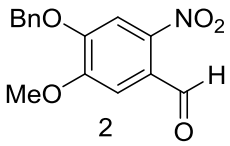
Supporting information

SG3249 synthesis, discovery route

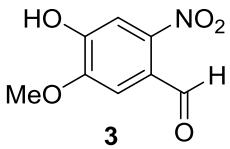
Warning: *Unprotected and N10 enzymatically-cleavable pyrrolobenzodiazepines should be treated as cytotoxic and handled as such.*



2

4-(benzyloxy)-5-methoxy-2-nitrobenzaldehyde (2)

Powdered *O*-benzylvanillin (31 g, 0.128 mol) was added to nitric acid (150 mL) and stirred at 12 °C. After 1 h the reaction mixture was poured into ice, filtered and washed copiously with water to give 35 g (95%) of compound **3** as pale, light-sensitive, yellow powder. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.39 (s, 1H), 7.65 (s, 1H), 7.44–7.35 (m, 6H), 5.25 (s, 2H), 3.91 (s, 3H).

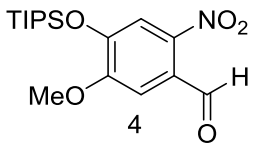


3

4-hydroxy-5-methoxy-2-nitrobenzaldehyde (3) (CAS 2454-72-0)

Crude *O*-benzyl-6-nitrovanillin **2** (140 g, 487 mmol) was suspended in TFA (200 mL) and heated at 85 °C for 1 h. LC/MS on a test sample confirmed completion of the reaction. The mixture was allowed to cool with an ice/water bath. The precipitate was

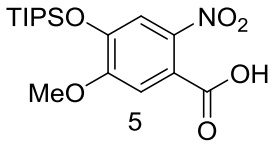
collected by vacuum filtration and washed with diethyl ether and dried to give pure 6-nitrovanillin **3** as a light-sensitive yellow powder. 48 g (50%). LC/MS (4.25 min (ES-) *m/z* (relative intensity) 195.79 ([*M* - H]$^{-\cdot}$, 100)); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.39 (s, 1H), 7.70 (s, 1H), 7.46 (s, 1H), 4.05 (s, 3H).



5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzaldehyde (4)

Neat triisopropylsilylchloride (56.4 mL, 262 mmol, 1.1 eq) was added to a mixture of imidazole (48.7 g, 715 mmol, 3 eq) and the previously prepared 4-hydroxy-5-methoxy-2-nitrobenzaldehyde **3** (47 g, 238 mmol, 1 eq). The mixture was heated until the phenol and imidazole melted to give a homogeneous solution (100 °C). The reaction mixture was allowed to stir for 15 min and was then allowed to cool, whereupon a precipitate was observed to form (imidazole chloride). The reaction mixture was diluted with 5% EtOAc/hexanes and loaded directly onto silica gel. The desired product was eluted with 5% EtOAc/hexanes. The solvent was removed by rotary evaporation under reduced pressure, followed by drying under high vacuum to afford **4** as a crystalline light-sensitive solid (74.4 g, 88 %)**.** LC/MS (4.22 min (ES+) *m/z* (relative intensity) 353.88 ([*M* + H]$^{+\cdot}$, 100)); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.43 (s, 1H), 7.60 (s, 1H), 7.40 (s, 1H), 3.96 (s, 3H), 1.35-1.24 (m, 3H), 1.10 (m, 18H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 187.85 , 155.32 , 149.68 , 143.60 , 125.76 , 116.25 , 110.51 , 56.17 , 17.76 , 12.86 .

HRMS (ESI) m/z Calc. $C_{17}H_{27}NO_5Si$ 353.17313, found 353.17365. FT-IR (ATR, cm^{-1}) 2867, 1691, 1567, 1510, 1462, 1299, 1223, 1153, 1061, 1009, 882, 840, 683.

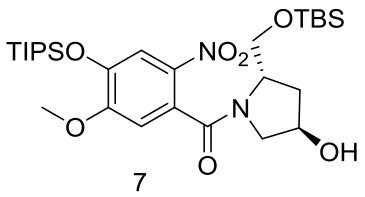


5

5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoic acid (5)

