C), 124.05 (phenyl 4-C), 119.84 (phenyl 2-C and 6-C), 79.95 (Boc 4 °C), 39.40 (CH_2NH), 34.80 (CH_2CONH), 28.51 ($(CH_3)_3$), 27.48 ($CH_2CH_2CH_2$).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{23}N_2O_3$ $[MH]^+$ calcd 279.1703; found 279.1695.

3-(Phenylcarbamoyl)propylammonium trifluoroacetate (97)



tert-Butyl 3-(phenylcarbamoyl)propyl carbamate **(96)** (653 mg, 2.35 mmol) was dissolved in TFA/DCM (20 mL 1:1) and stirred for 2 hours at room temperature. Removal of volatiles *in vacuo* gave 773 mg of semi-solid requiring no further purification.

Yield: 100%.

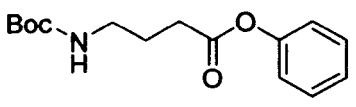
IR: 3061 (br, NH_3^+, str), 2937 (alkyl C-H, str), 1673 (amide I, C=O str), 1543 (amide II, N-H bend), 757, 700 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 10.03 (s, 1H, NH), 7.87 (br s, 3H, NH_3^+), 7.59 (d, *J* = 7.6 Hz, 2H, phenyl 2-H and 6-H), 7.29 (dd, *J* = 7.6/7.6 Hz, 2H, phenyl 3-H and 5-H), 7.02 (dd, *J* = 7.4/7.4 Hz, 1H, phenyl 4-H), 2.80 – 2.91 (m, 2H, $CH_2NH_3^+$), 2.42 (t, *J* = 7.2 Hz, 2H, $COCH_2$), 1.85 (tt, *J* = 7.3 Hz, 2H, $CH_2CH_2CH_2$).

^{13}C NMR (DMSO-d_6): δ 170.30 (amide C=O), 158.47 (J_{CF} = 34.1 Hz, formate C=O), 155.03 (J_{CF} = 341.0 Hz, CF_3), 139.20 (phenyl 1-C), 128.72 (phenyl 3-C and 5-C), 123.14 (phenyl 4-C), 119.14 (phenyl 2-C and 6-C), 38.55 (CH_2 NH_3^+), 32.95 (CH_2C=O), 23.00 ($CH_2CH_2CH_2$).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{15}N_2O$ $[MH]^+$ calcd 179.1179; found 179.1191.

tert-Butyl 3-(phenoxycarbonyl)propylcarbamate (98)



tert-Butyl 3-carboxypropylcarbamate **(95)** (2.000 g, 9.84 mmol) and DCC (2.232 g, 10.82 mmol, 1.1 eq) were dissolved in DCM (30 mL) and stirred for 30 minutes. Phenol (1.018 g, 10.82 mmol, 1.1 eq) was added and the mixture stirred for 48 hours. TLC monitoring indicated slow progression of the reaction, thus DMAP (122 mg, 1 mmol, 0.1 eq) was added and the reaction left to stir for a further 24 hours. The precipitated *N,N'*-dicyclohexylurea was filtered (suction) and the filtrate diluted to 50 mL with DCM before washing with acidified water (1 x 30 mL, water acidified to pH 4 with aqueous 1 M $KHSO_4$ solution) and aqueous 0.5 M NaOH (1 x 30 mL). After concentrating the organic layer, FCC (eluent EtOAc/hexanes 20:80) was required to afford 1.894 g of white crystalline solid.

Yield: 69%.

Mp: 62 – 65 °C.

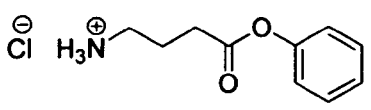
IR: 3389 (carbamate N-H, str), 2983, 2937, 2873 (alkyl C-H, str), 1756 (ester C=O, str), 1688 (carbamate C=O, str), 1526 (aryl, str), 1370 ($C(CH_3)_3$, str), 1186, 1137 (ester C-O, str), 755, 691 (aryl C-H bend, phenyl ring).

^{1}H NMR: δ 7.38 (ddd, *J* = 7.6/7.6/2.0 Hz, 2H, aryl 3-H and 5-H), 7.23 (ddd, *J* = 7.6/7.6/1.0 Hz, 1H, aryl 4-H), 7.08 (dd, *J* = 8.5/1.4 Hz, 2H, aryl 2-H and 6-H), 4.67 (br s, 1H, NH), 3.26 (dt, *J* = 7.0/6.2 Hz, 2H, NHCH_2), 2.61 (t, *J* = 7.0 Hz, 2H, CH_2C=O), 1.94 (tt, *J* = 7.0/7.0 Hz, 2H, $CH_2CH_2CH_2$), 1.45 (s, 9H, $(CH_3)_3$).

^{13}C NMR: δ 172.01 (C=O, ester), 156.14 (C=O, carbamate), 150.76 (aryl 1-C), 129.56 (aryl 3-C and 5-C), 125.97 (aryl 4-C), 121.68 (aryl 2-C and 6-C), 79.51 (Boc 4° C), 39.99 ($NHCH_2$), 31.79 (CH_2C=O), 28.55 ($(CH_3)_3$)

m/z: HRMS (TOF ES$^+$) $C_{15}H_{22}NO_4$ $[MH]^+$ calcd 280.1543; found 280.1515.

Phenyl 4-aminobutanoate hydrochloride (99)



tert-Butyl 3-(phenoxycarbonyl)propyl carbamate **(98)** was Boc-deprotected in a similar fashion to phenyl-2-(*tert*-butyloxycarbonyl)aminoethylcarbamate **(86)** as described in the method for phenyl 2-aminoethylcarbamate hydrochloride **(87)**, to give 1.289 g of the desired hydrochloride salt as a yellow semi-solid.

Yield: 98%.

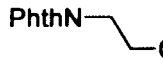
IR: 2973 (br, NH_3^+, str), 1751 (ester C=O, str), 1197, 1167 (ester C-O, str), 755, 693 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 8.22 (br s, 3H, NH_3^+), 7.42 (ddd, *J* = 7.6/7.6/2.0 Hz, 2H, aryl 3-H and 5-H), 7.26 (ddd, *J* = 7.6/7.6/1.0 Hz, 1H, aryl 4-H), 7.14 (dd, *J* = 8.5/1.4 Hz, 2H, aryl 2-H and 6-H), 2.82 – 2.92 (m, 2H, C$H_2NH_3^+$), 2.74 (t, *J* = 7.5 Hz, 2H, CH_2C=O), 1.93 (tt, *J* = 7.6/7.6 Hz, 2H, $CH_2CH_2CH_2$).

^{13}C NMR (DMSO-d_6): δ 171.13 (C=O, ester), 150.40 (aryl 1-C), 129.47 (aryl 3-C and 5-C), 125.82 (aryl 4-C), 121.77 (aryl 2-C and 6-C), 37.91 ($CH_2NH_3^+$), 30.45 (CH_2C=O), 22.25 ($CH_2CH_2CH_2$).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{14}NO_2$ $[MH]^+$ calcd 180.1019; found 180.1011.

2-(2-Hydroxyethyl)phthalimide (101)



Phthalic anhydride (12.125 g, 81.86 mmol) and ethanolamine **(100)** (4.94 mL, 81.86 mmol, 1 eq) were heated to 175 °C with stirring, under a water condenser for 2 hours. On cooling, the crude solid was crushed before collecting by filtration (suction) and washing with water to give 13.010 g of beige crystalline solid.

Yield: 83 %.

Mp: 134 – 137 °C.

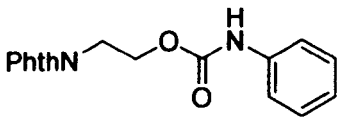
IR: 3472 (br, OH, str), 2948, 2886 (alkyl C-H, str), 1767, 1697 (phth C=O, str), 1606 (aryl, str), 1393 (O-H, bend), 1056 (C-OH, str).

^{1}H NMR ($CDCl_3$): δ 7.79 – 7.87 (2H, m, phth C-H), 7.67 – 7.76 (2H, m, phth C-H), 3.81 – 3.94 (m, 4H, 2 x CH_2), 2.40 (br s, 1H, OH).

^{13}C NMR ($CDCl_3$): δ 168.98 (C=O), 134.25, 123.43 (phth C-H), 132.09 (phth 4° C), 61.19 (CH_2OH), 40.97 (NCH_2).

m/z: HRMS (TOF ES^+) $C_{10}H_{10}NO_3$ $[MH]^+$ calcd 192.0655; found 192.0665.

2-Phthalimidoethyl phenylcarbamate (102)



2-(2-Hydroxyethyl)phthalimide (**101**) (2.000 g, 10.46 mmol) was dissolved in dry DCM (30 mL) under a nitrogen atmosphere. Phenyl isocyanate (1.137 ml, 10.46 mmol, 1 eq) was added and the mixture stirred for 48 hours. Hexanes were added to the mixture until a precipitation of a solid was observed. After collection by filtration (suction), this crude solid was purified by FCC (eluent EtOAc/hexanes 30:70 to 50:50 over 10 column volumes to give 400 mg of white solid.

Yield: 12%.

Mp: 109 – 111 °C (lit. 179 °C)[179].

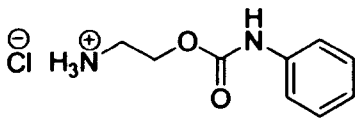
IR: 3286 (N-H, str), 1775 (phth C=O, str), 1711 (carbamate C=O, str), 1600 (aryl, str), 769, 720 (aryl C-H, bend, phenyl ring).

^{1}H NMR ($CDCl_3$): δ 7.81 – 7.90 (2H, m, phth C-H), 7.68 – 7.77 (2H, m, phth C-H), 7.33 (d, *J* = 8.1 Hz, 2H, 2-H and 6-H phenyl ring), 7.27 (dd, *J* = 8.8/8.8 Hz, 2H, 3-H and 5-H phenyl ring), 7.04 (dd, *J* = 7.5/7.5 Hz, 1H, 4-H phenyl ring), 6.69 (br s, 1H, NH), 4.42 (t, *J* = 5.2 Hz, 2H, CH_2O), 4.02 (t, *J* = 5.5 Hz, 2H, NCH_2).

^{13}C NMR ($CDCl_3$): δ 168.32 (phth C=O), 153.17 (carbamate C=O), 137.72 (phenyl 1-C), 134.23, 123.55 (phth C-H), 132.12 (phth 4° C), 129.14 (phenyl 3-C and 5-C), 123.71 (phenyl 4-C), 118.90 (phenyl 2-C and 6-C), 62.55 (CH_2O), 37.48 (NCH_2).

m/z: HRMS (TOF ES$^+$) $C_{17}H_{15}N_2O_4$ $[MH]^+$ calcd 311.1026; found 311.1014.

2-Aminoethyl phenylcarbamate hydrochloride (103)



Phthalimide deprotection of 2-phthalimidoethyl phenylcarbamate **(102)** (348 mg, 1.13 mmol) was carried out as described for 1-(2-hydroxyphenyl)-3-(2-phthalimidoethyl)urea **(69a)** to give 180 mg of white solid requiring no further purification.

Yield: 74 %.

Mp: 176 – 180 °C.

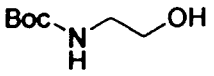
IR: 3361 (br, carbamate N-H, str), 3012 (br, $NH3^+$, str), 1711 (carbamate C=O, str), 1601 (aryl, str), 760, 688 (aryl C-H, bend, phenyl ring).

^{1}H NMR (DMSO-d_6): δ 9.73 (s, 1H, C=ONH), 8.26 (br s, 3H, $NH3^+$), 7.48 (d, *J* = 7.5 Hz, 2H, 2-H and 6-H phenyl ring), 7.28 (dd, *J* = 7.2/7.2 Hz, 2H, 3-H and 5-H phenyl ring), 7.00 (dd, *J* = 7.2/7.2 Hz, 1H, 4-H, phenyl ring), 4.29 (t, *J* = 5.6 Hz, 2H, CH_2O), 3.09 (dt, *J* = 5.6/5.2 Hz, 2H, $NH_3^+CH_2$).

^{13}C NMR (DMSO-d_6): δ 153.10 (C=O), 138.89 (4° C), 128.75, 122.57, 118.29 (phenyl C-H), 60.70 (CH_2O), 38.19 ($NH_3^+CH_2$).

m/z: HRMS (TOF ES$^+$) $C_9H_{13}N_2O_2$ $[MH]^+$ calcd 181.0972; found 181.0971.

tert-Butyl 2-hydroxyethylcarbamate (104)



Ethanolamine **(100)** (4.940 mL, 81.86 mmol) was dissolved in water (75 mL) and THF (35 mL). To this was added Boc_2O (19.650 g, 90.05 mmol, 1.1 eq) and the mixture stirred at room temperature overnight. After removal of THF *in vacuo*, the aqueous slurry was extracted with DCM (3 x 30 mL). The organic layer was concentrated to give 13.077 g of clear colourless oil.

Yield: 99%.

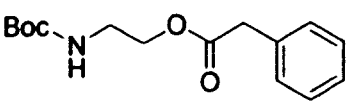
IR: 3360 (O-H, str), 2978, 2934 (alkyl C-H, str), 1689 (carbamate C=O, str), 1527 (carbamate-amide II* N-H bend), 1393, 1367 ($C(CH_3)_3$, str), 1252, 1173 (C-O, str). *nomenclature derived from hydrogen-bonded and non-hydrogen bonded bands shown with amides.

^{1}H NMR: δ 5.18 (br s, 1H, OH), 3.64 (dt, *J* = 5.5/4.9 Hz, 2H, CH_2OH), 3.26 – 3.35 (br m, 1H, NH), 3.23 (dt, *J* = 5.1/5.1 Hz, 2H, $NHCH_2$), 1.40 (s, 9H, $(CH_3)_3$).

^{13}C NMR: δ 156.91 (C=O), 79.67 (Boc 4° C), 62.31 (CH_2OH), 43.17 ($NHCH_2$), 28.46 ($(CH_3)_3$).

m/z: HRMS (TOF ES$^+$) $C_7H_{16}NO_3$ $[MH]^+$ calcd 162.1125; found 162.1129.

tert-Butyl 2-(2-phenylacetoyloxy)ethylcarbamate (105)



tert-Butyl 2-hydroxyethylcarbamate **(104)** was reacted with phenyl acetyl chloride as described in the procedure for *tert*-butyl 2-(2-phenylacetamido)ethylcarbamate **(88)**. The desired ester was recovered as 4.895 g of a yellow crystalline solid.

Yield: 94%.

Mp: 53 – 55 °C.

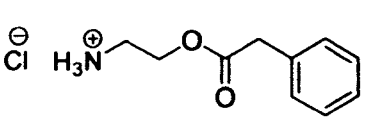
IR: 3399 (carbamate N-H, str), 2980, 2934, 2902 (alkyl C-H, str), 1730, (ester C=O, str), 1716 (carbamate C=O, str), 1522 (aryl, str), 1366 ($C(CH_3)_3$, str), 1251, 1162 (ester C-O, str), 762, 709 (aryl C-H bend, phenyl ring).

