Inhibition of HSP90 with pochoximes: SAR and structure-based insights

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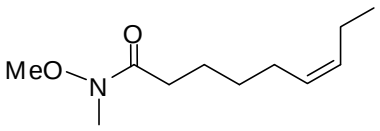
Supporting information

General Techniques. All reactions were carried out under a nitrogen atmosphere with dry (anhydrous) solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing them through commercially available alumina column (Innovative Technology, Inc., ® VA). All substituted polystyrene resins (100-200 mesh, 1% DVB) were purchased from Novabiochem® or Aldrich®. The Grubbs' II catalyst was purchased from Materia Inc.® Solid phase reactions were carried on a Quest® 210 or round bottom flasks and filtered in fritted funnels. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck® silica gel plates (60F-254) using UV light as visualizing agent and 10% ethanolic phosphomolybdic acid or vanillin solution and heat as developing agents or by LC-MS. E. Merck® silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. PTLC (preparative thin layer chromatography) were carried out on 0.25 mm E. Merck® silica gel plates. NMR spectra were recorded on a Bruker Advance-400® instrument and calibrated by using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. LC-MS were recorded using an Agilent 1100® HPLC with a Bruker® micro-TOF instrument (ESI). Unless otherwise stated, a Supelco® C8 (5 cm x 4.6mm, 5 μm particles) column was used with a linear elution gradient from 100% H_2O (0.5% HCO_2H) to 100% MeCN in 13 min at a flow rate of 0.5 ml/min. Unless otherwise stated, LDA was prepared at a concentration of 0.566 M by treating a solution of diisopropylamine (1.0 equiv.) in THF at -78 °C with *n*-butyllithium (1.0 equiv) and stirred for 30 min at this temperature before use.

Esters A1 and A2: Aromatic esters **A1** and **A2** were synthesized as previously reported.[1]

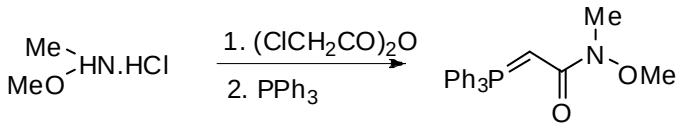
[1] S. Barluenga, C. Wang, J.G. Fontaine, K. Aouadi, K. Beebe, S. Tsutsumi, L. Neckers and N. Winssinger, *Angew. Chem., Int. Ed.* **47** (2008), p. 4432

Weinreb amides B1: Weinreb amide **B1** was synthesized as previously described. [1]



B2

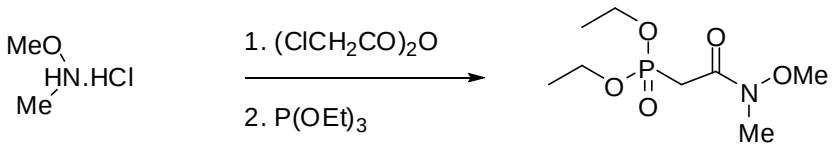
Weinreb Amide B2: N,O-dimethylhydroxylamine hydrochloride (**15**) (976 mg, 10.0 mmol, 1 equiv), DMAP (122 mg, 1 mmol, 0.1 equiv) and EDC (1.92 g, 10.0 mmol, 1 equiv) were sequentially added to a solution of (Z)-6-nonenoic acid (1.56 g, 10.0 mmol, 1 equiv) in anhydrous CH_2Cl_2 (30 mL). After stirring at room temperature for 4 hours, the reaction was diluted with EtOAc (30 mL), washed with saturated NH_4Cl_{aq}. (40 mL) and dried over Na_2SO_4. Concentration under reduced pressure and purification on silica chromatography (petroleum ether/EtOAc 12/1) afforded pure **B2** as a yellowish oil (1.69 g, 8.5 mmol) in an 85% yield. *Rf* = 0.45 (petroleum ether/EtOAc 4/1); ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 5.40-5.26 (m, 2H), 3.67 (s, 3H), 3.17 (s, 3H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.11-1.97 (m, 4H), 1.64 (tt, *J* = 8.7, 6.3 Hz, 2H), 1.39 (tt, *J* = 8.6, 6.7 Hz, 2H), 0.95 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 131.9, 128.7, 61.2, 31.8 (×2), 29.5, 26.9, 24.3, 20.5, 14.3, one quaternary carbon is not visible.



15 **16**

Synthesis of N-Methoxy-N-methyl-2-(triphenylphosphoranylidene)acetamide (16). To a solution of N,O-dimethylhydroxylamine hydrochloride (**15)** (48.8 g, 500 mmol, 1.0 equiv) in CH_2Cl_2 (1.0 L) at 0 °C were added sequentially pyridine (80.6 mL, 1.0 mol, 2.0 equiv) and chloroacetic anhydride (85.5 g, 500 mmol, 1.0 equiv). The resulting mixture was stirred for 15 min at 0 °C, then warm up to 23 °C and stirred for 3 hours. The reaction mixture was then poured carefully into sat. $NaHCO_{3aq}$ solution (1.0 L) and stirred 1 hour, and then the layers were separated, the aqueous phase was extracted with CH_2Cl_2 (400 mL) and the combined

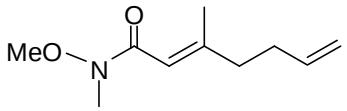
organic layers were washed with 6N HCl (200 mL x 2), brine (200 mL x 2), dried over Na_2SO_4 (10 g) and filtered. Evaporation of the solvents under reduced pressure afforded the corresponding acetamide (N-methoxy-N-methyl acetamide-2-chloride) as a green oil which was used in the next step without further purification. To a solution of this acetamide in CH_3CN (800 mL) was added Ph_3P (108.0 g, 411.7 mmol, 1 equiv from crude acetamide) and the resulting mixture was refluxed for 18 hours. Then the solvents were removed under vacuum and the resulting viscous oil was dissolved in CH_2Cl_2 (1.0 L), washed with 2N KOH (400 mL x 2), brine (400 mL) and dried over Na_2SO_4 (10.0 g). Filtration and evaporation of the solvents under reduced pressure afforded N-methoxy-N-methyl-2-(triphenylphosphoranylidene)acetamide **(16)** as a thick oil that solidified by standing (146.5 g, 403.3 mmol, 81% over two steps). This compound was used in the next step without further purification. *Rf* = 0.85 (Hexane/EtOAc 3/1); ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 7.71-7.65 (m, 6H), 7.55-7.50 (m, 3H), 7.48-7.42 (m, 6H), 3.74 (s, 3H), 3.53 (d, J_{PH} = 23.5 Hz, 1H) 3.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 133.3 (× 3), 133.2 (× 3), 131.9 (× 3), 128.9 (× 3), 128.8 (× 3), 127.9, 61.3, 35.9, four quaternary carbons are not visible.



15 **17**

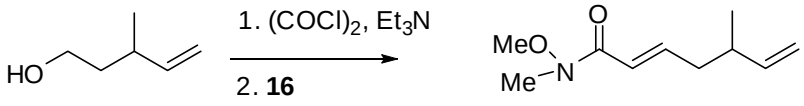
Synthesis of (*N*-methoxy-*N*-methylcarbamoyl)methylphosphonate (17). To a solution of N,O-dimethylhydroxylamine hydrochloride **(15)** (48.8 g, 500 mmol, 1.0 equiv) in CH_2Cl_2 (1.0 L) at 0°C were added pyridine (80.63 mL, 1.0 mol, 2.0 equiv) and chloroacetic anhydride (85.5 g, 500 mmol, 1.0 equiv). The resulting mixture was stirred for 15 min at 0 °C, then warm up to 23 °C and stirred overnight. The reaction mixture was then poured carefully into sat. $NaHCO_{3aq}$ solution (1.0 L) and stirred 1 hour, after which the layers were separated, the aqueous phase was extracted with CH_2Cl_2 (400 mL) and the combined organic layers were washed with 6N HCl (200 mL x 2), brine (200 mL x 2), dried over Na_2SO_4 (10 g) and filtered. Evaporation of the solvents under reduced pressure afforded the corresponding acetamide (N-methoxy-N-methyl acetamide-2-chloride) as a green oil which was used in the next step without further purification. The N-methoxy-N-methyl acetamide-2-chloride was heated with triethylphosphite (70.5 mL, 411.3 mmol, 1.0 equiv from crude acetamide) neat at

100 °C for 20 hours. The mixture was then distilled (110 °C at 0.5 Torr), to afford pure phosphonate **17** as a colorless oil (83.7 g, 70 % over two steps). R*f* = 0.63 (petroleum ether/EtOAc 7/3); ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 4.18-4.00 (m, 4H), 3.70 (s, 3H), 3.13 (s, 3H), 3.08 (d, J_{PH} = 21.8 Hz, 2H), 1.27 (2 x t, *J* = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 165.9, 62.4 (× 2), 61.4, 40.7, 32.0, 16.2 (× 2); ^{31}P NMR ($CDCl_3$, 162 MHz, 25 °C) δ = 21,16.



B3

Synthesis of Weinreb amide B3. To a suspension of NaH (4.8 g, 200 mmol, 1.2 equiv) in anhydrous THF (200 mL) was added dropwise at 0 °C diethyl (*N*-methoxy-*N*-methylcarbamoyl)methylphosphonate (**17**) (48.0 g, 200 mmol, 1.2 equiv) and the reaction mixture was stirred at this temperature for 30 min. Then, a solution of 5-hexen-2-one (19.4 mL, 167 mmol, 1.0 equiv) was added at 0 °C via cannula. After stirring at room temperature overnight, the reaction was quenched with saturated NH_4Cl_{aq} and extracted with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. Concentration under reduced pressure and purification on silica chromatography (petroleum ether/EtOAc 8/1 to 4/1 gradient) afforded pure Weinreb amide **B3** as a single isomer, in a 43% yield (13.0 g, 71 mmol). R*f* = 0.52 (petroleum ether/EtOAc 3/1); ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.11 (s, 1H), 5.87-5.73 (m, 1H), 5.04 (dd, *J* = 17.0, 1.7 Hz, 1H), 4.89 (dd, *J* = 10.1, 1.7 Hz, 1H), 3.66 (s, 3H), 3.20 (s, 3H), 2.27-2.23 (m, 4H), 2.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 167.9, 155.2, 137.5, 115.1, 114.2, 61.4, 40.3, 31.7, 18.7, one quaternary carbon is not visible.

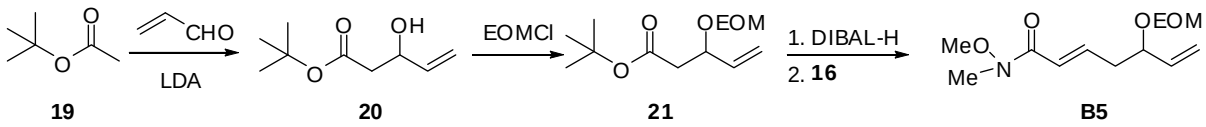


18 **B4**

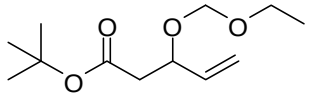
Synthesis of Weinreb amide B4. To the solution of $(COCl)_2$ (3.0 mL, 35.3 mmol, 1.5 equiv) in anhydrous CH_2Cl_2 (150 mL) at -78 °C was added DMSO (5.0 mL, 70.6 mmol, 3

equiv) dropwise under nitrogen atmosphere and kept stirring for 30 min. Then 3-methylpent-4-en-1-ol (**18**) (2.35 g, 23.5 mmol, 1 equiv) was added by syringe and stirred for 30 min at the same temperature. Finally NEt_3 (9.8mL mL, 70.5 mmol, 3 equiv) was added and the reaction was allowed to warm to 23°C during one hour, subsequently N-methoxy-N-methyl-2-(triphenylphosphoranylidene)acetamide (**16**) (12.8 g, 35.3 mmol, 1.5 equiv) was added to the reaction mixture in one portion and stirred overnight. The solvent was removed by evaporation under reduced pressure and the residue obtained was purified by flash chromatography (silica gel, petroleum ether/EtOAc 6/1) to give Weinreb amide **B4** as pale yellow oil (1.84 g, 10 mmol) in a 43 % yield. R*f* = 0.5 (petroleum ether/EtOAc 3/1); ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.93 (dt, *J* = 15.5, 7.6 Hz, 1H), 6.39 (d, *J* = 15.5 Hz, 1H), 5.79-5.70 (m, 1H), 5.02-4.94 (m, 2H), 3.69 (s, 3H), 3.23 (s, 3H), 2.18-2.36 (m, 3H), 1.02 (d, *J* = 9.9 Hz, 3H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ = 166.8, 145.8, 143.2, 120.0, 113.2, 61.6, 39.5, 36.9, 32.3, 19.6.

Synthesis of Weinreb amide B5.

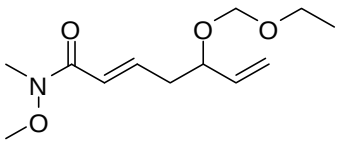


Synthesis of alcohol 20. To a solution of freshly prepared LDA (0.56 M, 60 mmol) at -78 ^{o}C was added a solution of *t*-butyl acetate (8.1 mL, 60 mmol, 1.0 equiv) in THF (10 mL) dropwise. After one hour at -78 ^{o}C, a solution of acrolein (4.0 mL, 60 mmol, 1.0 equiv) in THF (5 mL) was added and the reaction mixture was stirred at the same temperature for 5 more min. The reaction was then quenched with saturated NH_4Cl_{aq} solution (100 mL) and extracted with EtOAc (150 mL x 3), the combined organic phases were washed with brine (120 mL) and dried over anhydrous Na_2SO_4 (10.0 g). Filtration and evaporation of the solvents under reduced pressure followed by flash chromatography (silica gel, 1/8 EtOAc/petroleum ether) afforded desired compound **20** in a 81% yield (8.35 g). R*f* = 0.25 (EtOAc/petroleum ether 1/8);^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 5.96 (ddd, *J* = 15.9, 10.4, 5.2 Hz, 1H), 5.30 (dd, *J* = 15.9, 0.8 Hz, 1H), 5.14 (dd, *J* = 10.4, 0.8 Hz, 1H), 4.46-4.40 (m, 1H), 3.13 (d, *J* = 4.4 Hz, 1H), 2.46-2.34 (m, 2H), 1.46 (s, 9H). ^{13}C NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 171.5, 138.9, 114.9, 81.2, 68.9, 42.1, 28.0 (x3).



21

Synthesis of alcohol 21: To a solution of alcohol **20** (2.82 g, 16.4 mmol) in CH_2Cl_2 (50 mL) at 0 °C under nitrogen atmosphere, was added diisopropylethylamine (5.7 mL, 32.7 mmol, 2.0 equiv) and EOMCl (3.65 mL, 39.3 mmol, 2.4 equiv), and the reaction was allowed to warm to 23 °C and stirred for 5 hours. Then the mixture was quenchded with sat. NH_4Cl_{aq} solution and extracted with CH_2Cl_2, washed with brine and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure followed by flash chromatography (EtOAc/petroleum ether 1/10) afforded desired compound **21** (3.54 g, 15.4 mmol) in 94% yield. R*f* = 0.85 (petroleum ether/EtOAc 4/1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ 5.73 (ddd, *J* = 17.3, 10.3, 7.8 Hz, 1H), 5.29 (d, *J* = 16.9 Hz, 1H), 5.21 (d, *J* = 10.3 Hz, 1H), 4.71 (d, *J* = 6.8 Hz, 1H_A, AB system), 4.65 (d, *J* = 6.8 Hz, 1H_B, AB system), 4.46 (td, *J* = 7.5, 6.1 Hz, 1H), 3.73-3.61 (m, 1H), 3.56-3.45 (m, 1H), 2.55 (dd, *J* = 15.0, 7.5 Hz, 1$H_{A'}$, A'B' system), 2.55 (dd, *J* = 15.0, 5.4 Hz, 1$H_{B'}$, A'B' system), 1.45 (s, 9H), 1.21 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, 25 °C) δ 170.2, 137.3, 118.0, 92.9, 80.3, 74.4, 63.5, 42.4, 28.3, 15.2 (x 3).



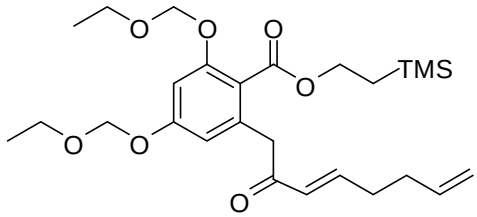
B5

Weinreb amide B5: To a solution of **21** (3.54 g, 15.4 mmol) in CH_2Cl_2 (100 mL) under nitrogen atmosphere, DIBAL (18.4 mL, 1M in toluene, 1.2 equiv.) was added at -78 ^{o}C and the reaction was kept stirring for 30 min. Then a saturated solution of tartrate salt (100 mL) was added to the reaction and stirred for 2 hours until the system turned clear. The two phases were separated and extracted with CH_2Cl_2 (100 mL x 2), washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue (2.59 g) obtained was used directly in the next step without further purification. To a solution of the previously obtained aldehyde (2.59 g) in CH_2Cl_2 (100 mL) N-methoxy-N-methyl-2-(triphenylphosphoranylidene) acetamide **(16)** (11.2 g, 30.8 mmol, 2.0 equiv.) was added at 23 ^{o}C and the reaction was

stirred overnight. After removal of the solvent under reduced pressure followed by flash chromatography (EtOAc/petroleum ether 1/10) desired compound **B5** (2.25 g) was isolated in a 60 % yield over two steps. R*f* = 0.23 (petroleum ether/EtOAc 4/1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ 6.95 (dt, *J* = 15.2, 7.6 Hz, 1H), 6.47 (d, *J* = 15.6 Hz, 1H), 5.71 (ddd, *J* = 17.0, 9.8, 6.9 Hz, 1H), 5.25 (d, *J* = 17.6 Hz, 1H), 5.22 (d, *J* = 10.8 Hz, 1H), 4.71 (d, *J* = 6.4 Hz, $1H_A$, AB system), 4.63 (d, *J* = 7.1 Hz, $1H_B$, AB system), 4.17 (td, *J* = 6.8, 6.8 Hz, 1H), 3.74-3.65 (m, 1H), 3.70 (s, 3H), 3.56-3.47 (m, 1H), 3.24 (s, 3H), 2.60-2.42 (m, 2H), 1.20 (t, *J* = 7.0 Hz, 3H), ^{13}C ($CDCl_3$, 400 MHz, 25 °C) δ 166.6, 143.1, 137.5, 121.1, 117.8, 92.4, 76.0, 63.5, 61.7, 38.6, 32.4, 15.1.

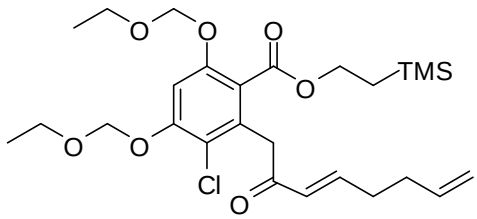
General procedure for the acylation reaction. Compounds 22:

To a solution of the corresponding aromatic **A** (1.0 equiv) in anhydrous THF (6 mL/mmol) at -78°C was added LDA (0.56 M, 2.0 equiv). Immediately, a precooled (-78°C) solution of the corresponding Weinreb amide **B** (0.9 equiv) in THF (1 mL/mmol) was added via a cannula. The reaction was stirred 20 minutes at -78°C and then quenched with sat. NH_4Cl_{aq} solution. Upon warming to room temperature, the resulting solution was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. Column chromatography (silica gel, hexane to hexane/EtOAc gradient) afforded the desired products **22** as pale yellow oils in 50 to 85% yield.



22_{A1B1}

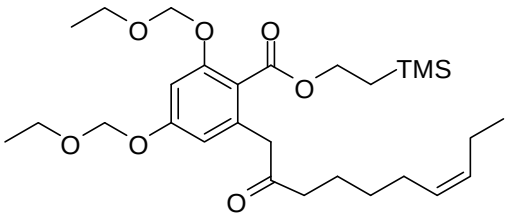
22_{A1B1}: ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.86 (dt, *J* = 15.6, 6.4 Hz, 1H), 6.79 (d, *J* = 1.8 Hz, 1H), 6.50 (d, *J* = 1.8 Hz, 1H), 6.14 (dt, *J* = 15.8, 1.5 Hz, 1H), 5.74 (ddt, *J* = 17.0, 10.2, 6.3 Hz, 1H), 5.18 (s, 2H), 5.16 (s, 2H), 5.03-4.93 (m, 2H), 4.33-4.26 (m, 2H), 3.81 (s, 2H), 3.70 (q, *J* = 6.9 Hz, 2H), 3.67 (q, *J* = 6.9 Hz, 2H), 2.31-2.13 (m, 4H), 1.19 (t, *J* = 7 Hz, 3H), 1.17 (t, *J* = 7 Hz, 3H), 1.06-1.00 (m, 2H), 0.03 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ =196.0, 167.5, 158.9, 156.2, 146.9, 136.8, 134.9, 129.3, 118.6, 115.4, 111.2, 102.7, 93.6, 92.9, 64.3, 64.2, 63.2, 45.5, 31.9, 31.6, 17.3, 14.9 (× 2), -1.7 (× 3).



