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

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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 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 (Fig. 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_{50} = 160 nM *vs.* IC_{50} = 20–23 nM for radicicol), cyclopropararadicicol **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,[32] a cyclic carbonate,[32] a diol[32] and a

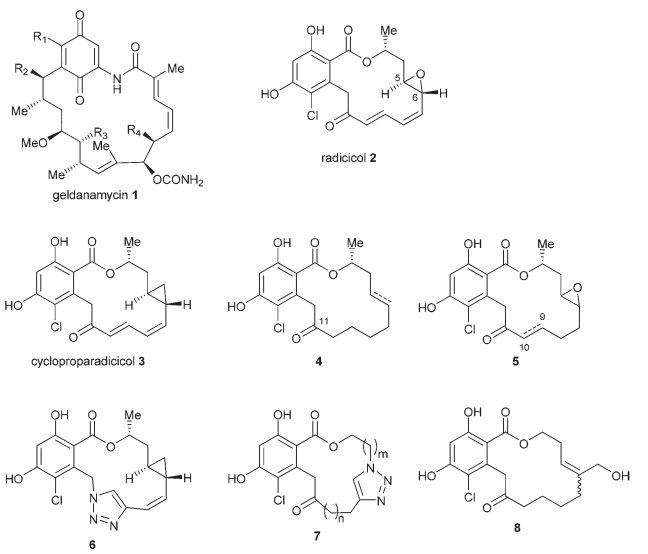

Fig. 1 Radicicol analogue Hsp90 inhibitors.

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carbon chain (both saturated and unsaturated).[33] Whilst the former three are significantly detrimental to the potency, a carbon chain is tolerated with only a two-fold drop in performance.[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 either a saturated carbon chain from C7–C10 or an enone (**4** and **5**, respectively).[32–34] Additionally, Danishefsky has replaced the dienone of cycloproparadicicol with a triazole **6**[35] and we have also incorporated a triazole into the macrocycle (compound **7**) to investigate the benefits of additional H-bonding potential.[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.[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 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 (Fig. 2).[2]

Our laboratory has been interested in developing novel Hsp90 inhibitors, including the synthesis of more potent and metabolically stable radicicol analogues, for some years.[25–27,33,34,36,42,43] 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,[43] a strategy widely employed in drug development and also utilised in the development of similar RAL analogues by Danishefsky[44] and Winssinger.[45] 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 CO_2, giving the isocoumarin **14** in 75% yield. This was readily converted into both the NH and NMe macrolactams **16** (see Fig. 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.[43]

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.[43] 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.

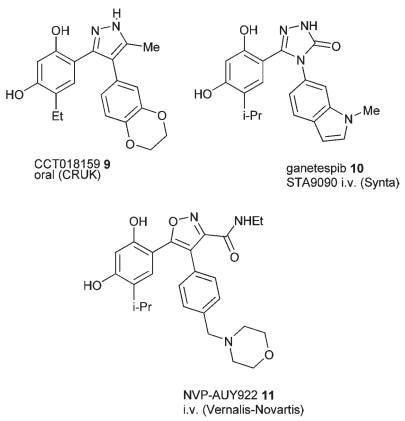
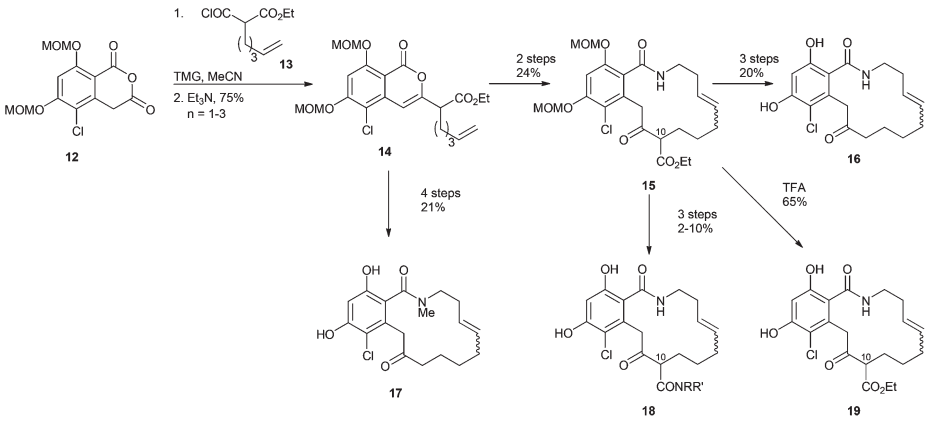


Fig. 2 Pyrazole and isoxazole-containing Hsp90 inhibitors.