^{1}H NMR: δ 7.24 – 7.37 (m, 5H, aromatic C-H), 4.72 (br s, 1H, NH), 4.14 (t, *J* = 5.3 Hz, 2H, CH_2O), 3.64 (s, 2H, $(C=O)CH_2$), 3.32 – 3.40 (br m, 2H, $NHCH_2$), 1.44 (s, 9H, $(CH_3)_3$).

^{13}C NMR: δ 171.61 (C=O, ester), 155.85 (C=O, carbamate), 133.93 (aryl 4° C), 129.31 (aryl 2-C and 6-C), 128.74 (aryl 3-C and 5-C), 127.30 (aryl 4-C), 79.66 (Boc 4° C), 64.08 (CH_2O), 41.34 ($(C=O)CH_2$), 39.70 ($NHCH_2$), 28.46 ($(CH_3)_3$).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{22}NO_4$ $[MH]^+$ calcd 280.1543; found 280.1537.

2-Aminoethyl 2-phenylacetate hydrochloride (106)



tert-Butyl 2-(2-phenylacetoyloxy)ethyl carbamate **(105)** was Boc-deprotected in a similar fashion to phenyl-2-(*tert*-butyloxycarbonyl)aminoethylcarbamate **(86)** as described in the method for phenyl 2-aminoethylcarbamate hydrochloride **(87)**, to give 3.260 g of the desired hydrochloride salt as a beige crystalline solid.

Yield: 91%.

Mp: 81 – 83 °C.

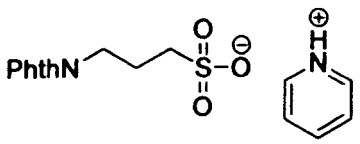
IR: 3059 (br, NH_3^+, str), 1748 (ester C=O, str), 1216, 1145 (ester C-O, str), 754, 704 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d$_6$): δ 8.30 (br s, 3H, NH_3^+), 7.19 – 7.42 (m, 5H, aromatic C-H), 4.25 (t, *J* = 4.9 Hz, 2H, CH_2O), 3.74 (s, 2H, $(C=O)CH_2$), 3.07 (br s, 2H, $CH_2NH_3^+$).

^{13}C NMR (DMSO-d$_6$): δ 171.11 (C=O), 134.15 (aryl 4° C), 129.51(aryl 2-C and 6-C), 128.30 (aryl 3-C and 5-C), 126.84 (aryl 4-C), 60.76 (CH_2O), 46.00 ($(C=O)CH_2$), 37.72 ($CH_2NH_3^+$).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{14}NO_2$ $[MH]^+$ calcd 180.1019; found 180.1031.

Pyridinium 3-phthalimidopropane-1-sulfonate (108)



3-Aminopropane sulfonic acid **(107)** (1.045 g, 7.51 mmol) and phthalic anhydride (1.112 g, 7.51 mmol, 1 eq) were dissolved in pyridine (20 mL) and refluxed at 120 °C for 2 hours. Removal of pyridine *in vacuo* and subsequent trituration of the crude solid with Et_2O, gave 2.369 g of white crystalline solid.

Yield: 91%.

Mp: 110 – 112 °C.

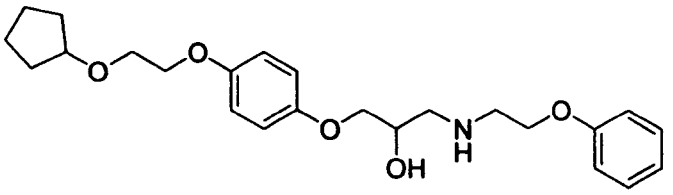
IR: 3068 (pyridinium N-H, str), 2981, 2940, 2877 (alkyl C-H, str), 1718 (phth C=O, str), 1613, 1544 (aryl, str), 1395, 1032 (sulfonate C-O, str), 756 (phth C-H bend), 770, 721 (pyridinium C-H, bend).

^{1}H NMR (DMSO-d_6): δ 8.93 (d, *J* = 5.1 Hz, 2H, pyridinium 2-H and 6-H), 8.57 (dddd, *J* = 7.8/7.8/1.6/1.6 Hz, 1H, pyridinium 4-H), 8.05 (dd, *J* = 7.8/6.6 Hz, 2H, pyridinium 3-H and 5-H), 7.78 – 7.89 (m, 4H, phth C-H), 3.62 (t, *J* = 7.4 Hz, 2H, NCH_2), 2.44 – 2.54 (m, 3H, $CH_2SO_3^-$, NH), 1.89 (tt, *J* = .4/7.4 Hz, 2H, $CH_2CH_2CH_2$).

^{13}C NMR (DMSO-d_6): δ 167.90 (phth C=O), 145.72 (pyridinium 4-C), 142.63 (pyridinium 2-C and 6-C), 134.30 (phth C-H), 131.59 (phth 4° C), 127.03 (pyridinium 3-C and 5-C), 122.94 (phth C-H), 49.05 ($CH_2SO_3^-$), 37.02 (NCH_2), 24.50 ($CH_2CH_2CH_2$).

m/z: HRMS (TOF ES$^+$) $C_{11}H_{12}NO_5S$ $[MH]^+$ calcd 270.0431; found 270.0404.

1-(2-Phenoxyethylamino)-3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)propan-2-ol (110a)



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (50 mg, 0.18 mmol) and 2-phenoxyethylamine (47 µL, 0.36 mmol, 2 eq) were dissolved in propan-2-ol (3 mL) before heating under reflux overnight. After removal of all solvent *in vacuo*, the crude residue was purified via PLC (eluent 37% aq NH_3/MeOH/DCM 2:5:93) to give 51 mg of white solid.

Yield: 68 %.

Mp: 155 – 157 °C.

IR: 3406 (O-H, str), 2930, 2864 (alkyl C-H, str), 1507 (aryl, str), 1109 (C-O, str), 762, 695 (aryl C-H bend, phenyl ring).

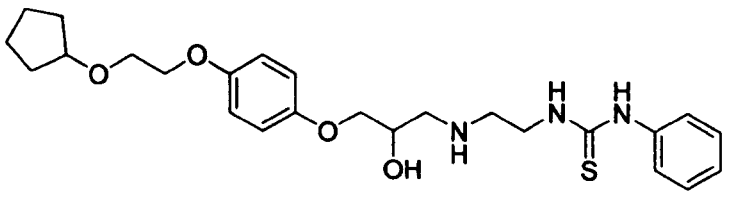
^{1}H NMR: δ 7.29 (dd, *J* = 8.7/7.5 Hz, 2H, phenoxy 3-H and 5-H), 6.96 (dd, *J* = 7.4/7.4 Hz, 1H, phenoxy 4-H), 6.91 (d, *J* = 7.7 Hz, 2H, phenoxy 2-H and 6-H), 6.80 – 6.86 (m, 4H, aryl-dioxy ring), 3.91 – 4.11 (m, 8H, OCH_2CH_2OAr, $NHCH_2CH_2OAr$, $ArOCH_2CH$, $CH(OH)$, ^{c}Pe CH), 3.72 (t, *J* = 5.2 Hz, 2H, $^c PeOCH_2$), 3.06 (t, *J* = 5.1 Hz, 2H, $NHCH_2CH_2OAr$), 2.94 (dd, *J* = 12.1/3.9 Hz, 1H, $CH(OH)CH_2NH$), 2.84 (dd, *J* = 12.2/7.9 Hz, 1H, $CH(OH)CH_2NH$), 1.44 – 1.84 (m, 8H, ^{c}Pe CH_2).

^{13}C NMR: δ 158.87 (phenoxy 1-C), 153.49, 153.04 (aryl-dioxy 4° C), 129.64 (phenoxy 3-C and 5-C), 121.07 (phenoxy 4-C), 115.83, 115.55 (aryl-dioxy C-H), 114.63 (phenoxy 2-C and 6-C), 82.10 (^{c}Pe CH), 71.17 ($ArOCH_2CH(OH)$), 68.57, 68.36 ($NHCH_2CH_2OAr$, $CH(OH)$), 67.44 ($^c PeOCH_2$), 67.32 ($^c PeOCH_2CH_2$), 51.76 ($CH(OH)CH_2NH$), 48.93 ($NHCH_2CH_2$), 32.41 (2-C and 5-C ^{c}Pe ring), 23.68 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{24}H_{34}NO_5$ $[MH]^+$ calcd 416.2431; found 416.2456.

HPLC R_t: 4.47 (System 1b), 12.64 (System 3).

1-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-phenylthiourea (110b)



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (50 mg, 0.18 mmol) was opened with 1-(2-aminoethyl)-3-phenylthiourea hydrochloride **(91)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:5:94) afforded 31 mg of white semi-solid.

Yield: 18 %.

IR: 3283 (O-H, str), 2931, 2869 (alkyl C-H, str), 1508 (aryl, str), 1315 (thiourea, str), 1110 (C=S, str), 810 (aryl C-H, bend, *para*-disubstituted ring), 765, 695 (aryl C-H bend, phenyl ring).

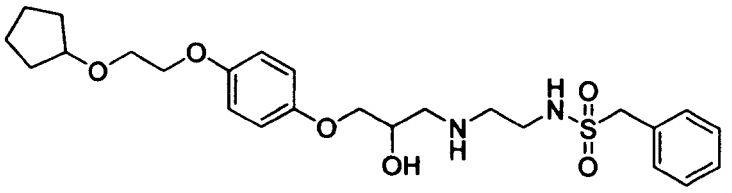
^{1}H NMR (DMSO-d_6): δ 9.66 (br s, 1H, NH(C=S)N*H*Ar), 7.73 (br s, 1H, N*H*(C=S)NHAr), 7.42 (d, *J* = 8.1 Hz, 2H, 2-H and 6-H phenyl ring), 7.29 (dd, *J* = 7.4/7.4 Hz, 2H, 3-H and 5-H phenyl ring), 7.08 (dd, *J* = 7.4/7.4 Hz, 1H, 4-H phenyl ring), 6.84 (s, 4H, aryl-dioxy ring), 4.99 (br s, 1H, OH), 3.75 – 4.03 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.62 (t, *J* = 4.9 Hz, 2H, ^{c}PeOCH_2), 3.55 (br s, 2H, NHCH$_2$CH_2), 2.75 (t, *J* = 6.3 Hz, 2H, NHCH_2CH$_2$), 2.67 – 2.73 (m, 1H, CH(OH)CH_2NH), 2.61 (dd, *J* = 11.8/5.9 Hz, 1H, CH(OH)CH_2NH), 1.39 – 1.76 (m, 8H, ^{c}Pe CH$_2$).

^{13}C NMR (DMSO-d_6): δ 180.26 (C=S), 152.72, 152.50 (aryl-dioxy 4° C), 141.37 (phenyl 1-C), 128.58 (phenyl 3-C and 5-C), 128.54 (phenyl 2-C and 6-C), 124.00 (phenyl 4-C), 115.34 (aryl-dioxy C-H), 80.83 (^{c}Pe CH), 71.18 (ArOCH$_2$), 68.03 (*C*H(OH)), 67.72 (CH$_2$OAr), 66.71 (^{c}PeOCH$_2$), 52.00 (CH(OH)CH$_2$NH), 51.10 (NHCH$_2$CH$_2$), 43.68 (NHCH$_2$CH$_2$), 31.78 (2-C and 5-C ^{c}Pe ring), 23.10 (3-C and 4-C ^{c}Pe ring),

m/z: HRMS (TOF ES$^+$) $C_{25}H_{36}N_3O_4S$ $[MH]^+$ calcd 474.2421; found 474.2433.

HPLC R_t: 4.25 (System 1b), 12.02 (System 3).

***N*-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-benzylsulfonamide (110c)**



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (50 mg, 0.18 mmol) was opened with *N*-(aminoethyl)benzylsulfonamide hydrochloride **(93)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:5:94) afforded 79 mg of white solid.

Yield: 45 %.

Mp: 104 – 106 °C.

IR: 3296 (sulfonamide N-H, str), 3239 (O-H, str), 2958, 2929, 2868 (alkyl C-H, str), 1510 (aryl, str), 1352, 1159 (sulfonamide, str), 1119 (C-O-C, str), 827 (aryl C-H, bend, *para*-disubstituted ring), 764, 697 (aryl C-H bend, phenyl ring)

^{1}H NMR (DMSO-d_6): δ 7.29 – 7.43 (m, 5H, C-H, phenyl ring), 6.84 (s, 4H, aryl-dioxy ring), 4.94 (br s, 1H, OH), 4.33 (s, 2H, SO_2CH_2), 3.74 – 4.02 (m, 6H, CH_2OAr, ^{c}Pe C*H*, C*H*(OH), ArOCH_2), 3.62 (t, *J* = 4.9 Hz, 2H, ^{c}PeOCH_2), 2.97 (t, *J* = 6.5 Hz, 2H, CH_2NHSO_2), 2.52 – 2.69 (m, 4H, NHCH_2CH_2, CH(OH)CH_2NH), 1.40 – 1.77 (m, 8H, ^{c}Pe CH_2).

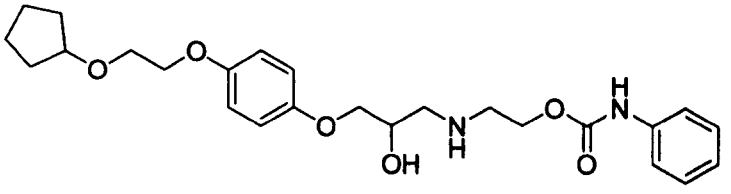
^{13}C NMR (DMSO-d_6): δ 152.75, 152.48 (aryl-dioxy 4° C), 130.78 (phenyl 2-C and 6-C), 130.46 (phenyl 1-C), 128.25 (phenyl 3-C and 5-C), 127.88 (phenyl 4-C), 115.33, 115.30 (aryl-dioxy C-H), 80.83 (^{c}Pe CH), 71.17 (ArOCH_2), 68.20 (CH(OH)), 67.71 (CH_2OAr), 66.71 (^{c}PeOCH_2), 57.20 (SO_2CH_2), 52.07 (CH(OH)CH_2NH), 49.35

($NHCH_2CH_2$), 42.69 ($NHCH_2CH_2$), 31.77 (2-C and 5-C ^cPe ring), 23.10 (3-C and 4-C ^cPe ring).

m/z: HRMS (TOF ES^+) $C_{25}H_{37}N_2O_6S$ $[MH]^+$ calcd 493.2367; found 493.2419.

HPLC R_t: 4.39 (System 1b), 12.05 (System 3).

2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl phenylcarbamate (110d)



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (50 mg, 0.18 mmol) was opened with 2-aminoethyl phenylcarbamate hydrochloride **(103)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:5:94) afforded 66 mg of white solid.

Yield: 40 %.

Mp: 94 – 96 °C.

IR: 3315 (O-H/N-H, str), 2957, 2926, 2871 (alkyl C-H, str), 1705 (carbamate C=O, str), 1539 (carbamate-amide II*, N-H bend), 1509 (aryl, str), 1092 (C-O, str), 824 (aryl C-H, bend, *para*-disubstituted ring), 765, 691 (aryl C-H bend, phenyl ring). *nomenclature derived from hydrogen-bonded and non-hydrogen bonded bands shown with amides.

