Synthesis of macrolactam analogues of radicicol and their binding to heat shock protein Hsp90†

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TOC Graphic

Macrolactam analogues of the natural lactone radicicol bind to Hsp90.

Abstract

A series of macrolactam analogues of the naturally occurring resorcylic acid lactone radicicol have been synthesised from methyl orsellinate in 7 steps, involving chlorination, protection of the two phenolic groups, and hydrolysis to the benzoic acid. Formation of the dianion and quenching with a Weinreb amide results in acylation of the toluene methyl group that is followed by amide formation and ring closing metathesis to form the macrocyclic lactam. Final deprotection of the phenolic groups gives the desired macrolactams whose binding to the N-terminal domain of yeast

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† Electronic supplementary information (ESI) available; copies of NMR spectra.
Hsp90 was studied by isothermal titration calorimetry and protein X-ray crystallography.

**Introduction**

In recent years, the chaperone heat shock protein 90 (Hsp90) has become one of the most attractive and widely studied biological targets for molecular cancer therapeutics.\(^1\), \(^2\) Ubiquitous in eukaryotic cells, Hsp90 utilises the energy released from ATP-hydrolysis to effect the maturation or activation of client proteins and has been found to play a pivotal role in numerous oncogenic pathways. More recently, the significance of Hsp90 has increased further following the discovery of its relevance in a number of other important medical conditions, ranging from neurodegenerative conditions,\(^3\)-\(^8\) to HIV/AIDS,\(^9\)-\(^11\) and malaria.\(^12\)-\(^14\) As a result, significant research efforts have been devoted to discovering and synthesising potent small molecule inhibitors of Hsp90, a topic which has been the focus of many reviews.\(^2\), \(^9\), \(^10\), \(^15\)-\(^22\)

Seminal research in this area was centred on the natural products geldanamycin 1,\(^23\), \(^24\) a benzoquinone ansamycin polyketide, which has been the subject of previously published research from our group,\(^25\)-\(^27\) and radicicol 2, a resorcylic acid lactone (RAL) first isolated in 1953,\(^28\) which is the most potent *in vitro* Hsp90 inhibitor found to date (IC\(_{50}\) =20–23 nM).\(^29\)-\(^31\) Unfortunately, radicicol exhibits no *in vivo* activity, which may be due to the highly sensitive functionality present in the molecule, including an epoxide and a conjugated dienone, both of which are readily metabolised. As a result, significant efforts have focussed on synthesising radicicol analogues which retain the potency, yet are more metabolically stable (Figure 1).\(^2\) Having established the importance of the epoxide stereochemistry for good activity, Danishefsky and co-workers replaced the C5-6 epoxide with a cyclopropyl group in order to improve the
stability of the molecule. Although a drop in potency was observed (IC\textsubscript{50} = 160 nM vs. IC\textsubscript{50} = 20-23 nM for radicicol), cycloproparadiciol 3 was still a very potent Hsp90 inhibitor. It is noteworthy that the stereochemistry of the cyclopropyl group was also crucial for the activity. Additionally, the epoxide has been replaced with a thiirane,\textsuperscript{32} a cyclic carbonate,\textsuperscript{32} a diol\textsuperscript{32} and a carbon chain (both saturated and unsaturated).\textsuperscript{33} Whilst the former three are significantly detrimental to the potency, a carbon chain is tolerated with only a two-fold drop in performance.\textsuperscript{33} The replacement of the dienone with more stable functionalities has also been investigated. Moody and Shinonaga have developed radicicol analogues with a C11 ketone and a either a saturated carbon chain from C7-C10 or an enone (4 and 5, respectively).\textsuperscript{32-34} Additionally, Danishefsky has replaced the dienone of cycloproparadicicol with a triazole 6\textsuperscript{35} and we have also incorporated a triazole into the macrocycle (compound 7) to investigate the benefits of additional H-bonding potential.\textsuperscript{36} Unfortunately, it appears that the conformational constraints of RALs is important for binding to the Hsp90 N-terminal domain and, as such, all of the analogues showed a significant drop in activity compared to radicicol itself, although potencies commensurate with other important Hsp90 inhibitors were achievable.\textsuperscript{35}
High throughput screening of simplified radicicol analogues, identified resorcinol-containing diaryl pyrazoles as potent Hsp90 inhibitors.\(^{37}\) The hydrophobic group at C5 (alkyl or halide), along with two free phenols were found to be essential for a high binding affinity from SAR analysis, whilst the heterocycle was found to be variable, with isoxazoles, triazoles and benzisoxazoles also potent Hsp90 inhibitors.\(^{17, 22, 38, 39}\) For such compounds, the heterocycle mimics the radicicol lactone unit, satisfying the same H-bonding interactions with the Hsp90 ATP-binding site. Lead optimisation gave compounds such as the resorcinol-pyrazole CCT018159 9 (IC\(_{50} = 7.1\) µM), reported by Workman and colleagues.\(^{40, 41}\) Presently, there are currently four closely related compounds in clinical trials.\(^2\) These include ganetespib 10, owned by Synta Pharmaceuticals, administered intravenously, which has been evaluated in the clinic against non-small cell lung carcinoma and advanced solid tumours, and NVP-AU922
11, another intravenous drug owned by Vernalis-Novartis, which is currently in phase 2 clinical trials.²

![Chemical structures of Hsp90 inhibitors](image)

**Figure 2.** Pyrazole and isoxazole-containing Hsp90 inhibitors.

Our laboratory has been interested in developing novel Hsp90 inhibitors, including the synthesis of more potent and metabolically stable radicicol analogues, for some years.⁵⁻²⁷, ³³, ³⁴, ³⁶, ⁴², ⁴³ As previously mentioned, despite still inhibiting Hsp90 effectively, replacement of the dienone with a C-11 ketone and the epoxide with a double bond or a saturated hydrocarbon chain (compound 4) led to a 2-fold decrease in activity compared to radicicol itself,³³ whilst the introduction of extra H-bonding, either at the 6-position, or from a triazole in the macrocycle,³⁴, ³⁶ gave a conformational change in the molecule and a loss in binding affinity. We also investigated the effect of varying the ring size and double bond position within the macrocycle and found that those analogues with 14 or 15-membered rings and the double bond located γ,δ- with respect to the lactone ring oxygen to be the most potent Hsp90 inhibitors, whilst a 12,13 or 16-membered ring gave depleted activity.³³ More
recently, we investigated the synthesis of a series of resorcylic acid lactams as more metabolically stable radicicol analogues, a strategy widely employed in drug development and also utilised in the development of similar RAL analogues by Danishefsky and Winssinger. Our original synthetic route is outlined in Scheme 1: addition of the malonate-derived acid chloride 13 to anhydride 12 gave the acylated intermediate, which spontaneously underwent a cyclisation/retrocyclisation, with the loss of CO₂, giving the isocoumarin 14 in 75% yield. This was readily converted into both the NH and NMe macrolactams 16 (see Figure 3 for bound X-ray structure) and 17, respectively, and deprotection of the β-keto ester intermediate 15 also allowed for the incorporation of amide and ester functional groups at C10 (18 and 19, respectively), giving additional H-bonding potential. Pleasingly, the macrolactams were indeed more metabolically stable (43% metabolism for macrolactam 17 vs. 84% for radicicol after 15 minutes with human liver microsomes and NADH/NADPH) and significantly, were also often superior to the corresponding macrolactones in terms of activity.

Analysis of the Hsp90-bound protein structures of compounds 18 and 19 showed by X-ray crystallography that the C10 substituents displaced a loop between Leu93 and Lys98 in the ATP-binding site, allowing access to a further hydrophobic pocket, favourable for binding, and enhancing the biological properties of these analogues. Since only one of these analogues contained a substituted lactam nitrogen, we wanted to extend our SAR and synthesise more of these analogues. We herein report a new and improved synthetic route to N-methylated resorcylic acid macrolactams, which is shorter and more convergent and allows us to vary the ring size and the position of the
double bond. We also report the binding of these analogues to Hsp90, as studied by protein crystallography.

**Scheme 1.** Original synthesis of 14-membered macrolactams, analogues of the RALs.\(^{43}\)

**Figure 3.** Hsp90-bound X-ray crystal structure of macrolactam 16.\(^{43}\) Image from the article by Day *et al.*,\(^{43}\) reprinted, with permission from *ACS Chem. Biol.*, 2011, 6, 1339-1347. J. E. H. Day, S. Y. Sharp, M. G. Rowlands, W. Aherne, A. Hayes, F. I.
Results and Discussion

The synthesis commenced from the methyl orsellinate 20, readily prepared via a self-condensation of methyl acetoacetate.\(^{25}\) In our hands, the success of the previously reported\(^{45-47}\) sulfuryl chloride-mediated chlorination was found to be highly temperature dependent; a reaction temperature of \(\sim 30 \, ^\circ\text{C}\) was crucial for selective monochlorination, with the product 21 isolated in 98% yield, whereas higher temperatures gave further chlorination. Protection of the resorcinol as the ethoxymethoxy ether proceeded smoothly, but saponification of the ester proved to particularly sluggish, with lengthy reaction times at reflux with potassium hydroxide required. Additionally, the orsellinic acid product 23\(^{34, 48}\) was incompatible with chromatography; any attempts led to deprotection of the hydroxyl group at the 6-position. Instead, a carefully controlled work-up procedure was performed, washing the basic solution with organic solvent to remove any organic impurities, then rapid acidification and extraction of the pre-cooled solution to minimise any undesired deprotection. Satisfactory yields of 60-69% were achievable via this method.
Scheme 2. The synthesis of orsenillic acid 23 [EOM = ethoxymethyl].