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[46–48] sulfuryl chloride-mediated chlorination was found to be highly temperature dependent; a reaction temperature of −30 °C was crucial for selective monochlorination, with the product **21**



Scheme 1 Original synthesis of 14-membered macrolactams, analogues of the RALs.[43]

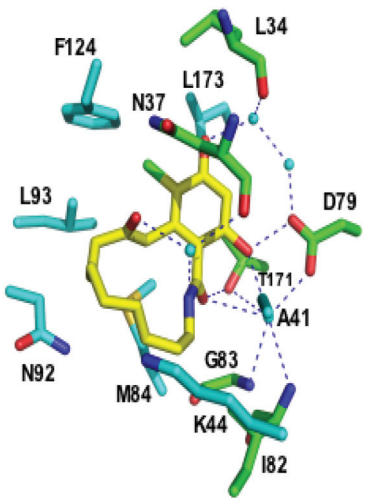
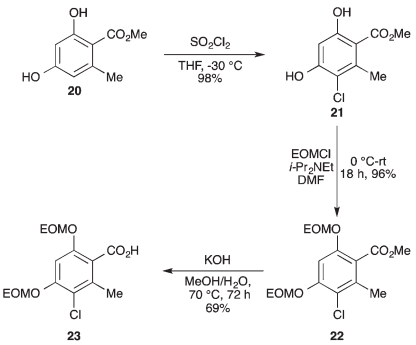


Fig. 3 Hsp90-bound X-ray crystal structure of macrolactam **16**.[43] Image from the article by Day *et al.*,[43] reprinted, with permission from J. E. H. Day, S. Y. Sharp, M. G. Rowlands, W. Aherne, A. Hayes, F. I. Raynaud, W. Lewis, S. M. Roe, C. Prodromou, L. H. Pearl, P. Workman, C. J. Moody, *ACS Chem. Biol.*, 2011, **6**, 1339–1347. Copyright (2011) American Chemical Society.

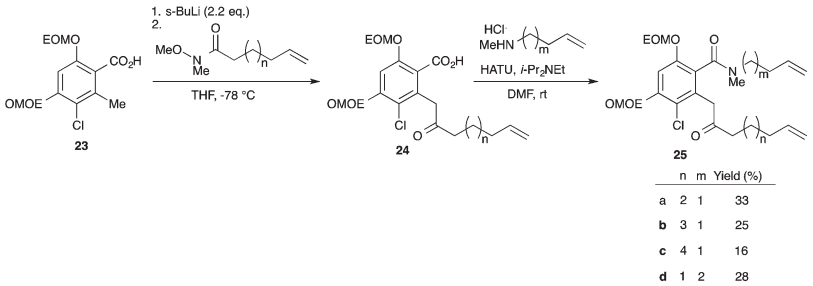


Scheme 2 The synthesis of orsellinic acid **23** [EOM = ethoxymethyl].

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**[34,49] 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).

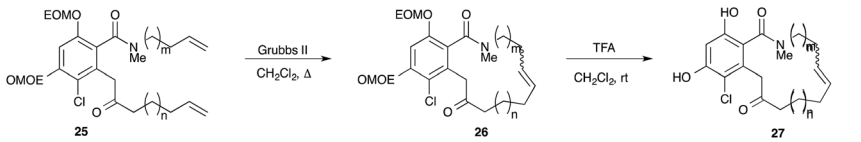
With the key intermediate **23** in hand, our focus now turned to the amide coupling and benzylic acylation steps. With the option of varying the order of the two steps, we initially performed a HATU-mediated amide coupling with *N*-methylbut-3-en-1-amine and obtained the product in quantitative yield. 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.[50] 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).

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 Hz). We postulated that this is a result of the *N*-methyl group being sufficiently bulky to hinder the rotation past the lower chain, creating pairs of rotamers.



	n	m	Yield (%)
a	2	1	33
b	3	1	25
c	4	1	16
d	1	2	28

Scheme 3 Acylation and coupling investigations.



	n	m	Yield (%)	E/Z
a	2	1	51	4:1
b	3	1	39	2:1
c	4	1	34	4:1
d	1	2	68	1:9

	n	m	Yield (%)	E/Z
a	2	1	72	9:1
b	3	1	45	3:1
c	4	1	33	5:1
d	1	2	28	1:4

Scheme 4 Formation of the radicicol analogues.

The macrocycles were formed *via* ring closing metathesis, utilising Grubbs' 2nd 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).

Next, the thermodynamics of the binding of macrocycles **27b–d** to Hsp90 was investigated through isothermal titration calorimetry (ITC) (Table 1). The results 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 μM). 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 1 Isothermal titration calorimetry for macrolactams **27b–d** with yeast Hsp90 N-terminal domain (*N* = stoichiometry of binding)

Ligand	*N*	K_d/μM	ΔH/kJ mol^{-1}	ΔS/J mol^{-1} K^{-1}
Radicicol	1.16	0.015 ± 0.06	−6726	13.6
27b	1.0 (fixed)	144 ± 25	−1012	14.2
27c	0.92	41.8 ± 8.7	−834.8	17.3
27d	1.1	110 ± 7.9	−1466	13.3

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 (Fig. 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 1.5σ 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 1.5σ 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 Fig. 5.