^{1}H NMR (DMSO-d_6): δ 9.64 (s, 1H, carbamate NH), 7.46 (d, *J* = 7.3 Hz, 2H, 2-H and 6-H phenyl ring, 7.26 (dd, *J* = 7.3/7.3 Hz, 2H, 3-H and 5-H phenyl ring), 6.99 (dd, *J* = 7.3/7.3 Hz, 1H, 4-H phenyl ring), 6.84 (s, 4H, aryl-dioxy CH), 4.99 (br s, 1H, OH), 4.14 (t, *J* = 5.7 Hz, 2H, $CH_2OC{=}O$), 3.76 – 4.00 (m, 6H, CH_2OAr, ^cPe *CH*, *CH*(OH), $ArOCH_2$), 3.61 (t, *J* = 4.8 Hz, 2H, $^cPeOCH_2$), 2.82 (t, *J* = 5.7 Hz, 2H, $NHCH_2CH_2O$), 2.71 (dd,

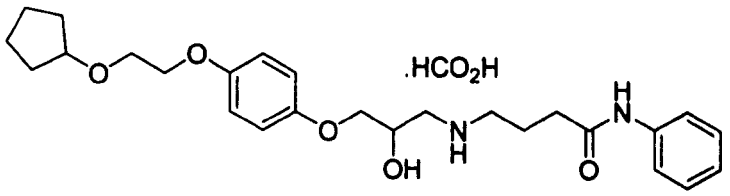
J = 11.9/3.8 Hz, 1H, CH(OH)C*H*$_2$), 2.61(dd, *J* = 11.5/6.1 Hz, 1H, CH(OH)C*H*$_2$), 1.41 – 1.75 (m, 8H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d$_6$): δ 153.56 (C=O), 152.72, 152.48 (aryl-dioxy 4° C), 139.18 (phenyl 1-C), 128.68 (phenyl 3-C and 5-C), 122.25 (phenyl 4-C), 118.11 (phenyl 2-C and 6-C), 115.32 (aryl-dioxy C-H), 80.82 (^{c}Pe CH), 71.16 (ArO*C*H$_2$), 68.18 (*C*H(OH)), 67.70 (*C*H$_2$OAr), 66.70 (^{c}PeO*C*H$_2$), 63.87 (NHCH$_2$*C*H$_2$O), 52.30 (CH(OH)*C*H$_2$NH), 48.20 (NH*C*H$_2$CH$_2$), 31.77 (2-C and 5-C ^{c}Pe ring), 23.10 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{25}H_{35}N_2O_6$ $[MH]^+$ calcd 459.2490; found 459.2128.

HPLC R$_t$: 4.47 (System 1b), 12.64 (System 3).

4-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)-N-phenylbutanamide hydroformate (110e)



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (50 mg, 0.18 mmol) was opened with 3-(phenylcarbamoyl)propylammonium trifluoroacetate **(97)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH$_3$/MeOH/DCM 2:8:90) and preparative HPLC afforded 19 mg of white semi-solid.

Yield: 23 %.

IR: 3419 (amide N-H, str), 2955, 2868 (alkyl C-H, str), 1662 (amide I, C=O str), 1509 (aryl, str), 1560 (amide II, N-H bend), 1111 (C-O, str), 757 (aryl C-H bend, phenyl ring).

^{1}H NMR (DMSO-d$_6$): δ 9.83 (s, 1H, amide NH), 8.28 (br s, 2H, NH_2^+), 7.58 (d, *J* = 7.6 Hz, 2H, phenyl 2-H and 6-H), 7.27 (dd, *J* = 7.8/7.8 Hz, 2H, phenyl 3-H and 5-H), 7.01 (dd, *J* = 7.4/7.4 Hz, 1H, phenyl 4-H), 6.84

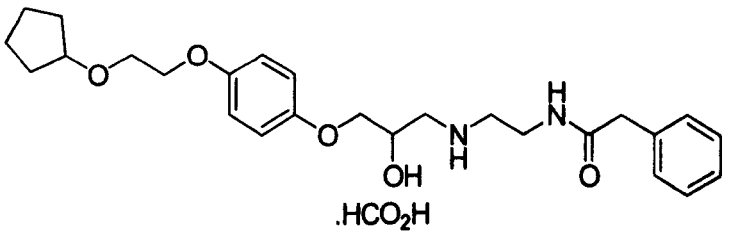
(s, 4H, aryl-dioxy CH), 3.98 (t, *J* = 4.8 Hz, 2H, CH_2OAr), 3.78 – 3.96 (m, 4H, $ArOCH_2$, C*H*(OH), ^cPe CH), 3.62 (t, *J* = 4.9 Hz, 2H, $^cPeOCH_2$), 2.76 (dd, *J* = 11.9/4.1 Hz, 1H, CH(OH)C*H*$_2$), 2.61 – 2.69 (m, 3H, CH(OH)C*H*$_2$, NHC*H*$_2$), 2.36 (t, *J* = 7.4 Hz, 2H, $CH_2C{=}O$), 1.72 – 1.82 (m, 2H, $CH_2CH_2CH_2$), 1.41 – 1.72 (m, 8H, ^cPe CH_2).

^{13}C NMR (DMSO-d$_6$): δ 170.95 (C=O), 152.66, 152.57 (aryl-dioxy 4° C), 139.30 (phenyl 1-C), 128.63 (phenyl 3-C and 5-C), 122.95 (phenyl 4-C), 119.04 (phenyl 2-C and 6-C), 115.36 (aryl-dioxy C-H), 80.85 (^cPe CH), 71.01 ($ArOCH_2$), 67.73 (CH_2OAr), 67.20 (CH(OH)), 66.72 ($^cPeOCH_2$), 51.60 ($CH(OH)CH_2NH$), 48.28 ($NHCH_2$), 33.97 ($CH_2C{=}O$), 31.79 (2-C and 5-C ^cPe ring), 24.26 ($CH_2CH_2CH_2$), 23.12 (3-C and 4-C ^cPe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{37}N_2O_5$ $[MH]^+$ calcd 457.2697; found 457.2716.

HPLC R$_t$: 4.30 (System 1b), 12.44 (System 3).

***N*-(2-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-2-phenylacetamide hydroformate (110f)**



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** (55 mg, 0.20 mmol) was opened with 2-(2-phenylacetamido)ethylammonium trifluoroacetate **(89)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 2:8:90) and preparative HPLC afforded 15 mg of white semi-solid.

Yield: 16 %.

IR: 3441 (amide N-H, str), 2956, 2870 (alkyl C-H, str), 1652 (amide I, C=O str), 1510 (aryl, str), 1539 (amide II, N-H bend), 1123 (C-O, str),

820 (aryl C-H, bend, *para*-disubstituted ring), 699 (aryl C-H bend, phenyl ring).

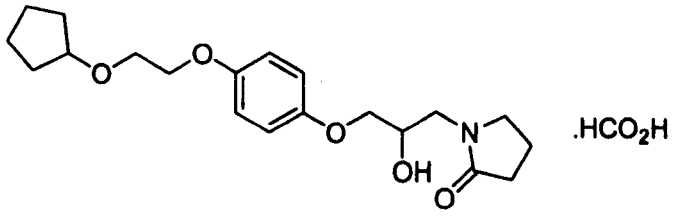
^{1}H NMR (DMSO-d_6): δ 8.08 (br s, 1H, amide NH), 7.17 – 7.32 (m, 5H, phenyl CH), 6.85 (s, 4H, aryl-dioxy CH), 3.98 (t, *J* = 4.8 Hz, 2H, CH_2OAr), 3.90 – 3.96 (m, 1H, ^{c}Pe CH), 3.77 – 3.90 (m, 3H, $ArOCH_2$, C*H*(OH)), 3.62 (t, *J* = 4.9 Hz, 2H, $^c PeOCH_2$), 3.40 (s, 2H, $C{=}OCH_2$), 3.12 – 3.20 (m, 2H, $CH_2NHC{=}O$), 2.56 – 2.77 (m, 4H, $CH(OH)CH_2NH$, CH_2NHCH_2), 1.41 – 1.76 (m, 8H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 170.17 (C=O), 152.71, 152.52 (aryl-dioxy 4° C), 136.44 (phenyl 1-C), 128.95, 128.15 (phenyl 2-C, 3-C, 5-C, 6-C), 126.25 (phenyl 4-C), 115.33 (aryl-dioxy C-H), 80.83 (^{c}Pe CH), 71.10 ($ArOCH_2$), 67.72 (CH_2OAr, CH(OH)), 66.71 ($^c PeOCH_2$), 51.85 ($CH(OH)CH_2NH$), 48.46 (CH_2NHCH_2), 42.36 ($C{=}OCH_2$), 38.45 ($CH_2NHC{=}O$), 31.77 (2-C and 5-C ^{c}Pe ring), 23.10 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{26}H_{37}N_2O_5$ $[MH]^+$ calcd 457.2697; found 457.2688.

HPLC R_t: 4.20 (System 1b), 12.14 (System 3).

1-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropyl)pyrrolidin-2-one hydroformate (113a)



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** was reacted with phenyl 4-aminobutanoate hydrochloride **(99)** under microwave conditions as described in the method for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-fluoro-4-hydroxyphenyl)urea hydroformate **(85a)**. Isolation of the major peak by preparative HPLC gave 10 mg of the title compound as white semi-solid. The desired phenyl 4-(3-(4-(2-

(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)butanoate **(112a)** was not isolated.

Yield: 7%.

IR: 3329 (O-H, str), 2933, 2870 (alkyl C-H, str), 1658 (lactam C=O, str), 1513 (aryl, str), 1025 (C-O-C, str), 828 (aryl C-H, bend, *para*-disubstituted ring).

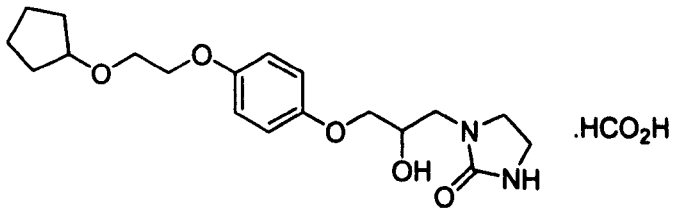
^{1}H NMR (DMSO-d_6): δ 6.82 – 6.87 (m, 4H, aryl-dioxy C-H), 5.16 (d, *J* = 5.4 Hz, 1H, OH), 3.98 (t, *J* = 4.7 Hz, 2H, CH_2OAr) 3.90 – 3.96 (m, 2H, ^{c}Pe CH, C*H*(OH)), 3.74 – 3.83 (m, 2H, $ArOCH_2$), 3.62 (t, *J* = 4.7 Hz, 2H, $^c PeOCH_2$), 3.37 – 3.49 (m, 2H, $CH(OH)CH_2NCH_2$), 3.28 – 3.36 (m, 1H, $CH(OH)CH_2N$), 3.23 (dd, *J* = 13.1/7.3 Hz, 1H, $CH(OH)CH_2N$), 2.20 (t, *J* = 8.0Hz, 2H, $(C=O)CH_2$), 1.90 (tt, *J* = 7.6/7.6 Hz, 2H, $NCH_2CH_2CH_2(C=O)$), 1.41 – 1.76 (m, 8H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 174.26 (C=O), 152.61 (aryl-dioxy 4° C), 115.39, 115.36 (aryl-dioxy C-H), 80.83 (^{c}Pe CH), 71.02 ($ArOCH_2$), 67.71 (CH_2OAr), 67.10 (CH(OH)), 66.70 ($^c PeOCH_2$), 47.92 ($CH(OH)CH_2NCH_2$), 45.75 ($CH(OH)CH_2N$), 31.77 (2-C and 5-C ^{c}Pe ring), 30.34 ($CH_2(C=O)$), 23.10 (3-C and 4-C ^{c}Pe ring), 17.72 ($NCH_2CH_2CH_2(C=O)$).

m/z: HRMS (TOF ES$^+$) $C_{20}H_{30}NO_5$ $[MH]^+$ calcd 364.2118; found 364.2124.

1-(3-(4-(2-(Cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropyl)imidazolidin-2-one

hydroformate (113b)



2-((4-(2-(Cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)** was reacted with phenyl 2-aminoethylcarbamate hydrochloride **(87)** under microwave conditions as described in the method for 1-(2-(3-(4-(2-

(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-fluoro-4-hydroxyphenyl)urea hydroformate **(85a)**. Isolation of the major peak by preparative HPLC gave 9 mg of the title compound as white semi-solid. The desired phenyl 2-(3-(4-(2-(cyclopentyloxy)ethoxy) phenoxy)-2-hydroxypropylamino)ethylcarbamate **(112b)** was not isolated.

Yield: 6%.

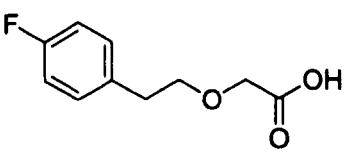
IR: 3390 (O-H, str), 2956, 2871 (alkyl C-H, str), 1667 (imidazolidinone C=O, str), 1509 (aryl, str), 1040 (C-O-C, str), 821 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 6.83 – 6.87 (m, 4H, aryl-dioxy C-H), 6.30 (br s, 1H, NH), 5.15 (d, *J* = 5.0 Hz, 1H, OH), 3.98 (t, *J* = 4.6 Hz, 2H, CH_2OAr) 3.85 – 3.96 (m, 2H, ^{c}Pe CH, C*H*(OH)), 3.73 – 3.85 (m, 2H, $ArOCH_2$), 3.62 (t, *J* = 4.6 Hz, 2H, $^c PeOCH_2$), 3.35 – 3.49 (m, 2H, $CH(OH)CH_2NCH_2$), 3.16 – 3.26 (m, 3H, $CH(OH)CH_2N$, $CH_2NH(C=O)$), 3.07 (dd, *J* = 13.6/6.8 Hz, 1H, $CH(OH)CH_2N$), 1.41 – 1.76 (m, 8H, ^{c}Pe CH_2).

^{13}C NMR (DMSO-d_6): δ 162.53 (C=O), 152.66, 152.56 (aryl-dioxy 4° C), 115.37, 115.33 (aryl-dioxy C-H), 80.83 (^{c}Pe CH), 70.94 ($ArOCH_2$), 67.88 (CH(OH)), 67.68 (CH_2OAr), 66.71 ($^c PeOCH_2$), 46.93 ($CH(OH)CH_2N$), 46.13 ($CH(OH)CH_2NCH_2$), 37.58 ($CH_2NH(C=O)$), 31.77 (2-C and 5-C ^{c}Pe ring), 23.12 (3-C and 4-C ^{c}Pe ring).

m/z: HRMS (TOF ES$^+$) $C_{19}H_{29}N_2O_5$ $[MH]^+$ calcd 365.2071; found 365.2090.