With the key intermediate 23 in hand, our focus now turned to the amide coupling and benzylic acylation steps. With the option of varying the order of the two steps, we initially performed a HATU-mediated amide coupling with N-methylbut-3-en-1-amine and obtained the product in quantitative yield. However, subsequent attempts to deprotonate and acylate the methyl group were unsuccessful with a variety of bases, additives and reaction conditions. We therefore turned to dianion chemistry, described previously in similar synthetic routes. Initial attempts to acylate the dianion formed from the treatment of orsellinic acid 23 with two equivalents of base (LDA or s-BuLi) were particularly low yielding, with only traces of product obtained and no purification possible without protecting group cleavage as described previously. Following significant investigative effort, it was established that the scale and concentration of the acylations were crucial for the success of the reaction. Following optimisation studies, the reactions were performed on scales in excess of 0.5 mmol of orsellinic acid, at a concentration of 0.2 M in THF at −78 °C, with the product 24 being coupled with the relevant amine salt without further purification. This protocol gave satisfactory yields over two steps (Scheme 3).
**Scheme 3.** Acylation and coupling investigations.

Interestingly, the products 25 exhibited magnetic inequivalence in the proton NMR spectrum, exemplified by the splitting of the benzylic protons, observed as a pair of roofed doublets \((J = 17 \text{ Hz})\). We postulated that this is a result of the \(N\)-methyl group being sufficiently bulky to hinder the rotation past the lower chain chain, creating pairs of rotamers.

The macrocycles were formed *via* ring closing metathesis, utilising Grubbs’ 2\(^{\text{nd}}\) generation catalyst, in moderate to good yield and as mixtures of \(E/Z\)-isomers. Deprotection of the EOM groups proceeded smoothly with trifluoroacetic acid, giving the \(N\)-methyl resorcylic acid macrolactams 27 in up to 12\% yield over four steps from the known precursor 23. The macrocycles were characterised as inseparable mixtures of \(E/Z\)-isomers, with the major component being the \(E\)-isomer in all cases with the exception of the 14-membered lactam 27d, which was predominantly the \(Z\)-isomer.

**Scheme 4.** Formation of the radicicol analogues.

Next, the thermodynamics of the binding of macrocycles 27b-d to Hsp90 was investigated through isothermal titration calorimetry (ITC) (Table 1). The results

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**Table 1.** Thermodynamic parameters for the binding of macrocycles 27b-d to Hsp90. 

<table>
<thead>
<tr>
<th>Sample</th>
<th>(n)</th>
<th>(m)</th>
<th>Yield (%)</th>
<th>(E/Z)</th>
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<tr>
<td>a</td>
<td>2</td>
<td>1</td>
<td>51</td>
<td>4:1</td>
</tr>
<tr>
<td>b</td>
<td>3</td>
<td>1</td>
<td>39</td>
<td>2:1</td>
</tr>
<tr>
<td>c</td>
<td>4</td>
<td>1</td>
<td>34</td>
<td>4:1</td>
</tr>
<tr>
<td>d</td>
<td>1</td>
<td>2</td>
<td>68</td>
<td>1:9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>(n)</th>
<th>(m)</th>
<th>Yield (%)</th>
<th>(E/Z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2</td>
<td>1</td>
<td>72</td>
<td>9:1</td>
</tr>
<tr>
<td>b</td>
<td>3</td>
<td>1</td>
<td>45</td>
<td>3:1</td>
</tr>
<tr>
<td>c</td>
<td>4</td>
<td>1</td>
<td>33</td>
<td>5:1</td>
</tr>
<tr>
<td>d</td>
<td>1</td>
<td>2</td>
<td>28</td>
<td>1:4</td>
</tr>
</tbody>
</table>
show that there is a significant enthalpic penalty compared to radicicol, although the binding of the macrolactams was found to be superior to the macrolactone analogues we have previously described\(33\) \((K_d = 210-1200 \ \mu M)\). It should be noted that compounds 27b-d are mixtures of geometric isomers, albeit with heavy predominance of one isomer in each case, that makes interpretation of the binding data less clear. Nevertheless the data suggest that the interaction of the epoxide plays a significant role in the interaction with the protein.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>N</th>
<th>(K_d/\mu M)</th>
<th>(\Delta H / \text{kJmol}^{-1})</th>
<th>(\Delta S / \text{Jmol}^{-1} \text{K}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radicicol</td>
<td>1.16</td>
<td>0.015±0.06</td>
<td>-6726</td>
<td>13.6</td>
</tr>
<tr>
<td>27b</td>
<td>1.0 (fixed)</td>
<td>144±25</td>
<td>-1012</td>
<td>14.2</td>
</tr>
<tr>
<td>27c</td>
<td>0.92</td>
<td>41.8±8.7</td>
<td>-834.8</td>
<td>17.3</td>
</tr>
<tr>
<td>27d</td>
<td>1.1</td>
<td>110±7.9</td>
<td>-1466</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 1. Isothermal titration calorimetry for macrolactams 27b-d with yeast Hsp90 N-terminal domain (N = stoichiometry of binding).

The radicicol analogues 27b-d were co-crystallised (major E/Z-isomer crystallised in all cases) with yeast Hsp90 in order to probe the interaction of the compounds with the Hsp90 N-terminal domain (Figure 4). The resorcylic acid macrolactams 27 bind to Hsp90 in a similar fashion to radicicol 2, with the same interactions exhibited by the resorcinol group in all cases. However, there is a clear conformational change in the macrocycle from radicicol to the macrolactams, emphasising the importance of the interaction between the radicicol epoxide and the Hsp90 backbone. For radicicol the electron density contoured at 1.5\(\sigma\) is clearly visible for the whole molecule. In contrast, for radicicol analogues 27b-d, electron density for the macrocycle was poor. Electron density contoured at 1.5\(\sigma\) for compound 27b and 27d was lacking for carbon
atoms 4 to 9 of the macrocycle ring. For compounds 27c the macrocycle was not represented by clear electron density for the carbon atoms 7 to 11. These observations are consistent with the idea that the epoxide group is important for radicicol binding. This is even more apparent on studying the overlay of radicicol 2 with the corresponding NH and NMe macrolactam analogues (17 and 27d, respectively), depicted in Figure 5.
**Figure 4.** Structures of resorcylic acid macrolactams (yellow) bound in the ATP site of yeast Hsp90 as determined by protein X-ray crystallography, showing the interactions of **a** radicicol 2 with Hsp90 (green and cyan residues); **b**, macrolactam 27b (E-alkene) with Hsp90 (green and cyan residues); **c**, macrolactam 27c (E-alkene) with Hsp90 (green and cyan residues); **d**, macrolactam 27d (Z-alkene) with Hsp90 (green and cyan residues). In general, the analogues exhibit a significant conformational change in the macrocycle. Dotted blue lines: hydrogen bonds, red-coloured spheres: water molecules. The structures for radicicol and the macrolactam analogues were obtained at 2.5 (radicicol), 2.4 (27b), 2.0 (27c) and 2.3 Å (27d) resolution and the atomic coordinates and structure factors deposited with the following PDB codes 4ce2, 4ce1 and 4ce3, respectively.
**Figure 5.** Overlaid crystal structures showing the difference in the conformation and binding of resorcylic acid macrolactams in comparison to radicicol. The different conformations of radicicol 2 (PDB code 1BGQ; green), NH macrolactam 16 (PDB code 2XX2; yellow) and NMe macrolactam 27d (PDB code 4ce3; cyan).

In conclusion we have devised a route to, and successfully synthesised, a series of novel N-methyl macrolactam radicicol analogues as Hsp90 inhibitors. Their binding to Hsp90 was investigated and, despite diminished affinity compared to radicicol itself, the binding was found to be superior to the corresponding macrolactones. Importantly, details of the binding to Hsp90 inhibitors through co-crystallisation with the protein, with a change in the conformation of the macrocycle was observed.

**General Experimental Details**

Unless specified, all reagents were purchased from commercial sources and were used without further purification. Tetrahydrofuran was distilled from sodium-benzophenone ketyl immediately before use. Water refers to deionised water, ether to diethyl ether, and light petroleum refers to the fraction with bp 40-60 °C.
Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60GF$_{254}$ pre-coated aluminium-backed plates. The compounds were visualised using UV light (254 nm) and basic aqueous potassium permanganate solution, with heating. Flash column chromatography was performed at medium pressure using slurry packed Davisil silica gel 35-70 µm, 60 Å with the specified eluant. Silver nitrate-impregnated silica TLC plates were prepared by soaking the Merck Kieselgel 60GF$_{254}$ pre-coated aluminium-backed plates in a 1:3 w/v solution of AgNO$_3$ in MeCN for 5 min, before being dried in an oven at 150 °C for 10 min. The TLC plates were covered in aluminium foil and stored in the dark. Compounds were visualised with basic aqueous potassium permanganate solution and heating.