A solution of sodium chlorite (47.3 g, 523 mmol, 80 % technical grade, 2.5 eq) and sodium phosphate monobasic (NaH_2PO_4) (35.2 g, 293 mmol, 1.4 eq) in water (800 mL) was added to a solution of compound **4** (74 g, 209 mmol, 1 eq) in tetrahydrofuran (500 mL) at room temperature. Hydrogen peroxide (60% *w/w*, 140 mL, 2.93 mol, 14 eq) was immediately added to the vigorously-stirred biphasic mixture. The reaction mixture evolved gas, the starting material dissolved and the temperature to 45 °C. After 30 min, LC/MS analysis revealed completion. The reaction mixture was cooled in an ice bath and the pH was adjusted to 3 with hydrochloric acid (1 M). The reaction mixture was then extracted with ethyl acetate (1 L) and the organic phase was washed with brine (2 x 100 mL) and dried over magnesium sulphate. The organic phase was filtered and the solvent removed by rotary evaporation under reduced pressure to afford the product **5** in quantitative yield as a yellow waxy solid. Trituration in hexane followed by filtration and drying afforded a whiter, powdery solid. 77 g (100%). LC/MS (3.93 min (ES-) *m/z* (relative intensity) 367.74 ([*M* - H]$^{-\cdot}$, 100)); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (s, 1H), 7.24 (s, 1H), 3.93 (s, 3H), 1.34 – 1.22 (m, 3H), 1.10 (m, 18H). ^{13}C NMR (101 MHz,

Chloroform-*d*) δ 170.62 , 153.91 , 148.49 , 142.40 , 119.20 , 115.96 , 112.24 , 56.10 , 17.77 , 12.86. HRMS (ESI) m/z Calc. $C_{17}H_{27}NO_6Si$ 369.16804, found 369.16751. FT-IR (ATR, cm^{-1}) 2945, 2867, 1704, 1573, 1527, 1463, 1337, 1301, 1224, 1051, 883, 838, 684.

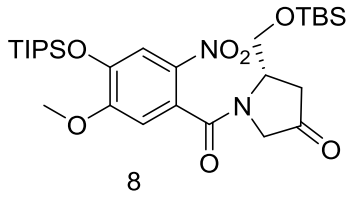


7

((*2S,4R*)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-hydroxypyrrolidin-1-yl)(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)phenyl)methanone (7)

DCC (29.2 g, 141 mmol, 1.2 eq) was added to a solution of acid **5** (43.5 g, 117.8 mmol, 1 eq), and hydroxybenzotriazole hydrate (19.8 g, 129.6 mmol, 1.1 eq) in dichloromethane (200 mL) at 0 °C. The cold bath was removed and the reaction was allowed to proceed for 30 min at room temperature, at which time a solution of C-ring amine **6** (30 g, 129.6 mmol, 1.1 eq) and triethylamine (24.66 mL, 176 mmol, 1.5 eq) in dichloromethane (100 mL) was added rapidly at -10 °C under argon. The reaction mixture was allowed to stir at room temperature for 40 to 60 min and monitored by LC/MS and TLC (EtOAc). The solids were removed by filtration over celite and the organic phase was washed with cold aqueous hydrochloric acid (0.1 M) until the pH was adjusted to 4 or 5. The organic phase was then washed with water, followed by saturated aqueous sodium bicarbonate and brine. The organic layer was dried over magnesium sulphate, filtered and the solvent removed by rotary evaporation under reduced pressure. The residue was subjected to flash chromatography (silica gel; gradient 40/60 *v/v* ethyl acetate/hexane to 80/20 ethyl

acetate/ hexane). The solvent was removed by rotary evaporation under reduced pressure to afford **7** (45.5 g of pure product 66%, and 17 g of slightly impure product, 90% in total). LC/MS 4.43 min (ES+) *m/z* (relative intensity) 582.92 ($[M + H]^{+\cdot}$, 100); $[\alpha]^{20}_{D}$ = -114° (*c* = 0.208, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 (s, 1H), 6.74 (s, 1H), 4.54 (s, 1H), 4.40 (s, 1H), 4.13 (s, 1H), 3.86 (s, 3H), 3.77 (d, *J* = 9.2 Hz, 1H), 3.36 (dd, *J* = 11.3, 4.5 Hz, 1H), 3.14 – 3.02 (m, 1H), 2.38 – 2.28 (m, 1H), 2.10 (ddd, *J* = 13.3, 8.4, 2.2 Hz, 1H), 1.36 – 1.19 (m, 3H), 1.15 – 1.05 (m, 18H), 0.91 (s, 9H), 0.17 – 0.05 (m, 6H). (presence of rotamers). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 166.8, 156.4, 146.1, 145.8, 137.4, 128.3, 116.0, 109.7, 70.5, 62.7, 57.1, 56.1, 55.0, 37.7, 36.5, 25.8, 18.2, 17.8, 12.8, -5.3, -5.5. HRMS (ESI) m/z Calc. $C_{28}H_{50}N_2O_7Si_2$ 582.32293, found 582.32300. FT-IR (ATR, cm^{-1}) 3373, 2948, 2868, 1611, 1572, 1526, 1470, 1337, 1288, 1227, 1121, 1051, 997, 882, 841, 786, 683.