2-(4-Fluorophenethyloxy)acetic acid (115)



NaH 60% suspension in mineral oil (2.400 g, equivalent to 1.440 g of NaH, 60 mmol, 2 eq) was weighed into a flame-dried flask and suspended in dry DMF (60 mL) with stirring, under a nitrogen atmosphere. To this was added 4-fluorophenethyl alcohol **(114)** (4.205 g, 3.751 mL, 30 mL) and the temperature was raised to 60 °C with stirring for 15 minutes. Chloroacetic acid (2.835 g, 30 mmol, 1 eq) was

added to the flask and the mixture allowed to stir at 60 °C for a further 2.5 hours. After cooling and removal of solvent, the residue was suspended in Et_2O (30 mL) and extracted with water (2 x 30 mL). The combined aqueous layers were acidified with aqueous 2 M HCl (to around pH 3) before extraction with EtOAc (3 x 30 mL). After removal of solvent, the crude solid was recrystallised from cyclohexane to yield 3.000 g of pink crystals.

Yield: 50%.

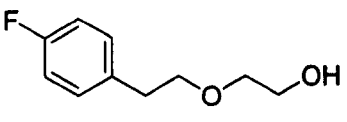
Mp: 84 – 86 °C (lit. 82 – 85 °C)[98].

IR: 2964, 2893 (alkyl C-H, str), 2772, 2677, 2571 (O-H, str, carboxylic acid), 1733 (carboxylic acid), 1510 (aryl, str), 1137 (C-F, str), 1103 (C-O-C, str), 840 (aryl C-H, bend, *para*-disubstituted ring), 770 (C-F, weak).

^{1}H NMR: δ 8.5 – 10.4 (br s, 1H, CO_2H), 7.19 (dd, J = 8.6/5.7 Hz, 2H, aryl 3-H and 5-H), 6.99 (dd, J = 8.6/8.6 Hz, 2H, aryl 2-H and 6-H), 4.12 (s, 2H, CH_2CO_2H), 3.76 (t, J = 6.8 Hz, 2H, CH_2O), 2.92 (t, J = 6.8 Hz, 2H, $ArCH_2$).

m/z: HRMS (TOF ES$^-$) $C_{10}H_{10}FO_3$ $[M-H]^-$ calcd 197.0619; found 197.0629.

2-(4-Fluorophenethyloxy)ethanol (116)



Lithium aluminium hydride (472 mg, 12.45 mmol, 1 eq) was suspended in anhydrous THF (15 mL) over ice with stirring. 2-(4-fluorophenethyloxy)acetic acid **(115)** (2.467 g, 12.45 mmol) in anhydrous THF (15 mL) was slowly dripped in the suspension over 10 minutes and the resulting mixture stirred overnight at room temperature under a nitrogen atmosphere. After quenching carefully with water, the suspension was filtered (gravity) and the filtrate concentrated to an oil. Purification was achieved by FCC (eluent EtOAc/hexanes 60:40), yielding 1.52 g of clear, colourless oil.

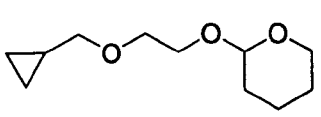
Yield: 67%.

IR: 3417 (br, O-H, str), 2922, 2870 (alkyl C-H, str), 1510 (aryl, str), 1117 (C-O-C, str), 830 (aryl C-H, *para*-disubstituted ring).

^{1}H NMR: δ 7.15 (dd, *J* = 8.6/5.5 Hz, 2H, aryl 3-H and 5-H), 6.95 (dd, *J* = 8.8/8.8 Hz, 2H, aryl 2-H and 6-H), 3.65 – 3.72 (m, 2H, CH_2OH), 3.65 (t, *J* = 7.0 Hz, 2H, $ArCH_2CH_2$), 3.53 (t, *J* = 4.8 Hz, 2H, OCH_2CH_2OH), 2.85 (t, *J* = 7.0 Hz, 2H, $ArCH_2$), 2.38 (br s, 1H, OH).

m/z: HRMS (TOF ES$^-$) $C_{11}H_{14}FO_4$ $[M+HCO_2]^-$ calcd 229.0882; found 229.0883.

2-(2-(Cyclopropylmethoxy)ethoxy)-tetrahydro-*2H*-pyran (118)



NaH 60% suspension in mineral oil (6.659 g, equivalent to 3.995 g of NaH, 0.166 mol, 1.2 eq) was weighed into a flame-dried flask and washed with hexanes (2 x 50 mL) under nitrogen atmosphere. Residual hexanes were allowed to evaporate under nitrogen flow before suspending the NaH in dry THF and cooling to 0 °C. Cyclopropylmethanol **(117)** (10.000 g, 0.139 mol) was dissolved in dry THF (20 mL) and dry DMF (30 mL) before adding dropwise over 30 minutes to the suspended NaH with stirring. The mixture was brought to room temperature before dropwise addition of 2-chloroethoxytetrahydro-*2H*-pyran (30.71 mL 0.208 mol 1.5 eq) in dry THF (20 mL) over 30 minutes. The mixture was stirred at room temperature overnight before quenching with MeOH (20 mL). All solvents were removed before dissolving the residue in Et_2O (200 mL) and washing with water (2 x 150 mL) and brine (150 mL). After removal of solvent, the resulting crude oil was purified by FCC (eluent DCM) to give 5.878 g of colourless oil.

Yield: 21%.

IR: 2941, 2869 (alkyl C-H, str), 1124 (C-O-C, str).

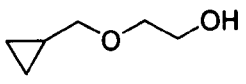
^{1}H NMR: δ 4.65 (t, *J* = 3.6 Hz, 1H, CH THP group), 3.85 – 3.90 (m, 2H, OCH_2 THP group, $THPOCH_2$), 3.59 – 3.67 (m, 3H, $THPOCH_2$, $CH_2OCH_2{}^cPr$), 3.48 – 3.53 (OCH_2 THP group), 3.34 (dd, *J* = 24.2/6.9 Hz, 1H, $OCH_2{}^cPr$), 3.34 (dd, *J* = 6.9/3.5 Hz, 1H, $OCH_2{}^cPr$), 1.49 – 1.86

(m, 6H, 3 x CH_2 THP group), 1.02 – 1.12 (m, 1H, ^cPr CH), 0.51 – 0.55 (m, 2H, ^cPr CH_2), 0.19 – 0.26 (m, 2H, ^cPr CH_2)*. *Refers to cis-protons of ^cPr ring.

^{13}C NMR: δ 98.88 (CH THP group), 75.92 ($OCH_2{}^cPr$), 69.65 ($CH_2OCH_2{}^cPr$), 66.66 ($THPOCH_2$), 62.16 (OCH_2 THP group), 30.53 ($OCHCH_2$ THP group), 25.41 (OCH_2CH_2 THP group), 19.46 (OCH CH_2CH_2 THP group), 10.54 (^cPr CH), 3.00, 2.91 (^cPr CH_2).

m/z: HRMS (TOF ES^+) $C_{11}H_{21}O_3$ $[MH]^+$ calcd 201.1485; found 201.1488.

2-(Cyclopropylmethoxy)ethanol (119)



2-(2-(Cyclopropylmethoxy)ethoxy)-tetrahydro-*2H*-pyran **(118)** (1.800 g, 8.99 mmol) was diluted in EtOH (60 mL). PPTS (226 mg, 0.90 mmol, 0.1 eq) in EtOH (15 mL) was added and the solution stirred at 55 °C for 4 hours. Excess solvent was removed and on dilution of the residue with PE/Et_2O (15:85), PPTS precipitated out. Following filtration of PPTS the remaining crude product was purified by FCC (eluent pet ether 40°-60°C/Et_2O 15:85) to afford 670 mg of colourless oil.

Yield: 79%.

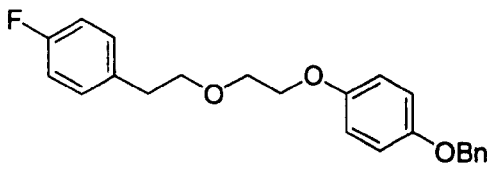
IR: 3409 (br, O-H, str), 3081 (^cPr C-H, str), 2865 (alkyl C-H, str), 1430 (C-H, deformation (def)), 1117 (C-O-C, str), 1070 (C-OH, str).

^1H NMR: δ 3.73 – 3.77 (m, 2H, CH_2OH), 3.58 (t, *J* = 4.9 Hz, 2H, CH_2CH_2OH), 3.33 (d, *J* = 7.2 Hz, 2H, $^cPrCH_2O$), 2.06 (t, *J* = 6.6 Hz, 1H, OH), 1.08 (m, 1H, CH), 0.53 – 0.58 (m, 2H, ^cPr CH_2)*, 0.20 – 0.24 (m, 2H, ^cPr CH_2)*. *Refers to cis-protons of ^cPr ring.

^{13}C NMR: δ 75.98 ($^cPrCH_2O$), 71.48 (OCH_2CH_2OH), 61.87 (CH_2OH), 10.53 (CH ^cPr), 2.95 (^cPr CH_2).

m/z: HRMS (TOF ES^+) $C_6H_{12}NaO_2$ $[MNa]^+$ calcd 139.0730; found 139.0745.

1-(2-(4-Fluorophenethyloxy)ethyloxy)-4-(benzyloxy)benzene (122a)



2-(4-Fluorophenethyloxy)ethanol **(116)** was reacted with 4-(benzyloxy)phenol as described in the method for 1-(2-(cyclopentyloxy)ethoxy)-4-(benzyloxy)benzene **(60)**. After work-up, the organic layer was concentrated and purified by FCC (eluent EtOAc/hexanes 15:85) to give 999 mg of white crystalline solid.

Yield: 35%.

Mp: 59 - 61°C.

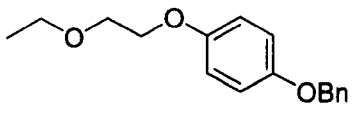
IR: 2864, 2929 (alkyl C-H, str), 1509 (aryl, str), 1119 (C-O-C, str). 827 (aryl C-H, bend, *para*-disubstituted ring), 739, 698 (aryl C-H, bend, phenyl ring).

^{1}H NMR: δ 7.30 – 7.45 (m, 5H, aromatic benzyl CH), 7.19 (dd, *J* = 8.6/5.5 Hz, 2H, 3-H and 5-H of fluorophenyl ring), 6.97 (dd, *J* = 8.8/8.8 Hz, 2H, 2-H and 6-H of flurophenyl ring), 6.91, 6.85 (d, *J* = 9.2 Hz, 2 x 2H, *para*-disubstituted aryl-dioxy ring), 5.02 (s, 2H, PhCH_2O), 4.06 (t, *J* = 4.7 Hz, 2H, CH_2OArOBn), 3.78 (t, *J* = 4.7 Hz, 2H, CH_2CH$_2$OArOBn), 3.72 (t, *J* = 7.1 Hz, 2H, FC$_6$H$_4$CH$_2$CH_2O), (t, *J* = 7.1 Hz, 2H, FC$_6$H$_4$CH_2)

^{13}C NMR: δ 161.64 (J_{CF} = 244.1 Hz, CF), 153.26 (2 x 4° C, aryl-dioxy ring), 137.38 (4° benzyl C), 134.69 (J_{CF} = 3.0 Hz, 4-C fluorophenyl ring), 130.44 (J_{CF} = 7.7 Hz, 3-C and 5-C fluorophenyl ring), 128.68, 128.02, 127.61 (benzyl CH), 115.77, 115.90 (CH aryl-dioxy ring), 115.22 (J_{CF} = 20.8 Hz, 2-C and 6-C fluorophenyl ring), 72.48 (FC$_6$H$_4$CH$_2$CH$_2$O), 70.78 (benzyl CH$_2$), 69.63 (CH$_2$CH$_2$OArOBn), 68.19 (CH$_2$OArOBn), 35.55 (FC$_6$H$_4$CH$_2$).

m/z: HRMS (TOF ES$^+$) $C_{23}H_{24}FO_3$ [MH]$^+$ calcd 367.1704; found 367.1698.

1-((4-(2-Ethoxyethoxy)phenoxy)methyl)benzene (122b)



2-Ethoxyethanol **(120)** was reacted with 4-(benzyloxy)phenol as described in the method for 1-(2-(cyclopentyloxy)ethoxy)-4-(benzyloxy)benzene **(60)**. DEAD was used instead of DBAD as the azodicarboxylate. After work-up, the organic layer was concentrated and purified by FCC (eluent EtOAc/hexanes 20:80) to give 2.314 g of white crystalline solid.

Yield: 85%.

Mp: 32.5 - 34.5 °C (lit: 35 – 37 °C)[180].

IR: 2975, 2925, 2864 (alkyl C-H, str), 1509 (aryl, str), 1126 (C-O-C, str), 826 (aryl C-H, bend, *para*-disubstituted ring), 732, 693 (aryl C-H, bend, phenyl ring).

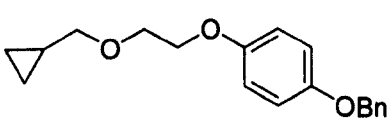
^{1}H NMR: δ 7.25 – 7.32 (m, 5H, aromatic benzyl CH), 6.90, 6.86 (d, J = 9.2 Hz, 2 x 2H, *para*-disubstituted aryl ring), 5.01 (s, 2H, PhCH_2O), 4.07 (t, J = 4.9 Hz, 2H, CH_2OArOBn), 3.77 (t, J = 4.9 Hz, 2H, CH_2CH$_2$OArOBn), 3.60 (q, J = 7.0 Hz, 2H, CH$_3$CH_2O), 1.24 (t, J = 7.0 Hz, 3H CH_3).

^{13}C NMR: δ 153.32, 153.21 (4° aryl C), 137.40 (4° benzyl C), 128.67, 128.01, 127.62 (benzyl CH), 115.86, 115.75 (aryl CH), 70.77 (benzyl CH$_2$), 69.20, 68.21, 66.96 (CH$_2$O), 15.31 (CH$_3$).

m/z: HRMS (TOF ES$^+$) $C_{17}H_{21}O_3$ [MH]$^+$ calcd 273.1485; found 273.1506.

1-(2-(Cyclopropylmethoxy)ethoxy)-4-(benzyloxy)benzene (122c)

Method A



2-(Cyclopropylmethoxy)ethanol **(119)** was reacted with 4-(benzyloxy)phenol as described in the method for 1-(2-(cyclopentyloxy)ethoxy)-4-(benzyloxy)benzene **(60)**. DEAD was used instead of DBAD as the azodicarboxylate. After work-up, the organic layer was concentrated and purified by FCC (eluent EtOAc/hexanes 15:85) to give 843 mg of white waxy solid.

Yield: 82%.

Method B

1-((4-(2-(Allyloxy)ethoxy)phenoxy)methyl)benzene **(122d)** (2.000 g, 7.03 mmol) underwent Simmons-Smith cyclopropanation as described for 5-(2-(allyloxy)ethoxy)-2-(4-methoxybenzyloxy)benzonitrile **(50)** in the synthesis of 5-(2-(cyclopropylmethoxy)ethoxy)-2-(4-methoxybenzyloxy) benzonitrile **(51)**. After overnight stirring, the reaction was quenched with aqueous saturated ammonium chloride solution (100 mL), before extraction with DCM (3 x 50 mL). The combined organic extracts were washed with aqueous saturated $NaHCO_3$ solution (1 x 30 mL) and water (1 x 30 mL). After removal of solvent *in vacuo*, the residue was passed through a silica plug (eluent EtOAc) to remove inorganic impurities. Concentration of these washings gave 2.044 g of yellow crystalline solid.