Infrared spectra were obtained on a Perkin Elmer 1600 series FT-IR spectrometer using NaCl solution cells in chloroform or acetone. $^1$H NMR and $^{13}$C NMR spectra were obtained using a JEOL EX270 operating at 270 MHz ($^{13}$C 67.5 MHz), or Bruker Avance III-400, Bruker Avance 400 or Bruker DPX 400 spectrometers operating at 400 MHz ($^{13}$C 100 MHz). All spectroscopic data was recorded at 295K. Chemical shifts are quoted in parts per million (ppm), using the residual solvent peak as an internal standard (7.26 ppm [$^1$H NMR] and 77.2 ppm [$^{13}$C NMR] for CDCl$_3$ and 2.05 ppm [$^1$H NMR] and 206.7, 29.9 ppm [$^{13}$C NMR] for acetone-d$_6$). Coupling constants ($J$) are quoted in Hz. Multiplicity abbreviations used: s singlet, d doublet, t triplet, q quartet, m multiplet. Signal assignment was achieved via analysis of DEPT, COSY, HMBC and HSQC experiments where needed. $^1$H NMR and $^{13}$C NMR spectra of final compounds were performed on a Bruker AV(III)800 at 800 and 200 MHz, respectively. Low and high-resolution mass spectra were performed for all novel
compounds. Electrospray ionisation (ESI) and high resolution mass spectrometric (HRMS) analyses were performed on a Bruker MicroTOF spectrometer.

**Methyl 2,4-dihydroxy-6-methylbenzoate 20**

Sodium hydride (20.67 g, 0.52 mol, 1.5 eq.) was prewashed with pentane (3 × 50 mL), before being suspended in THF (800 mL) and cooled to 0 °C under argon. Methyl acetoacetate (40.00 g, 0.34 mol, 1.0 eq.) was added dropwise over 2 h and the reaction mixture was warmed to room temperature over 1 h. After cooling to −78 °C, n-butyllithium (130.90 mL, 327.25 mmol, 0.95 eq.) was added via cannula over 1 h and the solution was allowed to warm to room temperature over 16 h, before being heated under reflux for 24 h. The mixture was re-cooled to 0 °C and hydrochloric acid (1 M; 400 mL) was added cautiously, the aqueous layer was acidified to pH 1 with concentrated hydrochloric acid and the mixture was warmed to room temperature and stirred for 18 h. The layers were separated and the aqueous layer was washed with ethyl acetate (2 × 400 mL). The combined organics were washed with water (400 mL) and saturated brine (400 mL), before being dried over magnesium sulfate and concentrated in vacuo. The residue was purified by recrystallisation (ethyl acetate/light petroleum) to give the title compound as a light brown, crystalline solid (16.31 g, 52%); δ_H (400 MHz; CDCl_3) 11.41 (1H, s, OH), 6.53 (1H, s, ArH), 6.08 (1H, s, ArH), 5.09 (1H, br. s, OH), 3.95 (3H, s, OMe), 2.63 (3H, s, CH_3); δ_C (100 MHz; CDCl_3) 171.4 (C), 162.9 (C), 156.0 (C), 139.5 (C), 107.1 (C), 102.0 (CH), 52.3 (Me), 30.9 (CH), 19.9 (Me); data consistent with those reported in the literature.
**Methyl 3-chloro-4,6-dihydroxy-2-methylbenzoate 21**

![Chemical Structure](image)

To a solution of methyl 2,4-dihydroxy-6-methylbenzoate 20 (5.73 g, 31.48 mmol, 1.0 eq.) in THF (315 mL) at −30 °C was added sulfuryl chloride (2.78 mL, 34.62 mmol, 1.1 eq.) slowly over 5 min, keeping the temperature between −20 and −30 °C throughout. After stirring for a further 30 min, the reaction mixture was quenched with ammonium chloride (200 mL) and the layers were stirred and separated. The aqueous layer was extracted with ether (2 × 200 mL) and the combined organics were washed with saturated brine (2 × 200 mL), before being dried over sodium sulfate and concentrated *in vacuo* to give a yellow solid. The product was purified by recrystallisation (dichloromethane/light petroleum) to give the *title compound* as a colourless solid (6.651 g, 98%); mp 133-135 °C (lit,\(^{50}\) mp 134 °C); \(^{1}H\) (270 MHz, CDCl\(_3\)) 11.41 (1H, s, 6-OH), 6.52 (1H, s, H-5), 6.03 (1H, s, 4-OH), 3.31 (3H, s, OMe), 2.61 (3H, s, Me); \(^{13}C\) (100 MHz, CDCl\(_3\)) 171.4 (C), 162.9 (C), 156.0 (C), 139.5 (C), 113.9 (C), 107.1 (C), 102.0 (CH), 52.3 (Me), 19.9 (Me). Data consistent with those reported in the literature.\(^{45}\)

**Methyl 3-chloro-4,6-di(ethoxymethoxy)-2-methylbenzoate 22**

![Chemical Structure](image)

To a solution of methyl 3-chloro-4,6-dihydroxy-2-methylbenzoate 21 (7.03 g, 32.52 mmol, 1.0 eq.) in dimethylformamide (108 mL) at 0 °C under argon was added ethyl chloromethyl ether (7.54 mL, 81.29 mmol, 2.5 eq.). \(N,N\)-Diisopropylethylamine
(16.99 mL, 97.55 mmol, 3.0 eq.) was added slowly and the reaction mixture was stirred for 30 min, before being allowed to warm to room temperature and stirred for a further 2 h. Saturated aqueous ammonium hydroxide solution (100 mL) was added and the reaction mixture was stirred for a further 10 min, before being extracted with ether (3 × 100 mL). The combined organics were washed with aqueous lithium chloride solution (5%; 3 × 100 mL), dried over magnesium sulfate and concentrated in vacuo to give a yellow oil. The product was purified by flash column chromatography, eluting with 9:1 light petroleum/ethyl acetate to give the title compound as a colourless oil (10.37 g, 96%); (Found: M+Na+, 355.0916. C_{13}H_{21}^{35}ClO_{6}+Na^+, requires 355.0919); \nu_{\text{max}} (\text{CHCl}_{3})/\text{cm}^{-1} 3526, 2981, 2955, 1728, 1660, 1596, 1476, 1433, 1321, 1265, 1114, 1042, 963, 932, 844; \delta_{\text{H}} (400 \text{ MHz;} \text{CDCl}_{3}) 7.02 (1H, s, ArH), 5.32 (2H, s, OCH_{2}O), 5.22 (2H, s, OCH_{2}O), 3.93 (3H, s, OMe), 3.82-3.76 (2H, q, J = 7.0, OCH_{2}), 3.76-3.71 (2H, q, J = 7.0, OCH_{2}), 2.34 (3H, s, Me), 1.27-1.23 (3H, t, J = 7.0, CH_{3}), 1.26-1.22 (3H, t, J = 7.0, CH_{3}); \delta_{\text{C}} (100 \text{ MHz;} \text{CDCl}_{3}) 167.9 (C), 154.3 (C), 153.1 (C), 135.1 (C), 119.8 (C), 117.1 (C), 101.7 (CH), 93.8 (CH_{2}), 93.8 (CH_{2}), 64.6 (CH_{2}), 64.4 (CH_{2}), 52.3 (Me), 17.5 (Me), 15.0 (Me), 15.0 (Me); m/z (ESI) 355 ([M^{35}Cl]+Na^+, 100%), 357 (33.0%).

3-Chloro-4,6-di(ethoxymethoxy)-2-methylbenzoic acid 23

To a stirred solution of methyl 3-chloro-4, 6-di(ethoxymethoxy)-2-methylbenzoate 22 (6.26 g, 18.82 mmol, 1.0 eq.) in 1:1 methanol/water (60 mL) was added potassium hydroxide (5.28 g, 94.09 mmol, 5.0 eq.) and the reaction mixture was heated to 70 °C
and stirred for 3 d. After cooling to room temperature, water (50 mL) was added and the mixture was extracted with ethyl acetate (50 mL). The aqueous layer was cooled to 0 °C, acidified to pH 1 with concentrated hydrochloric acid and quickly extracted with ethyl acetate (3 × 75 mL). The combined organic extracts were washed with brine (100 mL), dried over magnesium sulfate and concentrated in vacuo to give the title compound as a colourless solid (4.15 g, 69%); mp 78-80 °C; (Found: C, 52.8; H, 6.0%). C_{14}H_{19}^{35}\text{ClO}_6, requires C, 52.8; H, 6.0%; δ_H (400 MHz; acetone-\text{d}_6) 7.10 (1 H, s, ArH), 5.36 (2H, s, OCH_2O), 5.27 (2 H, s, OCH_2O), 3.78 (2H, q, J 7.1, OCH_2), 3.73 (2H, q, J 7.1, OCH_2), 2.36 (3 H, s, Me), 1.21 (3H, t, J 7.1, Me), 1.19 (3H, t, J 7.1, Me); δ_C (100 MHz; acetone-\text{d}_6) 169.1 (C), 154.9 (C), 153.7 (C), 136.9 (C), 117.8 (C), 110.0 (C), 101.7 (CH), 94.3 (CH_2), 93.8 (CH_2), 64.8 (CH_2), 64.8 (CH_2), 17.9 (Me), 15.0 (Me), 15.0 (Me). Data consistent with those reported in the literature.\textsuperscript{31}

\textbf{N-(But-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-N-methyl-2-(2-oxooct-7-en-1-yl)benzamide 25a}