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.

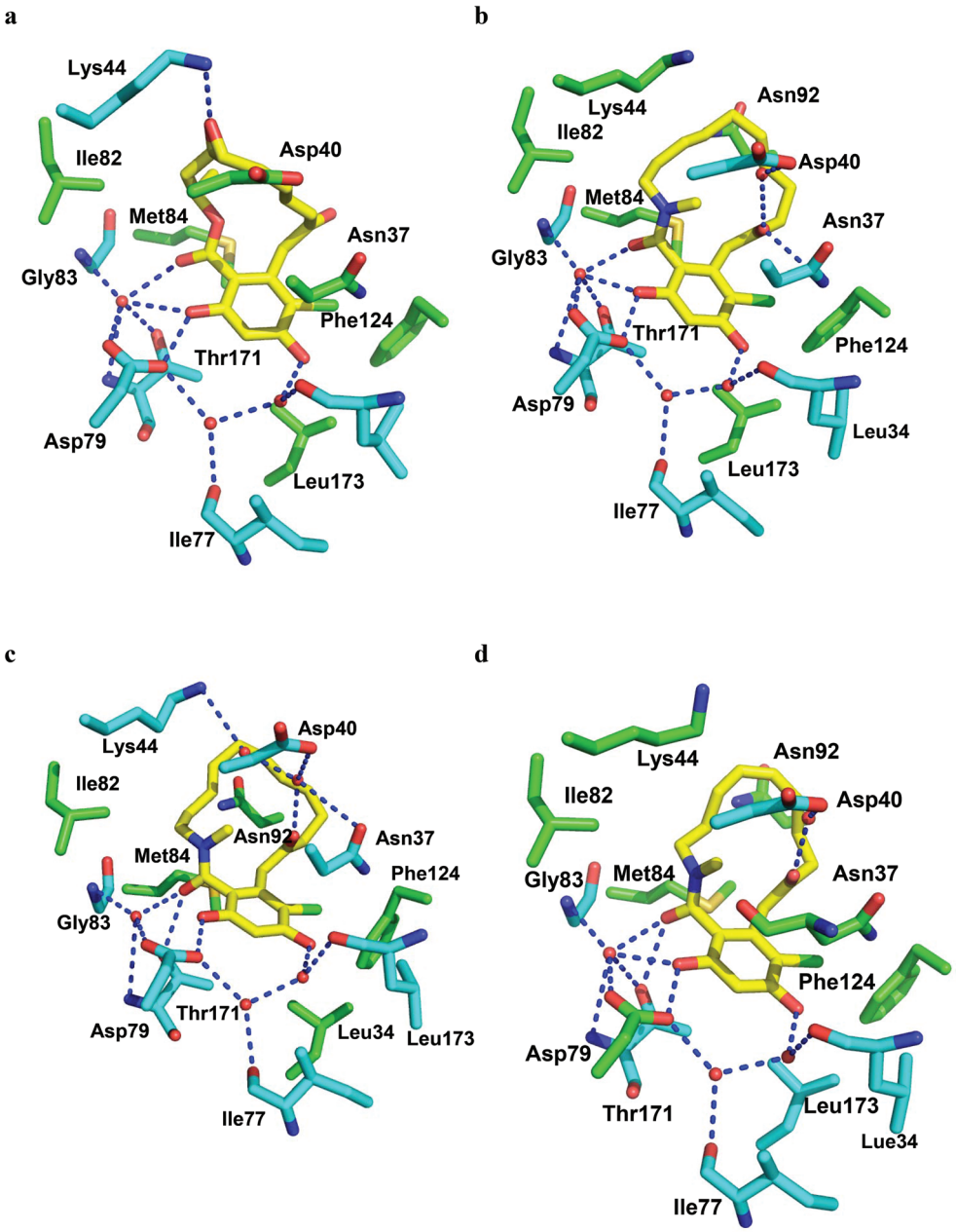


Fig. 4 Structures of resorcylic acid macrolactams (yellow) bound in the ATP site of yeast Hsp90 as determined by protein X-ray crystallography, showing the interactions of (a) radicicol **2** with Hsp90 (green and cyan residues); (b) macrolactam **27b** (*E*-alkene) with Hsp90 (green and cyan residues); (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.

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

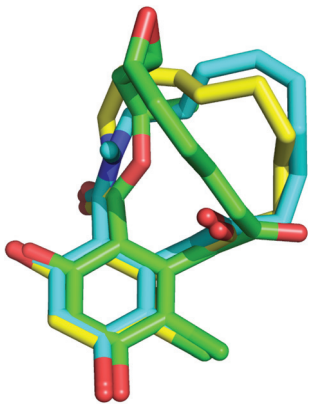
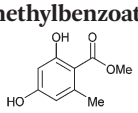


Fig. 5 Overlaid crystal structures showing the difference in the conformation and binding of resorcylic acid macrolactams in comparison to radicicol. The different conformations of radicicol **2** (PDB code 1BGQ; green), NH macrolactam **16** (PDB code 2XX2; yellow)[43] and NMe macrolactam **27d** (PDB code 4ce3; cyan).