Yield: 97%.

Mp: 30.5 – 32.5 °C

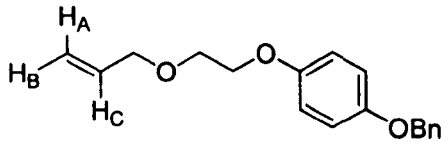
IR: 2920, 2862 (alkyl C-H, str), 1509 (aryl, str), 1137, 1114 (C-O-C, str), 827 (aryl C-H, bend, *para*-disubstituted ring), 732, 693 (aryl C-H, bend, phenyl ring).

^{1}H NMR: δ 7.33 – 7.45 (m, 5H, aromatic benzyl CH), 6.95, 6.91 (d, J = 9.2 Hz, 2 x 2H, *para*-disubstituted aryl ring), 5.01 (s, 2H, PhCH_2O), 4.10 (t, J = 5.0 Hz, 2H, CH_2OArOBn), 3.82 (t, J = 5.0 Hz, 2H, CH_2CH$_2$OArOBn) 3.42 (d, J = 6.8 Hz, 2H, ^{c}PrCH$_2$O) 1.10 – 1.20 (m, 1H, CH), 0.54 – 0.66 (m, 2H, ^{c}Pr CH$_2$)*, 0.23 – 0.34 (m, 2H, ^{c}Pr CH$_2$)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR: δ 153.30, 153.19 (4° aryl C), 137.39 (4° benzyl C), 128.67, 128.47, 128.00 (benzyl CH), 115.85, 115.73 (aryl CH), 76.36 (^{c}PrCH$_2$O), 70.76 (benzyl CH$_2$), 69.09 (CH$_2$CH$_2$OArOBn), 68.20 (CH$_2$CH$_2$OAr), 10.69 (^{c}Pr CH), 3.18 (^{c}Pr CH$_2$).

m/z: HRMS (TOF ES$^+$) $C_{19}H_{23}O_3$ [MH]$^+$ calcd 299.1642; found 299.1636.

1-((4-(2-(Allyloxy)ethoxy)phenoxy)methyl)benzene (122d)



2-Allyloxyethanol **(121)** was reacted with 4-(benzyloxy)phenol in similar conditions as described for 1-(2-(cyclopentyloxy)ethoxy)-4-(benzyloxy)benzene **(60)**. DIAD was used in the place of DBAD. The desired product was obtained as 4.021 g of white crystalline solid, after purification by FCC (eluent Et_2O/PE 25:75).

Yield: 71%.

Mp: 35 – 38 °C.

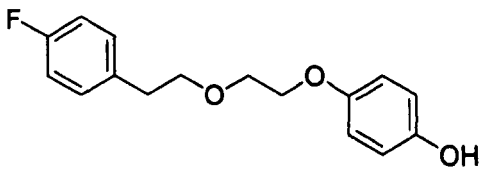
IR: 3064 (alkene C-H, str), 2928, 2863 (alkyl C-H, str), 1510 (aryl, str), 1115 (C-O-C, str), 919 (alkene C-H, deformation), 826 (aryl C-H, bend, *para*-disubstituted ring), 735, 694 (aryl C-H, bend, phenyl ring).

^{1}H NMR: δ 7.31 – 7.51 (m, 5H, aromatic benzyl C-H), 6.95, 6.91 (d, J = 9.1/1.9 Hz, 2 x 2H, *para*-disubstituted aryl ring), 5.93 – 6.06 (m, 1H, H_C), 5.37 (d, J = 17.2 Hz, 1H, H_A), 5.26 (d, J = 10.2 Hz, 1H, H_B), 5.04 (s, 2H, benzyl CH_2), 4.07 – 4.17 (m, 4H, CH_2OAr, CH_2=CHCH_2), 3.81 (t, J = 4.3 Hz, 2H, allyl-OCH_2).

^{13}C NMR: δ 153.19, 153.13 (aryl-dioxy 4° C), 137.31 (benzyl 4° C), 134.66 (CH_2=*CH*), 128.52 (aromatic benzyl 3-C and 5-C), 127.84 (aromatic benzyl 4-C), 127.45 (aromatic 2-C and 6-C), 117.19 (*CH_2*=CH), 115.77, 115.62 (aryl-dioxy C-H), 72.30 (CH_2=CH*CH_2*), 70.59 (benzyl CH_2), 68.63, 68.06 (OCH_2CH_2O).

m/z: HRMS (TOF ES$^+$) $C_{18}H_{21}O_3$ $[MH]^+$ calcd 285.1485; found 285.1490.

4-(2-(4-Fluorophenethyloxy)ethyloxy)phenol (123a)



1-(2-(4-Fluorophenethyloxy)ethyloxy)-4-(benzyloxy)benzene **(122a)** was hydrogenated according to the procedure for synthesis of 4-(2-(cyclopentyloxy)ethoxy)phenol **(61)**. After filtration over celite and evaporation of volatiles, no further workup

was required and the desired compound was isolated in quantitative yield as clear oil.

Yield: 100%.

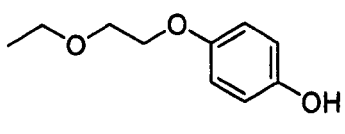
IR: 3366 (br, O-H, str), 2925, 2871 (alkyl C-H, str), 1510 (aryl, str), 1222 (C-F), 1121 (C-O-C, str), 828 (aryl C-H, bend, *para*-disubstituted ring), 738 (C-F)

^{1}H NMR: δ 7.18 (dd, *J* = 8.6/5.5 Hz, 2H, 3-H and 5-H of fluorophenyl ring), 6.96 (dd, *J* = 8.8/8.8 Hz, 2H, 2-H and 6-H of flurophenyl ring), 6.74, 6.76 (d, *J* = 9.2 Hz, 2 x 2H, *para*-disubstituted phenol), 6.00 (br s, 1H, OH), 4.05 (t, *J* = 4.7 Hz, 2H, CH_2OAr), 3.80 (t, *J* = 4.9 Hz, 2H, CH_2CH_2OAr), 3.75 (t, *J* = 7.2 Hz, 2H, $FC_6H_4CH_2CH_2O$), 2.90 (t, *J* = 7.1 Hz, 2H, $FC_6H_4CH_2$).

^{13}C NMR: δ 161.59 (J_{CF} = 245.0 Hz, CF), 152.64, 150.08 (4° C, aryl-dioxy ring), 134.43 (J_{CF} = 3.1 Hz, 4-C fluorophenyl ring), 130.40 (J_{CF} = 8.0 Hz, 3-C and 5-C fluorophenyl ring), 116.19, 115.92 (CH phenolic ring), 115.19 (J_{CF} = 21.1 Hz, 2-C and 6-C fluorophenyl ring), 72.48 ($FC_6H_4CH_2CH_2O$), 69.58 (CH_2CH_2OAr), 68.13 (CH_2OAr), 35.34 ($FC_6H_4CH_2$).

m/z: HRMS (TOF ES$^-$) $C_{16}H_{16}FO_3$ [M-H]$^-$ calcd 275.1089; found 275.1090.

4-(2-Ethoxyethoxy)phenol (123b)



1-((4-(2-Ethoxyethoxy)phenoxy)methyl) benzene **(122b)** (904 mg, 3.32 mmol) was dissolved in EtOH (60 mL) before hydrogenating over 10% Pd/C (168 mg) at room temperature and atmospheric pressure for 48 hours. The suspension was filtered over celite and washed with excess EtOH. Removal of excess solvent gave viscous amber oil. The crude oil was dissolved in DCM (20 mL) and washed with aqueous 2 M NaOH solution (3 x 20 mL). The combined aqueous extracts were acidified with concentrated HCl (until the pH was below 7) to effect an emulsion, before extracting with DCM (3 x 30 mL). The combined organic layers were washed with water (1 x 30 mL) and

brine (1 x 30 mL). Solvent removal afforded 413 mg of clear, colourless oil.

Yield: 68%.

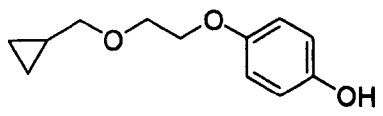
IR: 3365 (br, O-H, str), 2976, 2873 (alkyl C-H, str), 1602, 1511 (aryl, str), 1105 (C-O-C, str), 826 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 6.81, 6.74 (d, *J* = 9.0 Hz, 2H, aryl-H), 4.57 (br s, 1H OH); 4.06 (t, *J* = 4.9 Hz, 2H, C*H*$_2$OAr), 3.77 (t, *J* = 4.9 Hz, 2H, C*H*$_2$CH$_2$OAr), 3.60 (q, *J* = 7.0 Hz, 2H, CH$_3$C*H*$_2$O), 1.24 (t, *J* = 7.0 Hz, 3H C*H*$_3$).[132]

^{13}C NMR: δ 152.54, 150.05 (4° aryl C), 116.12, 115.80 (aryl CH), 69.11, 68.03, 66.99 (CH_2O), 15.06 (CH_3).

m/z: HRMS (TOF ES$^-$) $C_{10}H_{13}O_3$ $[M-H]^-$ calcd 181.0870; found 181.0890.

4-(2-(Cyclopropylmethoxy)ethoxy)phenol (123c)



1-(2-(Cyclopropylmethoxy)ethoxy)-4-(benzyloxy)benzene **(122c)** (840 mg, 2.82 mmol) was hydrogenated according to the method for 4-(2-(cyclopentyloxy)ethoxy)phenol **(61)**. After 4 hours of stirring, the suspension was filtered over celite and washed with excess EtOH. Excess solvent was removed to give amber oil. The crude oil was purified by FCC (eluent EtOAc/hexanes 30/70) to give 508 mg of colourless oil.

Yield: 100%.

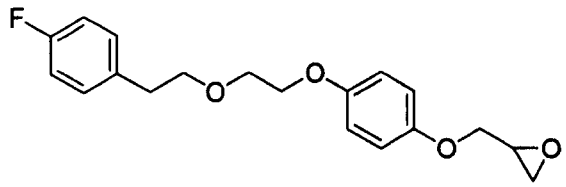
IR: 3367 (br, O-H, str), 2873 (alkyl C-H, str), 1510 (aryl, str), 1448 (C-H, def), 1101 (C-O-C, str), 826 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 6.72 – 6.77 (m, 4H, aryl-H), 5.70 – 5.50 (br s, 1H, OH), 4.06 (t, *J* = 5.0 Hz, 2H CH_2OAr), 3.81 (t, *J* = 5.0 Hz, 2H, C*H*$_2$CH$_2$OAr), 3.40 (d, *J* = 6.8 Hz, 2H, ^{c}PrC*H*$_2$O), 1.07 – 1.11 (m, 1H, ^{c}Pr CH), 0.48 – 0.60 (m, 2H, ^{c}Pr CH_2)*, 0.17 – 0.28 (m, 2H, ^{c}Pr CH_2)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR: δ 152.69, 149.90 (4° aryl C), 116.07, 115.76 (aryl CH), 76.38 (^{c}Pr*C*H$_2$O), 69.02 (*C*H$_2$CH$_2$OAr), 68.05 (CH$_2$OAr), 10.50 (^{c}Pr CH), 3.14 (^{c}Pr *C*H$_2$).

m/z: HRMS (TOF ES$^-$) $C_{12}H_{15}O_3$ [M-H]$^-$ calcd 207.1027; found 207.1026.

2-((4-(2-(4-Fluorophenethyloxy)ethyloxy)phenoxy)methyl)oxirane (124a)



NaH 60% suspension in mineral oil (13 mg, equivalent to 7.8 mg of NaH, 0.33 mmol, 1.1 eq) was suspended in dry DMF (2 mL) with stirring, under a nitrogen atmosphere. To this was added 4-(2-(4-fluorophenethyloxy)ethyloxy)phenol **(123a)** (82 mg, 0.30 mmol) in dry DMF (4 mL) and stirred until no further hydrogen gas evolution was visible. *rac*-Epichlorohydrin (800 µL, 10.22 mmol, 34 eq) was added and the reaction stirred overnight at room temperature. The reaction mixture was diluted with water (30 mL) before extraction with Et$_2$O (3 x 30 mL). The combined organic extracts were concentrated before purification over a silica plug (initial wash with hexanes, followed by EtOH/DCM 5:95) to give 70 mg of clear yellow oil.

Yield: 71%.

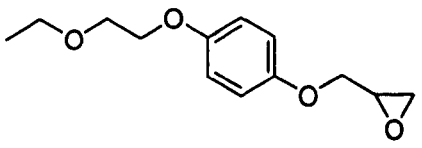
IR: 3051 (epoxide C-H, str, weak), 2871, 2923 (alkyl C-H, str), 1508 (aryl, str), 1229 (C-F), 1124 (C-O-C, str), 827 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 7.19 (dd, *J* = 8.6/5.6 Hz, 2H, 3-H and 5-H of fluorophenyl ring), 6.96 (dd, *J* = 8.8/8.8 Hz, 2H, 2-H and 6-H of flurophenyl ring), 6.85 (s, 4H, aryl-dioxy ring), 4.17 (dd, *J* = 11.1/3.2 Hz, 1H, ArOC*H*$_2$CH), 4.06 (t, *J* = 4.7 Hz, 2H, C*H*$_2$OAr), 3.90 (dd, *J* = 11.0/5.7 Hz, 1H, ArOC*H*$_2$CH), 3.78 (t, *J* = 4.9 Hz, 2H, C*H*$_2$CH$_2$OAr), 3.72 (t, *J* = 7.1 Hz, 2H, FC$_6$H$_4$CH$_2$C*H*$_2$O), 3.32 – 3.35 (m, 1H, epoxide CH), 2.89 (t, *J* = 7.1 Hz, 2H, FC$_6$H$_4$C*H*$_2$), 2.87 – 2.91 (m, 1H, epoxide CH$_2$), 2.74 (dd, *J* = 4.9/2.7 Hz, 1H, epoxide CH$_2$).

^{13}C NMR: δ 162.58 (J_{CF} = 243.7 Hz, CF), 153.43, 152.92 (4° C, aryl-dioxy ring), 134.66 (J_{CF} = 3.4 Hz, 4-C fluorophenyl ring), 130.40 (J_{CF} = 7.9 Hz, 3-C and 5-C fluorophenyl ring), 115.71 (CH aryl-dioxy ring), 115.39 (J_{CF} = 21.1 Hz, 2-C and 6-C fluorophenyl ring), 72.40 ($FC_6H_4CH_2CH_2O$), 69.54, 69.56 (CH_2CH_2OAr, $ArOCH_2CH$), 68.11 (CH_2OAr), 50.34 (epoxide CH), 44.77 (epoxide CH_2), 35.49 ($FC_6H_4CH_2$).

m/z: HRMS (TOF ES$^+$) $C_{19}H_{22}FO_4$ $[MH]^+$ calcd 355.1316; found 355.1315.

