Abstract

The application of a tandem condensation/cyclisation/[3+2]-cycloaddition/elimination reaction gives an sp³-rich tricyclic pyrazoline scaffold with two ethyl esters in a single step from a simple linear starting material. The successive hydrolysis and cyclisation (with Boc anhydride) of these 3-dimensional architectures, generates unprecedented 16-membered macrocyclic bisanhydrides (characterised by XRD). Selective amidations could then be achieved by ring opening with a primary amine followed by HATU-promoted amide coupling to yield an sp³-rich natural product-like library.
Keywords

- $sp^3$-rich;
- Natural product-like;
- Scaffold;
- Cycloaddition;
- Two-directional;
- Macrocycle;
- Library;
- Tandem

1. Introduction

The development of rapid access strategies towards novel 3-dimensional heterocyclic scaffolds is of high interest to organic and medicinal chemists alike.\(^1\) We have pioneered an approach to complex molecular architectures exploiting two-directional synthesis to make symmetrical polyelectrophilic linear molecules, which undergo tandem reactions when reacted with a range of nucleophiles. These diastereoselective processes yield polycyclic structures with multiple stereocentres in a single reaction pot.\(^2\) We have employed this strategy for the syntheses of several natural products over the past decade, including anatoxin-a, histrionicotoxin, hippodamine, xenovenine and halichlorine.\(^3\) More recently, our attention has been drawn to the synthesis of natural product-like scaffolds for chemical library synthesis.\(^4\) Our previous reports on the use of diester \(^1\) as a substrate for tandem cyclisations described access to a variety of novel scaffolds, including 1,2,3,3a,7,8,9,10-octahydrocyclopenta[4,5]pyrazolo[1,5-a]pyridine containing structure \(^2\).\(^4\) This complex fused heterocyclic core was synthesised via treatment of ketodiester \(^1\) with para-toluene sulfonyl hydrazide resulting in a tandem condensation/Michael addition/azamethineimine \([3+2]\) cycloaddition reaction sequence, followed by an elimination step, to deliver the desired scaffold. In order to exploit this useful strategy for the synthesis of derivatives for library synthesis, a method for the selective reaction of the pendant ester functionalities needed to be devised with the ultimate goal of producing a library of diamides. Herein we report our findings on the selective synthesis of this diamide functionalised polycyclic scaffold using a unique dimerisation strategy.

2. Results and discussion

The high yielding synthesis of diester \(^1\) can be easily performed on large scale (\(>10\) g) in three simple steps from 5-bromopent-1-ene (70% overall yield).\(^5\) As previously reported, the construction of the polycyclic core could be achieved via
reaction of 1 with \textit{para}-toluenesulfonyl hydrazide under reflux to provide compound 2. In this instance the reaction was carried out for 24 h resulting in a separable mixture of diastereomers 2a and 2b in 37% and 15% isolated yields, respectively (52% overall yield; Scheme 1).

Scheme 1.

The synthesis of 3a and 3b derived from 1. Inset—Ortep diagram of 3a, some hydrogen atoms are omitted for clarity, thermal ellipsoids shown at 50%.

It was proposed that the conversion of 1 to 2 initially proceeded via condensation with \textit{para}-toluenesulfonyl hydrazide to produce $N$-sulfonyl hydrazone A (Fig. 1). Nucleophilic attack of the imine nitrogen onto one of the $\alpha,\beta$-unsaturated esters, followed by proton transfer, then delivered intermediate B. The azomethine imine ylide B is ideally set up for an intramolecular [3+2]-dipolar cycloaddition with the remaining olefin (Fig. 1). This process sets the remaining stereochemistry of the scaffold core structure to produce intermediate C which underwent rapid elimination of \textit{para}-toluenesulfinic acid to produce 2 (Fig. 1). It was presumed that the formation of diastereomer 2b occurred post cycloaddition through a retro-Michael elimination of the pyrazoline followed by conjugate addition. A similar epimerisation has been noted in a related pyrrolidine system.\textsuperscript{5} Another possible reaction pathway is the initial [3+2]-dipolar cycloaddition via an in situ formed diazointermediate (see SI for details).
With large quantities of the diastereomeric compounds 2a and 2b in hand, the selective reaction of a single ester moiety was attempted (reduction, transesterification and transamidation) but met with only limited success. Reduction with multiple hydride reagents (NaBH$_4$, LiBH$_4$, LiAlH$_4$ and DIBAL) under a variety of conditions generally provided only the doubly reduced product or recovered starting material. The same trend was observed when attempting to obtain a monohydrolysed product. Treatment of 2awith 1 equiv of NaOH$_{(aq)}$ provided a 48% yield of the doubly hydrolysed product and the remainder of the material was untouched. Due to the apparent lack of selectivity, it was deemed that gaining a selective ester functional group interconversion was not possible without substantial further investigation. To circumvent this regioselectivity issue, a new strategy was devised involving full hydrolysis of 2 to the diacid 3, followed by a selective amidation reaction. Double hydrolysis of 2 was achieved with an excess of hydroxide to provide 3a and 3b, in 82% and 64% yields, respectively, after a simple trituration (Scheme 1). The recrystallisation of 3a and 3b was performed (MeOH; slow evaporation) to provide crystals of sufficient quality to allow analysis by X-ray crystallography, hence the relative stereochemistry of both 3a and 3b was determined unambiguously (Scheme 1, Inset and SI).