To a solution of 3-chloro-4,6-di(ethoxymethoxy)-2-methylbenzoic acid 23 (170 mg, 0.54 mmol, 1.0 eq.) in THF (2.67 mL) in an oven dried flask at −78 °C was added \textit{sec}-butyllithium (1.08 mL, 1.19 mmol, 2.2 eq.). The resulting red solution was stirred for 40 min before a solution of \textit{N}-methoxy-\textit{N}-methylhept-6-enamide (138 mg, 0.81 mmol, 1.5 eq.) in THF (0.3 mL) was added. The reaction mixture was stirred for a further 50 min at −78 °C. Water (5 mL) was added and the solution was washed with ether (2 × 5 mL). The aqueous layer was acidified to pH 1 with hydrochloric acid (1
M; 5 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organics were dried over magnesium sulfate and concentrated in vacuo to give a yellow oil (171 mg). The crude material was used without further purification. 3-Chloro-4,6-di(ethoxymethoxy)-2-(2-oxooct-7-en-1-yl)benzoic acid (171 mg, 0.40 mmol 1 eq.) was dissolved in DMF (2 mL). To the reaction solution was added N-methylbut-3-en-1-amine hydrochloride (73 mg, 0.60 mmol, 1.5 eq.), N,N-diisopropylethylamine (2.15 mL, 1.60 mmol, 4.0 eq.) and HATU (174 mg, 0.46 mmol, 1.15 eq.). The reaction mixture was stirred at room temperature for 18 h. A further 0.2 equivalents of N-methylbut-3-en-1-amine hydrochloride (10 mg) and 0.1 equivalents of HATU (15 mg) were added and the reaction mixture was stirred for a further 3 h. The solution was diluted with ethyl acetate (10 mL) and washed with hydrochloric acid (1 M; 5 mL), saturated aqueous sodium hydrogen carbonate (5 mL) and brine (5 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo to give a yellow oil. The residue was purified by flash column chromatography, eluting with 4:1, then 2:1 light petroleum/ethyl acetate to give the title compound as a colourless oil (0.056g, 33% over 2 steps) (3:1 mixture of isomers); (Found: M+Na+, 518.2287, C26H38ClNO6+Na+, requires 518.2280); νmax (CHCl3)/cm−1 3665, 3079, 3001, 1759, 1628, 1590, 1486, 1453, 1327, 1244, 1054, 1036, 1029; major isomer δH (400 MHz; CDCl3) 7.08 (1H, s, ArH), 5.89-5.73 (2H, m, CH), 5.29 (1H, d, J 12.8, OCH2O), 5.27 (1H, d, J 12.8, OCH2O), 5.18 (2H, s, OCH2O), 5.14-4.92 (4H, m, =CH2), 3.98 (1H, d, J 17.6, CH2), 3.79 (1H, d, J 17.6, CH2), 3.80-3.73 (2H, m, OCH2), 3.72-3.66 (2H, m, OCH2), 3.57 (1H, dt, J 13.1, 6.8, CH2), 2.83 (3H, s, NMe), 2.54-2.48 (2H, m, CH2), 2.39-2.34 (2H, m, CH2), 2.08-2.04 (2H, m, CH2), 1.65-1.57 (3H, m, CH2), 1.43-1.36 (2H, m, CH2), 1.23 (6H, 2 × t, J 14.5, 7.1, Me); δC (100 MHz; CDCl3) 206.5 (C), 167.6 (C), 153.8 (C), 152.1 (C), 138.5 (CH), 135.4 (CH), 134.7 (C), 132.3 (C), 122.8
13-Chloro-14,16-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10-hexahydrobenz[c][1]azacyclotetradecine-1,11(2H,12H)-dione 26a

A solution of N-(but-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-N-methyl-2-(2-oxooct-7-en-1-yl)benzamide 25a (56 mg, 0.11 mmol, 1.0 eq.) dichloromethane (35 mL) was sparged with nitrogen and Grubbs II catalyst (11 mg, 0.01 mmol, 0.11 eq.) was added. The reaction mixture was heated to reflux for 90 min. After cooling to room temperature, DMSO (0.1 mL) was added and the solution was stirred for 18 h. The reaction mixture was concentrated in vacuo and purified by flash column chromatography, eluting with 4:1 light petroleum/ethyl acetate to give the title compound as a colourless oil (0.027 g, 51%) (4:1 mixture of E/Z isomers); (Found: M+Na+, 490.1956. C_{24}H_{34}{^{35}}ClNO_{6}+Na+, requires 490.1967); $\nu_{\text{max}}$ (CHCl$_3$)/cm$^{-1}$ 3011, 2928, 2855, 1720, 1624, 1592, 1464, 1323, 1239, 1118, 1045, 929; major isomer $\delta_H$ (C), 116.6 (CH$_2$), 114.6 (CH$_2$), 102.5 (CH), 94.0 (CH$_2$), 93.7 (CH$_2$), 64.6 (CH$_2$), 64.4 (CH$_2$), 46.6 (CH$_2$), 44.9 (CH$_2$), 42.5 (CH$_2$), 36.1 (Me), 33.5 (CH$_2$), 31.6 (CH$_2$), 28.3 (CH$_2$), 23.2 (CH$_2$), 15.0 (Me), 15.0 (Me); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: $\delta_H$ (400 MHz; CDCl$_3$) 7.12 (1H, s, ArH), 5.68-5.56 (2H, m, CH), 5.30 (1H, d, $J_{15.1}$, O-CH$_2$-O), 5.28 (1H, d, $J_{15.1}$, O-CH$_2$-O), 3.87 (1H, d, $J_{17.5}$, CH$_2$), 3.04 (3H, s, NMe), 2.26-2.21 (2H, m, CH$_2$); $\delta_C$ (100 MHz; CDCl$_3$) 117.7 (CH$_2$), 117.0 (CH$_2$), 102.7 (CH), 64.5 (CH$_2$), 50.2 (CH$_2$), 45.3 (CH$_2$), 42.2 (CH$_2$), 32.6 (CH$_2$), 32.2 (CH$_2$); $m/z$ (ESI) 518 ([M{^{35}}Cl]+Na$^+$, 100%), 519 (27.0%), 520 (35.6%), 521 (9.5%).
(400 MHz; CDCl$_3$) 7.10 (1H, s, ArH), 5.57-5.39 (2H, m, CH=CH), 5.30 (1H, d, $J$ 15.1, OCH$_2$O), 5.28 (1H, d, $J$ 15.1, OCH$_2$O), 5.22-5.18 (2H, m, OCH$_2$O), 4.56 (1H, ddd, $J$ 19.1, 9.6, 5.6, CH$_2$), 4.03 (1H, d, $J$ 17.9, CH$_2$), 3.85 (1H, d, $J$ 17.9, CH$_2$), 3.78 (2H, dq, $J$ 14.2, 7.1, OCH$_2$O), 3.67 (2H, dq, $J$ 14.2, 4.1, OCH$_2$O), 2.77 (3H, s, NMe), 2.68 (1H, ddd, $J$ 16.9, 7.9, 1.9, CH$_2$), 2.59-2.50 (1H, m, CH$_2$), 2.46-2.31 (3H, m, CH$_2$), 2.20-2.11 (1H, m, CH$_2$), 2.07-1.99 (1H, m, CH$_2$), 1.83-1.75 (1H, m, CH$_2$), 1.68-1.59 (1H, m, CH$_2$) 1.56-1.47 (2H, m, CH$_2$), 1.23 (6H, 2 × t, $J$ 8.8, 7.1, Me), 0.97-0.88 (1H, m, CH$_2$); $\delta_C$ (100 MHz; CDCl$_3$) 205.8 (C), 168.2 (C), 153.7 (C), 152.0 (C), 132.7 (CH), 132.0 (C), 128.4 (CH), 123.0 (C), 117.9 (C), 102.7 (CH), 93.9 (CH$_2$), 93.7 (CH$_2$), 64.5 (CH$_2$), 64.4 (CH$_2$), 46.3 (CH$_2$), 44.8 (CH$_2$), 40.4 (CH$_2$), 36.1 (Me), 30.4 (CH$_2$), 30.2 (CH$_2$), 26.2 (CH$_2$), 21.6 (CH$_2$), 15.0 (Me), 15.0 (Me); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: $\delta_H$ (400 MHz; CDCl$_3$) 7.11 (1H, s, ArH), 4.06 (1H, d, $J$ 18.1, CH$_2$), 3.95 (1H, d, $J$ 18.1, CH$_2$), 2.82 (3H, s, NMe); $\delta_C$ (100 MHz; CDCl$_3$) 206.2 (C), 151.9 (C), 132.2 (CH), 131.2 (C), 129.3 (CH), 102.6 (CH), 47.3 (CH$_2$), 44.6 (CH$_2$), 41.6 (CH$_2$), 37.1 (Me), 29.7 (CH$_2$), 27.3 (CH$_2$), 25.4 (CH$_2$), 21.9 (CH$_2$); $m/z$ (ESI) 490 ([M$^{35}$Cl]+Na$^+$, 100%), 492 (33.0 %).