2-((4-(2-Ethoxyethoxy)phenoxy)methyl)oxirane (124b)



4-(2-Ethoxyethoxy)phenol **(123b)** (413 mg, 2.27 mmol) was dissolved in aqueous 2 M NaOH solution (4.0 mL) and stirred for 10 minutes. *rac*-Epichlorohydrin (533 µL, 6.81 mmol, 3 eq) was added and the mixture stirred at 60 °C for 24 hours. The cooled mixture was extracted with DCM (3 x 20 mL) and the organic layers combined. After solvent removal, the product purified by FCC (eluent Et_2O) to give 417 mg of colourless oil.

Yield: 84%.

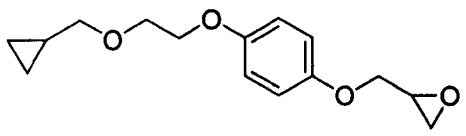
IR: 2975, 2927, 2872 (alkyl C-H, str), 1508 (aryl, str), 1231 (epoxide C-C, str), 1122 (C-O-C, str), 826 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 6.83 – 6.88 (m, 4H, *para*-disubstituted aryl ring), 4.17 (dd, *J* = 11.2/3.2 Hz, 1H, $ArOCH_2CH$), 4.07 (t, *J* = 4.9 Hz, 2H, CH_2OAr), 3.91 (dd, *J* = 10.08/5.6 Hz, 1H, $ArOCH_2CH$), 3.77 (t, *J* = 4.9 Hz, 2H, CH_2CH_2OAr), 3.60 (q, *J* = 7.0 Hz, 2H, CH_3CH_2O), 3.34 (m, 1H, epoxide CH), 2.89 (dd, *J* = 5.0/4.0 Hz, 1H, epoxide CH_2), 2.74 (dd, *J* = 5.0/2.6 Hz, 1H, epoxide CH_2), 1.25 (t, *J* = 7.0 Hz, 3H, CH_3).

^{13}C NMR: δ 153.41, 152.79 (4° aryl C), 115.63, 115.60 (aryl CH), 69.45 ($ArOCH_2CH$), 69.05 ($C_2H_5OCH_2$), 68.06 (CH_2OAr), 66.84 (CH_3CH_2O), 50.27 (epoxide CH), 44.74 (epoxide CH_2), 15.18 (CH_3).

m/z: HRMS (TOF ES$^+$) $C_{13}H_{19}O_4$ $[MH]^+$ calcd 239.1278; found 239.1262.

2-((4-(2-(Cyclopropylmethoxy)ethoxy)phenoxy)methyl)oxirane (124c)



4-(2-(Cyclopropylmethoxy)ethoxy) phenol **(123c)** (450 mg, 2.16 mmol) was dissolved in aqueous 2 M NaOH solution (1.5 mL) and stirred for 10 minutes. *rac*-Epichlorohydrin (507 µL, 6.481 mmol, 3 eq) was added and the mixture stirred at 60 °C for 24 hours. The cooled mixture was extracted with DCM (3 x 25 mL) and the organic layers combined. After solvent removal, the product purified by FCC (eluent EtOAc/hexanes 30:70) to give 356 mg of colourless oil.

Yield: 62%.

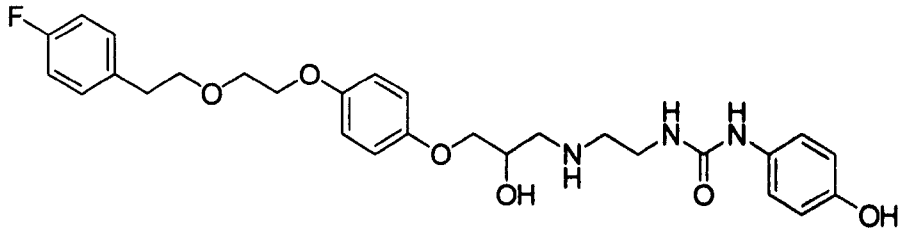
IR: 3080 (epoxide C-H, str), 2926, 2872 (alkyl C-H, str), 1508 (aryl, str), 1230 (epoxide C-C, str), 1113 (C-O-C, str), 826 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR: δ 6.83 (s, 4H, aryl CH), 4.15 (dd, *J* = 11.0/3.2 Hz, 1H, ArOCH_2CH), 4.06 (t, 4.9 Hz, 2H, CH_2OAr), 3.88 (dd, *J* = 11.0/5.7 Hz, 1H, ArOCH_2CH), 3.78 (t, *J* = 5.0 Hz, 2H, CH_2CH$_2$OAr), 3.36 (d, *J* = 6.8 Hz, 2H, ^{c}PrCH_2O), 3.29 – 3.33 (m, 1H, epoxide CH), 2.87 (dd, *J* = 4.8/4.3 Hz, 1H, epoxide CH$_2$), 2.72 (dd, *J* = 4.9/2.7 Hz, 1H, epoxide CH$_2$), 1.05 – 1.10 (m, 1H, ^{c}Pr CH), 0.51 – 0.55 (m, 2H, ^{c}Pr CH$_2$)*, 0.19 – 0.22 (m, 2H, ^{c}Pr CH$_2$)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR: δ 153.48, 152.86 (4° aryl C), 115.68 (aryl CH), 76.32 (^{c}PrCH$_2$O), 69.53 (ArOCH$_2$CH), 69.05 (CH$_2$CH$_2$OAr), 68.14 (CH$_2$OAr), 50.35 (epoxide CH), 44.80 (epoxide CH$_2$), 10.66 (^{c}Pr CH), 3.16 (^{c}Pr CH$_2$).

m/z: HRMS (TOF ES$^+$) $C_{15}H_{21}O_4$ $[MH]^+$ calcd 265.1434; found 265.1407.

1-(2-(3-(4-(2-(4-Fluorophenethyloxy)ethyloxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea (125a)



2-((4-(2-(4-Fluorophenethyloxy)ethyloxy)phenoxy)methyl)oxirane **(124a)** was opened with 1-(2-aminoethyl)-3-(4-hydroxyphenyl)urea **(56)** as described in the general procedure for synthesis of aromatically substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxy propylamino)ethyl)-3-(aryl)ureas.

Yield: 25%.

Mp: 113 – 115 °C.

IR: 3308 (br, O-H, str), 2926, 2868 (alkyl C-H, str), 1636 (urea C=O, str), 1510, 1572 (aryl, str), 1229 (C-F), 1120 (C-O-C, str), 831 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR (DMSO-d_6): δ 8.92 (br s, 1H, phenol), 8.22 (s, 1H, NH(C=O)N*H*Ar), 7.28 (dd, *J* = 8.3/5.8 Hz, 2H, 3-H and 5-H of fluorophenyl ring), 7.14 (d, *J* = 8.8 Hz, 2H, aryl C-H *ortho* to urea), 7.08 (dd, *J* = 8.9/8.9 Hz, 2H, 2-H and 6-H of flurophenyl ring), 6.82, 6.85 (d, *J* = 9.3 Hz, 2 x 2H, aryl-dioxy ring), 6.62 (d, *J* = 8.8 Hz, 2H, aryl C-H *ortho* to phenol), 6.03 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 5.01 (br s, 1H, NH), 3.99 (t, *J* = 4.3 Hz, 2H, CH_2OAr), 3.78 – 3.93 (m, 3H, C*H*(OH), ArOCH_2), 3.69 (t, *J* = 4.3 Hz, 2H, CH_2CH$_2$OAr), 3.64 (t, *J* = 6.9 Hz, 2H, FC$_6$H$_4$CH$_2$CH_2O), 3.11 – 3.19 (m, 2H, NHCH$_2$CH_2), 2.81 (t, *J* = 6.8 Hz, 2H, FC$_6$H$_4$CH_2), 2.58 – 2.76 (m, 4H, CH(OH)CH_2NH, NHCH_2CH$_2$).

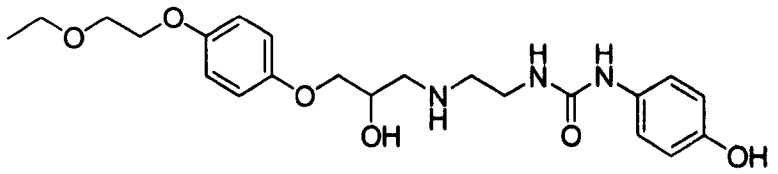
^{13}C NMR (DMSO-d_6): δ 160.80 (J_{CF} = 241.3 Hz, CF), 155.70 (C=O), 152.76, 152.47, 151.88, 132.12, (aryl 4° C), 135.18 (J_{CF} = 3.2 Hz, 4-C fluorophenyl ring), 130.59 (J_{CF} = 7.8 Hz, 3-C and 5-C fluorophenyl ring), 119.28 (aryl C-H *ortho* to urea), 115.33, 115.36 (CH aryl-dioxy ring), 115.06 (aryl C-H *ortho* to phenol), 114.84 (J_{CF} = 21.0 Hz, 2-C and 6-C

fluorophenyl ring), 71.21 ($FC_6H_4CH_2CH_2O$, $ArOCH_2$), 68.71 (CH_2CH_2OAr), 67.99 (CH(OH)), 67.49 (CH_2OAr), 51.13 ($CH(OH)CH_2NH$), 49.35 ($NHCH_2CH_2$), 39.05 ($NHCH_2CH_2$), 34.56 ($FC_6H_4CH_2$).

m/z: HRMS (TOF ES^+) $C_{28}H_{35}FN_3O_6$ $[MH]^+$ calcd 528.2510; found 528.2514.

HPLC R_t: 3.47 (System 1b), 11.50 (System 3).

1-(2-(3-(4-(2-Ethoxyethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea (125b)



2-((4-(2-Ethoxyethoxy)phenoxy)methyl)oxirane **(124b)** was opened with 1-(2-aminoethyl)-3-(4-hydroxyphenyl)urea **(56)** as described in the general procedure for synthesis of aromatically substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(aryl)ureas.

Yield: 21%.

Mp: 96 - 98°C.

IR: 3336 (br, O-H, str), 2926, 2866 (alkyl C-H, str), 1636 (urea C=O, str), 1513, 1574 (aryl, str), 1123 (C-O-C, str), 819, 835 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR (MeOD-d_4): δ 7.12 (d, *J* = 8.9 Hz, 2H, aryl C-H *ortho* to urea), 6.87 (d, *J* = 9.0 Hz, 2H, aryl-dioxy ring), 6.84 (d, *J* = 9.0 Hz, 2H, aryl-dioxy ring), 6.70 (d, *J* = 8.9 Hz, 2H, aryl C-H *ortho* to phenol), 4.01 – 4.08 (m, 1H, C*H*(OH)), 4.03 (t, *J* = 4.6 Hz, 2H, CH_2OArO), 3.87 – 3.95 (m, 2H, $ArOCH_2CH(OH)$), 3.75 (t, *J* = 4.7 Hz, 2H, OCH_2CH_2OArO), 3.59 (q, *J* = 7.0 Hz, 2H, CH_3CH_2), 3.34 (t, *J* = 6.1 Hz, 2H, CH_2CH_2NH), 2.74 – 2.90 (m, 4H, $CH(OH)CH_2NH$, $NHCH_2CH_2$), 1.21 (t, *J* = 7.0 Hz, 3H, CH_3).

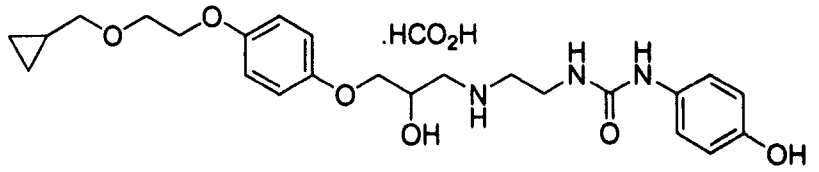
^{13}C NMR (MeOD-d_4): δ 159.22, 154.63, 154.60, 132.32 (aryl 4° C), 123.56 (aryl C-H *ortho* to urea), 116.59, 116.55 (*C*H aryl-dioxy ring), 116.34 (aryl C-H *ortho* to phenol), 72.38 ($ArOCH_2$), 70.29 (CH_2CH_2OAr), 69.65 (*C*H(OH)), 69.14 (CH_2OAr), 67.72 (CH_3CH_2)

52.94 ($CH(OH)CH_2NH$), 50.55 ($NHCH_2CH_2$), 40.26 ($NHCH_2CH_2$), 14.40 (CH_3).

m/z: HRMS (TOF ES^+) $C_{22}H_{32}N_3O_6$ $[MH]^+$ calcd 434.2286; found 434.2291; (TOF ES^-) $C_{22}H_{30}N_3O_6$ $[M-H]^-$ calcd 432.2140; found 432.2134.

HPLC R_t: 2.29 (System 1b), 8.72 (System 2).

1-(2-(3-(4-(2-(Cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(4-hydroxyphenyl)urea formate (125c)



2-((4-(2-(Cyclopropylmethoxy)ethoxy)phenoxy)methyl)oxirane **(124c)** was opened with 1-(2-aminoethyl)-3-(4-hydroxyphenyl)urea **(56)** as described in the general procedure for synthesis of aromatically substituted 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxy propylamino)ethyl)-3-(aryl)ureas.

Yield: 21%.

Mp: semi-solid.

IR: 3307 (br, O-H, str), 2929, 2870 (alkyl C-H, str), 1634 (urea C=O, str), 1509, 1570 (aryl, str), 1111 (C-O-C, str), 830 (aryl C-H, bend, *para*-disubstituted ring).

^{1}H NMR (MeOD-d_4): δ 7.09 – 7.16 (m, 2H, aryl C-H *ortho* to urea), 6.90 (d, *J* = 9.0 Hz, 2H, aryl-dioxy ring), 6.86 (d, *J* = 9.4 Hz, 2H, aryl-dioxy ring), 6.70 (d, *J* = 9.3 Hz, 2H, aryl C-H *ortho* to phenol), 4.18 – 4.27 (m, 1H, C*H*(OH)), 4.05 (t, *J* = 4.6 Hz, 2H, CH_2OArO), 4.00 (dd, *J* = 9.8/4.9,

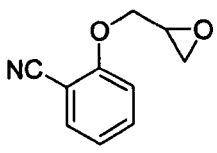
1H, ArOCH_2CH(OH)), 3.95 (dd, *J* = 9.8/5.3, 1H, ArOCH_2CH(OH)), 3.79 (t, *J* = 4.7 Hz, 2H, OCH_2CH_2OArO), 3.51 (t, *J* = 5.3 Hz, 2H, CH_2CH_2NH), 3.37 (d, *J* = 6.9 Hz, 2H, ^{c}PrCH_2O), 3.28 – 3.35, 3.15 – 3.24 (m, 4H, CH(OH)CH_2NH, NHCH_2CH_2), 1.01 – 1.13 (m, 1H, ^{c}Pr CH), 0.50 – 0.57 (m, 2H, ^{c}Pr CH_2)*, 0.19 – 0.26 (^{c}Pr CH_2)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR (MeOD-d$_4$): δ 159.81, 154.90, 154.20, 132.28, 131.90 (aryl 4° C), 123.71 (aryl C-H *ortho* to urea), 116.65, 116.64 (*C*H aryl-dioxy ring), 116.38 (aryl C-H *ortho* to phenol), 77.11 (^{c}PrCH_2O), 71.78 (ArOCH_2), 70.18 (*C*H_2CH_2OAr), 69.17 (*C*H_2OAr), 66.81 (*C*H(OH)), 51.54, 50.69 (CH(OH)CH_2NH, NHCH_2CH_2), 38.02 (NHCH_2CH_2), 11.36 (^{c}Pr CH), 3.47 (^{c}Pr CH_2).

m/z: HRMS (TOF ES$^+$) $C_{24}H_{34}N_3O_6$ [MH]$^+$ calcd 460.2442; found 460.2429.