22_{A2B1}

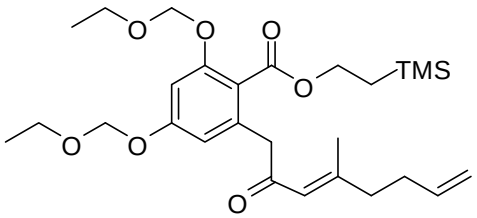
22_{A2B1}: ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 7.11 (s, 1H), 6.92 (dt, *J* = 15.7, 6.6 Hz, 1H), 6.18 (dt, *J* = 15.6, 1.3 Hz, 1H), 5.79 (ddt, *J* = 16.8, 10.2, 6.4 Hz, 1H), 5.30 (s, 2H), 5.21 (s,

2H), 5.08-4.96 (m, 2H), 4.34-4.27 (m, 2H), 4.03 (s, 2H), 3.76 (q, *J* = 6.8 Hz, 2H), 3.74 (q, *J* = 6.8 Hz, 2H), 2.36-2.28 (m, 2H), 2.26-2.18 (m, 2H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.06-1.00 (m, 2H), 0.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 194.3, 166.9, 154.4, 153.7, 146.7, 136.8, 132.6, 129.1, 120.2, 117.5, 115.4, 102.9, 93.8, 93.7, 64.5, 64.3, 63.5, 43.0, 31.9, 31.5, 17.2, 14.6 (× 2), -1.7 (× 3).



22$_{A1B2}$

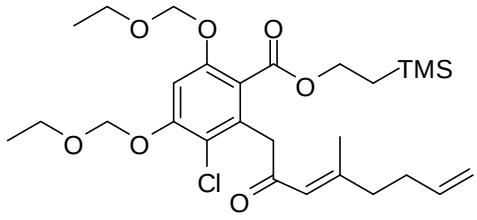
22$_{A1B2}$: ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.81 (d, *J* = 2.0 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H), 5.39-5.23 (m, 2H), 5.20 (s x 2, 4H), 4.36-4.28 (m, 2H), 3.73 (q, *J* = 7.6 Hz, 2H), 3.71 (q, *J* = 7.6 Hz, 2H), 3.68 (s, 2H), 2.44 (t, *J* = 7.6 Hz, 2H), 2.06-1.96 (m, 4H), 1.63-1.51 (m, 2H), 1.36-1.26 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.21 (t, *J* = 7.1 Hz, 3H), 1.10-1.03 (m, 2H), 0.94 (t, *J* = 7.6 Hz, 3H), 0.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 207.2, 167.7, 159.1, 156.4, 135.0, 131.9, 128.6 (× 2), 111.4, 102.9, 93.7, 93.0, 64.4 (× 2), 63.4, 48.1, 41.8, 29.2, 26.9, 23.2, 20.5, 17.4, 15.1, 15.0, 14.3, -1.5 (× 3).



22$_{A1B3}$

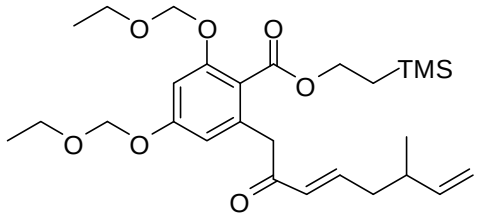
22$_{A1B3}$:^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.76 (d, *J* = 2.1 Hz, 1H), 6.48 (d, *J* = 2.1 Hz, 1H), 6.03 (s, 1H), 5.73-5.61 (m, 1H), 5.14 (s, 2H), 5.13 (s, 2H), 4.97-4.86 (m, 2H), 4.31-4.24 (m, 2H), 3.67 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 2H), 3.63 (q, *J* = 7.1 Hz, 2H), 2.15-2.12 (m, 4H), 2.06 (d, *J* = 1.2 Hz, 3H), 1.15 (t, *J* = 6.6 Hz, 3H), 1.14 (t, *J* = 6.6 Hz, 3H), 1.04-0.98 (m, 2H), 0.00 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 196.3, 167.4, 158.8, 158.4, 156.0,

136.9, 135.1, 122.3, 115.0, 111.2, 111.1, 102.5, 93.5, 92.8, 64.1, 64.0, 62.9, 49.0, 40.2, 31.3, 19.2, 17.1, 14.8 (× 2), -1.8 (× 3).



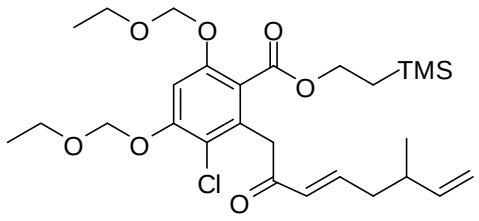
22$_{A2B3}$

22$_{A2B3}$: ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 7.09 (s, 1H), 6.03 (s, 1H), 5.75-5.62 (m, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 4.99-4.88 (m, 2H), 4.31-4.25 (m, 2H), 3.86 (s, 2H), 3.70 (q, *J* = 7.1 Hz, 2H), 3.67 (q, *J* = 7.1 Hz, 2H), 2.16 (s, 2H), 2.15 (s, 2H), 2.08 (d, *J* = 1.0 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 3H), 1.16 (t, *J* = 7.2 Hz, 3H), 1.02-0.96 (m, 2H), 0.00 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 195.0, 167.0, 158.7, 154.5, 153.7, 137.1, 133.1, 122.2, 120.3, 117.6, 115.2, 102.9, 93.8 (× 2), 64.6, 64.3, 63.6, 46.5, 40.3, 31.5, 19.4, 17.3, 15.0 (× 2), -1.6 (× 3).



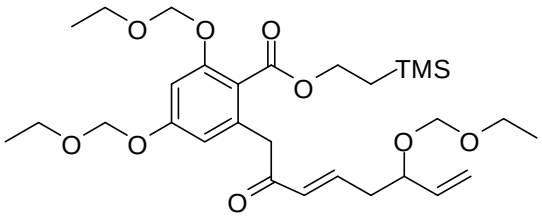
22$_{A1B4}$

22$_{A1B4}$: ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.83 (dt, *J* = 15.6, 7.4 Hz, 1H), 6.80 (d, *J* = 2.1 Hz, 1H), 6.50 (d, *J* = 2.1 Hz, 1H), 6.13 (dt, *J* = 16.1, 2.1 Hz, 1H), 5.75-5.63 (m, 1H), 5.19 (s, 2H), 5.17 (s, 2H), 4.99-4.90 (m, 2H), 4.34-4.27 (m, 2H), 3.82 (s, 2H), 3.71 (q, *J* = 7.1 Hz, 2H), 3.69 (q, *J* = 7.0 Hz, 2H), 2.35-2.10 (m, 3H), 1.20 (t, *J* = 7.4 Hz, 3H), 1.19 (t, *J* = 7.4 Hz, 3H), 1.08-0.95 (m, 5H), 0.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 196.1, 167.6, 159.0, 146.2, 142.8, 134.9, 130.4, 118.7, 113.4, 111.2, 102.7, 93.7, 92.9, 64.3 (× 2), 63.3, 45.5, 39.4, 36.8, 23.7, 19.6, 17.3, 15.0 (× 2), -1.6 (× 3).



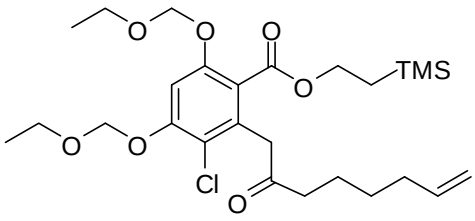
22_{A2B4}

22_{A2B4}: ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 7.10 (s, 1H), 6.86 (dt, *J* = 15.9, 7.5 Hz, 1H), 6.14 (dt, *J* = 15.7, 1.2 Hz, 1H), 5.71 (ddd, *J* = 17.1, 10.2, 6.8 Hz, 1H), 5.29 (s, 2H), 5.20 (s, 2H), 5.02-4.92 (m, 2H), 4.34-4.27 (m, 2H), 4.00 (s, 2H), 3.74 (q, *J* = 7.1 Hz, 2H), 3.71 (q, *J* = 7.0 Hz, 2H), 2.40-2.12 (m, 3H), 1.21 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.0 Hz, 3H), 1.05-0.99 (m, 5H), -0.03 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 194.5, 167.0, 154.5, 153.8, 145.9, 142.8, 132.8, 130.2, 120.3, 117.6, 113.4, 103.0, 93.9, 93.8, 64.6, 64.4, 63.6, 43.0, 39.3, 36.8, 19.6, 17.3, 14.9 (× 2), -1.6 (× 3).



22_{A1B5}

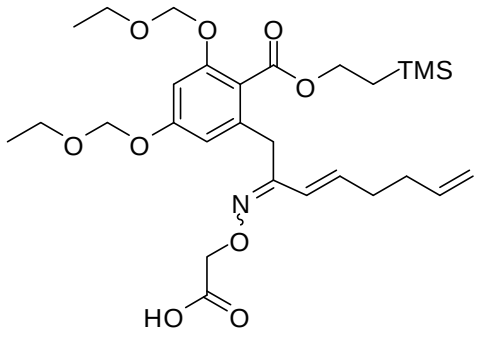
22_{A1B5}: ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ 6.79 (dt, *J* = 15.6, 7.3 Hz, 1H); 6.72 (d, *J* = 2.0 Hz, 1H); 6.42 (d, *J* = 2.2 Hz, 1H); 6.12 (dt, *J* = 15.7, 1.5 Hz, 1H); 5.57 (ddd, *J* = 17.0, 10.4, 7.7 Hz, 1H); 5.20-5.06 (m, 2H); 5.10 (s, 2H); 5.08 (s, 2H); 4.58 (d, *J* = 6.9 Hz, $1H_A$, AB system); 4.50 (d, *J* = 7.3 Hz, $1H_B$, AB system); 4.25-4.18 (m, 2H), 3.75 (s, 2H), 3.68-3.51 (m, 6H), 3.44-3.34 (m, 1H), 2.42-2.27 (m, 2H), 1.18-1.05 (m, 9H), 1.00-0.93 (m, 2H), -0.04 (3 × s, 9H); ^{13}C ($CDCl_3$, 400 MHz, 25 °C) δ 195.7, 167.5, 159.0, 156.3, 143.3, 137.2, 134.9, 131.1, 117.7, 111.3, 102.7, 93.6, 92.9, 92.2, 75.7, 64.3, 64.2, 63.3, 63.2, 45.5, 38.8, 23.8, 20.8, 17.3, 15.0, 14.1, -1.6 (× 3).



22_{A2B2}

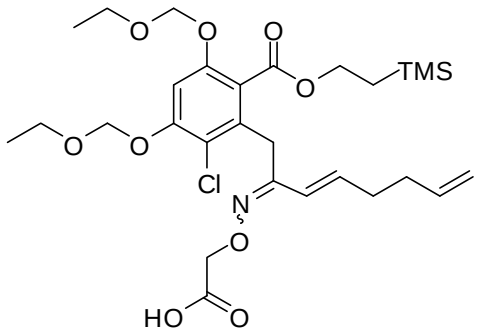
Preparation of the saturated ketone 22_{A2B2}: To a solution of **22_{A2B1}** (6.58 g, 12.48 mmol, 1 equiv) in CH_2Cl_2/AcOH mixture (10/1 125 mL/12.5 mL) at 0 °C was added $NaBH_3CN$ (1.57 g, 24.96 mmol, 2 equiv), and the reaction was warmed up to 23 °C and stirred for 3 hours. Then the reaction mixture was quenched with sat. NH_4Cl_{aq} solution, extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4 an evaporated under reduced pressure. Column chromatography (silica gel, hexane to hexane/EtOAc gradient) afforded the desired product **22_{A2B2}** (1.72 g, 3.26 mmol, 56%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 7.10 (s, 1H), 5.77 (ddt, *J* = 18.0, 10.4, 6.5 Hz, 1H), 5.30 (s, 2H), 5.20 (s, 2H), 5.02-4.88 (m, 2H), 4.36-4.29 (m, 2H), 3.85 (s, 2H), 3.76 (q, *J* = 7.1 Hz, 2H), 3.72 (q, *J* = 7.1 Hz, 2H), 2.45 (t, *J* = 7.3 Hz, 2H), 2.03 (qt, *J* = 7.2 Hz, 2H), 1.64-1.55 (m, 2H), 1.42-1.32 (m, 2H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H), 1.08-1.01 (m, 2H), 0.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 205.6, 167.0, 154.6, 153.9, 138.5, 132.8, 120.4, 117.6, 114.5, 103.2, 94.0 (× 2), 64.7, 64.5, 63.8, 45.4, 41.6, 33.5, 28.3, 23.0, 17.4, 15.0 (× 2), -1.6 (× 3).

General procedure for the oxime formation: To a solution of the corresponding carbonyl compound (1.0 equiv) in pyridine (2.0 mL/mmol) at 40 °C was added carboxymethoxylamine hemihydrochloride (5.0 equiv) and the reaction was stirred at such temperature followed by TLC till complete consuption of the starting material. After evaporation of the pyridine, the residue was dissolved in CH_2Cl_2 and washed with sat. NH_4Cl (x 2), brine (x 2) and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure followed by filtration on a short pad of silica gel, afforded desired oximes as mixtures of E/Z isomers in 85 to 95% yield.



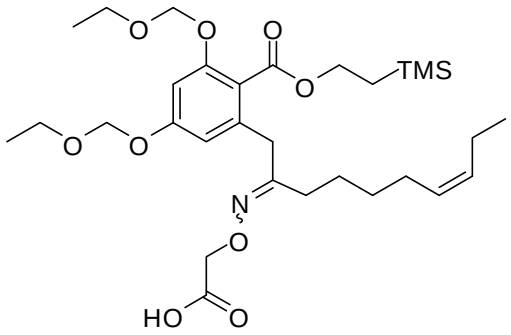
2_{A1B1}

2_{A1B2}: Mixture of two isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 8.65 (brs, 2H), 6.80-6.65 (m, 1H), 6.75 (d, *J* = 2.0 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 6.57 (d, *J* = 2.0 Hz, 1H), 6.49 (d, *J* = 2.0 Hz, 1H), 6.24 (dt, *J* = 16.3, 6.7, 1H), 6.15-6.02 (m, 2H), 5.72 (ddt, *J* = 17.1, 10.5, 6.6, 1H), 5.68 (ddt, *J* = 17.1, 10.5, 6.6, 1H), 5.19 (s, 4H), 5.17 (s, 2H), 5.16 (s, 2H), 4.98-4.86 (m, 4H), 4.67 (s, 2H), 4.66 (s, 2H), 4.42-4.35 (m, 4H), 3.87 (s, 2H), 3.71 (q, *J* = 7.2 Hz, 4H), 3.69 (q, *J* = 7.2 Hz, 4H), 3.65 (s, 2H), 2.25-2.05 (m, 8H), 1.21 (t, *J* = 7.1 Hz, 6H), 1.18 (t, *J* = 7.1 Hz, 6H), 1.12-1.06 (m, 4H), 0.07 (s, 9H), 0.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 173.7 (× 2), 168.3, 168.0, 159.1, 158.8, 158.1, 155.9, 155.6, 155.5, 142.0, 138.1, 137.4 (× 2), 137.3, 136.0, 125.8 (× 2), 118.7 (× 2), 118.5, 118.4, 115.1 (× 2), 109.0, 108.6, 102.3, 102.0, 93.6 (× 2), 93.0, 92.7, 70.4, 70.3, 64.5, 64.4 (× 2), 64.3, 63.6, 63.5, 34.3, 32.8, 32.6, 32.3, 28.4, 17.5, 15.0 (× 4), -1.54 (× 6).



2_{A2B1}

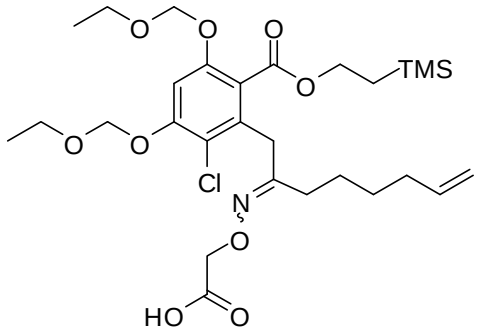
2_{A2B1}: Mixture of two isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 9.35 (brs, 2H), 7.04 (s, 2H), 6.73 (d, *J* = 16.4 Hz, 1H), 6.32 (dt, *J* = 16.4, 6.6 Hz, 1H), 6.05 (dt, *J* = 16.4, 6.6 Hz, 1H), 5.77 (d, *J* = 15.8 Hz, 1H), 5.66 (ddt, *J* = 17.0, 10.5, 6.5 Hz, 2H), 5.29 (s, 4H), 5.19 (s, 4H), 5.05-4.84 (m, 4H), 4.65 (s, 2H), 4.49 (s, 2H), 4.36-4.25 (m, 4H), 4.00 (s, 2H), 3.87 (s, 2H), 3.75 (q, *J* = 7.1 Hz, 4H), 3.70 (q, *J* = 7.0 Hz, 4H), 2.34-1.98 (m, 8H), 1.21 (t, *J* = 7.1 Hz, 6H), 1.20 (t, *J* = 7.1 Hz, 6H), 1.07-0.98 (m, 4H), 0.05 (s, 9H), 0.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 173.0, 172.9, 167.5, 167.1, 156.8, 155.0, 154.4, 154.3, 153.4, 140.6, 137.4, 137.2 (× 2), 134.3, 133.6, 124.2 (× 2), 120.3, 120.0, 119.2, 117.4, 117.3, 115.4, 114.9, 102.9, 102.6, 93.9, 93.8 (× 2), 93.7, 70.6, 70.2, 64.7 (× 2), 64.5, 64.4, 64.2, 63.7, 32.7 (× 2), 32.6, 32.5, 32.1, 29.4, 17.3, 17.1, 14.9 (× 4), -1.6 (× 6).



2_{A1B2}

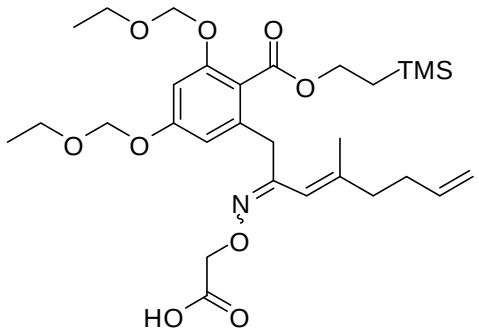
2_{A1B2}: Mixture of two isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 9.39 (brs, 2H), 6.78 (d, *J* = 2.0 Hz, 1H), 6.76 (d, *J* = 2.1 Hz, 1H), 6.62 (d, *J* = 2.0 Hz, 1H), 6.59 (d, *J* = 2.1 Hz, 1H), 5.40-5.21 (m, 4H), 5.20 (s, 4H), 5.19 (s, 4H), 4.65 (s, 2H), 4.62 (s, 2H), 4.40-4.32 (m, 4H), 3.76-3.66 (m, 10H), 3.47 (s, 2H), 2.30 (t, *J* = 7.7 Hz, 2H), 2.11 (t, *J* = 7.7 Hz, 2H),

2.05-1.90 (m, 8H), 1.51-1.38 (m, 4H), 1.37-1.25 (m, 4H), 1.24-1.18 (m, 12H), 1.12-1.05 (m, 4H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H), 0,07 (s, 18H);[13]C NMR (100 MHz, $CDCl_3$, 25°C) δ = 173.6, 173.4, 168.0, 167.7, 162.1, 160.6, 159.1, 158.8, 155.9, 155.7, 148.0, 137.5, 136.2, 136.1, 131.8, 128.7 (× 2), 124.3 (× 2), 119.3, 118.9, 110.4, 109.7, 102.7, 102.5, 93.6, 93.1, 92.8, 70.2, 70.1, 64.4 (× 2), 63.6, 63.5, 53.4, 37.3, 33.1, 31.8, 29.7, 29.2, 27.9, 26.7, 25.7, 25.4, 20.4 (× 2), 17.5, 17.4, 15.0 (× 4), 14.3 (× 2), -1.6 (× 6).