13-Chloro-14,16-dihydroxy-2-methyl-3,4,7,8,9,10-hexahydrobenzo[c][1]azacyclotetradecine-1,11(2H,12H)-dione 27a$^1$

![Chemical structure]

To a solution of 13-chloro-14,16-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10-hexahydrobenzo[c][1]azacyclotetradecine-1,11(2H,12H)-dione 26a (13 mg, 0.03 mmol, 1.0 eq.) in dichloromethane (2 mL) was added trifluoroacetic acid (0.32 mL,
0.04 mmol, 1.5 eq.) and the reaction mixture was stirred at room temperature for 30 min. Saturated aqueous sodium hydrogen carbonate (5 mL) was added and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined organics were washed with saturated brine (10 mL), dried over sodium sulfate and concentrated in vacuo to give a yellow oil. The residue was purified by flash column chromatography, eluting with 1% methanol/dichloromethane to give a colourless oil (7 mg, 72%) (9:1 mixture of isomers); major isomer $\delta_H$ (400 MHz; CDCl$_3$) 8.66 (1H, br. s, OH), 6.55 (1H, s, ArH), 5.51 (2H, dt, $J$ 15.4, 9.0, CH=CH$_2$), 4.46 (1H, ddd, $J$ 13.3, 9.3, 3.9, CH$_2$), 3.89 (2H, s, CH$_2$), 2.94-2.83 (1H, m, CH$_2$), 2.81 (3H, s, NMe), 2.77 (1H, ddd, $J$ 13.6, 6.4, 3.8, CH$_2$), 2.61 (1H, ddd, $J$ 16.3, 8.1, 2.0, CH$_2$), 2.52-2.33 (3H, m, CH$_2$), 2.15-2.09 (1H, m, CH$_2$), 1.74 (1H, m, CH$_2$), 1.68-1.58 (1H, m, CH$_2$), 1.53-1.46 (2H, m, CH$_2$); $\delta_C$ (100 MHz; CDCl$_3$) 204.8 (C), 167.9 (C), 153.3 (C), 152.2 (C), 132.0 (C), 129.1 (CH), 119.7 (C), 110.2 (C), 109.9 (C), 102.2 (CH), 46.8 (CH$_2$), 44.2 (CH$_2$), 40.2 (CH$_2$) 35.1 (Me), 30.3 (CH$_2$), 30.1 (CH$_2$), 26.3 (CH$_2$), 21.5 (CH$_2$); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: $\delta_H$ (400 MHz; CDCl$_3$) 6.57 (1H, s, ArH), 5.58-5.44 (2H, m, CH=CH$_2$), 4.06 (1H, ddd, $J$ 14.3, 8.05, 2.88, CH$_2$), 3.97, (2H, d, $J$ 9.3, CH$_2$) 2.82 (3H, s, Me); $\delta_C$ (100 MHz; CDCl$_3$) 130.7 (CH), 44.1 (CH$_2$). Data consistent with those reported in the literature.$^1$

$N$-(But-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-$N$-methyl-2-(2-oxonon-8-en-1-yl)benzamide 25b
As for the procedure for compound 25a, using 3-chloro-4,6-di(ethoxymethoxy)-2-methylbenzoic acid 23 (200 mg, 0.63 mmol, 1.0 eq.), N-methoxy-N-methyloct-7-enamide (187 mg, 0.94 mmol, 1.5 eq.), N-methylbut-3-en-1-amine hydrochloride (63 mg, 0.52 mmol, 1.5 eq), N,N-diisopropylethylamine (0.24 mL, 1.38 mmol, 4.0 eq.) and HATU (151 mg, 0.40 mmol, 1.15 eq.). The product was purified by flash column chromatography, eluting with 4:1 light petroleum/ethyl acetate to give the title compound as a colourless oil (0.070 g, 25% over 2 steps) (3:1 mixture of isomers); (Found: M+Na+, 532.2442. C_{27}H_{40}^{35}ClNO_{6}+Na+, requires 532.2436); ν_{\text{max}} (CHCl_3)/cm^{-1} 3685, 3001, 1719, 1630, 1466, 1407, 1321, 1274, 1264, 1151, 1120, 910; major isomer δ_{H} (400 MHz; CDCl_3) 7.08 (1H, s, ArH), 5.93-5.74 (2H, m, CH), 5.29 (1H, d, J 13.0, OCH_2O), 5.27 (1H, d, J 13.0, OCH_2O), 5.20-5.16 (2H, m, OCH_2O), 3.98 (1H, d, J 17.7, CH_2), 3.79 (1H, d, J 17.7, CH_2), 3.80-3.74 (2H, m, OCH_2O), 3.72-3.66 (2H, m, OCH_2O), 2.83 (3H, s, NMe), 2.50 (2H, dt, J 12.2, 7.2, CH_2), 2.39-2.34 (2H, m, CH_2), 2.26-2.20 (1H, m, CH_2), 2.06-2.01 (3H, m, CH_2), 1.64-1.57 (2H, m, CH_2), 1.37 (2H, ddd, J 14.9, 8.6, 2.7, CH_2), 1.34-1.28 (2H, m, CH_2), 1.23 (6H, 2 \times t, J 14.5, 7.3, Me); δ_{C} (100 MHz; CDCl_3) 196.5 (C), 167.6 (C), 138.8 (C), 138.7 (C), 135.4 (C), 135.0 (C), 134.8 (C), 116.6 (CH_2), 114.4 (CH_2), 110.6 (CH), 102.6 (CH), 102.5 (CH) 94.0 (CH_2), 93.7 (CH_2), 64.6 (CH_2), 64.4 (CH_2), 46.5 (CH_2), 44.8 (CH_2), 42.6 (CH_2), 36.1 (Me), 33.6 (CH_2), 31.6 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 23.6 (CH_2), 15.0 (Me), 15.0 (Me); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: δ_{H} (400 MHz; CDCl_3) 6.98 (1H, s, ArH), 5.68-5.55 (2H, m, CH), 5.30 (1H, d, J 15.3, OCH_2O), 5.28 (1H, d, J 15.3, OCH_2O), 3.88 (1H, d, J 17.5, CH_2), 3.57 (2H, dq, J 13.6, 9.4, OCH_2), 2.81 (3H, s, NMe), 1.27-1.16 (6H, m, Me); m/z (ESI) 532 ([M^{35}Cl]+Na]^+, 100%), 533 (34.6%), 534 (36.1%), 535 (10.5%).
14-Chloro-15,17-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10,11,13-octahydro-1H-benzo[c][1]azacyclopentadecine-1,12(2H)-dione 26b

As for the procedure for compound 26a, using N-(but-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-N-methyl-2-(2-oxonon-8-en-1-yl)benzamide 25b (70 mg, 0.14 mmol, 1.0 eq.). The product was purified by flash column chromatography, eluting with 2:1 light petroleum/ethyl acetate to give the title compound as a colourless oil (0.026 g, 39%) (2:1 mixture of isomers); (Found: M+Na+, 504.2124. C25H3635ClNO6Na+, requires 504.2123); νmax (CHCl3)/cm⁻¹ 3686, 3600, 2928, 2855, 1717, 1605, 1465, 1286, 1269, 1044, 910; major isomer δH (400 MHz; CDCl3) 7.10 (1H, s, ArH), 5.48-5.35 (2H, m, CH=CH), 5.30 (1H, d, J 16.6, OCH2O), 5.28 (1H, d, J 16.6, OCH2O), 4.64 (1H, ddd, J 14.9, 9.4, 5.9, CH2), 4.13 (1H, d, J 18.0, CH2), 3.83-3.74 (2H, m, OCH2O), 3.73-3.67 (2H, m, OCH2O), 3.69 (1H, d, J 18.0, CH2), 2.83 (3H, s, NMe), 2.82-2.73 (2H, m, CH2), 2.48-2.33 (3H, m, CH2), 2.09-2.00 (2H, m, CH2), 1.87-1.75 (1H, m, CH2), 1.63-1.52 (1H, m, CH2), 1.46-1.32 (4H, m, CH2), 1.24 (6H, 2 × t, J 13.1, 7.1, Me); δC (100 MHz; CDCl3) 207.5 (C), 168.8 (C), 153.7 (C), 152.2 (C), 132.5 (C), 132.3 (CH), 127.7 (CH), 123.7 (C), 117.4 (C), 102.7 (CH), 94.0 (CH2), 93.8 (CH2), 64.6 (CH2), 64.5 (CH2), 45.7 (CH2), 45.5 (CH2), 42.2 (CH2), 34.6 (Me), 30.8 (CH2), 29.9 (CH2), 26.7 (CH2), 25.9 (CH2), 23.4 (CH2), 15.1 (Me), 15.0 (Me); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: δH (400 MHz; CDCl3) 7.11 (1H, s, ArH), 5.31 (1H, d, J 17.3, OCH2O), 5.29 (1H, d, J 17.3, OCH2O), 4.14 (1H, d, J 18.8,
CH₂), 3.27-3.19 (2H, m, CH₂), 3.11-3.06 (1H, m, CH₂), 2.64-2.56 (1H, m, CH₂), 2.18-2.09 (2H, m, CH₂), 1.99-1.91 (2H, m, CH₂); δ C (100 MHz; CDCl₃) 207.0 (C), 154.1 (C), 152.7 (C), 133.3 (C), 131.8 (CH), 128.4 (CH), 122.4 (C), 118.2 (C), 102.4 (CH), 94.1 (CH₂), 94.0 (CH₂), 48.0 (CH₂), 46.8 (CH₂), 41.2 (CH₂), 31.1 (CH₂), 29.0 (CH₂), 26.0 (CH₂), 25.2 (CH₂), 22.0 (CH₂); m/z (ESI) 504 ([M(³⁵Cl)+Na]+, 100%), 505 (23.4%), 506 (32.4%), 507 (8.4%).