Using 3a as a model substrate, regioselective amidation of one of the carboxylic acid functions was probed. Using a variety of coupling reagents (HATU, CDI, T3P, DCC and DSC), stoichiometries, reaction conditions and hydrolytic workups were assessed but to no avail. The major problems encountered included double amidation, lack of selectivity and purification issues. These observations suggested that the issues stemmed from the high reactivity of activated esters formed in situ. The concurrent production of a non-polar dimeric species derived from the starting diacid 3a was noted as an interesting observation during some reactions. It was possible to synthesise dimer 4a by treatment of 3a by Boc anhydride (1.05 equiv), with a simple trituration providing macrocycle 4a in 83% yield and high purity.
This method was also used to produce the diastereomeric macrocycle 4b, albeit in a modest yield of 25% (Scheme 2). The structure of both 4a and 4b were confirmed by NMR and X-ray crystallographic analysis, demonstrating both the relative configuration and the 16-membered macrocyclic dianhydride functionality (Fig. 2).
Ortep diagrams of 4a (top) and 4b (bottom), hydrogen atoms and solvent molecules (CH₂Cl₂ for 4a) are omitted for clarity, thermal ellipsoids shown at 50%.

With 4a and 4b in hand, an attempt to selectively ring open the macrocycle by addition of a primary amine (2.5 equiv) was performed. Pleasingly, initial ring opening followed by the second addition of an amine to generate two molecules of acid-amide product occurred smoothly. Slow addition of the amine to 4 at low temperature was necessary to obtain good selectivities for the aliphatic amidation (Scheme 2). The use of benzylamine, n-butyramine and 4-aminomethyl tetrahydropyran all provided the acid-amides 5a–d in moderate yields after trituration from EtOAc/Et₂O. Unfortunately no significant regioselectivity was observed when using the secondary amines piperidine and dimethylaniline. In addition, anilines were also found to be poor reactants due to their low nucleophilicity. The reaction selectivity favoured amidation of the aliphatic carbonyl, presumably due the higher electrophilicity at this position, with only minor quantities of the regioisomer, diamide and regenerated diacid observed.

A structural analysis was performed where the 3 J HH coupling constants of the methylene-CH₂ were cross-correlated to the torsion angles observed in the solid state structures (Table 1). Firstly it was noted that the piperidinyl nitrogen was near planar in all of the X-ray crystal structures obtained. This demonstrates the level to which the nitrogen lone pair is conjugated through to the α,β-unsaturated ester functionality. A gauche–trans conformation is adopted by the carboxymethylene group in all the solid state structures which accounts for the difference in chemical shift in the methylene hydrogens (Δδ = 0.50 ppm). It appears that the proximity of one of the methylene hydrogens to the imine like nitrogen causes a downfield shift in the 1H NMR (Hb and Hc for the major and minor diastereomeric series, respectively, Table 1). Surprisingly large 3 J HH coupling constants were observed in both macrocycles, 11.7 Hz and 13.1 Hz for 4a and 4b, respectively (Table 1). This can be explained due to the macrocycle effectively locking the conformation of the carboxymethylene group (torsion angles of >170° are noted in the solid state, Table 1).

Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>δ Hₐ (ppm)</th>
<th>Jₐ-Hb (Hz)</th>
<th>δ Hb (ppm)</th>
<th>Jₐ-Hc (Hz)</th>
<th>δ Hc (ppm)</th>
<th>Hₐ-C-C-Hₐ torsion angle (°)</th>
<th>Hₐ-C-C-Hₐ torsion angle (°)</th>
</tr>
</thead>
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<tr>
<td>2a</td>
<td>3.80</td>
<td>6.1</td>
<td>3.05</td>
<td>7.9</td>
<td>2.65</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3a</td>
<td>3.86</td>
<td>8.0</td>
<td>3.03</td>
<td>5.9</td>
<td>2.66</td>
<td>172.2</td>
<td>70.8</td>
</tr>
<tr>
<td>4a</td>
<td>4.07</td>
<td>11.7</td>
<td>3.22</td>
<td>2.5</td>
<td>2.61</td>
<td>171.0</td>
<td>72.2</td>
</tr>
</tbody>
</table>
The second amide bond formation was found to proceed in good to quantitative yields when HATU was used as the coupling agent. Both 5a and 5b were coupled to piperidine, para-bromobenzylamine, n-butylamine and methylamine to provide the diamides 6a–h (Table 2). Figure 3 shows the C Log P versus MW for a proposed library of 133 members to demonstrate the areas of chemical space accessed by this approach (Fig. 3).