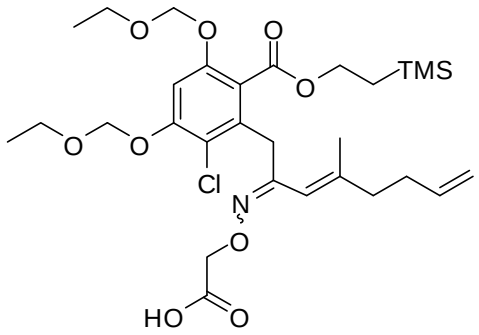
2_{A2B2}

2_{A2B2}: Mixture of two isomers (1:1) [1]H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 10.50 (brs, 2H), 7.08 (s, 1H), 7.07 (s, 1H), 5.84-5.65 (m, 2H), 5.31 (s, 2H), 5.30 (s, 2H), 5.21 (s, 2H), 5.19 (s, 2H), 5.04-4.83 (m, 4H), 4.65 (s, 2H), 4.52 (s, 2H), 4.38-4.29 (m, 4H), 3.96 (s, 2H), 3.82-3.65 (m, 8H), 3.74 (s, 2H), 2.32 (t, *J* = 7.7 Hz, 2H), 2.07-1.97 (m, 4H), 1.95 (t, *J* = 7.7 Hz, 2H), 1.55-1.35 (m, 6H), 1.35-1.27 (m, 2H), 1.26-1.18 (m, 12H), 1.10-1.02 (m, 4H), 0.06 (s, 18H); [13]C NMR (100 MHz, $CDCl_3$, 25°C) δ = 172.9 (× 2), 167.1, 166.9, 161.5, 159.6, 154.5, 154.4, 153.6, 153.5, 148.2, 138.7, 138.5, 137.4, 133.9, 133.1, 124.2 (× 2), 120.8, 120.6, 117.7 (× 2), 114.5, 114.3, 103.2, 103.0, 94.0, 93.9, 70.4, 70.1, 64.8, 64.7, 64.5 (× 2), 64.1, 63.8, 53.4, 35.5, 33.3 (× 2), 32.0, 30.9, 28.8, 28.3, 28.2, 25.2, 25.0, 17.3, 15.0 (× 4), -1.6 (× 6).



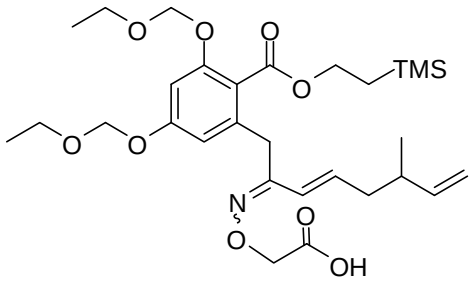
2_{A1B3}

2_{A1B3}: Mixture of two isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 9.15 (brs, 2H), 6.76 (d, *J* = 2.2 Hz, 1H), 6.74 (d, *J* = 2.2 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 6.58 (d, *J* = 2.0 Hz, 1H), 5.78-5.62 (m, 2H), 5.60 (s, 1H), 5.45 (s, 1H), 5.19 (s × 4, 8H), 5.01-4.85 (m, 4H), 4.69 (s, 2H), 4.62 (s, 2H), 4.39-4.31 (m, 4H), 3.78 (s, 2H), 3.76-3.65 (m, 8H), 3.60 (s, 2H), 2.39-1.58 (m, 14H), 2.24-2.16 (m, 12H), 1.11-1.05 (m, 4H), 0.06 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 173.9, 173.8, 168.1, 167.8, 159.1, 158.8, 157.4, 157.3, 155.9, 155.7, 146.3, 145.4, 137.8 (× 2), 136.6, 136.4, 118.8 (× 2), 116.1, 114.8 (x 3), 114.7 (× 2), 110.4, 109.9, 102.6, 102.4, 93.7, 93.0, 70.4, 70.3, 64.5 (× 4), 63.7, 63.5, 39.9, 39.1, 33.3 (× 2), 31.9, 31.8, 19.6, 19.1, 17.5 (× 2), 15.1 (× 4), -1.47 (× 6).



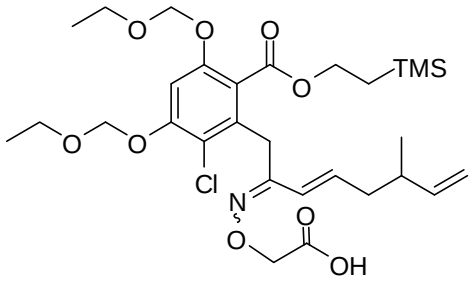
2_{A2B3}

2_{A2B3}: Mixture of two isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 9.94 (brs, 2H), 7.06 (s, 1H), 7.04 (s, 1H), 5.86-5.65 (m, 1H), 5.64-5.48 (m, 2H), 5.35-5.10 (m, 1H), 5.29 (s, 2H), 5.28 (s, 2H), 5.20 (s, 2H), 5.19 (s, 2H), 5.03-4.76 (m, 4H), 4.72 (s, 2H), 4.52 (s, 2H), 4.38-4.32 (m, 4H), 3.97 (s, 2H), 3.80 (s, 2H), 3.75 (q, *J* = 6.9 Hz, 2H), 3.73 (q, *J* = 6.9 Hz, 2H), 3.72 (q, *J* = 7.0 Hz, 2H), 3.71 (q, *J* = 7.0 Hz, 2H), 2.36-1.58 (m, 14H), 1.21 (t, *J* = 6.9 Hz, 6H), 1.20 (t, *J* = 6.9 Hz, 6H), 1.10-1.03 (m, 4H), 0.06 (s, 9H), 0.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 173.1 (x 2), 167.1 (x 2), 154.4 (x 2), 153.5 (x 2), 148.2 (× 2), 145.6, 145.5, 137.8, 134.1, 124.3, 124.2, 120.7 (× 2), 118.0 (x 2), 117.6 (x 2), 114.8, 114.5, 103.1, 102.9, 93.9, 93.8, 70.5, 70.3, 64.7 (× 2), 64.5 (× 2), 64.0, 63.8, 39.3, 38.7, 36.1 (× 2), 31.8 (x 2), 31.6, 31.5, 19.5, 18.7, 17.4, 17.3, 15.0 (× 4), -1.6 (× 6).



2_{A1B4}

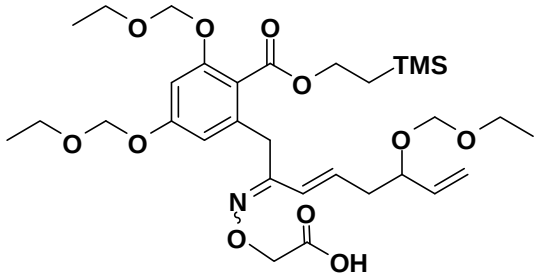
2_{A1B4}: Mixture of four isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 9.47 (brs, 2H), 6.72 (d, *J* = 2.1 Hz, 1H), 6.70 (d, *J* = 2.1 Hz, 1H), 6.67 (dt, *J* = 16.4, 1.4 Hz, 1H), 6.55 (d, *J* = 2.1 Hz, 1H), 6.48 (d, *J* = 2.0 Hz, 1H), 6.21 (dt, *J* = 16.4, 7.0 Hz, 1H), 6.05-6.00 (m, 2H), 5.65-5.49 (m, 2H), 5.16 (s, 4H), 5.13 (s, 4H), 4.85-4.73 (m, 4H), 4.66 (s, 2H), 4.65 (s, 2H), 4.39-4.33 (m, 4H), 3.86 (s, 2H), 3.72-3.59 (m, 10H), 2.20-1.98 (m, 6H), 1.17 (t, *J* = 6.5 Hz, 6H), 1.15 (t, *J* = 6.5 Hz, 3H), 1.14 (t, *J* = 6.5 Hz, 3H), 1.10-1.04 (m, 4H), 0.87 (d, *J* = 6.5 Hz, 3H), 0.82 (d, J = 6.5 Hz, 3H), 0.04 (s, 9H), 0.03 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 174.0 (× 2), 168.0, 167.9, 158.9, 158.7, 157.7, 155.4, 155.3 (× 2), 146.8, 143.2, 143.0, 140.9, 137.5, 136.8, 136.0, 126.6, 124.5, 119.3, 118.3 (× 2), 112.8, 109.0, 108.8, 101.9, 101.7, 93.4 (× 2), 92.9, 92.7, 70.2 (× 2), 64.2 (× 3), 64.1, 63.4 (× 2), 40.2, 39.9, 37.2, 37.1, 34.2, 28.1, 19.2, 19.1, 17.4, 14.9 (× 4), -1.66 (× 6).



2_{A2B4}

2_{A2B4}: Mixture of four isomers (1:1) ($CDCl_3$, 400 MHz, 25 °C) δ = 9.53 (brs, 2H), 7.04 (s, 2H), 6.69 (d, *J* = 16.1 Hz, 1H), 6.26 (dt, *J* = 16.1, 7.0 Hz, 1H), 5.97 (dt, *J* = 16.1, 7.0 Hz, 1H), 5.76 (d, *J* = 16.1 Hz, 1H), 5.71-5.53 (m, 2H), 5.28 (s, 4H), 5.18 (s, 2H), 5.17 (s, 2H), 4.96-4.84 (m, 4H), 4.65 (s, 2H), 4.49 (s, 2H), 4.33-4.27 (m, 4H), 4.02 (s, 2H), 3.87 (s, 2H), 3.77-3.67 (m, 8H), 2.26-1.98 (m, 6H), 1.20 (t, *J* = 7.1 Hz, 6H), 1.19 (t, *J* = 7.0 Hz, 6H), 1.06-0.99 (m, 4H), 0.96 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H), 0.05 (s, 9H), 0.03 (s, 9H); ^{13}C

NMR (100 MHz, $CDCl_3$, 25°C) δ = 173.5 (× 2), 167.4, 167.0, 156.7, 154.8, 154.4, 154.2, 153.4, 153.3, 143.2, 143.0, 139.4, 136.1, 134.4, 133.8, 128.9, 128.0, 125.2, 120.0, 117.4, 117.2, 113.1, 112.6, 102.8, 102.5, 93.8, 93.7 (× 2), 93.6, 70.5, 70.2, 64.6 (× 2), 64.4, 64.3, 64.1, 63.7, 40.2, 39.9, 37.2, 37.0, 32.7, 29.2, 19.4, 18.9, 17.2, 17.1, 14.8 (× 4), -1.69 (× 6).



2_{A1B5}

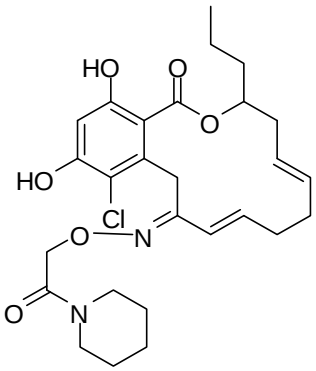
2_{A1B5}: Mixture of four isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.80 (dt, *J* = 16.2, 1.3 Hz, 1H); 6.76 (d, *J* = 2.0 Hz, 1H); 6.72 (d, *J* = 2.1 Hz, 1H); 6.58 (d, *J* = 2.2 Hz, 1H); 6.49 (d, *J* = 1.8 Hz, 1H); 6.26-6.04 (m, 3H); 5.60 (ddd, *J* = 17.2, 10.8, 7.6 Hz, 1H); 5.56 (ddd, *J* = 17.2, 10.8, 7.6 Hz, 1H); 5.19 (s × 2, 4H); 5.17 (s × 2, 4H); 5.14-5.01 (m, 4H); 4.72-4.50 (m, 8H); 4.41-4.34 (m, 4H); 4.04 (dt, *J* = 7.1, 7.1 Hz, 1H); 4.02 (dt, *J* = 7.1, 7.1 Hz, 1H); 3.89 (s, 2H); 3.77-3.64 (m, 10H); 3.62-3.53 (m, 2H); 3.51-3.41 (m, 2H); 2.49-2.28 (m, 4H); 1.22 (t × 2, *J* = 7.6 Hz, 12H); 1.18-1.13 (m, 6H); 1.13-1.06 (m, 4H); 0.07 (s × 2, 18H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 170.8 (× 2), 167.5 (× 2), 159.0 (× 4), 156.3 (× 2), 143.3 (× 2), 137.2 (× 2), 134.9 (× 2), 131.1 (× 4), 118.6, 117.7, 111.3 (× 2), 102.7 (× 2), 93.6, 92.9 (× 2), 92.2, 75.7 (× 2), 64.3 (× 2), 64.2 (× 2), 63.3, 63.2, 60.2 (× 2), 45.5, 44.7, 38.4 (× 4), 23.8, 17.3, 15.0 (× 4), 14.1 (× 2), -1.6 (× 6), 2 quaternary signals are not visible.

General procedure for the preparation of macrocyclic compounds. To a suspension of 3.0 equiv of polystyrene based chlorotrityl resin (1.1 mmol/g) in CH_2Cl_2 at room temperature were added 6.0 equiv of Hunig's base and 1.0 equiv of the corresponding acid **2**. After shaking the mixture for 24 hours, the different resins were capped with acetic acid for 24 hours more. Then the resins were washed with CH_2Cl_2, DMF, CH_2Cl_2 and Et_2O, dry and re-suspended in THF. To these suspensions, 4.0 equiv of TBAF (1M) were added and the mixtures were shaken for 4 hours. The resins were then filtered and washed thoroughly using THF, CH_2Cl_2, 1% AcOH in CH_2Cl_2, CH_2Cl_2, Et_2O several times. The completion of the deprotection and total elimination of the tetrabutyl ammonium salts was assessed by LC-MS after cleavage of an analytical aliquot of each resin using a solution of HFIP in CH_2Cl_2 1/4 for 30 min. The resins were split for further diversification with the different alcohols or amine **C**. For alcohols, the Mitsunobu reactions were carried out in dry toluene at room temperature using 5.0 equiv of the corresponding alcohol **C**, 4.0 equiv of Ph_3P and 4.0 equiv of DIAD for 12 h at room temperature. For the amine **C3**, the reaction was carried out in CH_2Cl_2 using 2 equiv of the amine, DIC (5.0 equiv), 4-DMAP (5 mol%) at room temperature for 12 h. The conversions were assessed by LC-MS after cleavage of an analytical aliquot of resin as describe before and the resins which had not proceeded to completion were re-subjected to the same conditions. After washing and drying the resins were suspended in toluene and submitted to the metathesis reaction. Grubbs' second generation catalyst was added to each suspension (0.06 equiv) and the reactions were heated at 120 ^oC in a CEM microwave reactor for 3 x 45 min (fresh catalyst was added in each cycle). For the ene-yne metathesis of resins including fragment C8 5-bromopent-1-ene (0.06 equiv) was added in the solution to facilitate catalyst turnover. The resins were then washed with CH_2Cl_2, DMF, CH_2Cl_2 and Et_2O several times. Then the compounds were cleaved from the resin using a solution of HFIP in CH_2Cl_2 1/4 for 3 hours (re-subjection of the resin to the cleavage conditions afforded minimal quantities of compound suggesting the original cleavage had proceeded to completion) and the corresponding products were purified by PTLC and isolated with yields in between 20 to 30 % after 5 steps.

Each compound was dissolved in CH_2Cl_2 and then aliquoted for further amidation. To each vial were added 2.0 equiv of the corresponding amine **D** (all amines used were commertially available), 3.0 equiv of PS-DCC (N-cyclohexylcarbodiimide, N'-methylpolystyrene HL, 200 - 400 mesh, 2% DVB, ≥ 1.30 mmol/g, Novabiochem, 01-64-0211) cat DMAP, and the suspensions were stirred for 72 h. The completion of each reaction was monitored by LC-MS. The corresponding amides were filtered, evaporated and re-

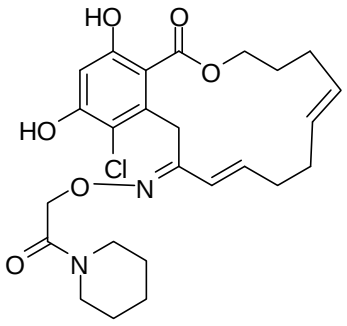
dissolved in methanol. To each solution were added 10 equiv of sulfonic acid polystyrene resin (sulfonic acid resin MP, 70-90 mesh, 3.0 mmol/g, Novabiochem, 01-64-0432) and the suspensions were stirred for 4 hours at room temperature. The final compounds were filtered, purified by PTLC and isolated in 75 to 85% yield.

Selected examples of final macrocycles. All compounds were obtained as E/Z mixtures 1:1 but for characterization proupouses, when possible, the E-isomer was isolated and its data reported.



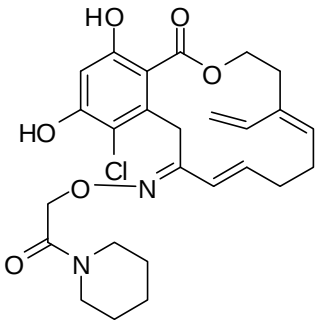
1$_{A2B1C2D1}$

1$_{A2B1C2D1}$: E isomer; ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 11.46 (bs, 1H), 6.63 (s, 1H), 6.03 (m, 1H), 5.30-5.05 (m, 2H), 5.13 (d, J = 16.1 Hz, 1H), 4.84 (s, 1H), 4.82 (s, 1H), 4.27 (s, 1H), 4.15 (s, 1H), 3.65-3.40 (m, 4H), 3.34-3.21 (m, 1H), 2.65-1.92 (m, 6H), 1.35-1.20 (m, 6H), 0.97 (t, J = 7.3 Hz, 3H), 0.91 (q, J = 7.3 Hz, 2H), 0.90 (q, J = 7.3 Hz, 2H), one OH is not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{27}H_{35}ClN_2O_6Na$: 541.2082; found: 541.2090.



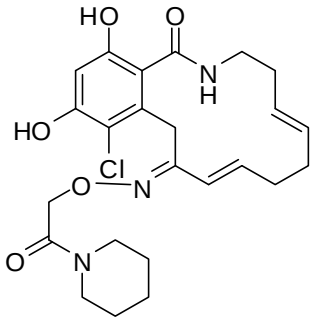
1$_{A2B1C5D1}$

$1_{A2B1C5D1}$: E isomer, ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 11.31 (s, 1H), 6.41 (s, 1H), 6.22 (m, 1H), 5.50 (d, J = 15.8Hz, 1H), 5.44-5.26 (m, 2H), 4.79 (s, 2H), 4.49 (t, J = 5.6 Hz, 2H), 4.21 (s, 2H), 3.62-3.37 (m, 4H), 2.33-1.93 (m, 8H), 1.34-1.21 (m, 4H), 0.93-0.79 (m, 2H), one OH signal is not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{31}ClN_2O_6Na$: 513.1769; found: 513.1753.



$1_{A2B1C8D1}$

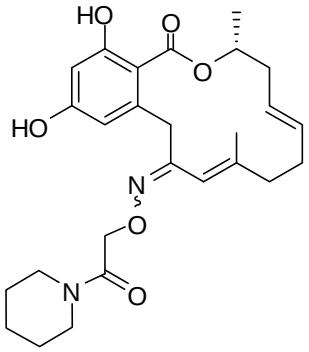
$1_{A2B1C8D1}$: E isomer ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 12.07 (s, 1H), 6.56 (s, 1H), 6.20-5.88 (m, 1H), 6.03 (d, J = 16 Hz, 1H), 5.60-5.46 (m, 1H), 4.92 (d, J = 23.1 Hz, 2H), 4.53-4.44 (m, 1H), 4.48 (s, 2H), 4.08 (s, 2H), 3.60-3.32 (m, 4H), 3.20 (m, 2H), 2.75 (t, J = 6.3 Hz, 2H), 2.50-2.39 (m, 2H), 2.28-2.15 (m, 2H), 1.37-1.23 (m, 4H), 0.93-0.78 (m, 2H), one OH signal is not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{31}ClN_2O_6Na$: 525.1769; found: 525.1747.