14-Chloro-15,17-dihydroxy-2-methyl-3,4,7,8,9,10,11,13-octahydro-1H-benz[c][1]aza cyclopentadecine-1,12(2H)-dione 27b

As for the procedure for compound 27a, using 14-chloro-15,17-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10,11,13-octahydro-1H-benzo[c][1]azacyclopentadecine-1,12(2H)-dione 26b (26 mg, 0.05 mmol, 1 eq.). Product purified by flash column chromatography, eluting with 2% methanol/dichloromethane to give the title compound as a colourless solid (9 mg, 45%) (3:1 mixture of isomers); mp 102-104 °C; (Found: M+Na⁺, 388.1296. C₁₉H₂₄³⁵ClNO₄⁺Na⁺, requires 388.1286); νmax (CHCl₃)/cm⁻¹ 3438, 3274, 2859, 1732, 1728, 1701, 1623, 1455, 1445, 1391, 1343, 1312, 1251, 1100; major isomer δH (800 MHz; acetone-d₆) 8.69 (1H, br. s, OH), 7.97 (1H, s, OH), 6.55 (1H, s, ArH), 5.46-5.41 (1H, m, CH), 5.40-5.34 (1H, m, CH), 4.51 (1H, ddd, J 14.7, 11.9, 3.4, CH₂), 3.98 (1H, d, J 17.9, CH₂), 3.76 (1H, d, J 17.9 CH₂), 2.94 (3H, s, NMe), 2.75-2.71 (2H, m, CH₂), 2.45-2.40 (1H, m, CH₂), 2.38-2.33 (1H, m, CH₂), 1.80-1.75 (1H, m, CH₂), 1.59-1.54 (1H, m, CH₂), 1.42-1.36 (2H, m, CH₂), 1.32-1.27 (3H, m, CH₂), 1.24-1.15 (2H, m, CH₂); δ C (200 MHz; acetone-d₆) 208.0 (C), 170.2 (C), 163.7 (C), 154.2 (C), 134.9 (C), 133.3 (CH), 130.1 (CH), 122.3 (C) 114.2
(C), 104.1 (CH), 47.1 (CH\textsubscript{2}), 47.0 (CH\textsubscript{2}), 46.9 (CH\textsubscript{2}), 43.5 (CH\textsubscript{2}), 37.1 (Me), 35.6 (CH\textsubscript{2}), 28.6 (CH\textsubscript{2}), 27.6 (CH\textsubscript{2}), 25.2 (CH\textsubscript{2}); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: \(\delta H\) (800 MHz; acetone-\(d_6\)) 5.27-5.21 (1H, m, CH), 5.22-5.19 (1H, m, CH), 4.38 (1H, ddd, \(J\) 14.7, 11.9, 3.4, CH\textsubscript{2}), 4.16 (1H, d, \(J\) 18.8, CH\textsubscript{2}), 3.99 (1H, d, \(J\) 18.8 CH\textsubscript{2}), 2.88 (3H, s, NMe), 2.62-2.56 (2H, m, CH\textsubscript{2}), 1.94-1.88 (1H, m, CH\textsubscript{2}); \(\delta C\) (200 MHz; acetone-\(d_6\)) 207.9 (C), 170.1 (C), 155.2 (C), 133.1 (CH), 131.2 (CH), 101.9 (CH), 49.3 (CH\textsubscript{2}), 47.8 (CH\textsubscript{2}), 42.6 (CH\textsubscript{2}), 32.4 (CH\textsubscript{2}), 28.1 (CH\textsubscript{2}), 26.8 (CH\textsubscript{2}), 23.6 (CH\textsubscript{2}); \(m/z\) (ESI) 388 ([M\textsuperscript{35}Cl+Na]\textsuperscript{+}, 100%), 389 (18.6%), 390 (31.6%), 391 (5.8%).

\textit{N-}-(\textit{But}-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-\textit{N-methyl}-2-(2-oxodec-9-en-1-yl)benzamide 25c

![Diagram of the compound](image)

As for the procedure for compound 25a, using 3-chloro-4,6-bis(ethoxymethoxy)-2-methylbenzoic acid 23 (200 mg, 0.63 mmol, 1.0 eq.), \(N\)-methoxy-\(N\)-methylnon-8-enamide (174 mg, 0.94 mmol, 1.5 eq.), \(N\)-methylbut-3-en-1-amine hydrochloride (74 mg, 0.61 mmol, 1.5 eq.). The product was purified by flash column chromatography, eluting with 4:1 light petroleum/ethyl acetate to give the title compound as a colourless oil (0.051 g, 16% over 2 steps) (4:1 mixture of isomers); (Found: M+Na\textsuperscript{+}, 546.2590 \(\text{C}_{28}\text{H}_{42}\text{Cl}\text{NO}_{6}\text{Na}\textsuperscript{+}\), requires 546.2593); \(v_{\text{max}}\) (CHCl\textsubscript{3}) cm\textsuperscript{-1} 3742, 3678, 3050, 2999, 2872, 1631, 1464, 1380, 1366, 1276, 1259, 1172, 1149, 1126, 1039, 851; \textit{major isomer} \(\delta H\) (400 MHz; CDCl\textsubscript{3}) 7.11 (1H, s, ArH), 5.92-5.77 (2H, m, CH), 5.32 (1H, d, \(J\) 13.0, OCH\textsubscript{2}O), 5.30 (1H, d, \(J\) 13.0, OCH\textsubscript{2}O), 5.23-5.19 (2H, m, OCH\textsubscript{2}O), 5.16-4.94 (4H, m, =CH\textsubscript{2}), 4.02 (1H, d, \(J\) 17.7, CH\textsubscript{2}), 3.82 (1H, d, \(J\) 17.7, CH\textsubscript{2}), 3.83-
3.76 (2H, m, OCH$_2$), 3.75-3.68 (2H, m, OCH$_2$), 3.64-3.53 (1H, dt, $J$ 15.6, 9.4, CH$_2$), 2.86 (3H, s, NMe), 2.52 (2H, ddd, $J$ 15.1, 7.1, 1.1, CH$_2$), 2.42-2.37 (1H, m, CH$_2$), 2.35-2.24 (2H, m, CH$_2$), 2.09-2.03 (2H, m, CH$_2$), 1.67-1.56 (2H, m, CH$_2$), 1.44-1.37 (3H, m, CH$_2$), 1.36-1.30 (3H, m, CH$_2$), 1.23 (6H, $2 \times t$, $J$ 14.5, 7.3, Me); $\delta_c$ (100 MHz; CDCl$_3$) 206.6 (C), 167.5 (C), 153.8 (C), 152.0 (C), 138.9 (CH), 135.4 (CH), 134.6 (C), 132.3 (C), 122.8 (C), 116.6 (CH$_2$), 114.2 (CH$_2$), 102.5 (CH), 93.9 (CH$_2$), 93.6 (CH$_2$), 64.5 (CH$_2$), 64.4 (CH$_2$), 46.5 (CH$_2$), 44.8 (CH$_2$), 42.6 (CH$_2$), 36.1 (Me), 33.6 (CH$_2$), 31.5 (CH$_2$), 28.9 (CH$_2$), 28.8 (CH$_2$), 28.6 (CH$_2$), 23.7 (CH$_2$), 15.0 (Me), 15.0 (Me);

For the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: $\delta_H$ (400 MHz; CDCl$_3$) 7.14 (1H, s, ArH), 5.71-5.76 (2H, m, CH), 5.31 (1H, d, $J$ 15.4, OCH$_2$O), 3.92 (1H, d, $J$ 17.6, CH$_2$), 3.10 (3H, s, NMe); $\delta_c$ (100 MHz; CDCl$_3$) 206.3 (C), 153.9 (C), 152.4 (C), 135.5 (CH), 134.2 (C), 132.4 (C), 122.3 (C), 117.8 (CH$_2$), 117.6 (CH$_2$), 102.7 (CH), 93.7 (CH$_2$), 93.5 (CH$_2$), 64.4 (CH$_2$), 46.3 (CH$_2$), 45.2 (CH$_2$), 42.4 (CH$_2$), 32.5 (CH$_2$), 32.1 (CH$_2$), 29.3 (CH$_2$), 28.8 (CH$_2$); $m/z$ (ESI) 546 ([M($^{35}$Cl)+Na$^+$], 100%), 547 (28.8%), 548 (34.9%), 549 (7.7%).

15-Chloro-16,18-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10,11,12-octahydrobenz[c][1]azacyclohexadecine-1,13(2H,14H)-dione 26c