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.

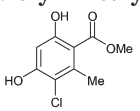
Infrared spectra were obtained on a Perkin Elmer 1600 series FT-IR spectrometer using NaCl solution cells in chloroform or acetone. ^{1}H NMR and ^{13}C NMR spectra were obtained using a JEOL EX270 operating at 270 MHz (^{13}C 67.5 MHz), or Bruker Avance III-400, Bruker Avance 400 or Bruker DPX 400 spectrometers operating at 400 MHz (^{13}C 100 MHz). All spectroscopic data was recorded at 295 K. 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[25]



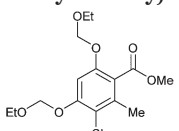
Sodium hydride (20.67 g, 0.52 mol, 1.5 eq.) was prewashed with pentane (3 × 50 mL), before being suspended in THF (800 mL) and cooled to 0 °C under argon. Methyl acetoacetate (40.00 g, 0.34 mol, 1.0 eq.) was added dropwise over 2 h and the reaction mixture was warmed to room temperature over 1 h. After 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.[25]

Methyl 3-chloro-4,6-dihydroxy-2-methylbenzoate 21



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.,[51] mp 134 °C); δ_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); δ_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.[46]

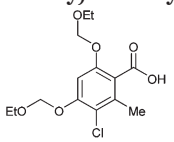
Methyl 3-chloro-4,6-di(ethoxymethoxy)-2-methylbenzoate 22



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

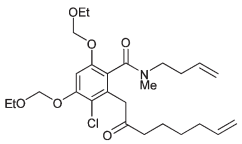
2 h. Saturated aqueous ammonium hydroxide solution (100 mL) was added and the reaction mixture was stirred for a further 10 min, before being extracted with ether (3 × 100 mL). The combined organics were washed with aqueous lithium chloride solution (5%; 3 × 100 mL), dried over magnesium sulfate and 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. $C_{15}H_{21}{}^{35}ClO_6$ + Na^+, requires 355.0919); ν_{max} ($CHCl_3$)/cm^{-1} 3526, 2981, 2955, 1728, 1660, 1596, 1476, 1433, 1321, 1265, 1114, 1042, 963, 932, 844; δ_H (400 MHz; $CDCl_3$) 7.02 (1H, s, ArH), 5.32 (2H, s, OCH_2O), 5.22 (2H, s, OCH_2O), 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); δ_C (100 MHz; $CDCl_3$) 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



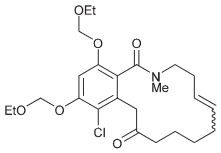
To a stirred solution of methyl 3-chloro-4, 6-di(ethoxymethoxy)-2-methylbenzoate **22** (6.26 g, 18.82 mmol, 1.0 eq.) in 1 : 1 methanol–water (60 mL) was added potassium hydroxide (5.28 g, 94.09 mmol, 5.0 eq.) and the reaction mixture was heated to 70 °C and stirred for 3 d. After cooling to room temperature, 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%. $C_{14}H_{19}{}^{35}ClO_6$, requires C, 52.8; H, 6.0%); δ_H (400 MHz; acetone-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-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.[31]

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



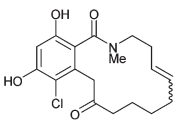
To a solution of 3-chloro-4,6-di(ethoxymethoxy)-2-methylbenzoic acid **23** (170 mg, 0.54 mmol, 1.0 eq.) in THF (2.67 mL) in an oven dried flask at −78 °C was added *sec*-butyllithium (1.08 mL, 1.19 mmol, 2.2 eq.). The resulting red solution was stirred for 40 min before a solution of *N*-methoxy-*N*-methylhept-6-enamide (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 (0.056 g, 33% over 2 steps) (3 : 1 mixture of isomers); (Found: M + Na^+, 518.2287. $C_{26}H_{38}{}^{35}ClNO_6$ + Na^+, requires 518.2280); ν_{max} ($CHCl_3$)/cm^{-1} 3665, 3079, 3001, 1759, 1628, 1590, 1486, 1453, 1327, 1244, 1054, 1036, 1029; *major isomer* δ_H (400 MHz; $CDCl_3$) 7.08 (1H, s, ArH), 5.89–5.73 (2H, m, CH), 5.29 (1H, d, J 12.8, OCH_2O), 5.27 (1H, d, J 12.8, OCH_2O), 5.18 (2H, s, OCH_2O), 5.14–4.92 (4H, m, $=CH_2$), 3.98 (1H, d, J 17.6, CH_2), 3.79 (1H, d, J 17.6, CH_2), 3.80–3.73 (2H, m, OCH_2), 3.72–3.66 (2H, m, OCH_2), 3.57 (1H, dt, J 13.1, 6.8, CH_2), 2.83 (3H, s, NMe), 2.54–2.48 (2H, m, CH_2), 2.39–2.34 (2H, m, CH_2), 2.08–2.04 (2H, m, CH_2), 1.65–1.57 (3H, m, CH_2), 1.43–1.36 (2H, m, CH_2), 1.23 (6H, 2 × t, J 14.5, 7.1, Me); δ_C (100 MHz; $CDCl_3$) 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 (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: δ_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); δ_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%).