$1_{A2B1C3D1}$

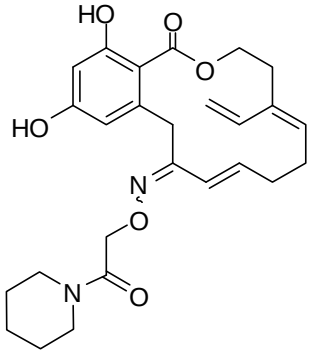
$1_{A2B1C3D1}$: E isomer ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.59 (s, 1H); 6.71 (d, J = 16.4 Hz, 1H); 6.64 (s, 1H); 6.24-6.13 (m, 1H); 5.41 (d, J = 15.3 Hz, 1H); 5.20-5.14 (m, 1H); 4.86 (s, 1H); 4.60 (s, 1H); 4.03 (s, 1H); 3.90 (s, 1H); 3.65-3.36 (m, 6H); 2.56-2.07 (m, 6H); 1.61-1.49 (m,

6H), 1 OH and 1 NH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{24}H_{30}ClN_3O_5Na$: 498.1772; found: 498.1720.



$1_{A1B3C1D1}$

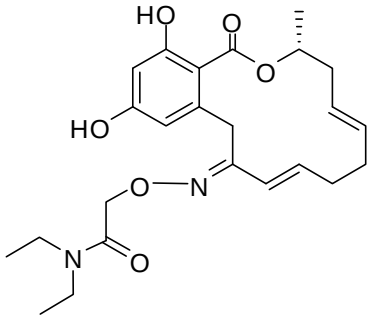
$1_{A1B3C1D1}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.92 (brs, 1H), 11.77 (brs, 1H), 6.56 (d, J = 2.4 Hz, 1H), 6.53 (d, J = 2.4 Hz, 1H), 6.36 (d, J = 2.4 Hz, 1H), 6.35 (d, J = 2.8 Hz, 1H), 5.43-5.17 (m, 6H), 5.11 (s, 1H), 5.02 (s, 1H), 4.88 (d, J = 14.4 Hz, 1H), 4.81-4.74 (m, 2H), 4.70 (d, J = 14.4 Hz, 1H), 4.46 (d, J = 13.6 Hz, 1H), 4.16-4.09 (m, 2H), 3.96 (d, J = 15.6 Hz, 1H), 3.64-3.37 (m, 8H), 2.78-2.60 (m, 4H), 2.23-2.12 (m, 8H), 1.79 (s, 3H), 1.65-1.56 (m, 12H), 1.53 (s, 3H), 1.35 (d, J = 6.4 Hz, 3H), 1.29 (d, J = 6.4 Hz, 3H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{34}N_2O_6Na$: 493.2315; found: 493.2375.



$1_{A1B1C8D1}$

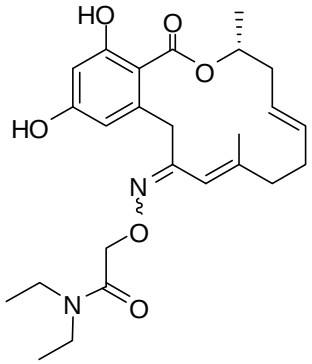
$1_{A1B1C8D1}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.95 (brs, 2H), 7.11 (d, J = 2.4 Hz, 2H), 6.32 (d, J = 2.4 Hz, 2H), 6.09-6.01 (m, 2H), 5.95 (d, J = 16.8 Hz, 2H), 5.91 (d, J = 16.4 Hz, 2H), 5.76-5.68 (m, 2H), 5.11 (s, 2H), 4.94 (s, 2H), 4.84 (s, 4H), 4.57 (t, J = 5.2 Hz, 4H), 4.26 (s, 4H), 3.59-3.53 (m, 4H), 3.39-3.33 (m, 4H), 2.67 (t, J = 5.2 Hz, 4H), 2.23-

2.17 (m, 8H), 1.60-1.52 (m, 8H), 1.36-1.28 (m, 4H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{32}N_2O_6Na$: 491.2158; found: 491.2162.



$1_{A1B1C1D18}$

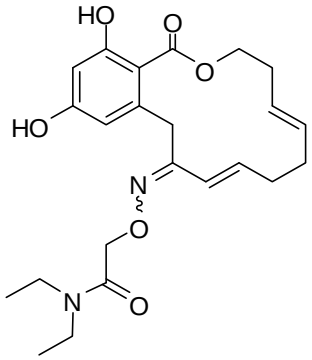
$1_{A1B1C1D18}$: E isomer. ^1H NMR ($CDCl_3$, 400 MHz) δ 11.21 (brs, 1H), 6.67 (d, J = 2.4 Hz, 1H), 6.62 (d, J = 16.4 Hz, 1H), 6.30 (d, J = 2.8 Hz, 1H), 6.23-6.15 (m, 1H), 5.52-5.44 (m, 1H), 5.40-5.31 (m, 1H), 4.82 (d, J = 14.8 Hz, 1H), 4.75 (d, J = 14.8 Hz, 1H), 4.51 (d, J = 14.8 Hz, 1H), 4.21 (t, J = 6.2 Hz, 1H), 3.66 (d, J = 15.2 Hz, 1H), 3.44-3.35 (m, 2H), 3.34-3.28 (m, 2H), 2.33-2.20 (m, 2H), 2.14-1.98 (m, 4H), 1.45 (d, J = 6.8 Hz, 3H), 1.14 (t, J = 7.2 Hz, 3H), 0.92 (t, J = 7.6 Hz, 3H), 1 OH signals are not visible. HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{24}H_{32}NaN_2O_6$: 467.2158; found: 467.2176



$1_{A1B3C1D18}$

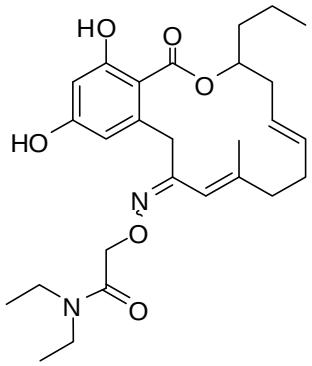
$1_{A1B3C1D18}$: Mixture of isomers 1:1. ^1H NMR ($CDCl_3$, 400 MHz) δ 11.92 (brs, 1H), 11.83 (brs, 1H), 6.98 (d, J = 2.4 Hz, 2H), 6.34-6.33 (m, 2H), 5.38-5.15 (m, 6H), 4.93 (d, J = 14.4 Hz, 2H), 4.79-4.68 (m, 2H), 4.75 (d, J = 14.4 Hz, 2H), 4.27 (d, J = 14.4 Hz, 2H), 4.04 (d, J = 14.4 Hz, 2H), 3.44-3.26 (m, 8H), 2.24-2.19 (m, 4H), 1.74-1.72 (m, 8H), 1.36 (d, J = 6.8 Hz, 6H), 1.34 (d, J = 6.8 Hz, 6H), 1.27-1.21 (m, 6H), 1.14 (t, J = 7.4 Hz, 6H), 2 OH signals are

not visible. HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{25}H_{35}N_2O_6$: 459.2495; found: 459.2499



$1_{A1B1C4D18}$

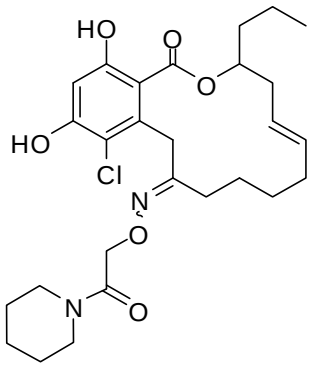
$1_{A1B1C4D18}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.70 (brs, 1H), 11.69 (brs, 1H), 6.99 (brs, 1H), 6.72 (d, J = 2.8 Hz, 1H), 6.64 (d, J = 16.4 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H), 6.31 (d, J = 2.4 Hz, 1H), 6.28-6.17 (m, 2H), 5.91 (d, J = 16 Hz, 1H), 5.38-5.33 (m, 4H), 4.87 (s, 2H), 4.79 (s, 2H), 4.57-4.53 (m, 4H), 4.34 (s, 2H), 4.07(s, 2H), 3.43-3.37 (m, 4H), 3.34-3.28 (q, J = 7.2 Hz, 4H), 2.53-2.48 (m, 4H), 2.16-1.97 (m, 8H), 1.29-1.24 (m, 12H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{31}N_2O_6$: 431.2182; found: 431.2186



$1_{A1B3C2D18}$

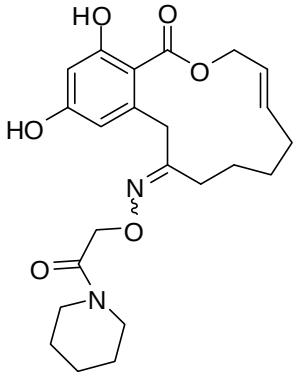
$1_{A1B3C2D18}$: E isomer, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.82 (brs, 1H), 7.75 (brs, 1H), 7.02 (d, J = 2.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 5.35-5.28 (m, 1H), 5.20 (s, 1H), 5.14-5.10 (m, 1H), 4.94 (d, J = 14.4 Hz, 1H), 4.74 (d, J = 14.4 Hz, 1H), 4.31 (d, J = 14.4 Hz, 1H), 4.07 (d, J = 14.4 Hz, 1H), 3.44-3.28 (m, 4H), 2.70-2.63 (m, 1H), 2.25-2.18 (m, 2H), 2.14-2.07 (m, 2H),

1.73-1.72 (m, 6H), 1.25-1.21 (m, 6H), 1.14 (t, J = 7.2 Hz, 3H), 0.95 (t, J = 7.2 Hz, 3H). HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{27}H_{38}NaN_2O_6$: 509.2627; found: 509.2652



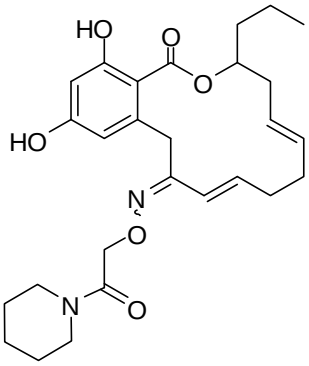
$1_{A2B2C2D1}$

$1_{A2B2C2D1}$: Mixture of isomers 1:1. ^1H NMR ($CDCl_3$, 400 MHz) δ 11.79 (brs, 1H), 11.03 (brs, 1H), 6.61 (s, 1H), 6.08 (s, 1H), 5.42-5.28 (m, 6H), 4.79 (d, J = 13.2 Hz, 2H), 4.74 (d, J = 13.6 Hz, 2H), 4.39 (m, 2H), 4.27-4.23 (m, 3H), 3.61-3.54 (m, 4H), 3.51-3.45 (m, 4H), 2.53-2.47 (m, 2H), 2.33-2.20 (m, 4H), 2.06-1.91 (m, 8H), 1.70-1.58 (m, 12H), 1.44-129 (m, 13H), 0.95 (t, J = 7.2 Hz, 6H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{27}H_{37}ClNaN_2O_6$: 543.2237; found: 543.2263.



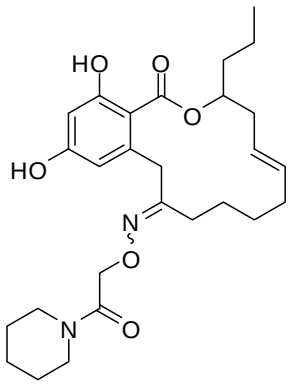
$1_{A1B2C6D1}$

$1_{A2B2C2D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{23}H_{30}NaN_2O_6$: 453.2001; found: 453.2010



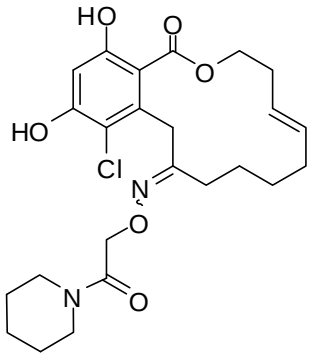
$1_{A1B1C2D1}$

$1_{A1B1C2D1}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.99 (brs, 1H), 6.68 (d, J = 2.4 Hz, 1H), 6.61 (d, J = 16.8 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H), 6.30 (d, J = 2.4 Hz, 1H), 6.25-6.10 (m, 2H), 5.89 (d, J = 16.4 Hz, 1H), 5.50-5.31 (m, 4H), 4.94 (d, J = 14.4 Hz, 2H), 4.84-4.76 (m, 4H), 4.50 (d, J = 15.2 Hz, 1H), 4.44 (d, J = 14.4 Hz, 1H), 4.29 (d, J = 14.4 Hz, 1H), 4.21 (t, J = 6.0 Hz, 1H), 3.70-3.37 (m, 8H), 2.72-2.61 (m, 4H), 2.35-2.19 (m, 6H), 2.15-1.96 (m, 8H), 1.93-1.82 (m, 3H), 1.38-1.28 (m, 11H), 0.96 (t, J = 7.6 Hz, 3H), 0.92 (t, J = 7.6 Hz, 3H), 4 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{27}H_{37}N_2O_6$: 485.2651; found: 485.2611.



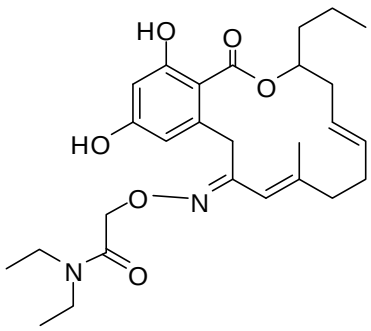
$1_{A1B2C2D1}$

$1_{A1B2C2D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{27}H_{38}NaN_2O_6$: 509.2628; found: 509.2639



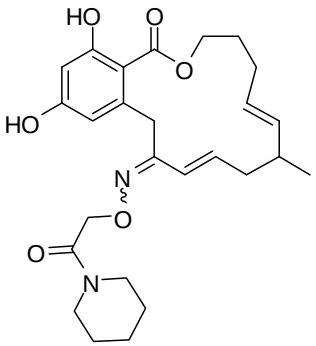
$1_{A2B2C4D1}$

$1_{A2B2C4D1}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.61 (s, 1H), 6.05 (s, 1H), 5.41-5.26 (m, 6H), 4.80-4.75 (m, 3H), 4.58-4.47 (m, 4H), 4.30-4.17 (m, 3H), 4.21 (t, J = 5.6 Hz, 2H), 4.05-4.01 (m, 2H), 3.61-3.54 (m, 4H), 3.49-3.42 (m, 4H), 2.46-2.40 (m, 2H), 2.24-2.20 (m, 3H), 2.07-1.90 (m, 8H), 1.34-1.28 (m, 15H), 4 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{27}H_{31}NaN_2O_6$: 501.1768; found: 501.1718



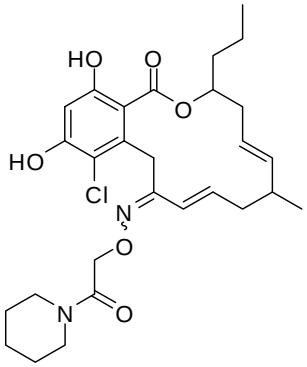
$1_{A1B3C2D18}$

$1_{A1B3C2D18}$: E isomer ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.85 (brs, 1H), 7.12 (d, J = 2.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 5.38-5.31 (m, 2H), 5.25 (s, 1H), 5.23-5.11 (m, 2H), 4.96 (d, J = 14.4 Hz, 1H), 4.74 (d, J = 14.4 Hz, 1H), 4.34 (d, J = 14 Hz, 1H), 4.21 (t, J = 6 Hz, 1H), 4.10 (d, J = 14 Hz, 1H), 3.43-3.27 (m, 4H), 1.70 (s, 3H), 1.61-1.59 (m, 4H), 1.37-1.29 (m, 7H), 1.13 (t, J = 7.2 Hz, 3H), 0.95 (t, J = 7.2 Hz, 3H), 1 OH singal is not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{27}H_{38}NaN_2O_6$: 509.2627; found: 509.2680.



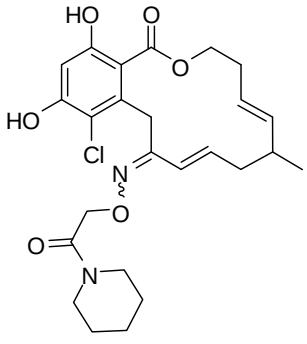
$1_{A1B4C5D1}$

$1_{A1B4C5D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{34}NaN_2O_6$: 493.2314; found: 493.2331.



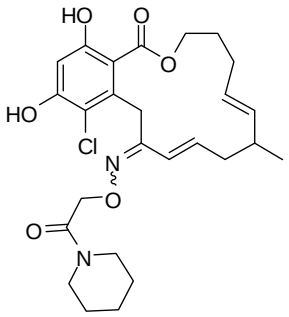
$1_{A2B4C2D1}$

$1_{A2B4C2D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{28}H_{37}ClNaN_2O_6$: 555.2238; found: 555.2242.



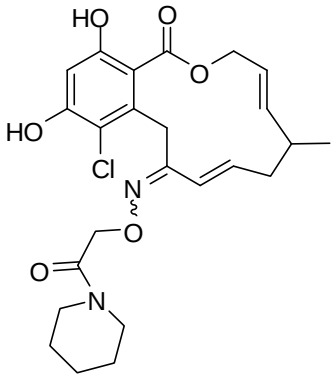
$1_{A2B4C4D1}$

$1_{A2B4C4D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{31}ClNaN_2O_6$: 513.1768; found: 513.1780.



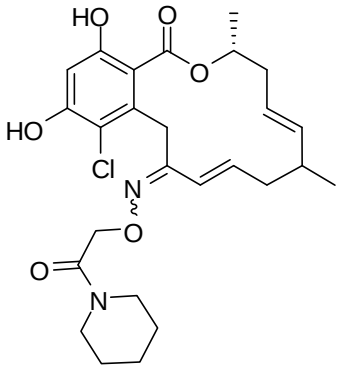
$1_{A2B4C5D1}$

$1_{A2B4C5D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{33}ClNaN_2O_6$: 527.1925; found: 527.1939.



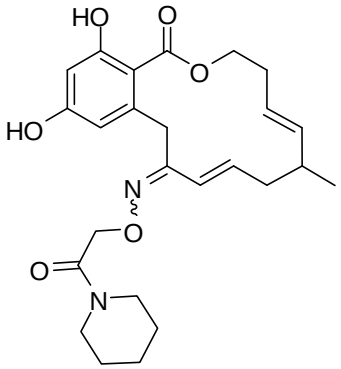
$1_{A2B4C6D1}$

$1_{A2B4C6D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{24}H_{29}ClNaN_2O_6$: 499.1612; found: 499.1626.



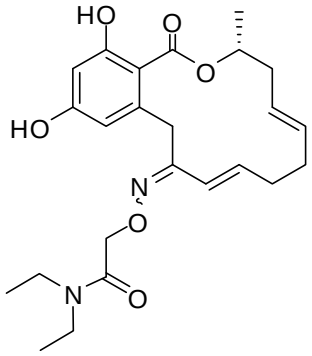
$1_{A2B4C1D1}$

$1_{A2B4C1D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{33}ClNaN_2O_6$: 527.1925; found: 527.1932.



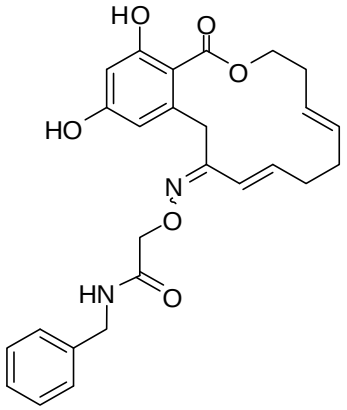
$1_{A1B4C4D1}$

$1_{A1B4C4D1}$: HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{32}NaN_2O_6$: 479.2158; found: 479.2174.



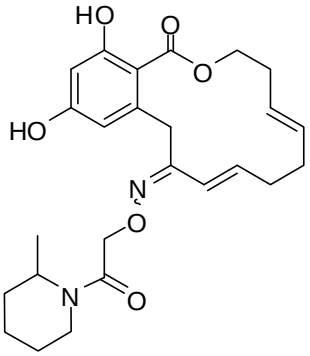
$1_{A1B1C1D18}$

$1_{A1B1C1D18}$: E isomer, ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40 (d, J = 2.4 Hz, 1H), 6.31 (d, J = 2.4 Hz, 1H), 6.19-6.11 (m, 1H), 5.89 (d, J = 16 Hz, 1H), 5.53-5.46 (m, 1H), 5.41-5.32 (m, 1H), 4.93 (d, J = 14.8 Hz, 1H), 4.81 (d, J = 14.8 Hz, 1H), 4.44 (d, J = 14.8 Hz, 1H), 4.28 (d, J = 14.8 Hz, 1H), 4.21 (t, J = 5.6 Hz, 1H), 3.44-3.29 (m, 2H), 2.35-2.20 (m, 2H), 2.13-2.01 (m, 2H), 1.45 (d, J = 6.8 Hz, 3H), 1.37-1.28 (m, 4H), 1.14 (t, J = 7.2 Hz, 3H), 0.92 (t, J = 7.6 H, 3H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{24}H_{32}NaN_2O_6$: 467.2158; found: 467.2147.