As for the procedure for compound 26a, using N-(but-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-N-methyl-2-(2-oxodec-9-en-1-yl)benzamide 25c (60 mg, 0.11 mmol, 1.0 eq.). The product was purified by flash column chromatography, eluting with 2:1 light petroleum/ethyl acetate to give the title compound as a colourless oil.
(19 mg, 34%) (4:1 mixture of isomers); (Found: M+Na\(^+\), 518.2283. C\(_{26}\)H\(_{38}\)\(^{35}\)ClNO\(_6\)+Na\(^+\), requires 518.2280; \(\nu\)\(_{\text{max}}\) (CHCl\(_3\))/cm\(^{-1}\) 3686, 2929, 2856, 1720, 1627, 1461, 1324, 1250, 1151, 1118, 940; major isomer \(\delta\)\(_{\text{H}}\) (400 MHz; CDCl\(_3\)) 7.08 (1H, s, ArH), 5.49-5.31 (2H, m, CH=CH), 5.30-5.24 (2H, m, OCH\(_2\)O), 5.19 (2H, s, OCH\(_2\)O), 4.40 (1H, ddd, J 13.2, 6.8, 3.2, CH\(_2\)), 4.03 (1H, d, J 17.3, CH\(_2\)), 3.75 (4H, dq, J 14.3, 7.6, OCH\(_2\)O), 3.71-3.66 (2H, m, CH\(_2\)O), 3.59 (1H, d, J 17.3, CH\(_2\)), 2.92 (3H, s, NMe), 2.79 (1H, ddd, J 17.8, 15.4, 2.6, CH\(_2\)), 2.71-2.60 (2H, m, CH\(_2\)), 2.55-2.46 (1H, m, CH\(_2\)), 2.37-2.25 (2H, m, CH\(_2\)), 2.09-1.98 (2H, m, CH\(_2\)), 1.97-1.83 (1H, m, CH\(_2\)), 1.56-1.43 (2H, m, CH\(_2\)), 1.41-1.33 (2H, m, CH\(_2\)), 1.22 (6H, dt, J 13.2, 7.0, Me); \(\delta\)\(_{C}\) (100 MHz; CDCl\(_3\)) 206.7 (C), 168.2 (C), 153.7 (C), 152.1 (C), 132.6 (C), 131.7 (CH), 128.0 (CH), 123.6 (C), 117.6 (C), 102.7 (CH), 94.0 (CH\(_2\)), 93.8 (CH\(_2\)), 64.6 (CH\(_2\)), 64.5 (CH\(_2\)), 48.2 (CH\(_2\)), 45.3 (CH\(_2\)), 40.9 (CH\(_2\)), 37.8 (Me), 31.1 (CH\(_2\)), 30.3 (CH\(_2\)), 27.0 (CH\(_2\)), 26.6 (CH\(_2\)), 25.0 (CH\(_2\)), 21.7 (CH\(_2\)), 15.1 (Me), 15.0 (Me); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: \(\delta\)\(_{\text{H}}\) (400 MHz; CDCl\(_3\)) 4.48 (1H, dt, J 13.3, 7.6, CH\(_2\)), 3.98 (1H, d, J 17.7, CH\(_2\)), 3.87 (1H, d, J 17.7, CH\(_2\)), 3.23-3.14 (1H, m, CH\(_2\)), 3.10-3.04 (1H, m, CH\(_2\)), 3.02 (3H, s, NMe), 2.46-2.37 (1H, m, CH\(_2\)), 1.14-1.03 (1H, m, CH\(_2\)); \(\delta\)\(_{C}\) (100 MHz; CDCl\(_3\)) 206.9 (C), 151.9 (C), 131.9 (CH), 102.7 (CH), 64.5 (CH\(_2\)), 45.0 (CH\(_2\)), 30.6 (CH\(_2\)), 30.6 (CH\(_2\)), 27.7 (CH\(_2\)), 26.3 (CH\(_2\)); m/z (ESI) 518 ([M\(^{35}\)Cl]+Na\(^+\), 100%), 519 (28.0%), 520 (33.5%), 521 (8.0%).

15-Chloro-16,18-dihydroxy-2-methyl-3,4,7,8,9,10,11,12-octahydrobenz[e][1]azacyclohexadecine-1,13(2H,14H)-dione 27c
As for the procedure for compound 27a, using 15-chloro-16,18-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10,11,12-octahydrobenzo[c][1]azacyclohexadecine-1,13(2H,14H)-dione 26c (15 mg, 0.33 mmol, 1 eq.). The product was purified by flash column chromatography, eluting with 2% methanol/dichloromethane to give the title compound as a colourless solid (4 mg, 33%) (5:1 mixture of isomers); mp 204-206 °C; (Found: M+Na+, 402.1442. C_{20}H_{26}^{35}ClNO_4^{+}Na^+, requires 402.1443); ν_max (CHCl_3)/cm\(^{-1}\) 3440, 3280, 2613, 1710, 1720, 1702, 1630, 1450, 1430, 1369, 1334, 1252, 1096; major isomer δ_H (800 MHz; acetone-d_6) 8.68 (1H, s, OH), 8.65 (1H, br. s, OH), 6.56 (1H, s, ArH), 5.44-5.41 (2H, m, CH=CH), 4.32-4.28 (1H, m, CH\(_2\)), 3.88 (1H, d, J 17.1, CH\(_2\)), 3.67 (1H, d, J 17.1, CH\(_2\)), 2.94 (3H, s, NMe), 2.77-2.72 (1H, m, CH\(_2\)), 2.67 (1H, ddd, J 17.5, 9.4, 2.6, CH\(_2\)), 2.59-2.53 (1H, m, CH\(_2\)), 2.32-2.28 (1H, m, CH\(_2\)), 2.02-1.98 (1H, m, CH\(_2\)), 1.84-1.77 (1H, m, CH\(_2\)), 1.54-1.48 (1H, m, CH\(_2\)), 1.48-1.42 (1H, m, CH\(_2\)), 1.41-1.34 (2H, m, CH\(_2\)), 1.32-1.25 (2H, m, CH\(_2\)), 1.25-1.21 (1H, m, CH\(_2\)), 1.17-1.10 (1H, m, CH\(_2\)); δ_C (200 MHz; acetone-d_6) 207.4 (C), 169.8 (C), 155.3 (C), 154.3 (C), 134.8 (C), 132.8 (C), 130.7 (CH), 129.4 (CH) 114.4 (C), 104.2 (CH), 49.5 (CH\(_2\)), 47.0 (CH\(_2\)), 41.7 (CH\(_2\)), 38.6 (Me), 32.9 (CH\(_2\)), 32.1 (CH\(_2\)), 28.8 (CH\(_2\)), 28.3 (CH\(_2\)), 26.6 (CH\(_2\)), 23.3 (CH\(_2\)); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: δ_H (800 MHz; acetone-d_6) 8.70 (1H, s, OH), 6.55 (1H, s, ArH), 3.96 (1H, d, J 17.8, CH\(_2\)), 2.91 (3H, s, NMe), 2.63-2.55 (1H, m, CH\(_2\)), 2.47-2.40 (1H, m, CH\(_2\)), 2.40-2.35 (1H, m, CH\(_2\)), 2.20-2.14 (1H, m, CH\(_2\)), 1.80-1.72 (1H, m, CH\(_2\)), 1.64-1.57 (1H, m, CH\(_2\)), 1.36-1.28 (1H, m, CH\(_2\)); δ_C (200 MHz; acetone-d_6) 122.2 (CH), 104.1 (CH), 101.9 (C), 56.0 (CH\(_2\)), 31.6 (CH\(_2\)), 31.3 (CH\(_2\)), 27.9 (CH\(_2\)), 26.8 (CH\(_2\)); m/z (ESI) 402 ([M^{35}Cl]+Na^+, 100%), 403 (21.9%), 404 (32.4%), 405 (6.8%).
\( N\)-(Pent-4-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-N-methyl-2-(2-oxohept-6-en-1-yl)benzamide 25d

As for the procedure for compound 25a, using 3-chloro-4,6-di(ethoxymethoxy)-2-methylbenzoic acid 23 (200 mg, 0.63 mmol, 1 eq.), \( N\)-methoxy-\( N\)-methylhex-5-enamide (148 mg, 0.94 mmol, 1.5 eq.) and \( N\)-methylpent-4-en-1-amine (91 mg, 0.68 mmol, 1.5 eq.). The product was purified by flash column chromatography, eluting with 4:1 light petroleum/ethyl acetate to give the title compound as a yellow oil (81 mg, 28% over 2 steps) (3:1 mixture of isomers); (Found: M+Na\(^+\), 518.2281 \( \text{C}_{26}\text{H}_{38}\text{ClNO}_6\text{+Na}^+\), requires 518.2280); \( \nu_{\text{max}} \) (CHCl\(_3\))/cm\(^{-1}\) 3686, 3600, 3058, 2996, 1719, 1628, 1597, 1458, 1408, 1319, 1278, 1265, 1152, 1119, 1042, 941, 896; \textit{major isomer} \( \delta \text{H} \) (400 MHz; CDCl\(_3\)) 7.11 (1H, s, ArH), 5.92-5.72 (2H, m, CH), 5.32 (1H, d, J 13.2, OCH\(_2\)O), 5.30 (1H, d, J 13.2, OCH\(_2\)O), 5.22 (2H, s, OCH\(_2\)O), 5.10-4.93 (4H, m, =CH\(_2\)), 4.03 (1H, d, J 17.7, CH\(_2\)), 3.86 (1H, d, J 17.7, CH\(_2\)), 3.82-3.77 (2H, m, OCH\(_2\)), 3.76-3.66 (2H, m, OCH\(_2\)), 2.85 (3H, s, NMe), 2.54 (2H, ddd, J 15.0, 7.4, 3.0 CH\(_2\)), 2.35-2.29 (1H, m, CH\(_2\)), 2.20-2.06 (6H, m, CH\(_2\)), 1.77-1.68 (6H, m, CH\(_2\)), 1.23 (6H, dt, J 8.3, 7.1, Me); \( \delta \text{C} \) (100 MHz; CDCl\(_3\)) 206.4 (C), 167.5 (C), 153.8 (C), 152.1 (C), 137.9 (CH), 137.8 (CH), 132.3 (C), 122.9 (C), 117.7 (C), 115.3 (CH\(_2\)), 115.1 (CH\(_2\)), 102.6 (CH), 94.0 (CH\(_2\)), 93.7 (CH\(_2\)), 64.6 (CH\(_2\)), 64.5 (CH\(_2\)), 46.5 (CH\(_2\)), 44.8 (CH\(_2\)), 41.8 (CH\(_2\)), 35.9 (Me), 33.0 (CH\(_2\)), 30.9 (CH\(_2\)), 26.2 (CH\(_2\)), 22.9 (CH\(_2\)), 15.0 (Me), 15.0 (Me); for the \textit{minor isomer}, the following NMR signals are discernable, the rest overlap with the major isomer: \( \delta \text{H} \) (400 MHz; CDCl\(_3\)) 7.15 (1H, s, ArH), 5.33
(1H, d, J 13.8, O-CH₂-O), 5.31 (1H, d, J 13.8, O-CH₂-O), 5.20 (2H, s, O-CH₂-O), 3.91 (1H, d, J 17.0, CH₂), 3.64-3.55 (2H, m, O-CH₂), 3.50-3.40 (2H, m, O-CH₂), 3.07 (3H, s, NMe), 2.54 (2H, ddd, J 15.0, 7.4, 3.0 CH₂), 1.98-1.91 (1H, m, CH₂); δC (100 MHz; CDCl₃) 206.6 (C), 138.0 (CH), 137.4 (CH), 115.2 (CH₂), 102.8 (CH), 41.5 (CH₂), 30.8 (CH₂), 22.8 (CH₂); m/z (ESI) 518 ([M(³⁵Cl)+Na]⁺, 100%), 519 (24.9%), 520 (32.6%), 521 (7.6%).