13-Chloro-14,16-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10-hexahydrobenz[*c*][1]azacyclotetradecine-1,11(2*H*,12*H*)-dione 26a



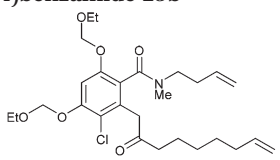
A solution of *N*-(but-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-*N*-methyl-2-(2-oxooct-7-en-1-yl)benzamide **25a** (56 mg, 0.11 mmol, 1.0 eq.) dichloromethane (35 mL) was sparged with nitrogen and Grubbs II catalyst (11 mg, 0.01 mmol, 0.11 eq.) was added. The reaction mixture was heated to reflux for 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); ν_{max} ($CHCl_3$)/cm^{-1} 3011, 2928, 2855, 1720, 1624, 1592, 1464, 1323, 1239, 1118, 1045, 929; *major isomer* δ_H (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_2O), 5.28 (1H, d, *J* 15.1, OCH_2O), 5.22–5.18 (2H, m, OCH_2O), 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), 3.70 (2H, dq, *J* 14.2, 4.1, OCH_2), 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); δ_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: δ_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); δ_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(2*H*,12*H*)-dione 27a[1]



To a solution of 13-chloro-14,16-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10-hexahydrobenzo[*c*][1]azacyclotetradecine-1,11-(2*H*,12*H*)-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* δ_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), 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); δ_C (100 MHz; $CDCl_3$) 204.8 (C), 167.9 (C), 153.3 (C), 152.2 (C), 132.0 (CH), 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: δ_H (400 MHz; $CDCl_3$) 6.57 (1H, s, ArH), 5.58–5.44 (2H, m, CH=CH), 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); δ_C (100 MHz; $CDCl_3$) 130.7 (CH), 44.1 (CH_2). Data consistent with those reported in the literature.[1]

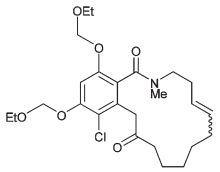
***N*-(But-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-*N*-methyl-2-(2-oxonon-8-en-1-yl)benzamide 25b**



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_{max} ($CHCl_3$)/cm^{-1} 3685, 3001, 2930, 1719, 1630, 1596, 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), 5.14–4.91 (4H, m, $=CH_2$), 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_2), 3.72–3.66 (2H, m, OCH_2), 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 × t, *J* 14.5, 7.3, Me); δ_C (100 MHz; $CDCl_3$) 196.5 (C), 167.6 (C), 138.8

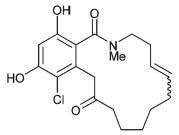
(C), 138.7 (C), 135.4 (C), 135.0 (C), 134.8 (C), 116.6 (CH_2), 114.4 (CH_2), 110.6 (CH), 102.6 (CH), 102.5 (CH) 94.0 (CH_2), 93.7 (CH_2), 64.6 (CH_2), 64.4 (CH_2), 46.5 (CH_2), 44.8 (CH_2), 42.6 (CH_2), 36.1 (Me), 33.6 (CH_2), 31.6 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 23.6 (CH_2), 15.0 (Me), 15.0 (Me); 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-1*H*-benzo[*c*][1]azacyclopentadecine-1,12(2*H*)-dione 26b