$1_{A1B1C4D22}$

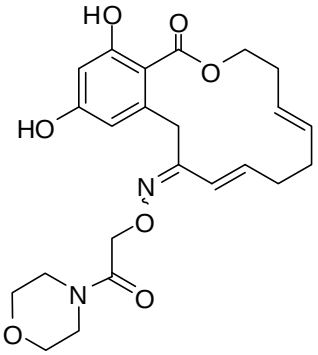
$1_{A1B1C4D22}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.60 (brs, 1H), 11.42 (brs, 1H), 7.35-7.28 (m, 11H), 6.69-6.62 (m, 2H), 6.49 (d, J = 16 Hz, 1H), 6.36 (d, J = 2.4 Hz, 1H), 6.33 (d, J = 2.4 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H), 6.28-6.24 (m, 1H), 6.23 (d, J = 2.8 H, 1H), 6.10 (dt, J = 16, 7.2 Hz, 1H), 5.73 (d, J = 16 Hz, 1H), 5.36-5.31 (m, 2H), 5.27-5.18 (m, 2H), 4.70 (s, 2H), 4.65 (s, 2H), 4.55-4.51 (m, 5H), 4.45 (t, J = 5.3 Hz, 2H), 4.27 (s, 2H), 4.08 (s, 2H), 2.50-2.42 (m, 4H), 2.17-2.07 (m, 8H), 1 NH signal is not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{26}H_{29}N_2O_6$: 465.2025; found: 465.1981.



$1_{A1B1C4D2}$

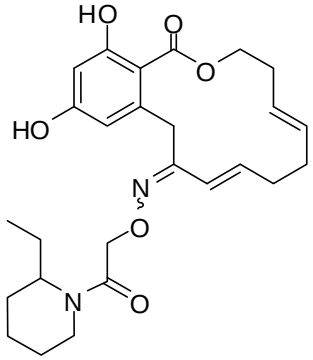
$1_{A1B1C4D2}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.67 (brs, 1H), 11.65 (brs, 1H), 8.00 (brs, 1H), 7.77 (brs, 1H), 7.13 (brs, 1H), 6.63-6.59 (m, 2H), 6.32 (2d, J = 2.8 Hz, 2H), 6.27-6.12 (m, 2H), 5.86 (d, J = 16.4 Hz, 1H), 5.37-5.33 (m, 4H), 4.95-4.72 (m, 4H), 4.57-4.51 (m, 4H), 4.34 (s, 2H), 4.10 (d, J = 15.2 Hz, 1H), 4.03 (d, J = 15.2 Hz, 1H), 3.58-3.48 (m, 2H), 3.20-3.09 (m, 2H), 2.77-2.69 (m, 2H), 2.53-2.45 (m, 4H), 2.17-2.05 (m, 8H),

1.71-1.32 (m, 12H), 1.30-1.12 (m, 6H). HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{25}H_{33}N_2O_6$: 457.2338; found: 457.2332.



$1_{A1B1C4D10}$

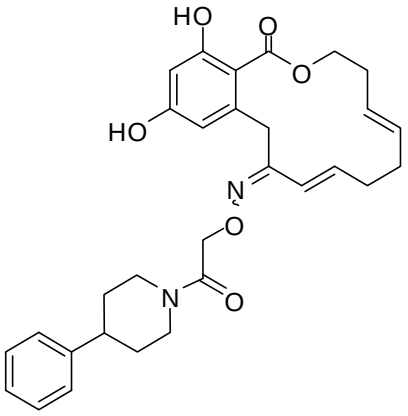
$1_{A1B1C4D10}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.66 (brs, 1H), 11.64 (brs, 1H), 7.56 (brs, 1H), 7.36 (brs, 1H), 6.95 (d, J = 2.8 Hz, 1H), 6.59-6.54 (m, 2H), 6.33 (d, J = 2.8 Hz, 1H), 6.32 (d, J = 2.8 Hz, 1H), 6.28-6.13 (m, 2H), 5.84 (d, J = 16.1 Hz, 1H), 5.38-5.32 (m, 4H), 4.85 (s, 2H), 4.78 (s, 2H), 4.54 (t, J = 5.2 Hz, 4H), 4.33 (s, 2H), 4.07 (s, 2H), 3.71-3.64 (m, 12H), 3.52-3.48 (m, 4H), 2.50 (brt, J = 4.8 Hz, 4H), 2.17-2.07 (m, 8H). HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{23}H_{28}NaN_2O_7$: 467.1794; found: 467.1765.



$1_{A1B1C4D5}$

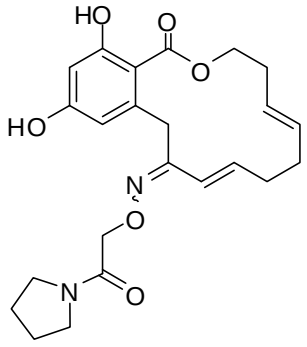
$1_{A1B1C4D5}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.62 (brs, 2H), 6.96 (d, J = 2 Hz, 1H), 6.60 (d, J = 2.8 Hz, 1H), 6.58 (d, J = 2.8 Hz, 1H), 6.32 (brd, J = 2.4 Hz, 2H), 6.24-6.10 (m, 2H), 5.81 (d, J = 16 Hz, 1H), 5.35-5.32 (m, 5H), 4.92-4.72 (m, 4H), 4.56-4.45 (m, 4H), 4.31 (brs, 2H), 4.04 (brs, 2H), 3.75-3.67 (m, 1H), 3.59-3.51 (m, 1H), 3.12-3.03 (m, 1H), 2.64 (t, J = 12.8 Hz, 1H), 2.52-2.46 (m, 4H), 2.14-2.04 (m, 8H), 1.70-1.58 (m, 10H), 1.60-1.34 (m, 5H), 1.19-1.13 (m, 1H), 1.07-1.01 (m, 1H), 0.98-0.82 (m, 6H), 2 OH signals are not

visible; HRMS (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{26}H_{34}NaN_2O_6$: 493.2314; found: 493.2332.



1$_{A1B1C4D7}$

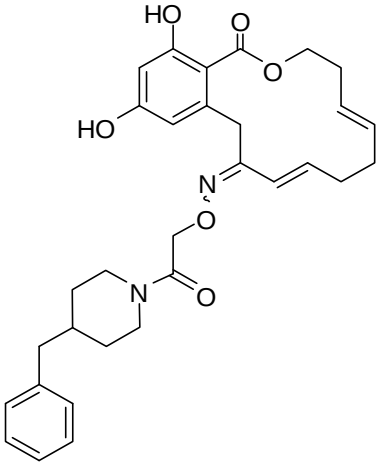
1$_{A1B1C4D7}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.65 (brs, 2H), 7.32-7.27 (m, 4H), 7.24-7.10 (m, 6H), 7.01 (d, J = 2.4 Hz, 1H), 6.64-6.59 (m, 2H), 6.34-6.33 (m, 2H), 6.28-6.18 (m, 2H), 5.85 (d, J = 16 Hz, 1H), 5.37-5.32 (m, 4H), 4.85 (s, 2H), 4.72-4.69 (m, 2H), 4.56-4.51 (m, 4H), 4.15 (d, J = 15.2 Hz, 2H), 4.02 (d, J = 15.2 Hz, 2H), 3.96-3.92 (m, 2H), 3.21-3.11 (m, 2H), 2.76-2.67 (m, 4H), 2.54-2.45 (m, 4H), 2.12-2.02 (m, 10H), 1.90-1.87 (m, 4H), 1.67-1.58 (m, 4H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{30}H_{34}NaN_2O_6$: 541.2314; found: 541.2314.



1$_{A1B1C4D17}$

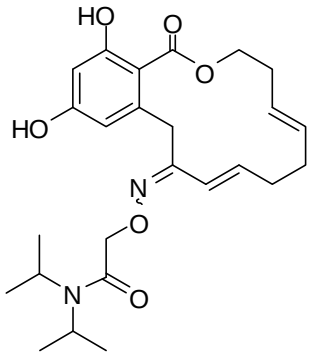
1$_{A1B1C4D17}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.65 (brs, 1H), 11.64 (brs, 1H), 8.70 (brs, 1H), 8.27 (brs, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.64-6.60 (m, 2H), 6.26-6.11 (m, 2H), 6.32 (2d, J = 2.6 Hz, 2H), 5.82 (d, J = 16 Hz, 1H), 5.36-5.32 (m, 4H), 4.76 (s, 2H), 4.70 (s, 2H), 4.53 (brt, J = 5.2 Hz, 4H), 4.33 (s, 2H), 4.05 (s, 2H), 3.51 (brt, J = 6.8 Hz, 4H), 3.44

(q, J = 7.2 Hz, 4H), 2.52-2.46 (m, 4H), 2.16-2.05 (m, 8H), 2.02-1.93 (m, 4H), 1.90-1.84 (m, 4H). HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{29}N_2O_6$: 429.2025; found: 429.2003.



$1_{A1B1C4D8}$

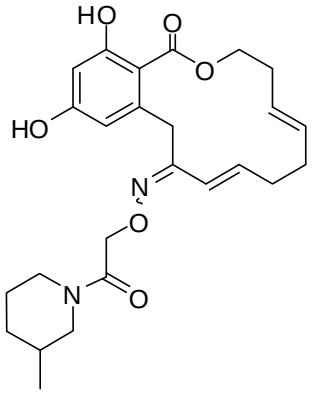
$1_{A1B1C4D8}$: Mixture of isomers 1:1.^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.66 (brs, 1H), 11.64 (brs, 1H), 8.15 (brs, 1H), 7.86 (brs, 1H), 7.31-7.27 (m, 4H), 7.21-7.18 (m, 2H), 7.15-7.10 (m, 4H), 7.00 (brs, 1H), 6.60 (d, J = 15.6 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 6.32 (2 x d, J = 2.4 Hz, 2H), 6.27-6.11 (m, 2H), 5.85 (d, J = 16 Hz, 1H), 5.36-5.32 (m, 4H), 4.84 (s, 2H), 4.77 (s, 2H), 4.59-4.48 (m, 4H), 4.33 (s, 2H), 4.11 (d, J = 15.2 Hz, 1H), 4.02 (d, J = 15.6 Hz, 1H), 3.78-3.71 (m, 2H), 3.01-2.92 (m, 2H), 2.59-2.47 (m, 8H), 2.12-2.07 (m, 8H), 1.82-1.68 (m, 6H), 1.36-1.30 (m, 8H). HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{31}H_{37}N_2O_6$: 533.2651; found: 533.2625.



$1_{A1B1C4D20}$

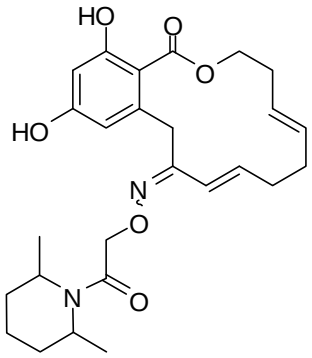
$1_{A1B1C4D20}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.72 (brs, 1H), 11.71 (brs, 1H), 6.71 (d, J = 2.4 Hz, 1H), 6.67 (d, J = 16.4 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 6.35 (2 x d,

J = 3.0 Hz, 2H,), 6.31-6.18 (m, 2H), 5.93 (d, *J* = 16.4 Hz, 1H), 5.41-5.36 (m, 4H), 4.86 (s, 2H), 4.77 (s, 2H), 4.60-4.54 (m, 4H), 4.37 (s, 2H), 4.10 (s, 2H), 3.94-3.86 (m, 2H), 3.62-3.49 (m, 2H), 2.55-2.49 (m, 4H), 2.20-2.10 (m, 8H), 1.44-1.25 (m, 24H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{25}H_{35}N_2O_6$: 459.2495; found: 459.2514



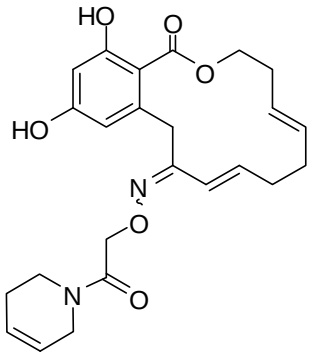
$1_{A1B1C4D3}$

$1_{A1B1C4D3}$: Mixture of isomers 1:1. ^1H NMR ($CDCl_3$, 400 MHz) δ 11.65 (brs, 1H), 11.64 (brs, 1H), 7.00 (d, *J* = 2.4 Hz, 1H), 6.61-6.57 (m, 2H), 6.33-6.32 (m, 2H), 6.25-6.10 (m, 2H), 5.82 (d, *J* = 16 Hz, 1H), 5.36-5.31 (m, 4H), 4.85 (s, 2H), 4.78 (s, 2H), 4.58-4.48 (m, 4H), 4.41-4.32 (m, 4H), 4.12-3.98 (m, 2H), 3.72-3.58 (m, 2H), 3.02-2.93 (m, 1H), 2.72-2.62 (m, 2H), 2.53-2.45 (m, 4H), 2.35-2.28 (m, 1H), 2.16-2.04 (m, 8H), 1.86-1.39 (m, 8H), 1.17-1.07 (m, 2H), 0.93-0.88 (m, 6H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{25}H_{33}N_2O_6$: 457.2338; found: 457.2380.



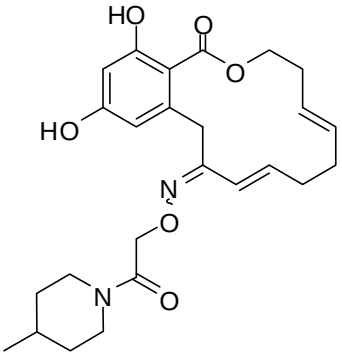
$1_{A1B1C4D6}$

$1_{A1B1C4D6}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.67 (brs, 2H), 6.99 (s, 1H), 6.68-6.63 (m, 2H), 6.32 (d, J = 2.4 Hz, 1H), 6.31 (d, J = 2.8 Hz, 1H), 6.27-6.14 (m, 2H), 5.90 (d, J = 16 Hz, 1H), 5.37-5.34 (m, 4H), 4.77 (s, 2H), 4.63-4.46 (m, 4H), 4.36 (brs, 2H), 4.21 (t, J = 6 Hz, 4H), 4.05-3.92 (m, 4H), 2.55-2.46 (m, 4H), 2.17-2.06 (m, 8H), 1.37-1.28 (m, 12H), 0.93-0.86 (m, 12H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{26}H_{34}NaN_2O_6$: 493.2314; found: 493.2314.



$1_{A1B1C4D9}$

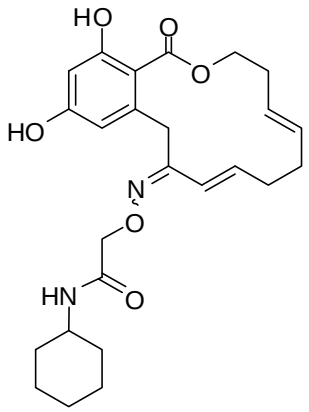
$1_{A1B1C4D9}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.62 (brs, 2H), 6.98 (m, 2H), 6.57 (m, 2H), 6.32 (m, 2H), 6.18 (m, 2H), 5.82 (d, J = 15.6 Hz, 1H), 5.67 (m, 2H), 5.33 (m, 4H), 4.87 (d, J = 13.6 Hz, 2H), 4.80 (d, J = 10.8 Hz, 2H), 4 .53 (m, 3H), 4.33 (s, 2H), 4.05 (brs, 4H), 3.73 (m, 2H), 3.69 (q, J = 6 Hz, 2H), 3.52 (q, J = 5.2 Hz, 2H), 2.48 (m, 5H), 2.13 (m, 13H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z [M + Na]$^+$ calcd for $C_{24}H_{28}NaN_2O_6$: 463.1845; found: 463.1870.



$1_{A1B1C4D4}$

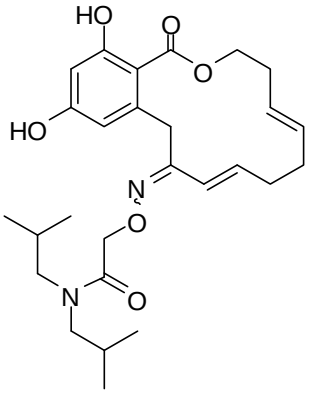
$1_{A1B1C4D4}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.66 (brs, 1H), 11.64 (brs, 1H), 8.29 (brs, 2H), 6.89 (d, J = 2.8 Hz, 1H), 6.59 (d, J = 16 H, 1H), 6.57 (d, J = 2.4 Hz, 1H),

6.33-6.32 (m, 2H), 6.24-6.09 (m, 2H), 5.79 (d, J = 16 Hz, 1H), 5.34-5.30 (m, 4H), 4.85 (s, 2H), 4.78 (s, 2H), 4.56-4.48 (m, 4H), 4.31 (s, 2H), 4.10 (d, J = 15.6 Hz, 1H), 4.05 (d, J = 15.6 Hz, 1H),3.80-3.71 (m, 2H), 3.06-2.96 (m, 2H), 2.61 (dt, J = 12.8, 2.6 Hz, 2H), 2.52-2.46 (m, 4H), 2.16-2.05 (m, 8H), 1.71-1.58 (m, 6H), 1.17-1.05 (m, 4H), 0.94 (d, J = 5.6 Hz, 3H), 0.93 (d, J = 5.6 Hz, 3H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{32}NaN_2O_6$: 479.2158; found: 479.2182.



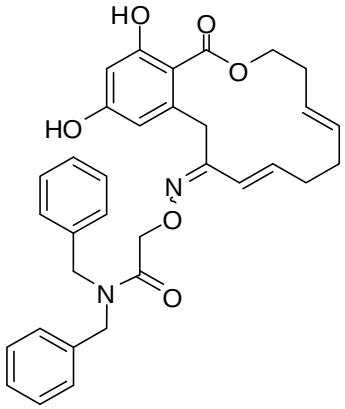
$1_{A1B1C4D24}$

$1_{A1B1C4D24}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.60 (brs, 1H), 11.47 (brs, 1H), 8.58 (brs, 2H), 6.53 (d, J = 16.4 Hz, 1H), 6.50 (d, J = 2.4 Hz, 1H), 6.35 (d, J = 2.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 6.30 (d, J = 2.4 Hz, 1H), 6.31-6.27 (m, 2H), 6.14 (m, 1H), 5.39-5.31 (m, 4H), 4.61 (s, 2H), 4.56-4.53 (m, 6H), 4.33 (s, 2H), 4.09 (s, 2H), 3.86-3.75 (m, 2H), 2.53-2.48 (m, 4H), 2.17-2.09 (m, 8H),1.93-1.86 (m, 4H), 1.70-1.55 (m, 8H), 1.39-1.11 (m, 8H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{32}NaN_2O_6$: 479.2158; found: 479.2157.



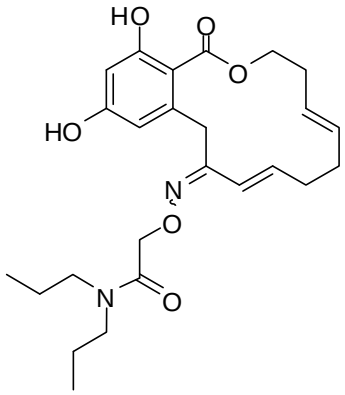
$1_{A1B1C4D19}$

$1_{A1B1C4D19}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.65 (brs, 2H), 7.06 (d, J = 2.4 Hz, 1H), 6.62-6.57 (m, 2H), 6.33 (d, J = 2.4 Hz, 2H), 6.21-6.11 (m, 2H), 5.81 (d, J = 16.4 Hz, 1H), 5.37-5.29 (m, 4H), 4.86 (s, 2H), 4.79 (s, 2H), 4.52 (brt, J = 5.2 Hz, 4H), 4.31 (s, 2H), 4.02 (s, 2H), 3.21 (m, 4H), 3.09 (dd, J = 10.8, 8.0 Hz, 4H), 2.52-2.44 (m, 4H), 2.11-1.91 (m, 12H), 0.95 (d, J = 6.8 Hz, 6H), 0.93 (d, J = 6.8 Hz, 6H), 0.88 (d, J = 6.8 Hz, 6H), 0.86 (d, J = 6.8 Hz, 6H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{27}H_{38}NaN_2O_6$: 509.2627; found: 509.2626.