### Table 2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R'</th>
<th>R''</th>
<th>Yield (%)</th>
<th>Product</th>
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<tbody>
<tr>
<td>1</td>
<td>Bn</td>
<td>Me</td>
<td>H</td>
<td>83</td>
<td>6a</td>
</tr>
<tr>
<td>2</td>
<td>‘Bu</td>
<td>H</td>
<td>Quant.</td>
<td></td>
<td>6b</td>
</tr>
<tr>
<td>3</td>
<td>(4-BrC₆H₄)CH₂</td>
<td>H</td>
<td>Quant.</td>
<td></td>
<td>6c</td>
</tr>
<tr>
<td>4</td>
<td>–CH₂(CH₂)₃CH₂–</td>
<td>76</td>
<td></td>
<td>6d</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>‘Bu</td>
<td>Me</td>
<td>H</td>
<td>63</td>
<td>6e</td>
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<tr>
<td>6</td>
<td>‘Bu</td>
<td>H</td>
<td>88</td>
<td>6f</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>(4-BrC₆H₄)CH₂</td>
<td>H</td>
<td>98</td>
<td>6g</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>–CH₂(CH₂)₃CH₂–</td>
<td>88</td>
<td></td>
<td>6h</td>
<td></td>
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</tbody>
</table>
3. Conclusion
The use of a rapid access route to the interesting 3-dimensional polycyclic
1,2,3,3a,7,8,9,10-octahydrocyclopenta[4,5]pyrazolo[1,5-a]pyridine scaffold has been
employed to provide material for use in library synthesis. The double hydrolysis of
the ethyl esters of 2 followed by a Boc anhydride mediated macrocyclisation was
used to prepare bisanhydrides 4a and 4b. These novel, highly interesting
macrocycles have been fully characterised and the X-ray crystal structures
determined. This is the first report of the synthesis and use of such macrocyclic
bisamidinhydrides. The selective amidation to generate compounds 5a and 5b was
achieved by addition of primary amines into the bisanhydride 4a at low temperature.
This novel route provided access to the regioselective acid-amides 5 in only 3 steps
from the cyclised products 2 with no column chromatography necessary. Further
derivatisation of acid-amides 5a and 5b was achieved using a HATU mediated
amide coupling to provide access to the desired diamide derivatives (6a–h).

4. Experimental
4.1. General experimental details
Nuclear Magnetic Resonance (NMR) spectra were recorded on a 400 (Bruker®
DPX400, or AV400) or 300 MHz (Bruker® DPX300) NMR spectrometers in CDCl₃ or
CD₂OD at 300 K (unless stated otherwise). For proton NMR, samples were prepared
using ca. 5–10 mg of compound dissolved in 1.0 mL of deuterated solvent and for
carbon NMR using ca. 20 mg of compound dissolved in 1.0 mL of deuterated
solvent. All spectra were referenced to CHCl₃ or CHD₂OD the residual hydrogen
solvent peaks for 1H NMR (CHCl₃δ = 7.26 ppm, CHD₂OD δ = 3.31) and the solvent
peak for 13C{1H} NMR (CDCl₃δ = 77.0 ppm, CD₂OD δ = 49.0). NMR Chemical shifts
(δ) are reported in ppm; coupling constants (J) are reported in Hz; splitting patterns
are assigned s = singlet, d = doublet, t = triplet, q = quartet, br = broad signal and
app = the apparent multiplicity. High resolution mass spectrometry (HRMS) data was
obtained using a Bruker® MicroTOF spectrometer measured using electrospray
ionization (ESI) in the positive mode. Solvents, unless otherwise stated, were
purchased in reagent grade or anhydrous quality and used as received. THF was
distilled from Na/benzophenone immediately prior to use. Reagents were either
purchased directly from commercial suppliers or prepared according to literature procedures. All reactions were carried out in round bottomed flasks and sealed with a glass stopper or a rubber septum equipped with an N₂ balloon and heated in oil baths with a thermocouple temperature control. Reactions were monitored using aluminium backed silica thin layer chromatography with a fluorescent dye (λ = 254 nm) and visualized under UV illumination or staining with basic KMnO₄ or bromocresol green dips. Flash column chromatography was performed manually on silica gel eluting with hexane/ethyl acetate under pressurised air flow.

4.2. General procedure for the cyclisation of 1

In a 1 L round bottomed flask, diester 1 (6.0 g, 19.4 mmol, 1.0 equiv) was dissolved in toluene (800 mL) and a large stirrer bar was added. To the reaction mixture was added para-toluenesulfonyl hydrazide (4.2 g, 22.6 mmol, 1.2 equiv) and the reaction setup was equipped with a Dean–Stark apparatus and condenser. The reaction was heated at reflux for 15 h then allowed to cool to room temperature prior to filtration through a cotton wool plug. The crude reaction mixture was then reduced down in vacuo to yield a thick oil which was subjected to purification via silica gel chromatography (1:9 through to 2:3 mixtures were then added aqueous HCl (0.5 M, 46 mL) to yield the product 2a (2.32 g, 7.2 mmol, 37% yield) and 2b (953 mg, 3.0 mmol, 15% yield) in an overall yield of 52% (2a and 2b elute in this order and are easily separable).