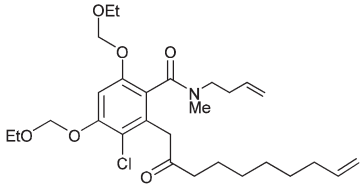
As for the procedure for compound **26a**, using *N*-(but-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-*N*-methyl-2-(2-oxonon-8-en-1-yl)benzamide **25b** (70 mg, 0.14 mmol, 1.0 eq.). The product was purified by flash column chromatography, eluting with 2 : 1 light petroleum–ethyl acetate to give the *title compound* as a colourless oil (0.026 g, 39%) (2 : 1 mixture of isomers); (Found: M + Na$^+$, 504.2124. $C_{25}H_{36}{}^{35}ClNO_6$ + Na$^+$, requires 504.2123); ν_{max} ($CHCl_3$)/cm^{-1} 3686, 3600, 2928, 2855, 1717, 1605, 1465, 1286, 1269, 1044, 910; *major isomer* δ_H (400 MHz; $CDCl_3$) 7.10 (1H, s, ArH), 5.48–5.35 (2H, m, CH=CH), 5.30 (1H, d, *J* 16.6, OCH_2O), 5.28 (1H, d, *J* 16.6, OCH_2O), 5.22–5.18 (2H, m, OCH_2O), 4.64 (1H, ddd, *J* 14.9, 9.4, 5.9, CH_2), 4.13 (1H, d, *J* 18.0, CH_2), 3.83–3.74 (2H, m, OCH_2), 3.73–3.67 (2H, m, OCH_2), 3.69 (1H, d, *J* 18.0, CH_2), 2.83 (3H, s, NMe), 2.82–2.73 (2H, m, CH_2), 2.48–2.33 (3H, m, CH_2), 2.09–2.00 (2H, m, CH_2), 1.87–1.75 (1H, m, CH_2), 1.63–1.52 (1H, m, CH_2), 1.46–1.32 (4H, m, CH_2), 1.24 (6H, 2 × t, *J* 13.1, 7.1, Me); δ_C (100 MHz; $CDCl_3$) 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 (CH_2), 93.8 (CH_2), 64.6 (CH_2), 64.5 (CH_2), 45.7 (CH_2), 45.5 (CH_2), 42.2 (CH_2), 34.6 (Me), 30.8 (CH_2), 29.9 (CH_2), 26.7 (CH_2), 25.9 (CH_2), 23.4 (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: δ_H (400 MHz; $CDCl_3$) 7.11 (1H, s, ArH), 5.31 (1H, d, *J* 17.3, OCH_2O), 5.29 (1H, d, *J* 17.3, OCH_2O), 4.14 (1H, d, *J* 18.8, CH_2), 3.27–3.19 (2H, m, CH_2), 3.11–3.06 (1H, m, CH_2), 2.64–2.56 (1H, m, CH_2), 2.18–2.09 (2H, m, CH_2), 1.99–1.91 (2H, m, CH_2); δ_C (100 MHz; $CDCl_3$) 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 (CH_2), 94.0 (CH_2), 48.0 (CH_2), 46.8 (CH_2), 41.2 (CH_2), 31.1 (CH_2), 29.0 (CH_2), 26.0 (CH_2), 25.2 (CH_2), 22.0 (CH_2); *m*/*z* (ESI) 504 ([M(^{35}Cl) + 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-1*H*-benz[*c*][1]azacyclopentadecine-1,12(2*H*)-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-1*H*-benzo[*c*][1]azacyclopentadecine-1,12(2*H*)-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. $C_{19}H_{24}{}^{35}ClNO_4$ + Na$^+$, requires 388.1286); ν_{max} ($CHCl_3$)/cm^{-1} 3438, 3274, 2859, 1732, 1728, 1701, 1623, 1455, 1445, 1391, 1343, 1312, 1251, 1100; *major isomer* δ_H (800 MHz; acetone-d_6) 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, CH_2), 3.98 (1H, d, *J* 17.9, CH_2), 3.76 (1H, d, *J* 17.9 CH_2), 2.94 (3H, s, NMe), 2.75–2.71 (2H, m, CH_2), 2.45–2.40 (1H, m, CH_2), 2.38–2.33 (1H, m, CH_2), 1.80–1.75 (1H, m, CH_2), 1.59–1.54 (1H, m, CH_2), 1.42–1.36 (2H, m, CH_2), 1.32–1.27 (3H, m, CH_2), 1.24–1.15 (2H, m, CH_2); δ_C (200 MHz; acetone-d_6) 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_2), 47.0 (CH_2), 46.9 (CH_2), 43.5 (CH_2), 37.1 (Me), 35.6 (CH_2), 28.6 (CH_2), 27.6 (CH_2), 25.2 (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) 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_2), 4.16 (1H, d, *J* 18.8, CH_2), 3.99 (1H, d, *J* 18.8 CH_2), 2.88 (3H, s, NMe), 2.62–2.56 (2H, m, CH_2), 1.94–1.88 (1H, m, CH_2); δ_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_2), 47.8 (CH_2), 42.6 (CH_2), 32.4 (CH_2), 28.1 (CH_2), 26.8 (CH_2), 23.6 (CH_2); *m*/*z* (ESI) 388 ([M(^{35}Cl) + Na]$^+$, 100%), 389 (18.6%), 390 (31.6%), 391 (5.8%).