HPLC R$_t$: 2.88 (System 1a), 9.84 (System 2).

2-((Oxiran-2-yl)methoxy)benzonitrile (127a)



2-Hydroxybenzonitrile **(126a)** was alkylated with *rac*-epichlorohydrin in a similar manner to 4-(2-(cyclopentyloxy)ethoxy)phenol **(61)** as described in the preparation of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)**. Purification by FCC (eluent EtOAc/hexanes 50:50) gave 623 mg of a pale yellow waxy solid.

Yield: 21%.

Mp: 62 – 64 °C (lit. 65 °C)[143].

IR: 3080 (epoxide C-H, str), 2957, 2931 (alkyl C-H, str), 2228 (CN, str), 1599, 1581, 1494 (aryl, str), 1258 (epoxide C-O, str), 1112 (C-O-C), 752 (aryl 1,2-disubstituted ring C-H, bend).

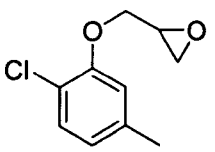
^{1}H NMR: δ 7.45 – 7.54 (m, 2H, aryl 4-H and 6-H), 6.93 – 7.03 (m, 2H, aryl 3-H and 5-H), 4.36 (dd, *J* = 11.4/2.6 Hz, 1H, ArOCH_2CH), 4.04 (dd, *J* = 11.4/5.3 Hz, 1H, ArOCH_2CH), 3.33 – 3.89 (m, 1H, epoxide CH),

2.88 (dd, *J* = 4.7/4.4 Hz, 1H, epoxide CH_2), 2.81 (dd, *J* = 4.7/2.6 Hz, 1H, epoxide CH_2).

^{13}C NMR: δ 160.01 (aryl 2-C), 134.45 (aryl 4-C), 133.69 (aryl 6-C), 121.33 (aryl 5-C), 116.21 (CN), 112.63 (aryl 3-C), 101.97 (aryl 1-C), 69.31 ($ArOCH_2$), 49.82 (epoxide CH), 44.37 (epoxide CH_2).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{10}NO_2$ $[MH]^+$ calcd 176.0706; found 176.0709.

2-((2-chloro-5-methylphenoxy)methyl)oxirane (127b)



2-Chloro-5-methylphenol **(126b)** was alkylated with *rac*-epichlorohydrin in a similar manner to 4-(2-(cyclopentyloxy)ethoxy)phenol **(61)** as described in the preparation of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)**. Purification by FCC (eluent EtOAc/hexanes 20:80) gave 1.406 g of a pale yellow crystalline solid.

Yield: 50%.

Mp: 43 – 45 °C.

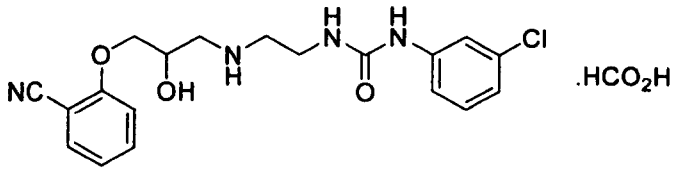
IR: 3064 (epoxide C-H, str), 3017 (aryl C-H, str), 2929 (alkyl C-H, str), 1585, 1493 (aryl, str), 1065 (C-O, str), 796 (C-Cl, bend).

^{1}H NMR: δ 7.17 (d, *J* = 7.9 Hz, 1H, aryl 3-H), 6.71 – 6.75 (m, 1H, aryl 6-H), 6.65 – 6.71 (m, 1H, aryl 4-H), 4.23 (dd, *J* = 11.2/2.9 Hz, 1H, $ArOCH_2CH$), 3.95 (dd, *J* = 11.3/5.5 Hz, 1H, $ArOCH_2CH$), 3.30 – 3.36 (m, 1H, epoxide CH), 2.77 (dd, *J* = 4.7/4.4 Hz, 1H, epoxide CH_2), 2.81 (dd, *J* = 4.8/2.5 Hz, 1H, epoxide CH_2), 2.27 (s, 3H, CH_3).

^{13}C NMR: δ 153.62 (aryl 1-C), 137.88 (aryl 5-C), 129.71 (aryl 3-C), 122.58 (aryl 4-C), 119.83 (aryl 2-C), 114.85 (aryl 6-C), 69.57 ($ArOCH_2$), 49.96 (epoxide CH), 44.36 (epoxide CH_2), 21.12 (CH_3).

m/z: HRMS (TOF ES$^+$) $C_{10}H_{12}ClO_2$ $[MH]^+$ calcd 199.0520; found 199.0507.

1-(2-(2-Hydroxy-3-((2-cyano)phenoxy)propylamino)ethyl)-3-(3-chlorophenyl)urea hydroformate (128a)



2-((Oxiran-2-yl)methoxy)benzonitrile **(127a)** was opened with 1-(2-aminoethyl)-3-(3-chlorophenyl)urea hydrochloride **(66k)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy) phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:10:89) and preparative HPLC afforded 14 mg of white solid.

Yield: 11%.

Mp: 51 – 53 °C.

IR: 3394 (br, OH, str), 2228 (CN, str), 1683 (urea C=O, str), 1596, 1549 (aryl, str), 1111 (C-O-C, str), 755 (C-Cl, bend).

^{1}H NMR (DMSO-d_6): δ 9.11 (s, 1H, NH(C=O)N*H*Ar), 8.26 (br s, 1H, formate HCO_2^-), 7.71 (dd, *J* = 7.5/1.6 Hz, 1H, cyanophenyl ring 3-H), 7.67 (dd, *J* = 1.8/1.8 Hz, 1H, chlorophenyl ring 2-H), 7.64 (ddd, *J* = 7.9/7.9/1.5 Hz, 1H, cyanophenyl ring 5-H), 7.26 (d, *J* = 8.6 Hz, 1H, cyanophenyl ring 6-H), 7.21 (dd, *J* = 8.3/8.3 Hz, 1H, chlorophenyl 5-H), 7.18 (ddd, *J* = 8.3/1.8/1.8 Hz, 1H, chlorophenyl ring 6-H), 7.08 (ddd, *J* = 7.5/7.5/1.0 Hz, 1H, cyanophenyl ring 4-H), 6.91 (ddd, *J* = 7.5/2.0/2.0 Hz, 1H, chlorophenyl ring 4-H),6.63 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 4.07 – 4.18 (m, 2H, $ArOCH_2$), 3.97 – 4.06 (m, 1H, CH(OH)), 3.23 (dt, *J* = 5.8/5.8 Hz, 2H, $NHCH_2CH_2$), 2.87 (dd, *J* = 12.3/4.6 Hz, 1H, $CH(OH)CH_2NH$), 2.72 – 2.81 (m, 3H, $CH(OH)CH_2NH$, $NHCH_2CH_2$).

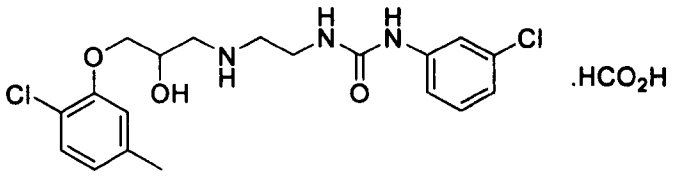
^{13}C NMR (DMSO-d_6): δ 164.39 (formate C=O)160.20 (cyanophenyl ring 1-C), 155.22 (C=O), 142.21 (chlorophenyl ring 1-C), 135.02 (cyanophenyl ring 5-C), 133.66 (cyanophenyl ring 3-C), 133.04 (chlorophenyl ring 3-C), 130.17 (chlorophenyl ring 5-C), 121.08 (cyanophenyl ring 4-C), 120.45 (chlorophenyl ring 4-C), 116.89

(chlorophenyl ring 2-C), 116.41 (CN), 115.91 (chlorophenyl ring 6-C), 113.17 (cyanophenyl ring 6-C), 100.64 (cyanophenyl ring 2-C), 71.30 ($ArOCH_2$), 66.96 (CH(OH)), 51.21(CH(OH)CH_2NH), 48.69 (NHCH_2CH_2), 38.24 (NHCH$_2CH_2$).

m/z: HRMS (TOF ES^+) $C_{19}H_{22}ClN_4O_3$ $[MH]^+$ calcd 389.1375; found 389.1410.

HPLC R_t: 3.65 (System 1b), 10.62 (System 3).

1-(2-(3-(2-Chloro-5-methylphenoxy)-2-hydroxypropylamino)ethyl)-3-(3-chlorophenyl)urea hydroformate (128b)



2-((2-chloro-5-methylphenoxy)methyl)oxirane **(127b)** was opened with 1-(2-aminoethyl)-3-(3-chlorophenyl)urea hydrochloride **(66k)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy) phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:10:89) and preparative HPLC afforded 16 mg of white solid.

Yield: 14%.

Mp: 43 – 45 °C.

IR: 3370 (br, OH, str), 3070 (aryl C-H, str), 1675 (urea C=O, str), 1594, 1550 (aryl, str), 1065 (C-O, str), 774 (C-Cl, bend).

^{1}H NMR (DMSO-d_6): δ 9.15 (s, 1H, NH(C=O)N*H*Ar), 8.27 (br s, 1H, formate HCO_2^-), 7.67 (dd, *J* = 2.1/1.1 Hz, 1H, chlorophenyl ring 2-H), 7.26 (d, *J* = 8.0 Hz, 1H, *p*-chlorotolyl ring 3-H), 7.22 (dd, *J* = 8.0/8.0 Hz, 1H, chlorophenyl 5-H), 7.19 (ddd, *J* = 8.3/1.8/1.8 Hz, 1H, chlorophenyl ring 6-H), 6.98 (d, *J* = 1.4 Hz, 1H, *p*-chlorotolyl ring 6-H), 6.91 (ddd, *J* = 6.6/2.3/2.3 Hz, 1H, chlorophenyl ring 4-H), 6.76 (ddd, *J* = 8.0/1.7/0.6 Hz, 1H, *p*-chlorotolyl ring 4-H), 6.70 (t, *J* = 5.4 Hz, 1H, N*H*(C=O)NHAr), 4.00 (br s, 3H, $ArOCH_2$, CH(OH)), 3.23 (dt, *J* = 5.9/5.7 Hz, 2H,

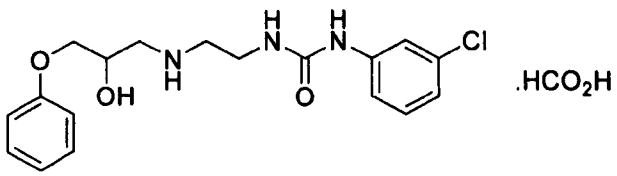
$NHCH_2CH_2$), 2.87 (dd, *J* = 11.9/3.7 Hz, 1H, $CH(OH)CH_2NH$), 2.72 – 2.82 (m, 3H, $CH(OH)CH_2NH$, $NHCH_2CH_2$), 2.28 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6): δ 164.52 (formate C=O), 155.24 (C=O), 153.56 (*p*-chlorotolyl ring 1-C), 142.24 (chlorophenyl ring 1-C), 138.06 (*p*-chlorotolyl ring 5-C), 133.03 (chlorophenyl ring 3-C), 130.16 (chlorophenyl ring 5-C), 129.40 (*p*-chlorotolyl ring 3-C), 122.06 (*p*-chlorotolyl ring 4-C), 120.42 (chlorophenyl ring 4-C), 118.31 (*p*-chlorotolyl ring 2-C), 116.88 (chlorophenyl ring 2-C), 115.90 (chlorophenyl ring 6-C), 114.71 (*p*-chlorotolyl ring 6-C), 71.19 ($ArOCH_2$), 67.03 (CH(OH)), 51.44 ($CH(OH)CH_2NH$), 48.67 ($NHCH_2CH_2$), 38.22 ($NHCH_2CH_2$), 20.83 (CH_3).

m/z: HRMS (TOF ES^+) $C_{19}H_{24}Cl_2N_3O_3$ $[MH]^+$ calcd 412.1189; found 412.1157.

HPLC R_t: 4.20 (System 1b), 12.78 (System 3).

1-(2-(2-Hydroxy-3-phenoxypropylamino)ethyl)-3-(3-chlorophenyl)urea hydroformate (128c)



Glycidyl phenyl ether **(127c)** was opened with 1-(2-aminoethyl)-3-(3-chlorophenyl)urea hydrochloride **(66k)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:10:89) and preparative HPLC afforded 16 mg of white solid.

Yield: 12%.

Mp: 142 – 144 °C.

IR: 3278 (br, OH, str), 3066 (aryl C-H, str), 1690 (urea C=O, str), 1594, 1496 (aryl, str), 1073 (C-O, str), 775 (C-Cl, bend), 755, 689 (aryl C-H, bend, phenyl ring).

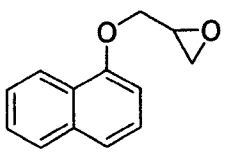
^{1}H NMR (DMSO-d_6): δ 9.14 (s, 1H, NH(C=O)N*H*Ar), 8.27 (br s, 1H, formate HCO_2^-), 7.67 (d, *J* = 1.7 Hz, 1H, chlorophenyl ring 2-H), 7.27 (ddd, *J* = 6.9/6.9/2.0 Hz, 2H, phenyl ring 3-H and 5-H), 7.22 (dd, *J* = 8.0/8.0 Hz, 1H, chlorophenyl 5-H), 7.19 (ddd, *J* = 8.3/1.8/1.8 Hz, 1H, chlorophenyl ring 6-H), 6.88 - 6.96 (m, 4H, phenyl ring 2-H, 4-H and 6-H, chlorophenyl ring 4-H), 6.66 (t, *J* = 5.2 Hz, 1H, N*H*(C=O)NHAr), 3.86 - 4.00 (m, 3H, $ArOCH_2$, CH(OH)), 3.22 (dt, *J* = 6.1/5.8 Hz, 2H, $NHCH_2CH_2$), 2.83 (dd, *J* = 12.1/4.0 Hz, 1H, $CH(OH)CH_2NH$), 2.66 – 2.78 (m, 3H, $CH(OH)CH_2NH$, $NHCH_2CH_2$).