4.2.1. Compound 2a

A light yellow oil; ¹H NMR (CDCl₃, 300 MHz) δ = 4.24 (q, J = 7.1, 2H), 4.14 (qd, J = 7.1, 1.4, 2H), 3.80 (dddd, J = 11.6, 7.9, 6.1, 2.7, 1H), 3.17 (dd, J = 9.2, 4.1, 1H), 3.05 (dd, J = 16.3, 6.1, 1H), 2.65 (dd, J = 16.3, 7.9, 1H), 2.22 (m, 1H), 1.93 (m, 1H), 1.86–1.34 (m, 10H), 1.31 (t, J = 7.1, 3H), 1.25 (t, J = 7.1, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ = 171.7, 163.8, 138.2, 80.2, 60.4, 60.2, 55.5, 53.2, 38.2, 36.1, 33.4, 32.9, 32.7, 26.5, 21.8, 14.4, 14.1; MS (EI) m/z = 667.4 (2 M+H⁺, 100), 345.2 (M+Na⁺, 31), 323.2 (M+H⁺, 61); HRMS = 323.1970 (calcd = 323.1965 C₁₇H₁₂N₂O₄⁺).

4.2.2. Compound 2b

Yellow oil; ¹H NMR (CDCl₃, 300 MHz) δ = 4.48 (m, 1H), 4.27 (qd, J = 7.1, 1.1, 2H), 4.13 (q, J = 7.1, 2H), 3.23 (dd, J = 9.0, 4.8, 1H), 2.78 (dd, J = 15.0, 6.5, 1H), 2.62 (dd, J = 15.0, 9.0, 1H), 2.12 (m, 1H), 1.97 (m, 1H), 1.88–1.49 (m, 10H), 1.33 (t, J = 7.1, 3H), 1.24 (t, J = 7.1, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ = 171.0, 163.4, 138.3, 78.2, 60.6, 60.5, 57.1, 55.1, 40.0, 39.0, 34.4, 32.8, 28.7, 26.3, 16.9, 14.5, 14.2; MS (EI) m/z = 667.4 (2 M+H⁺, 100), 345.2 (M+Na⁺, 46), 323.2 (M+H⁺, 26); HRMS = 323.1964 (calcd = 323.1965 C₁₇H₁₂N₂O₄⁺).

4.3. Procedure for the hydrolysis of 2a

In a 50 mL round bottomed flask, compound 2a (1.0 g, 3.1 mmol, 1.0 equiv) was dissolved in THF (23 mL) and a large stirrer bar was added. To the reaction mixture was added aqueous NaOH solution (1.0 M, 15.5 mL, 15.5 mmol, 5.0 equiv) and the reaction setup was stirred vigorously at room temperature for 24 h. To the reaction mixture was then added aqueous HCl (0.5 M, 40 mL) and the mixture extracted with EtOAc (3 × 80 mL). The organic extracts were then dried (MgSO₄), filtered, and reduced in vacuo to yield a crude semisolid which was triturated (EtOAc/Pet Ether 40–60—1:1, 10 mL) with sonication. The suspended solid was then filtered and washed (Pet Ether 40–60, 2 mL) to yield the product 3a (675 mg, 2.53 mmol, 82% yield).

4.3.1. Compound 3a

Colourless crystalline solid; mp = 161–163 °C; ¹H NMR (CD₂OD, 400 MHz) δ = 3.83 (dddd, J = 11.7, 7.7, 6.5, 2.8, 1H), 3.14 (dd, J = 9.3, 3.8, 1H), 2.98 (dd, J = 16.3, 7.7,
1H), 2.59 (dd, J = 16.3, 6.5, 1H), 2.29 (m, 1H), 1.94 (m, 1H), 1.85–1.50 (m, 9H), 1.31 (m, 1H); \(^{13}\text{C}\) NMR (CD$_2$OD, 101 MHz) δ = 175.2, 167.1, 139.3, 82.4, 56.5, 54.8, 39.0, 37.0, 34.4, 34.2, 34.1, 27.6, 22.9; MS (ESI) $m/z$ = 289.1 (M+Na$^+$, 100), 267.1 (M+H$^+$, 19); HRMS = 289.1158 (calcd = 289.1159 C$_{15}$H$_{18}$N$_2$NaO$_x$-$^+\) Crystals of sufficient quality to obtain an X-ray crystal structure (CCDC 1039024) were obtained by slow evaporation of 3a from a solution of methanol.

4.4. Procedure for the hydrolysis of 2b

In a 100 mL round bottomed flask, compound 2b (953 mg, 3.0 mmol, 1.0 equiv) was dissolved in THF (50 mL) and a large stirrer bar was added. To the reaction mixture at −5°C was added aqueous NaOH solution (1.0 M, 20.0 mL, 20.0 mmol, 6.8 equiv) and the reaction setup was stirred vigorously at room temperature for 24 h. To the reaction mixture was then added aqueous HCl (0.5 M, 40 mL) and the mixture extracted with EtOAc (3 × 80 mL). The organic extracts were then dried (MgSO$_4$), filtered, and reduced down in vacuo to yield a crude semisolid which was triturated (EtOAc/Pet Ether 40–60–1:1, 10 mL) with sonication. The suspended solid was then filtered and washed (Pet Ether 40–60, 2 mL) to yield the product 3b (504 mg, 1.89 mmol, 64% yield).