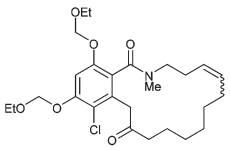
***N*-(But-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-*N*-methyl-2-(2-oxodec-9-en-1-yl)benzamide 25c**



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);

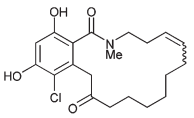
(Found: M + Na^+, 546.2590 $C_{28}H_{42}{}^{35}ClNO_6$ + Na^+, requires 546.2593); ν_{max} ($CHCl_3$)/cm^{-1} 3742, 3678, 3050, 2999, 2872, 1631, 1464, 1380, 1366, 1276, 1259, 1172, 1149, 1126, 1039, 851; *major isomer* δ_H (400 MHz; $CDCl_3$) 7.11 (1H, s, ArH), 5.92–5.77 (2H, m, CH), 5.32 (1H, d, *J* 13.0, OCH_2O), 5.30 (1H, d, *J* 13.0, OCH_2O), 5.23–5.19 (2H, m, OCH_2O), 5.16–4.94 (4H, m, $=CH_2$), 4.02 (1H, d, *J* 17.7, CH_2), 3.82 (1H, d, *J* 17.7, CH_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 × t, *J* 14.5, 7.3, Me); δ_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: δ_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_2O), 3.92 (1H, d, *J* 17.6, CH_2), 3.10 (3H, s, NMe); δ_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(2*H*,14*H*)-dione 26c



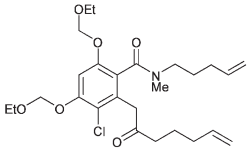
As for the procedure for compound **26a**, using *N*-(but-3-en-1-yl)-3-chloro-4,6-di(ethoxymethoxy)-*N*-methyl-2-(2-oxodec-9-en-1-yl)benzamide **25c** (60 mg, 0.11 mmol, 1.0 eq.). The product was purified by flash column chromatography, eluting with 2 : 1 light petroleum–ethyl acetate to give the *title 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); ν_{max} ($CHCl_3$)/cm^{-1} 3686, 2929, 2856, 1720, 1627, 1461, 1324, 1278, 1273, 1250, 1151, 1118, 940; *major isomer* δ_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_2O), 5.19 (2H, s, OCH_2O), 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), 3.71–3.66 (2H, m, CH_2), 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); δ_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: δ_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); δ_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[*c*][1]azacyclohexadecine-1,13(2*H*,14*H*)-dione 27c



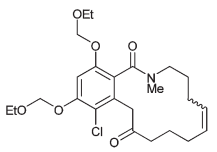
As for the procedure for compound **27a**, using 15-chloro-16,18-di(ethoxymethoxy)-2-methyl-3,4,7,8,9,10,11,12-octahydrobenzo-[*c*][1]azacyclohexadecine-1,13(2*H*,14*H*)-dione **26c** (15 mg, 0.33 mmol, 1 eq.). The product was purified by flash column chromatography, eluting with 2% methanol–dichloromethane 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, 1413, 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), 2.53–2.45 (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