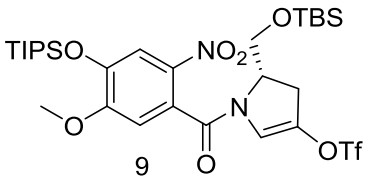


8

(*S*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoyl)pyrrolidin-3-one (8).

TCCA (8.82 g, 40 mmol, 0.7 eq) was added to a stirred solution of **7** (31.7 g, 54 mmol, 1 eq) and TEMPO (0.85 g, 5.4 mmol, 0.1 eq) in dry dichloromethane (250 mL) at 0 °C. The reaction mixture was vigorously stirred for 20 min, at which point TLC analysis

(50/50 ethyl acetate/hexane) revealed complete consumption of the starting material. The reaction mixture was filtered through celite and the filtrate was washed with aqueous saturated sodium bicarbonate (100 mL), aqueous sodium thiosulphate (9 g in 300 mL), brine (100 mL), and dried over magnesium sulphate. Rotary evaporation under reduced pressure afforded **8** in quantitative yield. LC/MS 4.52 min (ES+) *m/z* (relative intensity) 581.08 ([M + H]$^{+\cdot}$, 100); $[\alpha]^{25}_{D}$ = -34.9° (*c* = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.78 – 7.60 (m, 1H), 6.85 – 6.62 (m, 1H), 4.94 (dd, *J* = 30.8, 7.8 Hz, 1H), 4.50 – 4.16 (m, 1H), 3.99 – 3.82 (m, 3H), 3.80 – 3.34 (m, 3H), 2.92 – 2.17 (m, 2H), 1.40 – 1.18 (m, 3H), 1.11 (t, *J* = 6.2 Hz, 18H), 0.97 – 0.75 (m, 9H), 0.15 – -0.06 (m, 6H), (presence of rotamers). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 208.30 , 166.79 , 156.50 , 146.17 , 137.38 , 127.00 , 116.11 , 109.17 , 66.71 , 65.00 , 56.15 , 56.06 , 55.02 , 53.13 , 40.88 , 39.89 , 25.70 , 25.68 , 18.10 , 17.78 , 12.78 , -5.58 , -5.69 , -5.80 , -5.83. HRMS (ESI) m/z Calc. $C_{28}H_{48}N_2O_7Si_2$ 580.30728, found 580.30719. FT-IR (ATR, cm^{-1}) 2947, 2867, 1767, 1652, 1525, 1426, 1338, 1291, 1226, 1101, 1058, 1013, 840, 684

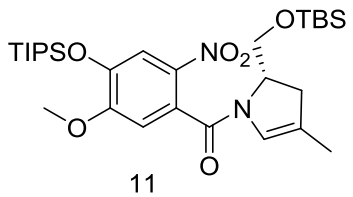


9

(*S*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-1-(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)benzoyl)-4,5-dihydro-1H-pyrrol-3-yl trifluoromethanesulfonate (9).

Triflic anhydride (27.7 mL, 46.4 g, 165 mmol, 3 eq) was injected (temperature controlled) to a vigorously stirred suspension of ketone **9** (31.9 g, 55 mmol, 1 eq) in dry

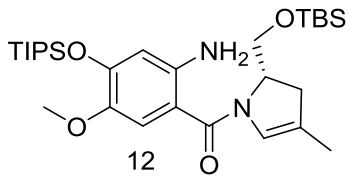
dichloromethane (900 mL) in the presence of 2,6-lutidine (25.6 mL, 23.5 g, 220 mmol, 4 eq, dried over 4 Å molecular sieves) at -50 °C (acetone/dry ice bath). The reaction mixture was allowed to stir for 1.5 h when LC/MS, following a mini work-up (water/dichloromethane), revealed the reaction to be complete. Water was added to the cold reaction mixture and the organic layer was separated and washed with saturated sodium bicarbonate, brine and dried over magnesium sulphate. The organic phase was filtered and the solvent was removed by rotary evaporation under reduced pressure. The residue was subjected to flash column chromatography (silica gel; 10/90 *v/v* ethyl acetate/hexane). Removal of excess eluent afforded the product **9** (37.6 g, 78 %). Reactive intermediate **9** was not stored for long period of times and was rapidly used in the next step. LC/MS, method 2, 4.32 min (ES+) *m/z* (relative intensity) 712.89 ([*M* + H]$^{+\cdot}$, 100); $[\alpha]^{25}_{D}$ = -65.7° (*c* = 2.13, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.71 (s, 1H), 6.75 (s, 1H), 6.05 (d, *J* = 1.8 Hz, 1H), 4.78 (dd, *J* = 9.8, 5.5 Hz, 1H), 4.15 – 3.75 (m, 5H), 3.17 (ddd, *J* = 16.2, 10.4, 2.3 Hz, 1H), 2.99 (ddd, *J* = 16.3, 4.0, 1.6 Hz, 1H), 1.45 – 1.19 (m, 3H), 1.15 – 1.08 (m, 18H), 1.05 (s, 6H), 0.95 – 0.87 (m, 9H), 0.15 – 0.08 (m, 6H). FT-IR (ATR, cm^{-1}) 1750, 1658, 1579, 1524, 1418, 1337, 1278, 1222, 1135, 1063, 823, 758, 609.