4.4.1. Compound 3b

Off white solid (CCDC 1039026); mp = 154–155 °C; \(^1\)H NMR (CDCl$_3$, 300 MHz) δ = 4.49 (m, 1H), 3.23 (dd, J = 9.0, 4.7, 1H), 2.78 (dd, J = 15.0, 8.2, 1H), 2.60 (dd, J = 15.0, 7.4, 1H), 2.16 (m, 1H), 2.03–1.51 (m, 11H); \(^{13}\text{C}\) NMR (CDCl$_3$, 75 MHz) δ = 176.5, 166.8, 137.4, 79.4, 56.3, 55.4, 40.1, 38.7, 34.7, 32.6, 29.2, 26.3, 16.9; MS (ESI) $m/z$ = 555.2 (2 M+Na$^+$, 100), 289.1 (M+Na$^+$, 51), 267.1 (M+H$^+$, 29); HRMS = 289.1158 (calcd = 289.1154 C$_{15}$H$_{18}$N$_2$NaO$_x$-$^+\).

4.5. Procedure for the macrocyclisation of 3a

In a 50 mL round bottomed flask, compound 3a (1.25 g, 4.69 mmol, 1.0 equiv) was dissolved in MeCN/DMF (10:1, 44 mL) along with DMAP (120 mg, 20 mol %) and a stirrer bar was added. To the stirred reaction mixture at −5 °C was added a solution of di-tert-butyl dicarbonate (1.08 g, 4.9 mmol, 1.05 equiv dissolved in 20 mL of MeCN) dropwise over 10 min and the reaction mixture was allowed to warm up to room temperature over 18 h with stirring. The reaction mixture was then worked up with aqueous HCl (0.5 M, 120 mL) and extracted with CH$_2$Cl$_2$ (3 × 200 mL). The organic extracts were then dried (MgSO$_4$), filtered, and reduced down in vacuo to yield a crude solid which was triturated (EtOAc/Pet Ether 40–60–3:1, 5 mL) with sonication. The suspended solid was then filtered and washed (EtOAc, 2 mL) to yield the product 4a (963 mg, 1.94 mmol, 83% yield).

4.5.1. Compound 4a

Colourless crystalline solid; mp = 192–194 °C; \(^1\)H NMR (CDCl$_3$, 400 MHz) δ = 4.07 (app tt, J = 11.7, 2.5, 1H), 3.30 (app t, J = 6.1, 1H), 3.22 (dd, J = 17.5, 11.7, 1H), 2.61 (dd, J = 17.5, 2.5, 1H), 2.32 (m, 1H), 1.99–1.89 (m, 2H), 1.87–1.35 (m, 9H); \(^{13}\text{C}\) NMR (CDCl$_3$, 101 MHz) δ = 167.2, 159.7, 133.8, 81.1, 53.6, 52.9, 37.0, 36.9, 35.4, 33.5, 33.4, 25.8, 21.7; MS (ESI) $m/z$ = 519.2 (M+Na$^+$, 100), 497.2 (M+H$^+$, 17), 289 (C$_{16}$H$_{20}$N$_2$O$_4$+Na$^+$, 32), 193.1 (C$_{11}$H$_{14}$N$_2$O$_2$+H$^+$, 43); HRMS = 519.2240 (calcd = 519.2214 C$_{15}$H$_{22}$N$_2$NaO$_x$-$^+\). Crystals of sufficient quality to obtain an X-ray crystal structure (CCDC 1039023) were obtained by slow evaporation of 4a from a solution of CH$_3$Cl$_2$/EtOAc—2:1.

4.6. Procedure for the macrocyclisation of 3b

In a 25 mL round bottomed flask, compound 3b (375 mg, 1.41 mmol, 1.0 equiv) was dissolved in MeCN/DMF (10:1, 13.2 mL) along with DMAP (36 mg, 20 mol %) and a stirrer bar was added. To the stirred reaction mixture at −15 °C was added a solution
of di-tert-butyl dicarbonate (324 mg, 1.48 mmol, 1.05 equiv dissolved in 6.0 mL of MeCN) dropwise over 10 min and the reaction mixture was allowed to warm up to room temperature over 18 h with stirring. The progress of the reaction was monitored by TLC (basic KMnO₄ stain) which showed that the reaction was not as clean as observed in the synthesis of 4a, hence the reaction was allowed to equilibrate for a further 72 h. After this time a further quantity of di-tert-butyl dicarbonate (97 mg, 0.44 mmol, 0.30 equiv dissolved in 2.0 mL of MeCN) was added dropwise over 10 min at −15 °C and the reaction mixture was then allowed to warm up to room temperature over the subsequent 18 h. At this point little difference to the initial analysis was observed so the reaction mixture was then worked up with aqueous HCl (0.05 M, 100 mL) and extracted with CH₂Cl₂ (3 × 80 mL) and EtOAc (2 × 50 mL). The organic extracts were combined then dried (MgSO₄), filtered, and reduced down in vacuo to yield a crude solid which purified via silica gel chromatography (1:9 through to 2:3—EtOAc/Pet Ether 40–60). The product containing fractions were reduced down to yield compound 4b (88 mg, 0.18 mmol, 25% yield).