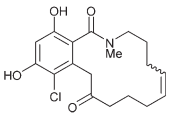
As for the procedure for compound **25a**, using 3-chloro-4,6-di(ethoxymethoxy)-2-methylbenzoic acid **23** (200 mg, 0.63 mmol, 1 eq.), *N*-methoxy-*N*-methylhex-5-enamide (148 mg, 0.94 mmol, 1.5 eq.) and *N*-methylpent-4-en-1-amine (91 mg, 0.68 mmol, 1.5 eq.). The product was purified by flash column 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 $C_{26}H_{38}{}^{35}ClNO_6$ + Na^+, requires 518.2280); ν_{max} ($CHCl_3$)/cm^{-1} 3686, 3600, 3058, 2996, 1719, 1628, 1597, 1458, 1408, 1319, 1278, 1265, 1152, 1119, 1042, 941, 896; *major isomer* δ_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_2O), 5.30 (1H, d, *J* 13.2, OCH_2O), 5.22 (2H, s, OCH_2O), 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); δ_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 *minor isomer*, the following NMR signals are discernable, the rest overlap with the major isomer: δ_H (400 MHz; $CDCl_3$) 7.15 (1H, s, ArH), 5.33 (1H, d, *J* 13.8, O–CH_2–O), 5.31 (1H, d, *J* 13.8, O–CH_2–O), 5.20 (2H, s, O–CH_2–O), 3.91 (1H, d, *J* 17.0, CH_2), 3.64–3.55 (2H, m, O–CH_2), 3.50–3.40 (2H, m, O–CH_2), 3.07 (3H, s, NMe), 2.54 (2H, ddd, *J* 15.0, 7.4, 3.0 CH_2), 1.98–1.91 (1H, m, CH_2); δ_C (100 MHz; $CDCl_3$) 206.6 (C), 138.0 (CH), 137.4 (CH), 115.2 (CH_2), 102.8 (CH), 41.5 (CH_2), 30.8 (CH_2), 22.8 (CH_2); *m*/*z* (ESI) 518 ([M(^{35}Cl) + 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(8*H*,12*H*)-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. $C_{24}H_{34}{}^{35}ClNO_6$ + Na^+, requires 490.1967); ν_{max} ($CHCl_3$)/cm^{-1} 3688, 3005, 2930, 2361, 2341, 1719, 1626, 1597, 1456, 1422, 1319, 1150, 1051, 941, 909; *major isomer* δ_H (400 MHz; $CDCl_3$) 7.10 (1H, s, ArH), 5.52 (2H, dt, *J* 9.9, 5.8, CH=CH), 5.28 (1H, d, *J* 15.8 OCH_2O), 5.27 (1H, d, *J* 15.8 OCH_2O), 5.20 (2H, s, OCH_2O), 4.45 (1H, ddd, *J* 13.3, 6.9, 3.7, CH_2), 4.08 (1H, d, *J* 18.6, CH_2), 3.90 (1H, d, *J* 18.6, CH_2), 3.76 (2H, q, *J* 7.1, OCH_2), 3.69 (2H, q, *J* 7.1, OCH_2), 2.86 (3H, s, NMe), 2.69–2.61 (2H, m, CH_2), 2.45 (1H, ddd, *J* 18.5, 6.0, 2.2 CH_2), 2.36–2.27 (1H, m, CH_2), 2.11–2.00 (2H, m, CH_2), 1.93–1.77 (2H, m, CH_2), 1.73–1.61 (3H, m, CH_2), 1.22 (6H, dt, *J* 8.3, 7.1, Me); δ_C (100 MHz; $CDCl_3$) 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 (CH_2), 93.7 (CH_2), 64.5 (CH_2), 64.4 (CH_2), 47.4 (CH_2), 44.8 (CH_2), 38.5 (CH_2), 37.5 (Me), 28.5 (CH_2), 25.1 (CH_2), 24.6 (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: δ_H (400 MHz; $CDCl_3$) 7.08 (1H, s, ArH), 5.32–5.25 (2H, m, OCH_2O), 5.19 (2H, s, OCH_2O), 4.05 (1H, d, *J* 18.0, CH_2), 2.88 (3H, s, NMe); *m*/*z* (ESI) 490 ([M(^{35}Cl) + 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(8*H*,12*H*)-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,12*H*)-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. $C_{18}H_{22}{}^{35}ClNO_4$ + Na^+, requires 374.1130); ν_{max} ($CHCl_3$)/cm^{-1} 3483, 3280, 1730, 1723, 1708, 1630, 1435, 1419, 1396, 1376, 1334, 1249, 12191, 1094; *major isomer* δ_H (800 MHz; acetone-d_6) 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, CH_2), 4.05 (1H, d, *J* 18.6, CH_2), 3.97 (1H, d, *J* 18.6, CH_2), 2.91 (3H, s, NMe), 2.72–2.64 (2H, m, CH_2), 2.57–2.52 (1H, m, CH_2), 2.39–2.34 (1H, dt, *J* 11.4, 9.9, CH_2), 2.10–2.05 (1H, m, CH_2), 2.02–1.96 (1H, m, CH_2), 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); δ_C (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: δ_H (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); δ_C (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.[52] 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[53,54] 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, $P4_322$. The model was refined using REFMAC[55] and rebuilt using COOT.[56] The inhibitors were built using the PRODRG server.[57] The inhibitor molecule and the waters were added in the final stages. Various programs from the CCP4[58] suite were used during the refinement. All refinements proceeded smoothly. The crystallographic statistics are given in Table 2.

Table 2 Crystallographic data

Data set (highest shell in parentheses)	27b	27c	27d
a (Å)	74.32	74.16	74.18
b (Å)	74.32	74.16	74.18
c (Å)	110.46	110.43	110.49
α (°)	90	90	90
β (°)	90	90	90
γ (°)	90	90	90
Space group	*P*4322	*P*4322	*P*4322
Wavelength (Å)	0.97961	0.97961	0.97961
Resolution limit (Å)	47.5–2.38 (2.45–2.38)	52.4–2.01 (2.06–2.01)	52.5–2.31 (2.37–2.31)
Number of obs.	12939 (927)	21192 (1511)	13834 (1002)
Completeness (%)	99.9 (100.0)	99.9 (99.8)	98.8 (98.3)
Multiplicity	8.5 (9.0)	8.5 (9.0)	3.8 (3.9)
R_{merge}	0.111 (0.803)	0.065 (0.720)	0.071 (0.676)
$R_{pim}(I)$	0.043 (0.301)	0.024 (0.263)	0.035 (0.318)
$I/\sigma I$	14.2 (2.8)	20.4 (3.5)	17.5 (2.5)
Refinement			
Resolution range (Å)	47.5–2.38	52.4–2.01	52.5–2.32
R_{cryst}	0.1930	0.2058	0.1933
R_{free}	0.2394	0.2660	0.2613
Number of protein atoms	1691	1690	1675
Number of ligand atoms	25	26	24
Number of solvent atoms	126	115	113
Mean B	42.4	36.5	39.7
Rmsd bond lengths (Å)	0.016	0.018	0.016
Rmsd bond angles (°)	1.848	1.935	1.802

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