13-Chloro-14,16-di(ethoxymethoxy)-2-methyl-2,3,4,5,9,10-hexahydrobenz[c][1]azacyclotetradecine-1,11(8H,12H)-dione 26d

As for the procedure for compound 26a, using N-(pent-4-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-N-methyl-2-(2-oxohept-6-en-1-yl)benzamide 25d (0.081 g, 0.176 mmol, 1 eq.). The product was purified by flash column chromatography, eluting with 1% methanol/dichloromethane to give the title compound as a yellow oil (0.056 g, 68%) (9:1 mixture of isomers); (Found: M+Na⁺, 490.1965. C₂₄H₃₄³⁵ClNO₆⁺Na⁺, requires 490.1967); νmax (CHCl₃)/cm⁻¹ 3688, 3005, 2930, 2361, 2341, 1719, 1626, 1597, 1456, 1422, 1319, 1150, 1051, 941, 909; major isomer δH (400 MHz; CDCl₃) 7.10 (1H, s, ArH), 5.52 (2H, dt, J 9.9, 5.8, CH=CH), 5.28 (1H, d, J 15.8 OCH₂O), 5.27 (1H, d, J 15.8 OCH₂O), 5.20 (2H, s, OCH₂O), 4.45 (1H, ddd, J 13.3, 6.9, 3.7, CH₂), 4.08 (1H, d, J 18.6, CH₂), 3.90 (1H, d, J 18.6, CH₂), 3.76 (2H, q, J 7.1, OCH₂), 3.69 (2H, q, J 7.1, OCH₂), 2.86 (3H, s, NMe), 2.69-2.61 (2H, m, CH₂), 2.45 (1H, ddd, J 18.5, 6.0, 2.2 CH₂), 2.36-2.27 (1H, m, CH₂), 2.11-2.00 (2H, m, CH₂), 1.93-1.77 (2H, m, CH₂), 1.73-1.61 (3H, m, CH₂), 1.22 (6H, dt, J 8.3, 7.1, Me); δC (100 MHz; CDCl₃)
205.6 (C), 167.9 (C), 153.7 (C), 151.8 (C), 131.9 (C), 130.7 (CH), 128.7 (CH), 123.0 (C), 118.0 (C), 102.6 (CH), 93.9 (CH2), 93.7 (CH2), 64.5 (CH2), 64.4 (CH2), 47.4 (CH2), 44.8 (CH2), 38.5 (CH2), 37.5 (Me), 28.5 (CH2), 25.1 (CH2), 24.6 (CH2), 21.6 (CH2), 15.0 (Me), 15.0 (Me); for the minor isomer, the following NMR signals are discernable, the rest overlap with the major isomer: δH (400 MHz; CDCl3) 7.08 (1H, s, ArH), 5.32-5.25 (2H, m, OCH2O), 5.19 (2H, s, OCH2O), 4.05 (1H, d, J 18.0, CH2), 2.88 (3H, s, NMe); m/z (ESI) 490 ([M(35Cl)+Na]+, 100%), 491 (23.5%), 493 (33.3%), 494 (6.6%).

13-Chloro-14,16-dihydroxy-2-methyl-2,3,4,5,9,10-hexahydrobenz[c][1]azacyclotetradecine-1,11(8H,12H)-dione 27d

As for the procedure for compound 27a, using 13-chloro-14,16-di(ethoxymethoxy)-2-methyl-2,3,4,5,9,10-hexahydrobenz[c][1]azacyclotetradecine-1,11(8H,12H)-dione 26d (52 mg, 0.111 mmol, 1 eq.). The product was purified by flash column chromatography, eluting with 2% methanol/dichloromethane to give the title compound as a yellow solid (11 mg, 28%) (4:1 mixture of isomers); mp 178-180 °C; (Found: M+Na+, 374.1138. C18H2235ClNO4+Na+, requires 374.1130); νmax (CHCl3)/cm⁻¹ 3483, 3280, 1730, 1723, 1708, 1630, 1435, 1419, 1396, 1376, 1334, 1249, 12191, 1094; major isomer δH (800 MHz; acetone-d6) 8.75 (1H, s, OH), 8.66 (1H, s, OH), 6.58 (1H, s, ArH), 5.55 (1H, dt, J 10.6, 5.6, CH), 5.33 (1H, dt, J 10.6, 5.6, CH), 4.35-4.31 (1H, m, CH2), 4.05 (1H, d, J 18.6, CH2), 3.97 (1H, d, J 18.6, CH2), 2.91 (3H, s, NMe), 2.72-2.64 (2H, m, CH2), 2.57-2.52 (1H, m, CH2), 2.39-2.34 (1H, dt, J 11.4, 9.9, CH2), 2.10-2.05 (1H, m, CH2), 2.02-1.96 (1H, m, CH2), 1.92-1.85 (2H,
m, CH$_2$), 1.71-1.66 (1H, m, CH$_2$), 1.64-1.58 (1H, m, CH$_2$), 1.34-1.29 (1H, m, CH$_2$); δC (200 MHz; acetone-$d_6$) 205.8 (C), 168.6 (C), 154.2 (C), 152.8 (C), 133.3 (C), 131.9 (CH), 129.3 (CH), 120.9 (C), 113.9 (C), 103.1 (CH), 47.9 (CH$_2$), 45.1 (CH$_2$), 38.9 (CH$_2$), 37.5 (Me), 29.5 (CH$_2$), 25.9 (CH$_2$), 25.5 (CH$_2$), 22.5 (CH$_2$); for the **minor isomer**, the following NMR signals are discernable, the rest overlap with the major isomer: δH (800 MHz; acetone-$d_6$) 8.86 (1H, s, OH), 8.57 (1H, s, OH), 6.82 (1H, s, ArH), 5.42-5.36 (1H, m, CH), 5.30-5.22 (1H, m, CH), 4.08 (1H, d, J 18.6, CH$_2$), 2.51-2.46 (1H, m, CH$_2$), 2.23-2.19 (1H, m, CH$_2$), 2.16-2.13 (1H, m, CH$_2$); δC (200 MHz; acetone-$d_6$) 154.3 (C), 152.6 (C), 133.4 (C), 103.2 (CH), 30.6 (CH$_2$), 21.5 (CH$_2$); m/z (ESI) 374 ([M($^{35}$Cl)+Na]$^+$, 100%), 375 (17.7%), 376 (30.6%), 377 (6.2%).

**Isothermal Titration Calorimetry (ITC)**

ITC was carried out as previously described by Proisy and co-workers.$^{16}$ Briefly, heats of the interaction were measured at 30 °C on an ITC200 (GE healthcare), with a cell volume of 200 µL. Ten injections of 3.8 µL of 600 µM of radicicol or radicicol analogue, in 20 mM Tris, 5 mM NaCl, 1 mM EDTA and 2% DMSO, were injected into 40 µM yeast Hsp90 N-terminal domain in the same buffer. Heats of dilution were determined in a separate experiment by injecting compound into buffer containing 2% DMSO, and the corrected data were fit with a nonlinear least square curve-fitting algorithm (Microcal Origin) with three floating variables: stoichiometry, binding constant, and change in enthalpy of interaction.

**Protein co-Crystallisation Experimental**

The expression, purification and co-crystallisation of the N-terminal domain of yeast Hsp90 have been previously described.$^{51}$ Single crystals appeared overnight of approximate dimensions 0.3 mm × 0.2 mm × 0.2 mm. These were flash frozen after
stepwise addition of glycerol to 30%, and the data were collected on station I02 at Diamond Light Source. The data were integrated and scaled using the automatic Xia2$^{52,53}$ processing at Diamond.

The complexes were initially solved by isomorphous replacement using a previously determined N-terminal structure (PDB 1AH6) in the usual space group, $P_{43}2_{2}$. The model was refined using REFMAC$^{54}$ and rebuilt using COOT.$^{55}$ The inhibitors were built using the PRODRG server.$^{56}$ The inhibitor molecule and the waters were added in the final stages. Various programs from the CCP4$^{57}$ suite were used during the refinement. All refinements proceeded smoothly. The crystallographic statistics are given in Table 2.

**Table 2.** Crystallographic data

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<td>8.5 (9.0)</td>
<td>3.8 (3.9)</td>
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<td>0.065 (0.720)</td>
<td>0.071 (0.676)</td>
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<td>Rpim(I)</td>
<td>0.043 (0.301)</td>
<td>0.024 (0.263)</td>
<td>0.035 (0.318)</td>
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<td>I/σI</td>
<td>14.2 (2.8)</td>
<td>20.4 (3.5)</td>
<td>17.5 (2.5)</td>
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### Refinement

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<th>Resolution Range (Å)</th>
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<th>Rfree</th>
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<td>Rmsd bond lengths (Å)</td>
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<td>Rmsd bond angles (°)</td>
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### Acknowledgements

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### References