4.6.1. Compound 4b
White crystalline solid; mp = 193–195 °C; ¹H NMR (CDCl₃, 400 MHz) δ = 4.76 (dd, J = 13.1, 5.8, 3.3, 1H), 3.29 (dd, J = 8.7, 5.0, 1H), 2.92 (app t, J = 13.1, 1H), 2.47 (dd, J = 12.9, 3.3, 1H), 2.37 (app dt, J = 12.5, 6.2, 1H), 2.07–1.91 (m, 2H), 1.84–1.48 (m, 9H); ¹³C NMR (CDCl₃, 101 MHz) δ = 166.7, 159.2, 135.0, 79.8, 57.1, 55.7, 40.2, 39.6, 36.2, 32.5, 31.2, 26.0, 17.3; MS (ESI) m/z = 519.2 (M+Na⁺, 100), 497.2 (M+H+, 18), 289 (C₁₃H₁₈N₂O₅Na⁺, 4), 193.1 (C₁₁H₁₆N₂O⁺, 11); HRMS = 519.2233 (calcd = 519.2214 C₂₉H₂₆N₂NaO₅⁺). Crystals of sufficient quality to obtain an X-ray crystal structure (CCDC 1039025) were obtained by slow evaporation of 4b from a solution of CH₂Cl₂/EtOAc—2:1.

4.7. General procedure for the macrocyclic ring opening of 4a and 4b with an amine
A round bottomed flask, containing a 20 mM solution of macrocycle 4a or 4b (1.0 equiv) in MeCN/DMF—1:1 and a stirrer bar was cooled to around −18 °C using a salt/ice bath. To the stirred reaction mixture a 0.2 M solution of the primary amine (2.5 equiv) in MeCN/DMF 1:1 was added dropwise over 10 min and the reaction mixture was allowed to warm up to room temperature over 18 h with stirring. The reaction mixture was then worked up with aqueous HCl (0.05 M) and extracted thrice with EtOAc. The organic extracts were then dried (MgSO₄), filtered, and reduced down in vacuo to yield a crude gummy product which was thoroughly dried under high vacuum (ca. 1 mbar). The purified products could then be obtained by trituration of the crude product with various solvents and solvent mixtures (e.g., Et₃O, EtOAc/Pet Ether 40–60—1:1, EtOAc/Et₃O—1:1) where the suspended solid was then filtered and washed (Et₃O) to yield the desired product 5.

4.7.1. Compound 5a
The general protocol was performed on 0.40 mmol of 4a which (after trituration with EtOAc/Et₃O—1:1) provided compound 5a (163 mg, 0.46 mmol, 57% yield) as a white solid; mp = 96–98 °C; ¹H NMR (CDCl₃, 400 MHz) δ = 7.29–7.18 (m, 5H), 6.65 (br dd, J = 6.7, 5.0, 1H), 4.55 (dd, J = 14.8, 6.7, 1H), 4.25 (dd, J = 14.8, 5.0, 1H), 3.87 (dddd, J = 12.1, 9.5, 4.3, 2.7, 1H), 3.16 (dd, J = 9.4, 3.9, 1H), 2.95 (dd, J = 14.0, 9.5, 1H), 2.45 (dd, J = 14.0, 4.3, 1H), 2.19 (ddd, J = 12.8, 6.1, 3.2, 1H), 1.90 (m, 1H), 1.82–1.61 (m, 6H), 1.60–1.45 (m, 2H), 1.42–1.26 (m, 2H); ¹³C NMR (CDCl₃, 101 MHz) δ = 170.1, 166.5, 138.2, 137.2, 128.5, 127.6, 127.3, 81.5, 54.7, 54.5, 43.5, 40.4, 36.1, 33.7, 33.6, 33.0, 26.6, 21.9; MS (ESI) m/z = 378.2 (M+Na⁺, 89), 356.2 (M+H+, 100); HRMS = 356.1979 (calcd = 356.1969 C₂₉H₂₆N₂O⁺).
The general protocol was performed on 0.40 mmol of 4a which (after trituration with Et₂O) provided compound 5b (116 mg, 0.36 mmol, 45% yield) as a white solid; mp = 182–185 °C; ¹H NMR (CDCl₃, 400 MHz) δ = 6.32 (br m, 1H), 3.86 (dd, dd, J = 12.0, 9.2, 4.5, 2.9, 1H), 3.32 (m, 1H), 3.19 (dd, J = 9.3, 3.9, 1H), 3.10 (m, 1H), 2.90 (dd, J = 13.9, 9.2, 1H), 2.41 (dd, J = 13.9, 4.5, 1H), 2.24 (m, 1H), 1.94 (m, 1H), 1.84–1.21 (m, 14H), 0.87 (t, J = 7.2, 3H); ¹³C NMR (CDCl₃, 101 MHz) δ = 171.0, 166.8, 137.1, 81.5, 54.7, 45.2, 40.6, 36.2, 35.3, 33.8, 33.7, 33.1, 30.5, 26.7, 21.9, 19.9, 13.7; MS (ESI) m/z = 344.2 (M+H⁺, 68), 322.2 (M+H⁺, 100); HRMS = 322.2123 (calcd 322.2125 C₁₁H₁₆N₂O₂⁺).