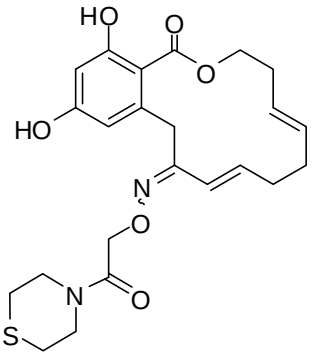
$1_{A1B1C4D21}$

$1_{A1B1C4D21}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.67 (brs, 1H), 11.66 (brs, 1H), 7.38-7.28 (m, 12H), 7.22-7.14 (m, 8H), 6.94 (d, J = 2.4 Hz, 1H), 6.57-6.53 (m, 2H), 6.33 (brd, J = 2.4 Hz, 2H), 6.22-6.12 (m, 2H), 5.85 (d, J = 16 Hz, 1H), 5.35-5.32 (m, 4H), 4.93 (s, 2H), 4.86 (s, 2H), 4.62 (s, 2H), 4.60 (s, 2H), 4.53 (brt, J = 5.2 Hz, 4H), 4.46 (s, 2H), 4.43 (s, 2H), 4.30 (s, 2H), 4.07 (s, 2H), 3.52-3.46 (m, 4H), 2.16-2.07 (m, 8H),), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{33}H_{34}NaN_2O_6$: 577.2314; found: 577.2278



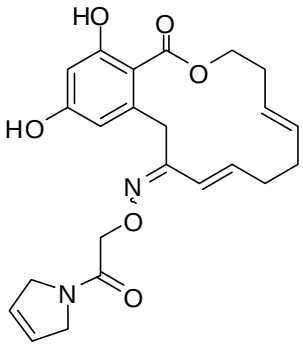
$1_{A1B1C4D25}$

$1_{A1B1C4D25}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.66 (brs, 1H), 11.65 (brs, 1H), 7.05 (d, J = 2.4 Hz, 1H), 6.62-6.55 (m, 2H), 6.32-6.29 (m, 2H), 6.23-6.11 (m, 2H), 5.82 (d, J = 16 Hz, 1H), 5.34-5.29 (m, 4H), 4.85 (s, 2H), 4.77 (s, 2H), 4.54 (brt, J = 5.2 Hz, 4H), 4.32 (s, 2H), 4.04 (s, 2H), 3.32-3.27 (m, 4H), 3.21-3.15 (m, 4H), 2.52-2.42 (m, 4H), 2.10-2.04 (m, 8H), 1.67-1.51 (m, 8H), 0.96-0.86 (m, 12H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{34}NaN_2O_6$: 481.2314; found: 481.2307.



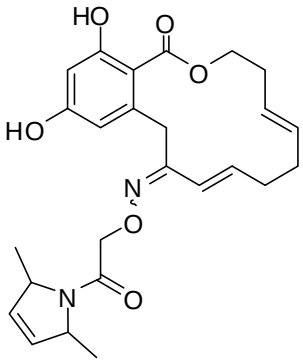
$1_{A1B1C4D11}$

$1_{A1B1C4D11}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.63 (brs, 2H), 6.88 (d, J = 2.0 Hz, 1H), 6.55 (d, J = 16.4 Hz, 1H), 6.43 (d, J = 2.4 Hz, 1H), 6.32 (d, J = 2.4 Hz 1H), 6.30 (d, J = 2 Hz, 1H), 6.28-6.13 (m, 2H), 5.84 (d, J = 16.1 Hz, 1H), 5.36-5.33 (m, 4H), 4.82 (s, 2H), 4.73 (s, 2H), 4.54 (t, J = 5.5 Hz, 4H), 4.32 (s, 2H), 4.09 (s, 2H), 3.91-3.85 (m, 4H), 3.79-3.70 (m, 4H), 2.65-2.60 (m, 8H), 2.49 (brt, J = 5.1 Hz, 4H), 2.17-2.04 (m, 8H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{29}N_2O_6S$: 461.1746; found: 461.1765.



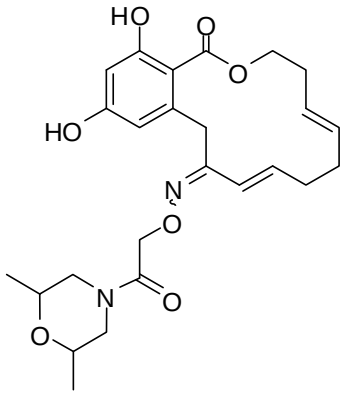
$1_{A1B1C4D15}$

$1_{A1B1C4D15}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.64 (brs, 2H), 6.98 (d, J = 2.3 Hz, 1H), 6.61 (d, J = 16.3 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 6.31 (2d, J = 2.2 Hz, 2H), 6.27-6.12 (m, 2H), 5.87-5.78 (m, 5H), 5.45-5.33 (m, 4H), 4.74 (s, 2H), 4.69 (s, 2H), 4.53 (t, J = 5.3 Hz, 4H), 4.33 (s, 2H), 4.28-4.25 (m, 8H), 4.05 (s, 2H), 2.52-2.46 (m, 4H), 2.17-2.05 (m, 8H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{23}H_{27}N_2O_6$: 427.1869; found: 427.1902.



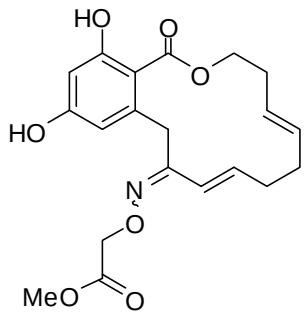
$1_{A1B1C4D16}$

$1_{A1B1C4D16}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.68 (brs, 2H), 7.16 (d, J = 2.8 Hz, 1H), 6.63 (d, J = 16.3 Hz, 1H), 6.60 (d, J = 2.0 Hz, 1H), 6.31 (brs, 2H), 6.26-6.13 (m, 2H), 5.85 (d, J = 16.0 Hz, 1H), 5.77-5.76 (m, 4H), 5.44-5.34 (m, 4H), 4.87-4.59 (m, 10H), 4.48-4.43 (m, 2H), 4.33 (brs, 2H), 4.19 (d, J = 15.3 Hz, 1H), 3.91 (d, J = 15.3 Hz, 1H), 2.54-2.44 (m, 4H), 2.19-2.05 (m, 8H), 1.36-1.30 (m, 12H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{25}H_{31}N_2O_6$: 455.2182; found: 455.2195.



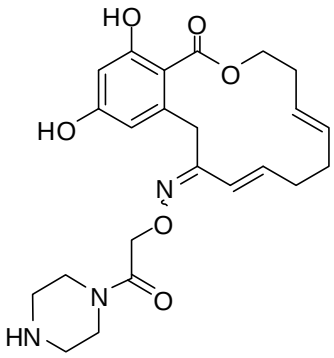
$1_{A1B1C4D14}$

$1_{A1B1C4D14}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.66 (brs, 1H), 11.63 (brs, 1H), 6.93 (d, J = 2.4 Hz, 1H), 6.57 (d, J = 16.0 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H), 6.31 (d, J = 2.4 Hz, 1H), 6.29-6.12 (m, 2H), 5.84 (d, J = 16.0 Hz, 1H), 5.38-5.32 (m, 4H), 4.91-4.71 (m, 4H), 4.59-4.49 (m, 4H), 4.37-4.26 (m, 4H), 4.13-3.97 (m, 4H), 3.78-3.68 (m, 2H), 3.62-3.52 (m, 2H), 3.35-3.15 (m, 4H), 2.50 (brt, J = 5.2 Hz, 4H), 2.18-2.08 (m, 8H), 1.24-1.18 (m, 12H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{32}NaN_2O_7$: 495.2107; found: 495.2067.



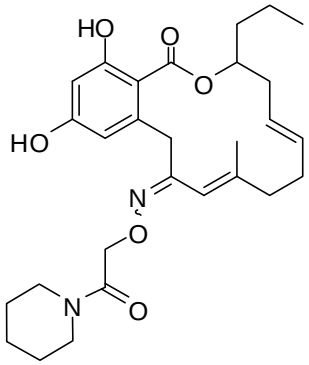
$1_{A1B1C4D26}$

$1_{A1B1C4D26}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.70 (brs, 1H), 11.68 (brs, 1H), 6.84 (d, J = 2.4 Hz, 1H), 6.61 (d, J = 16.4 Hz, 1H), 6.53 (d, J = 2.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H), 6.28-6.13 (m, 2H), 5.86 (d, J = 16.4 Hz, 1H), 5.38-5.33 (m, 4H), 4.75 (s, 2H), 4.68 (s, 2H), 4.57-4.54 (m, 4H), 4.36 (s, 2H), 4.08 (s, 2H), 3.79 (2s, 6H), 2.54-2.48 (m, 4H), 2.17-2.04 (m, 8H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{20}H_{23}NaNO_7$: 412.1373; found: 412.1322.



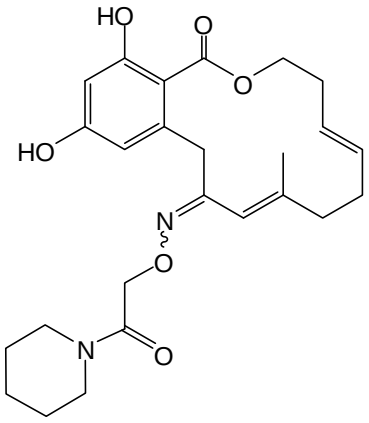
$\mathbf{1}_{A1B1C4D12}$

$\mathbf{1}_{A1B1C4D12}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (d, J = 2.4 Hz, 1H), 6.52 (d, J = 16.0 Hz, 1H), 6.34 (d, J = 2.8 Hz, 1H), 6.32 (d, J = 2.0 Hz, 1H), 6.29 (d, J = 2.4 Hz, 1H), 6.25-6.12 (m, 2H), 5.86 (d, J = 16.0 Hz, 1H), 5.38-5.32 (m, 4H), 4.85 (s, 2H), 4.71 (s, 2H), 4.57-4.52 (m, 4H), 4.35 (s, 2H), 4.14 (s, 2H), 3.73-3.58 (m, 8H), 3.02-2.87 (m, 8H), 2.54-2.48 (m, 4H), 2.14-2.09 (m, 8H), 4 OH signals and 2 NH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{23}H_{29}NaN_3O_6$: 466.1954; found: 466.1938.



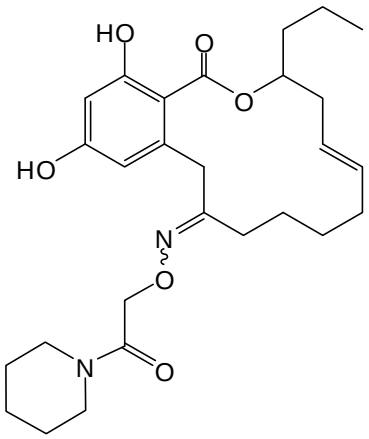
$\mathbf{1}_{A1B3C2D1}$

$\mathbf{1}_{A1B3C2D1}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.94 (brs, 1H), 11.80 (brs, 1H), 6.80 (d, J = 2.4 Hz, 2H), 6.35 (d, J = 2.4 Hz, 2H), 5.45-5.09 (m, 6H), 4.93 (d, J = 14.4 Hz, 2H), 4.82-4.68 (m, 2H), 4.76 (d, J = 14.4 Hz, 2H), 4.28 (d, J = 14.8 Hz, 2H), 4.06 (d, J = 14.4 Hz, 2H), 3.63-3.33 (m, 8H), 2.34-1.96 (m, 10H), 1.70-1.55 (m, 12H), 1.45-1.29 (m, 10H), 1.75 (s, 3H), 1.25 (s, 3H), 0.94 (t, J = 7.2 Hz, 6H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{28}H_{38}NaN_2O_6$: 521.2627; found: 521.2630.



$1_{A1B3C4D1}$

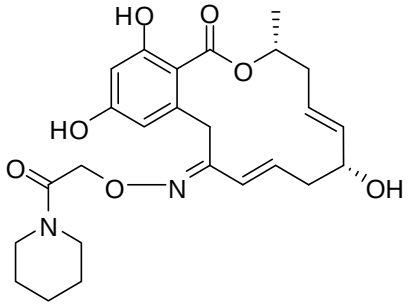
$1_{A1B3C4D1}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.92 (brs, 1H), 11.84 (brs, 1H), 7.08-7.05 (m, 2H), 6.35-6.33 (m, 2H), 5.50-5.20 (m, 6H), 4.87 (s, 2H), 4.73 (s, 2H), 4.44-4.35 (m, 4H), 3.92 (s, 2H), 3.60-3.49 (m, 4H), 3.44-3.33 (m, 4H), 2.47-2.38 (m, 4H), 2.21-1.99 (m, 8H), 1.81-1.76 (m, 6H), 1.70-1.62 (m, 14H), 2 OH signals are not visible; HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{25}H_{33}N_2O_6$: 457.2338; found: 457.2339.



$1_{A1B2C2D1}$

$1_{A1B2C2D1}$: Mixture of isomers 1:1. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.61 (brs, 1H), 11.57 (brs, 1H), 7.86 (brs, 1H), 7.64 (brs, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.51 (d, J = 2.4 Hz, 1H), 6.34 (brd, J = 2.0 Hz, 2H), 5.46-5.32 (m, 4H), 5.28-5.22 (m, 2H), 4.90 (d, J = 14.8 Hz, 1H), 4.83 (d, J = 14.4 Hz, 1H), 4.72 (d, J = 14.4 Hz, 1H), 4.71 (d, J = 14.8 Hz, 1H), 4.35 (d, J = 16.0 Hz, 1H), 4.25-4.17 (m, 3H), 4.09 (d, J = 15.2 Hz, 1H), 3.62-3.51 (m, 4H), 3.43-3.38 (m, 4H), 2.67-2.61 (m, 2H), 2.35-2.28 (m, 2H), 2.06-1.95 (m, 4H), 1.90-1.78 (m, 6H), 1.68-1.58 (m,

14H), 1.44-1.31 (m, 11H), 0.94 (m, 6H). HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{27}H_{39}N_2O_6$: 487.2808; found: 487.2806.

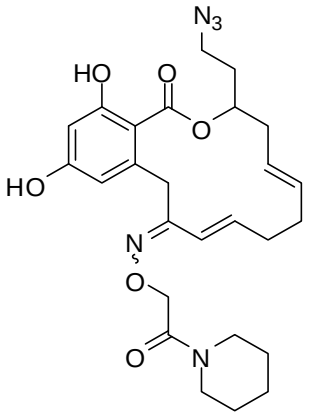


$1_{A1B5C1D1}$

$1_{A1B5C1D1\text{-}1}$: ^{1}H (Z-isomer, $CDCl_3$, 400 MHz, 25 °C) δ 10.4 (s, 1H), 6.67 (d, *J* = 14.3 Hz, 1H), 6.55 (d, *J* = 2.3 Hz, 1H), 6.31 (d, *J* = 2.4 Hz, 1H), 5.90-5.81 (m, 1H), 5.66 (dt, *J* = 16.0, 7.9 Hz, 1H), 5.54-5.39 (m, 2H), 4.78 (s, 2H), 4.17 (m, 2H), 3.82 (d, *J* = 14.6 Hz, 1H), 3.56-3.51 (m, 2H), 3.40-3.38 (m, 2H), 2.47-2.41 (m, 2H), 2.35-2.29 (m, 2H), 1.64-1.56 (m, 6H), 1.46 (d, *J* = 2.4 Hz, 3H), 2 OH signals are not visible.

^{1}H (E-isomer, $CDCl_3$, 400 MHz, 25 °C) δ 6.98 (d, *J* = 2.49 Hz, 1H), 6.30 (d, *J* = 2.5 Hz, 1H), 5.95 (d, *J* = 16.1 Hz, 1H), 5.85-5.77 (m, 1H), 5.67 (dt, *J* = 16.0, 7.9 Hz, 1H), 5.32-5.43 (m, 2H), 4.79 (d, *J* = 3.9 Hz, 2H), 4.17 (m, 2H), 3.82 (d, *J* = 14.6 Hz, 1H), 3.56-3.51 (m, 2H), 3.40-3.38 (m, 2H), 2.47-2.41 (m, 2H), 2.35-2.29 (m, 2H), 1.64-1.56 (m, 6H), 1.46 (d, *J* = 2.4 Hz, 3H), 3 OH signals are not visible.

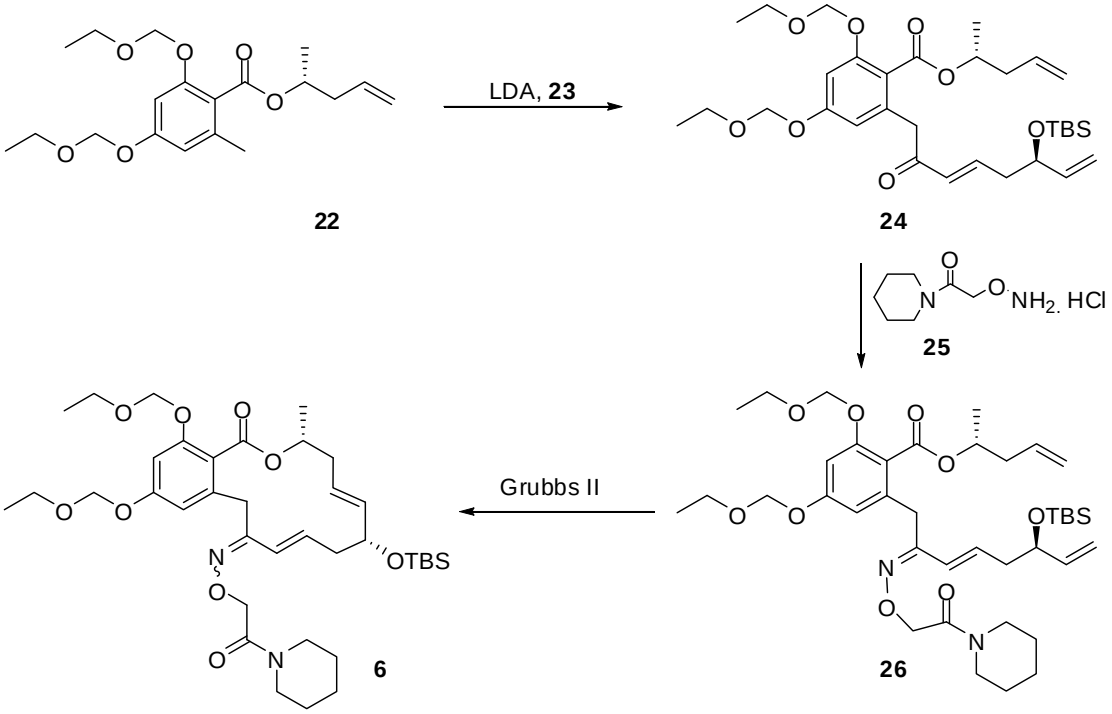
$1_{A1B5C1D1\text{-}1}$: ^{1}H (E-isomer, MeOD, 400 MHz, 25 °C) δ 8.43 (s, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 6.24 (d, *J* = 2.4 Hz, 1H), 6.05 (d, *J* = 16.4 Hz, 1H), 5.75-5.60 (m, 2H), 5.51-5.47 (m, 1H), 5.50-5.44 (td, *J* = 15.6, 5.6 Hz, 1H), 4.88 (d, *J* = 16.4 Hz, 2H), 4.44 (d, *J* = 16.4 Hz, 1H), 3.54-3.51 (m, 2H), 3.36-3.34 (m, 2H), 2.66-2.58 (m, 2H), 2.35-2.31 (m, 2H), 2.04-1.96 (m, 2H), 1.64-1.53 (m, 6H), 1.44 (d, *J* = 6.4 Hz, 3H), two OH signals are not visible.



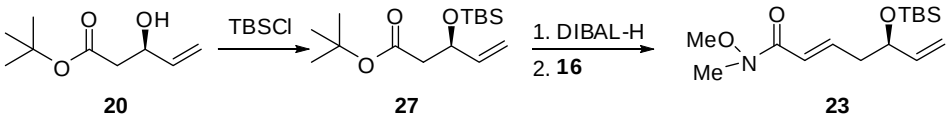
$1_{A1B1C7D1}$

$1_{A1B1C7D1}$: HRMS (MALDI-TOF) m/z $[M + H]^+$ calcd for $C_{26}H_{34}N_5O_6$: 512.2431; found: 512.2406.