^{13}C NMR (DMSO-d_6): δ 164.49 (formate C=O), 158.55 (phenyl 1-C), 155.23 (C=O), 142.25 (chlorophenyl ring 1-C), 133.04 (chlorophenyl ring 3-C), 130.17 (chlorophenyl ring 5-C), 129.44 (phenyl 3-C and 5-C), 120.52 (phenyl 4-C), 120.43 (chlorophenyl ring 4-C), 116.88 (chlorophenyl ring 2-C), 115.90 (chlorophenyl ring 6-C), 114.46 (phenyl 2-C and 6-C), 70.38 ($ArOCH_2$), 67.32 (CH(OH)), 51.59 ($CH(OH)CH_2NH$), 48.76 ($NHCH_2CH_2$), 38.36 ($NHCH_2CH_2$).

m/z: HRMS (TOF ES$^-$) $C_{18}H_{21}ClN_3O_3$ $[M-H]^-$ calcd 362.1277; found 362.1258.

HPLC R_t: 3.65 (System 1b), 11.10 (System 3).

2-((Naphthalen-1-yloxy)methyl)oxirane (130)



1-Naphthol **(129)** was alkylated with *rac*-epichlorohydrin in a similar manner to 4-(2-(cyclopentyloxy)ethoxy) phenol **(61)** as described in the preparation of 2-((4-(2-(cyclopentyloxy)ethoxy)phenoxy)methyl)oxirane **(62)**. Purification by FCC (eluent EtOAc/PE 10:90) gave clear, colourless oil in quantitative yield.

Yield: 100%.

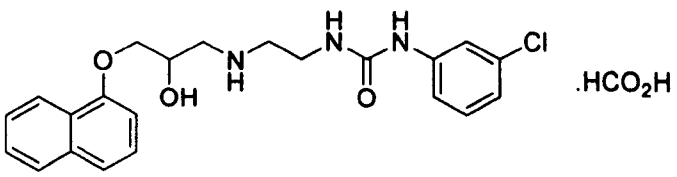
IR: 3054 (epoxide C-H, str), 3005 (aryl C-H, str), 2926, 2874 (alkyl C-H, str), 1595, 1580, 1509 (aryl, str), 1241 (epoxide C-O, str), 1101 (C-O-C), 793, 772 (aryl C-H bend)

^{1}H NMR: δ 8.35 – 8.42 (m, 1H, naphthyl 8-H), 7.82 – 7.88 (m, 1H, naphthyl 5-H), 7.51 – 7.58 (m, 2H, naphthyl 6-H and 7-H), 7.50 (d, *J* = 8.3 Hz, 1H, naphthyl 4-H), 7.40 (dd, *J* = 8.1/8.1 Hz, 1H, naphthyl 3-H), 6.80 (d, *J* = 7.5 Hz, 1H, naphthyl 2-H), 4.37 (dd, *J* = 11.1/3.2 Hz, 1H, $ArOCH_2$), 4.09 (dd, *J* = 11.1/5.5 Hz, 1H, $ArOCH_2$), 3.44 – 3.50 (m, 1H, epoxide CH), 2.94 (dd, *J* = 4.7/4.7 Hz, 1H, epoxide CH_2), 2.83 (dd, *J* = 4.8/2.8 Hz, 1H, epoxide CH_2).

^{13}C NMR: δ 154.20 (naphthyl 1-C), 134.51 (naphthyl 4a-C), 127.44 (naphthyl 5-C), 126.49 (naphthyl 6-C), 125.74 (naphthyl 7-C), 125.58 (naphthyl 8a-C), 125.29 (naphthyl 3-C), 122.02 (naphthyl 8-C), 120.78 (naphthyl 4-C), 105.00 (naphthyl 2-C), 68.89 ($ArOCH_2$), 50.15 (epoxide CH), 44.55 (epoxide CH_2).

m/z: HRMS (TOF ES$^+$) $C_{13}H_{13}O_2$ $[MH]^+$ calcd 201.0910; found 201.0924.

1-(2-(2-Hydroxy-3-(naphthalen-1-yloxy)propylamino)ethyl)-3-(3-chlorophenyl)urea hydroformate (131)



2-((Naphthalen-1-yloxy)methyl)oxirane **(130)** was opened with 1-(2-aminoethyl)-3-(3-chlorophenyl)urea hydrochloride **(66k)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy) phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:10:89) and preparative HPLC afforded 18 mg of white solid.

Yield: 16%.

Mp: 64 – 66 °C.

IR: 3369 (br, O-H, str), 3057 (aryl C-H, str), 1669 (urea C=O, str), 1598, 1549 (aryl, str), 1103 (C-O-C, str), 794 (aryl 1,3-disubstituted ring C-H, bend), 772 (C-Cl, bend).

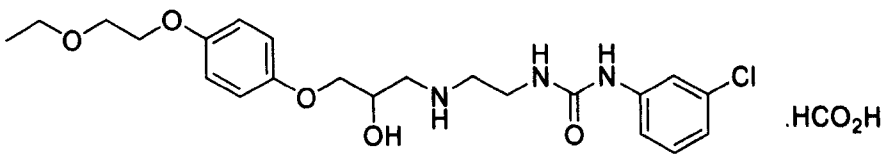
^{1}H NMR (DMSO-d_6): δ 9.13 (s, 1H, NH(C=O)N*H*Ar), 8.65 (br s, 2H, NH_2^+), 8.29 (dd, *J* = 8.3/1.9 Hz, 1H, naphthyl 8-H), 7.87 (dd, *J* = 7.5/1.9 Hz, 1H, naphthyl 5-H), 7.69 (d, *J* = 1.7 Hz, 1H, chlorophenyl ring 2-H), 7.47 – 7.57 (m, 3H, naphthyl 4-H, 6-H and 7-H), 7.42 (dd, *J* = 7.9/7.9 Hz, 1H, naphthyl 3-H), 7.20 – 7.29 (m, 2H, chlorophenyl ring 6-H, naphthyl 2-H), 6.92 – 7.03 (m, 2H, chlorophenyl ring 4-H and 5-H), 6.57 (t, *J* = 5.9 Hz, 1H, N*H*(C=O)NHAr), 4.30 – 4.40 (m, 1H, CH(OH)), 4.11 – 4.20 (m, 2H, $ArOCH_2$), 3.42 – 3.49 (m, 2H, $NHCH_2CH_2$), 3.10 – 3.32 (m, 4H, $CH(OH)CH_2NH$, $NHCH_2CH_2$).

^{13}C NMR (DMSO-d_6): δ 155.54 (C=O), 153.70 (naphthyl 1-C), 141.81 (chlorophenyl ring 1-C), 134.01 (naphthyl 4a-C), 133.06 (chlorophenyl ring 3-C), 130.26 (chlorophenyl ring 5-C), 127.41 (naphthyl 5-C), 126.52 (naphthyl 6-C), 126.14 (naphthyl 7-C), 125.24 (naphthyl 8a-C), 124.85 (naphthyl 3-C), 121.83 (naphthyl 8-C), 120.90 (naphthyl 4-C), 120.27 (chlorophenyl ring (4-C), 117.18 (chlorophenyl ring 2-C), 116.17 (chlorophenyl ring 6-C), 105.23 (naphthyl 2-C), 69.92 ($ArOCH_2$), 64.99 (CH(OH)), 49.64 ($CH(OH)CH_2NH$), 47.64 ($NHCH_2CH_2$), 37.40 ($NHCH_2CH_2$).

m/z: HRMS (TOF ES$^+$) $C_{22}H_{25}ClN_3O_3$ $[MH]^+$ calcd 414.1579; found 414.1568.

HPLC R_t: 4.49 (System 1b), 13.44 (System 3).

1-(2-(3-(4-(2-Ethoxyethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-chlorophenyl)urea hydroformate (132a)



.HCO_2H

2-((4-(2-Ethoxyethoxy)phenoxy)methyl)oxirane **(124b)** was opened with 1-(2-aminoethyl)-3-(3-chlorophenyl)urea hydrochloride **(66k)** according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy) phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:10:89) and preparative HPLC afforded 11 mg of beige semi-solid.

Yield: 11%.

IR: 3339 (br, OH, str), 2975, 2928, 2868 (alkyl C-H, str), 1632 (urea C=O, str), 1592, 1508 (aryl, str), 1121 (C-O-C, str), 823 (aryl C-H, bend, *para*-disubstituted ring), 774 (C-Cl, bend).

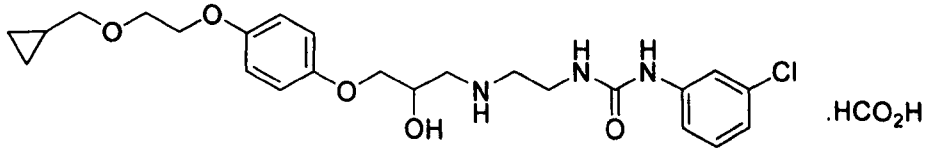
^{1}H NMR (DMSO-d_6): δ 9.02 (s, 1H, NH(C=O)N*H*Ar), 8.27 (br s, 1H, formate HCO_2^-), 7.67 (dd, *J* = 2.0/2.0 Hz, 1H, aryl 2-H), 7.22 (dd, *J* = 7.7/7.7 Hz, 1H, aryl 5-H), 7.18 (ddd, *J* = 8.3/1.8/1.8 Hz, 1H, aryl 6-H), 6.91 (ddd, *J* = 7.4/1.8/1.8 Hz, 1H, aryl 4-H), 6.86, 6.83 (d, *J* = 9.2 Hz, 2 x 2H, aryl-dioxy C-H), 6.51 (t, *J* = 5.3 Hz, 1H, N*H*(C=O)NHAr), 3.99 (t, *J* = 4.7 Hz, 2H, CH_2OAr), 3.78 – 3.92 (m, 3H, C*H*(OH), ArOCH_2), 3.66 (t, *J* = 4.7 Hz, 2H, CH_2CH$_2$OAr), 3.48 (q, *J* = 7.0 Hz, 2H, CH$_3$CH_2), 3.19 (dt, *J* = 6.2/5.8 Hz, 2H, NHCH$_2$CH_2), 2.74 (dd, *J* = 12.1/4.3 Hz, 1H, CH(OH)CH_2NH), 2.68 (t, *J* = 6.2 Hz, 2H, NHCH_2CH$_2$), 2.63 (dd, *J* = 12.1/6.6 Hz, 1H, CH(OH)CH_2NH), 1.20 (t, *J* = 6.6 Hz, 3H, CH$_3$).

^{13}C NMR (DMSO-d_6): δ 155.14 (C=O), 152.70, 152.49 (aryl-dioxy 4° C), 142.24 (aryl 1-C), 133.05 (aryl 3-C), 130.18 (aryl 5-C), 120.41 (aryl 4-C), 116.84 (aryl 2-C), 115.87 (aryl 6-C), 115.34, 115.25 (aryl-dioxy C-H), 71.14. (ArOCH$_2$), 68.42 (*C*H$_2$CH$_2$OAr), 67.79 (CH(OH)), 67.51 (*C*H$_2$OAr), 65.64 (CH$_3$*C*H$_2$), 51.94 (CH(OH)*C*H$_2$NH), 48.96 (NH*C*H$_2$CH$_2$), 38.90 (NHCH$_2$*C*H$_2$), 15.09 (CH$_3$).

m/z: HRMS (TOF ES$^-$) $C_{22}H_{29}ClN_3O_5$ $[M-H]^-$ calcd 450.1801; found 450.1801.

HPLC R_t: 3.93 (System 1b), 11.69 (System 3).

1-(2-(3-(4-(2-(Cyclopropylmethoxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(3-chlorophenyl)urea hydroformate (132b)



2-((4-(2-(Cyclopropylmethoxy)ethoxy)phenoxy)methyl)oxirane **(124c)** was opened with 1-(2-aminoethyl)-3-(3-chlorophenyl)urea hydrochloride

(66k) according to the method described for 1-(2-(3-(4-(2-(cyclopentyloxy)ethoxy)phenoxy)-2-hydroxypropylamino)ethyl)-3-(2-hydroxyphenyl)urea **(71t)**. Purification via PLC (eluent 37% aq NH_3/MeOH/DCM 1:10:89) and preparative HPLC afforded 9 mg of beige semi- solid.

Yield: 9%.

IR: 3403 (br, OH, str), 3078 (^{c}Pr C-H, str), 2927, 2867 (alkyl C-H, str), 1632 (urea C=O, str), 1592, 1508 (aryl, str), 1117 (C-O-C, str), 822 (aryl C-H, bend, *para*-disubstituted ring), 768 (C-Cl, bend).

^{1}H NMR (DMSO-d$_6$): δ 8.95 (s, 1H, NH(C=O)N*H*Ar), 8.26 (br s, 1H, formate HCO_2^-), 7.67 (dd, *J* = 2.0/2.0 Hz, 1H, aryl 2-H), 7.22 (dd, *J* = 7.7/7.7 Hz, 1H, aryl 5-H), 7.18 (ddd, *J* = 8.3/1.8/1.8 Hz, 1H, aryl 6-H), 6.91 (ddd, *J* = 7.4/1.8/1.8 Hz, 1H, aryl 4-H), 6.80 – 6.88 (m, 4H, aryl-dioxy C-H), 6.43 (t, *J* = 5.1 Hz, 1H, N*H*(C=O)NHAr), 3.99 (t, *J* = 4.7 Hz, 2H, C*H*$_2$OAr), 3.78 – 3.92 (m, 3H, C*H*(OH), ArOC*H*$_2$), 3.66 (t, *J* = 4.7 Hz, 2H, C*H*$_2$CH$_2$OAr), 3.28 (d, *J* = 7.1 Hz, 2H, ^{c}PrC*H*$_2$O), 3.18 (dt, *J* = 5.9/5.6 Hz, 2H, NHCH$_2$C*H*$_2$), 2.72 (dd, *J* = 11.9/4.0 Hz, 1H, CH(OH)C*H*$_2$NH), 2.66 (t, *J* = 6.2 Hz, 2H, NHC*H*$_2$CH$_2$), 2.61 (dd, *J* = 12.1/6.8 Hz, 1H, CH(OH)C*H*$_2$NH), 1.06 – 0.94 (m, 1H, ^{c}Pr CH), 0.42 – 0.49 (m, 2H, ^{c}Pr CH$_2$)*, 0.13 – 0.19 (^{c}Pr CH$_2$)*. *Refers to cis-protons of ^{c}Pr ring.

^{13}C NMR (DMSO-d$_6$): δ 155.10 (C=O), 152.72, 152.49 (aryl-dioxy 4° C), 142.22 (aryl 1-C), 133.05 (aryl 3-C), 130.18 (aryl 5-C), 120.42 (aryl 4-C), 116.84 (aryl 2-C), 115.87 (aryl 6-C), 115.34, 115.28 (aryl-dioxy C-H), 74.75 (^{c}PrCH$_2$O), 71.17. (ArOCH$_2$), 68.33 (CH$_2$CH$_2$OAr), 67.91 (CH(OH)), 67.58 (CH$_2$OAr), 52.04 (CH(OH)CH$_2$NH), 49.02 (NHCH$_2$CH$_2$), 38.90 (NHCH$_2$CH$_2$), 10.48 (^{c}Pr CH), 2.86 (^{c}Pr CH$_2$).

m/z: HRMS (TOF ES$^-$) $C_{24}H_{31}ClN_3O_5$ $[M-H]^-$ calcd 476.1958; found 476.1991.

HPLC R$_t$: 3.95 (System 1b), 11.73 (System 3).

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