4.7.3. Compound 5c
The general protocol was performed on 96 μmol of 4a which (after trituration with Et₂O) provided compound 5c (37 mg, 107 μmol, 54% yield) as a white solid; ¹H NMR (CDCl₃, 270 MHz) δ = 6.50 (br m, 1H), 3.97–3.82 (m, 3H), 3.39–3.24 (m, 3H), 3.19 (dd, J = 9.3, 3.6, 1H), 2.98–2.85 (m, 2H), 2.43 (dd, J = 13.8, 4.1, 1H), 2.24 (m, 1H), 1.49 (m, 1H), 1.84–1.17 (m, 15H); ¹³C NMR (CDCl₃, 90 MHz) δ = 171.1, 166.9, 137.0, 81.5, 67.6, 54.7, 45.2, 40.6, 36.2, 35.3, 33.8, 33.7, 33.1, 30.5, 26.7, 21.9 (16 out of a possibly 17 resonances observed); MS (ESI) m/z = 386.2 (M+Na⁺, 100), 364.2 (M+H⁺, 84); HRMS = 364.2225 (calcd 364.2223 C₁₀H₁₆N₂O₂⁺).

4.7.4. Compound 5d
The general protocol was performed on 0.10 mmol of 4b which (after trituration with EtOAc/Et₂O—3:1) provided compound 5d (33 mg, 0.09 mmol, 46% yield) as a white solid; ¹H NMR (CDCl₃, 400 MHz) δ = 7.36–7.22 (m, 5H), 6.10 (br m, 1H), 4.48 (dd, J = 14.6, 5.9, 1H), 4.38 (dd, J = 14.6, 5.5, 1H), 4.29 (m, 1H), 3.23 (dd, J = 8.8, 4.5, 1H), 2.73 (dd, J = 15.0, 8.0, 1H), 2.55 (dd, J = 15.0, 6.9, 1H), 2.03–1.52 (m, 11H), 1.39 (m, 1H); ¹³C NMR (CDCl₃, 101 MHz) δ = 170.2, 165.5, 138.5, 138.0, 128.7, 127.9, 127.5, 79.2, 56.3, 55.6, 43.7, 40.8, 40.4, 34.2, 32.6, 28.7, 26.1, 16.8; MS (ESI) m/z = 378.2 (M+Na⁺, 100), 356.2 (M+H⁺, 22); HRMS = 356.1971 (calcd = 519.1969 C₂₉H₄₅N₃O₄⁺).

4.8. General procedure for the coupling of 5a and 5b with an amine
To a round bottomed flask equipped with a stirrer bar, containing a 100 mM solution of carboxylic acid 5a or 5b (1.0 equiv) in DMF, the amine (2.0 equiv) and DIPEA (2.0 equiv: note if a hydrochloride salt of the amine was used a larger excess of DIPEA was as used as added) was added. The reaction vessel was cooled to around −18 °C using a salt/ice bath and a solution of HATU (ca. 0.5 M) was added, the reaction mixture was allowed to warm up to room temperature over 18 h with stirring. The reaction mixture was then worked up with the addition of aqueous 1 M HCl (30 mL) and extracted with EtOAc (100 mL). The organic extract was washed sequentially aqueous 1 M HCl (30 mL), saturated NaHCO₃ (30 mL) and 20% brine (2 × 30 mL). The organic solution was then dried (MgSO₄), filtered, and reduced down in vacuo and dried under high vacuum (ca. 1 mbar) to yield the desired product 6.

4.8.1. Compound 6a
The general protocol was performed on 0.11 mmol of 5a which provided compound 6a (34 mg, 93 mmol, 83% yield) as a clear gum; ¹H NMR (CDCl₃, 300 MHz) δ = 7.33–7.17 (m, 5H), 6.33 (br m, 1H), 6.23 (br m, 1H), 4.50 (dd, J = 14.6, 6.0, 1H), 4.33 (dd, J = 14.6, 5.1, 1H), 3.65 (dd, J = 11.8, 9.9, 4.4, 2.8, 1H), 3.18 (dd, J = 9.7, 4.5, 1H), 2.94 (dd, J = 15.0, 9.9, 1H), 2.81 (d, J = 5.5, 3H), 2.45 (dd, J = 15.0, 4.4, 1H), 2.06 (dd, J = 12.2, 6.3, 2.9, 1H), 1.90 (m, 1H), 1.74–1.20 (m, 10H); ¹³C NMR (CDCl₃, 101 MHz) δ = 170.1, 163.4, 145.9, 138.1, 128.7, 127.7, 127.5, 80.6, 55.7, 53.7, 49.3, 43.6, 40.4, 35.9, 32.1, 32.0, 27.2, 25.8, 21.6; MS
The general protocol was performed on 0.11 mmol of 4a which provided compound 6b (16.1 mg, 0.038 mmol, 76% yield) as a clear gum; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ = 7.32–7.18 (m, 5H), 6.43 (br m, 1H), 4.48 (dd, $J$ = 14.7, 5.9, 1H), 4.31 (dd, $J$ = 14.7, 5.2, 1H), 3.69–3.52 (m, 5H), 3.22 (dd, $J$ = 9.6, 5.0, 1H), 2.97 (dd, $J$ = 14.8, 10.3, 1H), 2.40 (dd, $J$ = 14.8, 4.0, 1H), 2.04 (m, 1H), 1.89 (m, 1H), 1.76–1.22 (m, 16H); $^{13}$C NMR (CDCl$_3$, 101 MHz) $\delta$ = 171.3, 162.7, 146.9, 138.1, 128.7, 127.7, 127.5, 127.3, 127.0, 121.2, 120.8, 85.6, 53.7, 43.6, 40.7, 35.7, 31.9, 31.8, 31.6, 29.7, 27.1, 24.7, 21.5 (20 out of a possibly 21 resonances observed); MS (ESI) $m/z$ = 445.3 (M+Na$^+$, 95), 423.3 (M+H$^+$, 100); HRMS = 423.2774 (calcd = 423.2755 C$_{25}$H$_{28}$N$_2$O$_7$).
1H), 3.36–3.15 (m, 4H), 2.90 (dd, J = 15.0, 9.2, 1H), 2.37 (dd, J = 15.0, 4.8, 1H), 2.13 (m, 1H), 1.98 (m, 1H), 1.83–1.47 (m, 8H), 1.47–1.20 (m, 6H), 0.94 (t, J = 7.3, 3H), 0.89 (t, J = 7.3, 3H); 13C NMR (CDCl₃, 101 MHz) δ = 171.2, 162.8, 145.6, 80.6, 55.6, 53.8, 40.5, 39.0, 38.8, 35.9, 32.2 (2×C), 32.1, 32.0, 31.6, 27.2, 21.6, 20.1, 20.0, 13.8, 13.7; MS (ESI) m/z = 399.3 (M+Na⁺, 89), 377.3 (M+H⁺, 100); HRMS = 377.2925 (calcd = 377.2911 C₂₁H₃₅N₄O₂⁺).