General Scheme for the synthesis of 6:



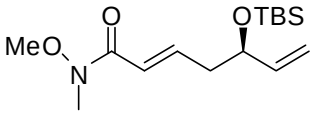
Synthesis of Weinreb amide 23.



Synthesis of TBS protected alcohol 27. To the solution of the previously prepared **20** (8.35g, 48.5 mmol) in vinyl acetate (120 mL) was added Amano lipase PS-C II (750mg, 15 mg/mmol) at 30 °C.[2] The reaction was stirred for 60 hours. After filtration, the solution was concentrated and the crude was purified by flash chromatography (petroleum ether/EtOAc, 15/1 to 5/1) to give the desired compound (3.86 g) in a 46% yield. To a solution of this chiral alcohol (3.75 g, 21.7 mmol) in DMF (60 mL) at 0 °C under nitrogen atmosphere, was added imidazole (2.96 g, 43.5 mmol, 2.0 equiv.) and TBSCl (3.93 g, 26.0 mmol, 1.2 equiv), then the reaction was allowed to warm to 23 °C and stirred for 5 hours. The reaction was then quenched with sat. NH_4Cl_{aq} solution (100 mL) and extracted with EtOAc (100 mL x 3), the combined organic phases were washed with brine (100 mL) and dried over anhydrous Na_2SO_4 (10.0 g). Filtration and evaporation of the solvents under reduced pressure followed

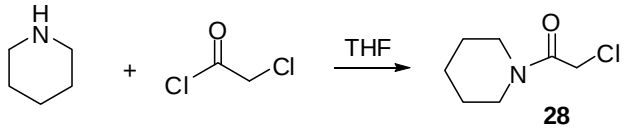
[2] C.-H. Tan, A. B. Holmes *Chem. Eur. J.* **2001**, *7*,1845

by flash chromatography (silica gel, 1/50 EtOAc/petroleum ether) afforded desired compound **27** (5.85 g) in a 93% yield. R*f* = 0.39 (silica gel, EtOAc/petroleum ether 1:8); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ 5.86 (ddd, *J* = 15.9, 10.4, 5.2 Hz, 1H), 5.20 (dd, *J* = 16.0, 2.8 Hz, 1H), 5.05 (dd, *J* = 10.4, 2.8 Hz, 1H), 4.56-4.51 (m, 1H), 2.46 (dd, *J* = 14.8, 7.6 Hz 1H), 2.34 (dd, *J* = 14.8, 6.0 Hz 1H), 1.44 (s, 9H), 0.88 (s, 9H), 0.05 (2 x s, 6H). ^{13}C NMR ($CDCl_3$, 400 MHz, 25 °C) δ 170.3, 140.5, 114.4, 80.4, 70.9, 44.8, 28.1 (x 3), 25.8 (x 3), 18.1, -4.4, -5.0.



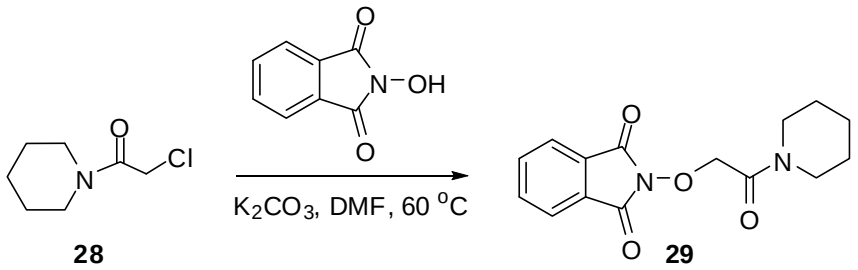
23

Synthesis of TBS protected hydroxyWeinreb amide 23. To a solution of **27** (5.85 g, 20.4 mmol) in toluene (100 mL) DIBAL (24.5 mL, 1M in toluene, 1.2 equiv) was added at -78 ^{o}C and the reaction was kept stirring at the same temperature for half an hour. Then an aqueous saturated tartrate salt solution (100 mL) was added to the reaction and stirred for 2 hours until the mixture became clear. The two phases were separated and extracted with CH_2Cl_2 (100 mL x 2), washed with brine (100 mL x 2) and dried over Na_2SO_4 (10 g). After removal of the solvent, the residue (4.33 g) obtained was used for the next step without any further purification. To a solution of the previously obtained aldehyde (4.33 g) in CH_2Cl_2 (100 mL) was added N-methoxy-N-methyl-2-(triphenylphosphoranylidene)acetamide (7.33 g, 20.2 mmol, 1.0 equiv) at 23 ^{o}C. The reaction was stirred overnight. Evaporation of the solvents under reduced pressure followed by flash chromatography (silica gel, 1/20, 1/10, 1/3 EtOAc/petroleum ether) afforded the desired compound **23** (4.25 g) in a 71 % yield over two steps. R*f* = 0.20 (silica gel, EtOAc/petroleum ether 1:9); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ 6.95 (dt, *J* = 15.2, 7.2 Hz, 1H), 6.41 (d, *J* = 15.6 Hz, 1H), 5.84 (ddd, *J* = 15.9, 10.4, 5.2 Hz, 1H), 5.18 (dd, *J* = 16.0, 2.8 Hz, 1H), 5.05 (dd, *J* = 10.4, 2.8 Hz, 1H), 4.26 (q, *J* = 6.0 Hz 1H), 3.67 (s, 3H), 3.22 (s, 3H), 2.42 (t, *J* = 7.2 Hz, 2H), 0.88 (s, 9H), 0.03 (2 x s, 6H).^{13}C ($CDCl_3$, 400 MHz, 25 °C) δ 166.6, 143.5, 140.6, 120.9, 114.3, 72.7, 61.6, 41.4, 32.3, 25.8 (x 3), 18.2, -4.5, -4.9.



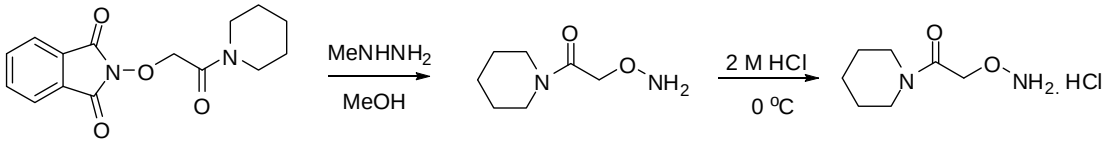
28

Synthesis of compound 28: To a solution of piperidine (51.9 mL, 525 mmol, 2.1 equiv) in THF (1000 mL) was added chloroacetyl chloride (19.9 mL, 250 mmol, 1.0 equiv) slowly at 0 ^{o}C under nitrogen atmosphere. The reaction was allowed to warm to 23 ^{o}C, and stirred for 1 hour. The mixture was then extracted with EtOAc (2 x 500 mL) from sat. NH_4Cl_{aq} solution, the combined organic layers were washed with brine (300 mL), dried over anhydrous Na_2SO_4. After filtration and evaporation under reduced pressure, the crude product (36.4 g) was used directly for the next step without further purification: ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 4.06 (s, 2H), 3.54 (t, *J* = 5.5 Hz, 2H), 3.43 (t, *J* = 5.2 Hz, 2H), 1.70-1.60 (m, 4H), 1.60-1.51 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) 164.9, 47.5, 43.4, 41.3, 26.4, 25.5, 24.4.



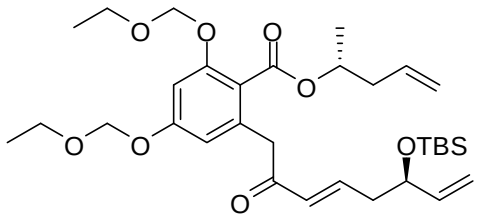
Synthesis of compound 29: To a suspension of N-hydroxyphthalimide (44.0 g, 270 mmol, 1.2 equiv) in DMF (600 mL), K_2CO_3 (46.6 g, 337 mmol, 1.5 equiv) was added in portions. Then a solution of **28** (36.4 g, 225 mmol, 1.0 equiv) in DMF (50 mL) was added by syringe. The reaction was heated to 60-65 ^{o}C for 3 hours. Then, the solvent was evaporated under reduced pressure, and the residue obtained was dissolved in CH_2Cl_2 (200 mL x 3) and

washed with sat. NH_4Cl_{aq} solution (200 mL). The combined organic layers were washed with brine (200 mL) and dried over Na_2SO_4. The desired product precipitated during the evaporation, after filtration, gave **29** as the white solid in 70% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 7.86-7.71 (m, 4H), 4.84 (s, 2H), 3.63 (t, *J* = 5.3 Hz, 2H), 3.57 (t, *J* = 5.4 Hz, 2H), 1.74-1.65 (m, 4H), 1.65-1.57 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) 164.0, 163.0 (x 2), 134.7 (x 2), 128.8 (x 2), 123.7 (x 2), 75.9, 46.9, 43.1, 26.3, 25.3, 24.5.



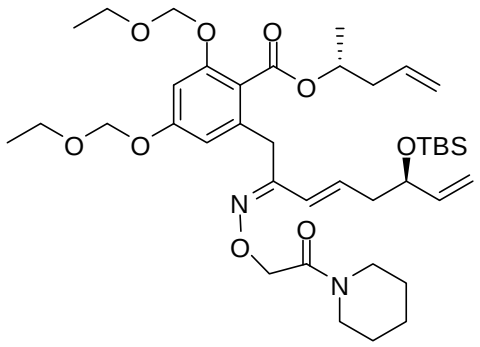
29 **30** **25**

Synthesis of hydroxylamine 25. To a suspension of **29** (38.9 g, 135 mmol, 1.0 equiv) in MeOH (270 mL) was added $MeNHNH_2$ (7.48 mL, 142 mmol, 1.05 equiv) by syringe at 0 °C. The reaction was allowed to warm up to 23 °C and stirred for 1 hour. The solvent was evaporated under reduced pressure, water (150 mL) was added and the precipitated obtained was filtered. After removal of water, the crude product was redissolved in MeOH (150 mL), concentrated HCl (25.0 mL, 12 mmol/mL, 2.0 equiv) was added dropwise at 0 °C. After stirring for 1 hour, the solvent was evaporated and the residue obtained was recrystalized from MeOH and ether to give **25** as a white solid (20.2 g, 77%). ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 4.89 (bs, 4H), 3.60 (t, *J* = 5.4 Hz, 2H), 3.34 (t, *J* = 5.4 Hz, 2H), 1.77-1.68 (m, 2H), 1.68-1.56 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz, 25 °C) 167.4, 71.5, 46.6, 44.0, 27.3, 26.6, 25.3.



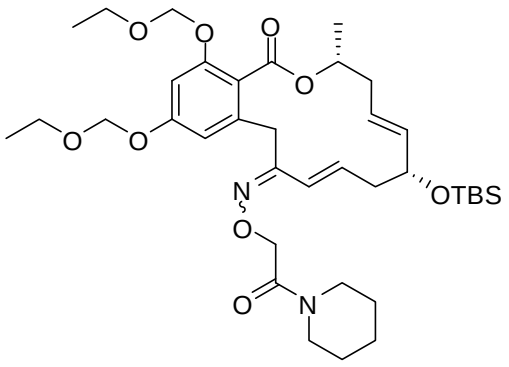
24

Synthesis of ketone 24. A solution of ester **22** (3.29 g, 9.3 mmol) in anhydrous THF (60 mL) at -78 ^{o}C was treated with freshly prepared LDA (0.56 M, 18.6 mmol, 2.0 equiv) via cannula. After 3min, a solution of Weinreb amide **23** (2.70 g, 8.8 mmol, 0.95 equiv) in THF (3 mL) at -78 ^{o}C was added by syringe. The resulting mixture was then stirred for 15 minutes and the reaction was quenched by addition of sat. NH_4Cl_{aq} solution. Upon warming to 23 ^{o}C, the reaction mixture was extracted with EtOAc (30 mL x 2), and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (petroleum ether/EtOAc, 8/1) to give **24** (2.3 g) in 42% yield. R_f = 0.39 (EtOAc/petroleum ether 1:9); ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 6.85 (dt, J = 15.2, 7.2 Hz, 1H), 6.80 (d, J = 2.4 Hz, 1H), 6.48 (d, J = 2.4 Hz, 1H), 6.16 (d, J = 15.2 Hz, 1H), 5.85-5.76 (m, 2H), 5.18 (s, 2H),5.15 (s, 2H), 5.11-5.03 (m, 5H), 4.25-4.23 (m, 1H), 3.91 (d, J = 16.4 Hz, 1H), 3.83 (d, J = 16.4 Hz, 1H), 3.71-3.65 (m, 4H), 2.45-2.31 (m, 4H), 1.27 (d, , J = 6.4 Hz, 3H), 1.22-1.17 (m, 6H), 0.87 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, 25 °C) δ 195.7, 167.1, 159.0, 156.2, 143.9, 142.2, 140.4, 134.8, 133.8, 131.2, 117.5, 114.5, 114.0, 111.2, 102.5, 93.5, 93.0, 72.6, 71.0, 64.3, 60.9, 44.7, 41.3, 40.1, 25.7 (x 3), 23.1, 19.4, 15.0, -4.5, -4.9.



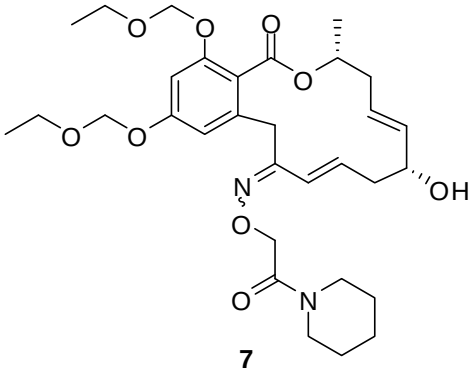
26

Synthesis of oxime 26. To a solution of ketone **2** (9.50 g, 16.3 mmol) in pyridine (35 mL) at 40 ^{o}C was added the corresponding hydroxyl amine **25** (6.20 g, 32.5 mmol, 2.0 equiv) and the reaction was stirred overnight. After evaporation of the pyridine, the residue was dissolved in CH_2Cl_2 (300 mL) and washed with sat. NH_4Cl_{aq} (100 mL x 2), brine (150 mL) and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure followed by flash chromatography (silica gel, 0-30% EtOAc/petroleum ether) afforded desired compound **26** (9.40 g, 80%) as a mixture of *E*/*Z* isomers in the ratio of 1 /1. R_f = 0.23 (EtOAc/petroleum ether 3:7); ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 6.76 (d, *J* =1.6 Hz, 1H), 6.75 (d, *J* =1.6 Hz, 1H), 6.73 (d, *J* =16.0 Hz, 1H), 6.52 (d, *J* =1.6 Hz, 1H), 6.41 (d, *J* =1.6 Hz, 1H), 6.12 (d, *J* =16.0 Hz, 1H), 6.10 (dt, *J* =16.0, 7.0 Hz, 1H), 6.04 (dt, *J* =16.0, 7.0 Hz, 1H), 5.87-5.79 (m, 2H), 5.70-5.73 (m, 2H), 5.23-5.18 (m, 2H), 5.15-5.12 (m, 10H), 5.10-5.05 (m, 4H), 4.98-4.90 (m, 4H), 4.74 (d x 2, *J* =12.0 Hz, 4H), 4.08-4.05 (dt, *J* =12.0, 6.0 Hz, 2H), 3.82 (s, 2H), 3.71-3.63 (m, 8H), 3.56 (m, 2H), 3.51 (m, 2H), 3.41 (m, 2H), 3.27 (m, 2H), 2.47-2.41 (m, 2H), 2.37-2.20 (m, 6H), 1.62-1.56 (m, 12H), 1.33 (d, *J* = 6.8 Hz, 3H), 1.32 (d, *J* = 6.8 Hz, 3H), 1.23-1.18 (m, 12H), 0.84 (s, 9H), 0.83 (s, 9H), -0.05 (s, 3H), -0.06 (s, 3H), -0.07 (s, 3H), -0.07 (s, 3H).



6

Synthesis of compound 6. A solution of **26** (9.40 g, 12.8 mmol) in toluene (450 mL) was degassed for 20 min under nitrogen atmosphere and then heated to 80ºC. Catalyst Grubbs' II (545 mg, 0.64 mmol, 0.05 equiv.) was added and stirred at 80ºC for 5 hours. After cooling down to 23 ºC, the reaction was treated with DMSO (3 mL, 60 equiv. to catalyst) for 1 day. The mixture was passed through silica pad, and washed with petroleum ether/EtOAc 1:1, and then1:2. The combined filtrates were concentrated under reduced pressure and the residue was purified by flash chromatography (petroleum ether/EtOAc, 2/1) to afford **6** (9.0 g, quantitative) as a mixture of *E*/*Z* isomers in the ratio of 1 to 1. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ 6.78 (d, *J* =17.6 Hz, 1H), 6.70 (s, *J* =1.6 Hz, 1H), 6.69 (d, *J* =1.6 Hz, 1H), 6.62 (d, *J* =1.6 Hz, 1H), 6.53 (d, *J* =1.6 Hz, 1H), 6.08 (d, *J* =16.8 Hz, 1H), 5.79-5.70 (m, 2H), 5.36-5.34 (m, 4H), 5.18-5.05 (m, 10H), 4.80 (s x 2, 4H), 3.86-3.83 (m, 4H), 3.72-3.66 (m, 12H), 3.58 (m, 2H), 3.49 (m, 2H); 3.43-3.38 (m, 4H), 2.45-2.13 (m, 8H), 1.63-1.55 (m, 12H), 1.41 (d x 2, *J* =6.4 Hz, 6H), 1.23-1.17 (m, 12H), 0.84 (2 x s, 18H), 0.01 (s x 2, 6H), -0.01 (s x 2, 6H).

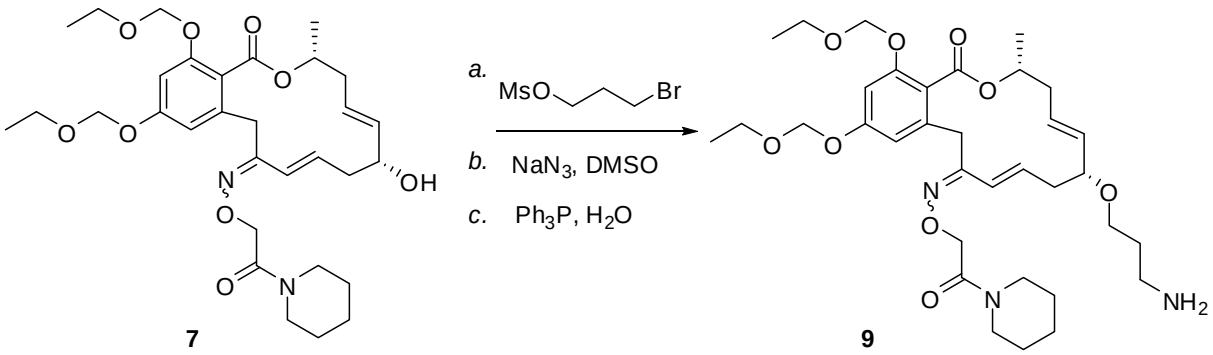


7

Deprotection of the TBS group, synthesis of compound 7. A solution of **6** (140 mg, 0.2 mmol) in THF (3.0 mL) was treated with a solution of TBAF in THF (0.3 mL, 1M in THF, 1.5 equiv) at 0 ^{o}C. The reaction was allowed to reach room temperature and was stirred for 3 hours. Then the mixture was extracted from sat. NH_4Cl_{aq} solution with EtOAc (10 mL x 3), washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated. Flash chromatography column (EtOAc) afforded desired compound **7** (104 mg) in 88% yield. Mixture of two isomers (1:1) ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C) δ = 6.81 (d, *J* = 16.5 Hz, 1H), 6.70 (s, 2H), 6.62 (s, 1H), 6.54 (s, 1H), 6.10 (d, *J* = 16.1 Hz, 1H), 5.82-5.64 (m, 2H), 5.55-5.44 (m, 2H), 5.44-5.34 (m, 2H), 5.18 (s × 2, 8H), 5.15-5.07 (m, 2H), 4.80 (s, 4H), 4.47 (d, *J* = 15.7 Hz, 1H), 3.97-3.89 (m, 2H), 3.71 (q, *J* = 6.8 Hz, 4H), 3.68 (q, *J* = 6.8 Hz, 4H), 3.60-3.35 (m, 10H), 3.12 (d, *J* = 15.7 Hz, 1H), 2.48-2.24 (m, 6H), 2.22-2.19 (m, 2H), 1.69-1.50 (m, 12H), 1.42 (d, *J* = 6 Hz, 6H), 1.22 (t, *J* = 6.9 Hz, 6H), 1.19 (t, *J* = 6.9 Hz, 6H), 2 OH signals are not visible; ^{13}C NMR (100 MHz, $CDCl_3$, 25°C) δ = 168.1, 168.0, 167.1, 166.9, 159.4, 159.2, 157.6, 155.8, 155.6, 154.7, 137.9, 137.5, 136.5, 135.9 (× 4), 129.6, 129.5, 127.6, 120.5 (× 2), 109.2, 108.5, 101.9, 101.8, 93.7 (× 2), 93.5, 93.3, 73.8, 73.6, 72.7, 72.4, 71.0, 70.9, 64.7 (× 2), 64.6 (× 2), 46.2, 46.1, 43.1 (× 2), 40.8, 40.3, 39.9 (× 2), 35.1 (× 2), 29.1, 26.6, 25.7, 25.6, 24.7 (× 2), 20.5 (× 2), 15.3 (× 2), 15.2 (× 2).