4.8.7. Compound 6g
The general protocol was performed on 75 μmol of 5b which provided compound 6g (36 mg, 74 μmol, 98% yield) as a white solid; mp = 61–63 °C; 1H NMR (CDCl₃, 300 MHz) δ = 7.45 (d, J = 8.4, 2H), 7.20 (d, J = 8.4, 2H), 6.68 (br m, 1H), 5.86 (br m, 1H), 4.48 (dd, J = 15.0, 6.4, 1H), 4.41 (dd, J = 15.0, 6.0, 1H), 3.65 (ddddd, J = 11.6, 8.6, 5.4, 2.7, 1H), 3.33–3.08 (m, 3H), 2.83 (dd, J = 14.8, 5.4, 1H), 2.33 (dd, J = 14.8, 5.4, 1H), 2.15 (m, 1H), 1.99 (m, 1H), 1.86–1.50 (m, 8H), 1.48–1.34 (m, 2H), 1.32–1.17 (m, 4H), 0.86 (t, J = 7.3, 3H) (Note: signals at 4.48 and 4.41 are heavily roofed); 13C NMR (CDCl₃, 101 MHz) δ = 170.9, 163.0, 144.5, 137.8, 131.7, 129.6, 121.3, 80.8, 55.5, 53.8, 42.5, 40.6, 39.1, 35.9, 32.3 (2×C), 31.6, 27.2, 21.7, 20.0, 13.7 (Note: 21 out of a possible 22 resonances observed); MS (ESI) m/z = 513.2 (MBr⁺Na⁺, 45), 511.2 (MBr⁺Na⁺, 44), 491.2 (MBr⁺H⁺, 98), 489.2 (MBr⁺H⁺, 100); HRMS = 489.1897 (calcd = 489.1860 C₂₄H₃₄N₄O₂Br⁺).

4.8.8. Compound 6h
The general protocol was performed on 75 μmol of 5b which provided compound 6h (25.6 mg, 66 μmol, 88% yield) as a white solid; 1H NMR (CDCl₃, 300 MHz) δ = 6.09 (br m, 1H), 3.90–3.50 (br m, 4H), 3.60 (ddddd, J = 11.6, 9.8, 4.2, 2.7, 1H), 3.30–3.16 (m, 3H), 2.85 (dd, J = 14.8, 9.8, 1H), 2.33 (dd, J = 14.8, 4.2, 1H), 2.12 (m, 1H), 1.98 (m, 1H), 1.81–1.48 (m, 14H), 1.46–1.36 (m, 2H), 1.35–1.21 (m, 4H), 0.89 (t, J = 7.2, 3H); 13C NMR (CDCl₃, 101 MHz) δ = 171.3, 162.8, 146.5, 78.9, 58.5, 53.7, 40.7, 39.0, 35.7, 32.1, 31.9, 31.6, 29.6, 27.2, 24.7, 21.5, 20.0, 13.7 (18 out of a possibly 20 resonances observed); MS (ESI) m/z = 411.3 (M+Na⁺, 100), 389.3 (M+H⁺, 97); HRMS = 389.2926 (calcd = 389.2911 C₂₂H₃₅N₄O₂⁺).

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Supplementary data
Supplementary data.

Supplementary data 1.
Spectral data.
Supplementary data 2.
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6. Plot calculated using the European Lead Factory web tool, designed by ChemAxon, a free to use submission tool for the design of libraries and submission to the European Lead Factory.