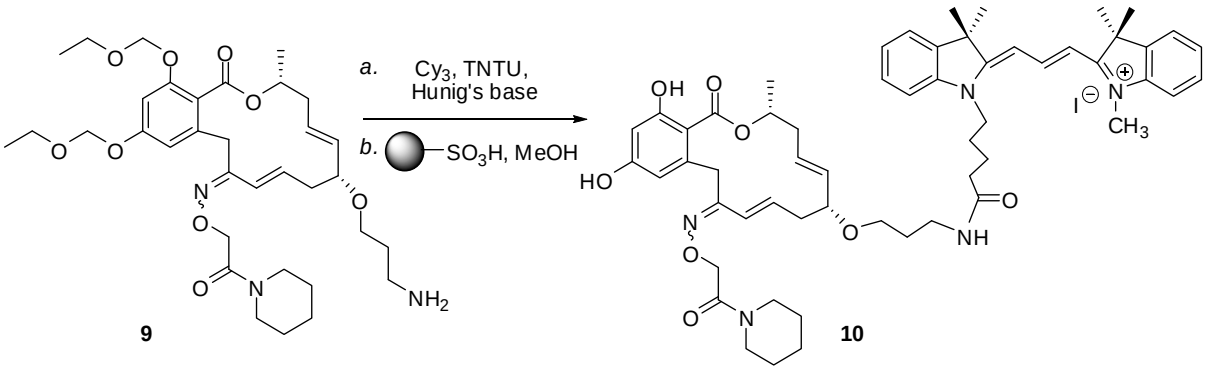
Deprotection of the EOM groups, synthesis of compound $\mathbf{1_{A1B5C1D1}}$ 1-4 To a solution of **7** (100 mg) were added 10 equiv of sulfonic acid polystyrene resin (sulfonic acid resin MP, 70-90 mesh, 3.0 mmol/g, Novabiochem, 01-64-0432) and the suspension was stirred for 4 hours at room temperature. The mixture was then filtered, and passed through a pad of silica gel. The four single isomers were separated on an HPLC (20-80% CH_3CN in Water gradient in 50 min, flow: 2 mL/min, Discovery^{R} HS C18, 5µm, 5 cm x 10.0 mm). The two first fractions were assigned by nmr (vide supra) to be Z isomers of the oxime and the two last fractions E

isomers by analogy to the nmr data of pochoxime A that was assigned inequivolcaly by x-ray. [1] The NMR spectra of fractions 1 and 2 were indistinguiseble as well as the NMR spectra of fractions 3 and 4 making imposible for us to determine the stereochemistry of the hydroxyl group.

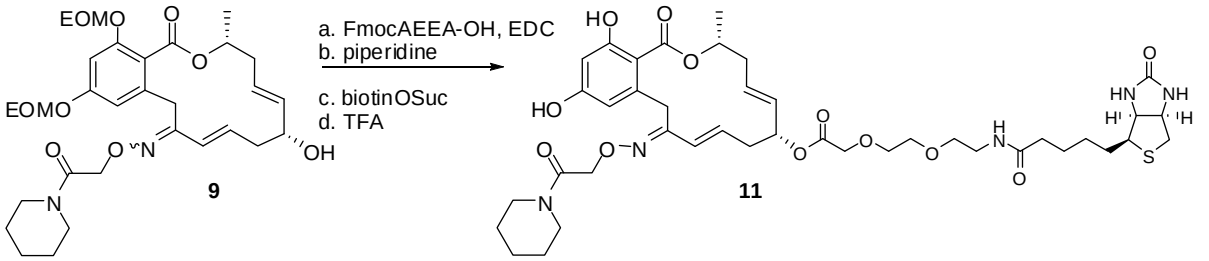


7 9

Preparation of amino-substituted macrocycle 9. To the solution of alcohol **7** (52 mg, 0.088 mmol) in THF (1.0 mL) at 0 ^{o}C under nitrogen atmosphere, NaH (15.4 mg, 0.64 mmol, 7.2 equiv) was added and the reaction stirred for 30 min at this temperature. Then Bu_4NI (34 mg, 0.1mmol, 1.1 equiv) and bromide (90 mg, 0.41 mmol, 4.7 equiv) were added sequentially. The reaction was warmed up slowly and then heated to 40^{o}C for 4 hours. The mixture was then quentched with sat. NH_4Cl_{aq} and extracted with EtOAc. The organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue obtained was submitted to the next step without further purification. To a solution of the crude mixture obtained previously in DMSO (1.5 mL) was added NaN_3 (65 mg, 1.0 mmol, 11 equiv) at 60 ^{o}C and stirred for 2 hours. The reaction was quentched with sat. NH_4Cl_{aq} and extracted with EtOAc; the organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4, Filtration and evaporation of the solvents under reduced pressure followed by filtration in a short pad of silica (silica gel, 1/1 EtOAc/petroleum ether) afforded crude azido substituted macrocycle (around 30 mg, 50% yield over two steps). To a solution of this compound (30 mg, 0.044 mmol) in THF/H_2O (0.9/0.1 mL) was added triphenyl phosphine (23 mg, 0.088 mmol, 2.0 equiv) at 40^{o}C and the reaction was stirred for 24 hours. Evaporation followed by flash chromatography (silica gel/ petroleum ether/EtOAc 1/1 then MeOH/NEt_3, 20/1) afforded the desired macrocycle **9** in 54% yield (16 mg, 0.024 mmol). HRMS (MALDI-TOF) m/z $[M + Na]^+$ calcd for $C_{34}H_{51}N_3O_9Na$: 668.3523; found: 668.3327.



Synthesis of Cy3 labeled macrocycle 10. To a solution of amine **9** (11 mg, 0.017 mmol) in DMF (1 mL) was added TNTU (10 mg, 1.35 equiv), Hunig's base (20 μL, 3.0 equiv), and fluorophore (15 mg, 1.5 equiv) sequentially at 0 ^{o}C under nitrogen atmosphere and the reaction was warmed up to 23 ^{o}C and kept stirring for 1 hour. The reaction was concentrated, the residue dissolved in MeOH (2 mL) and then treated with sulfonic acid resin (30 mg, 3.0 mmol/g) at 40 ^{o}C. After stirring for 2 hours, the reaction was filtered, rinsed with MeOH and CH_2Cl_2. The filtrate was concentrated and a purification by preparative TLC (silica gel, CH_2Cl_2/MeOH, 10/1) afforded the desired Cy3-labeled compound **10**. MS (ES) m/z $[M]^+$ calcd for $C_{57}H_{72}N_5O_8$: 954.54; found: 954.53.



Procedure for the synthesis of biotinelated pochoxime 11. To a solution of **6** (100 mg, 0.17 mmol) in CH_2Cl_2 (4 mL) at room temperature were added seqencially FmocAEEA-OH (130 mg 0.34 mmol, 2.0 equiv), EDC (64 mg, 0.34 mmol, 2.0 equiv) and DMAP (2 mg, 0.1 equiv). The mixture was stirred for 22 hours at room temperature, then was diluted with EtOAc and washed with sat. NH_4Cl solution extracted, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Flash chromatography column (silica gel, CH_2Cl_2/EtOAc 6/4) afforded the desired compound **12** (81 mg, 0.17 mmol) in 55% yield. This compound was directly dissolved in 20% piperidine in dimethyl formamide and the mixture was sitirred for 10 min at room temperature. The solvent was then evaporated and the mixture was filtered through a short pad of silica using 10% methanol in CH_2Cl_2 to isolate

the corressponding amine **13**. To a solution of this material in DMF was added biotinOSu (70 mg, 0.20 mmol, 1.2 equiv), and Et_3N (110 μL, 0.8 mmol, 4.6 equiv) dropwise and the mixture was stirred at room temperature for 12 hours. Then, the reaction was evaporated and the crude was purified by flash chromatography (silica gel, 10 % methanol in CH_2Cl_2) to afford the biotinilated EOM protected macrocycle **14** (97 mg, 0.10 mmol) in a 60% yield. This compound was dissolved in a mixture of TFA/cresol 4/1 (400 μL/100 μL) Conversion of the deprotection reaction was followed by LC-MS. Once the reaction was completed (10 minutes) ethyl ether (10 mL) were added and desired compound **11** precipited in a pure form (HPLC) as a white powder (80 mg). MS (ES) m/z $[M]^+$ calcd for $C_{41}H_{57}N_5O_{12}SH$: 844.98; found: 844.92.

HSP90 affinity assay, Fluorescence Polarization Hsp90α Competition Assay. The assays were performed as previously reported[1] according to slight modification of Chiosis et al's protocol[3]. Fluoresceine-GA was purchased from InvivoGen and dissolved in DMSO to form a 1 mM solution. HSP90α was purchased from Stressgen (SPP-776F). The assay buffer contained 20 mM HEPES (K), pH 7.3, 50 mM KCl, 5 mM $MgCl_2$, 20 mM Na_2MoO_4, 0.01% Tergitol® solution Type NP-40, 70% in H_2O (Sigma-Aldrich, NP40S). Before each use, 0.1 mg/mL bovine gamma globulin (BGG; Calbiochem, 345876) and 2 mM DTT (Fluka, 43817) were freshly added. Fluorescence polarizations measurements were performed on a Molecular Devices instrument, reading black 96-well plates (Corning, 3650) from the top of the wells. Measurements were made with excitation at 485 nm and emission at 538 nm with a cutoff of 530nm. The polarization values were calculated using the equation mP = 1000 x $[(I_S-I_{SB}) - (I_P-I_{PB})]/ [(I_S-I_{SB}) + (I_P-I_{PB})]$, where I_S is the parallel emission intensity, I_P is the perpendicular emission intensity and I_{SB} and I_{PB} are the values for the background. Stocks solutions of the compounds were made in DMSO at concentrations of 10 mM. The drugs were serially diluted over a threefold dilution in assay buffer starting from 30 μM, and 1 μM. GA-FITC and Hsp90 were added at 5 and 25 nM concentrations respectively. Total reaction mixture was of 100 μL. The plates were shaked at 4 ^oC for 5 h in the dark and then the FP values were recorded. A window of 100 mP was observed between wells containing protein and tracer and wells containing tracer only. The measured FP values (mP) were plotted

[3] J. Kim, S. Felts, L. Llauger, H. He, H. Huezo, N. Rosen, G. Chiosis, *J. Biomol. Screen.* **2004**, *9*, 375

against the competitor concentration. EC50 values were determined as the competitor concentration where 50 % of GA was displaced.

Client protein depletion assay

The protein depletion assays were performed as previously described (W. Xu, E. Mimnaugh, M. F. Rosser, C. Nicchitta, M. Marcu, Y. Yarden, L. Neckers, *J. Biol. Chem.* **2001**, *276*, 3702).

Pochoxime – HSP90 cocrystal structure

The N-terminal domain of Hsp90α was cocrystalized in the presence of a 2-fold molar excess of the inhibitors pochoxime A and B. Complete datasets were collected at the ESRF in Grenoble on beamline ID144.

Data processing. Diffraction images were processed with MOSFLM and SCALA (Table S1).

Table S1: Data collection statistics

Inhibitor	**Pochoxime A**	**Pochoxime B**
Spacegroup	I222	I222
Unit cell parameters [Å]	68.05, 90.69, 98.75	67.20, 89.62, 98.39
Resolution [Å]	19.30-1.75 (1.84-1.75)	20.00-1.75 (1.84-1.75)
# Unique reflections	31086 (4497)	30015 (4338)
Wilson B	24.8	15.9
I/σ(I) 14.5 (2.5)	12.0 (2.2)	14.5 (2.5)
Completeness [%]	99.8 (100.0)	99.3 (99.9)
Multiplicity	4.7 (4.8)	3.3 (3.3)
Rmeas	0.09 (0.60)	0.10 (0.61)

Structure determination. The structures were solved by molecular replacement methods using the structure of the Hsp90 N-terminal domain (PDB ID: 1YER) as a starting model. All water molecules in the vicinity of the nucleotide binding site were removed prior to molecular replacement calculations by our automatic pipeline. Rigid body refinement and

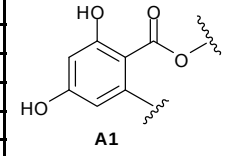
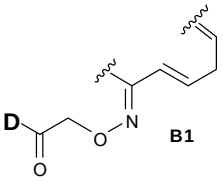
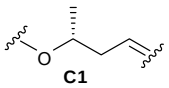
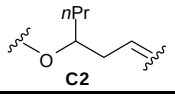
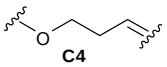
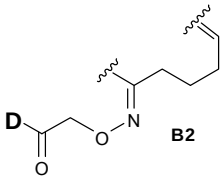
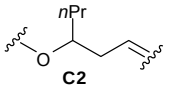
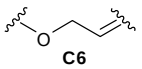
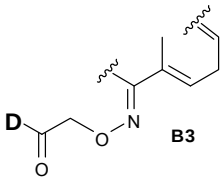
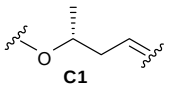
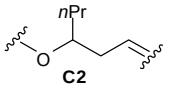
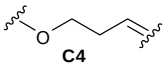
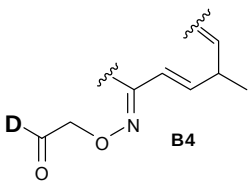
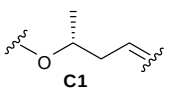
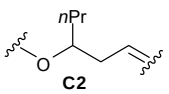
subsequent cycles of alternating manual re-building and maximum likelihood refinement with Refmac5 resulted in the current model (Table S2). The inhibitors could be unambiguously placed in the calculated FoFc difference electron densities. Water molecules were included where stereochemically plausible and difference electron densities of more than 4.0 σ where observed.

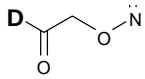
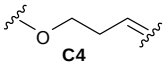
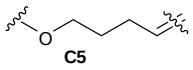
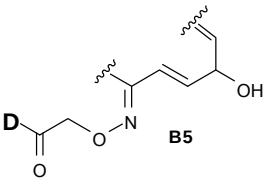
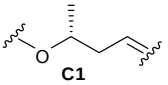
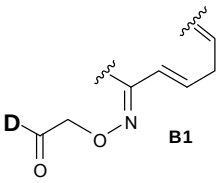
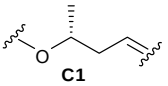
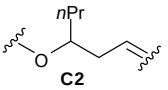
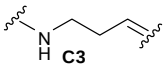
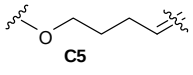
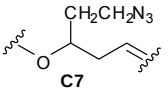
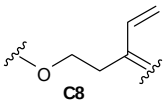
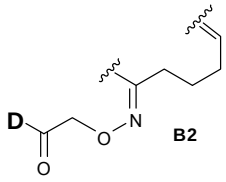
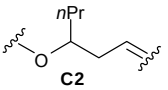
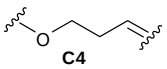
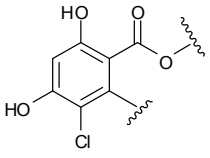
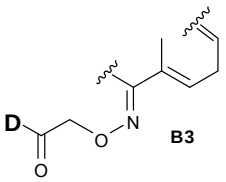
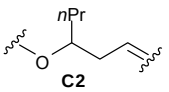
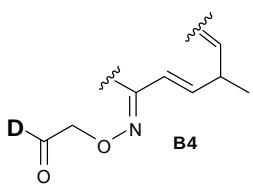
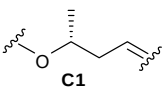
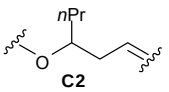
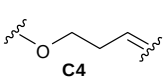
Table S2: Refinement statistics

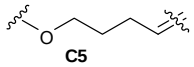
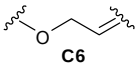
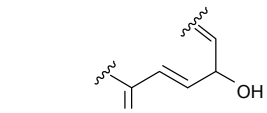
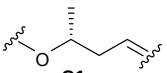
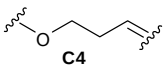
Inhibitor	**Pochoxime A**	**Pochoxime B**
Resolution	20.0-1.95 (2.00-1.95)	20.0-1.75 (1.79-1.75)
Rwork	0.185 (0.251)	0.168 (0.244)
Rfree	0.240 (0.405)	0.217 (0.346)
Completeness [%]	97.2 (97.6)	99.1 (99.9)
r.m.s.d. bonds [Å]	0.020	0.018
r.m.s.d. angles [°]	2.001	1.594

In both structures, single residues at the protein surface are disordered and did not allow an unambiguous placement of their sidechains (e.g. in the vicinity of Gln85, Gln123, Gly177). The only outlier in the Ramachandran plot is Ala166, which displays well defined electron density in both structures. The electron density for peptide segment from Ile104 through Ala111 is also unambiguous.

Table 1 with structures of fragments A-C

entry	compound				affinity (μM)	r^2	PD (μM)
1	 A1	 B1	 C1	D1	0.034	0.996	≤5.0
2				D18	0.540	0.994	
3			 C2	D1	0.588	0.991	>10
4			 C4	D2	0.886	0.997	
5				D3	0.060	0.991	≤0.1
6				D4	0.070	0.983	≤0.5
7				D5	0.243	0.994	<1.0
8				D6	0.238	0.990	<5.0
9				D7	2.240	0.990	>10
10				D8	5.757	0.995	>10
11				D9	0.022*	0.988	≤0.5
12				D10	0.124	0.995	≤0.5
13				D11	0.070	0.998	≤0.5
14				D12	0.640	0.997	≤5.0
15				D13	>10	0.991	
16				D14	0.337	0.995	≤5.0
17				D15	0.128	0.997	≤1.0
18				D16	0.119	0.982	≤1.0
19				D17	0.219	0.998	≤0.5
20				D18	0.196	0.991	≤1.0
21				D19	0.094	0.982	≤5.0
22				D20	0.162	0.993	≤1.0
23				D21	2.992	0.993	>10
24				D22	0.057	0.984	≤1.0
25				D23	0.140	0.991	
26				D24	0.155	0.987	≤5.0
27				D25	0.116	0.980	≤1.0
28				D26	0.372	0.994	≤10
29		 B2	 C2	D1	1.936	0.997	>10
30			 C6		3.027	0.989	>10
31		 B3	 C1	D18	0.200	0.989	≤1.0
32			 C2	D1	0.196	0.994	≤1.0
33				D18	4.663	0.993	>10
34			 C4	D1	0.046	0.976	≤0.5
35		 B4	 C1		0.032	0.910	
36			 C2	D1	0.811	0.988	

37		 B4	 C4		0.021*	0.988	≤0.5
38			 C5		0.587	0.987	≤5.0
39		 B5	 C1	D1	0.020*	0.965	
40		 B1	 C1		0.039	0.996	
41			 C2		0.096	0.994	≤5.0
42			 C3		0.198	0.980	≤1.0
43			 C5	D1	0.182	0.984	≤5.0
44			 C7		0.079	0.931	
45			 C8		0.432	0.985	≤1.0
46		 B2	 C2	D1	0.543	0.981	≤5.0
47			 C4		0.510	0.986	≤10
48	 A2	 B3	 C2	D18	0.900	0.976	>10
49		 B4	 C1		0.154	0.995	≤1.0
50			 C2		1.751	0.998	≤10
51			 C4	D1	0.170	0.976	≤5.0

52			 C5	1.722	0.996	>10
53			 C6	0.599	0.995	>10
54		 B5	 C1	0.010*	0.980	
55			 C4	0.018*	0.980	
56	radicicol			0.156	0.997	
57	17-AAG			0.033	0.978	

* Based on the fact that the assay is performed using 20 nM of HSP90,

IC_{50} below 20 nM can not be reliably measured