11

(*S*)-(2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)(5-methoxy-2-nitro-4-((triisopropylsilyl)oxy)phenyl)methanone (11)

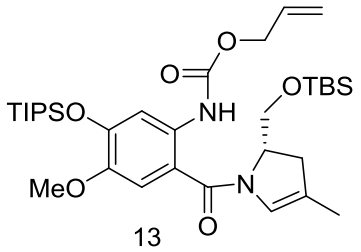
Triphenylarsine (1.71 g, 5.60 mmol, 0.4 eq) was added to a mixture of triflate **9** (10.00 g, 14 mmol, 1 eq), methylboronic acid (2.94 g, 49.1 mmol, 3.5 eq), silver oxide (13 g, 56 mmol, 4 eq) and potassium phosphate tribasic (17.8 g, 84 mmol, 6 eq) in toluene (80 mL) under an argon atmosphere. The reaction was flushed with argon 3 times and bis(benzonitrile)palladium(II) chloride (540 mg, 1.40 mmol, 0.1 eq) was added. The reaction was flushed with argon 3 more times before being rapidly warmed to 70 °C. After 30 min, the reaction was cooled to room temperature, diluted with ethyl acetate/hexane (50/50 *v/v*, 100 mL), decanted, filtered through a pad of celite and further washed with ethyl acetate/hexane. The solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to column flash chromatography (silica gel; 10 % ethyl acetate / hexane). The pure fractions were combined, and excess eluent was removed by rotary evaporation under reduced pressure to afford the product **11** (5.68 g, 70 %). LC/MS, method 2, 4.27 min (ES+) *m/z* (relative intensity) 579.18 ([*M* + H]$^{+\cdot}$, 100); $[\alpha]^{26}_{D}$ = -100.2° (*c* = 0.201, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.70 (s, 1H), 6.77 (s, 1H), 5.51 (d, *J* = 1.7 Hz, 1H), 4.77 – 4.59 (m, 1H), 3.89 (s, 3H), 2.92 – 2.65 (m, 1H), 2.55 (d, *J* = 14.8 Hz, 1H), 1.62 (d, *J* = 1.1 Hz, 3H), 1.40 – 1.18 (m, 3H), 1.11 (s, 9H), 1.10 (s, 9H), 0.90 (s, 9H), 0.11 (d, *J* = 2.3 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 156.16 , 137.74 , 127.15 , 123.15 , 123.01 , 116.05 , 110.29 , 59.02 , 56.05 , 36.75 , 25.83 , 18.21 , 17.80 , 13.74 , 12.79 , -5.30. HRMS (ESI) m/z Calc. $C_{75}H_{101}N_9O_{23}$ 578.32802, found 578.32813. FT-IR (ATR, cm^{-1}) 2927, 1639, 1569, 1523, 1426, 1376, 1333, 1284, 1223, 1056, 1006, 882, 837, 776, 682.



(*S*)-(2-amino-5-methoxy-4-((triisopropylsilyl)oxy)phenyl)(2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrol-1-yl)methanone (12)

Zinc powder (28 g, 430 mmol, 37 eq) was added to a solution of compound **11** (6.7 g, 11.58 mmol) in 5% formic acid in ethanol *v/v* (70 mL) at around 15 °C. The resulting exotherm was controlled using an ice bath to maintain the temperature of the reaction mixture below 30 °C. After 30 min, the reaction mixture was filtered through a pad of celite. The filtrate was diluted with ethyl acetate and the organic phase was washed with water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over magnesium sulphate, filtered and the solvent removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 10/90 *v/v* ethyl acetate/hexane). The pure fractions were collected and combined and the solvent was removed by rotary evaporation under reduced pressure to afford the product **12** (5.1 g, 80 %). Reactive intermediate **12** was not stored for long period of times and was rapidly used in the next steps. LC/MS, method 2, 4.23 min (ES+) *m/z* (relative intensity) 550.21 ($[M + H]^{+\cdot}$, 100); $[\alpha]^{21}_{D}$ = -96.9° (*c* = 0.124, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.72 (s, 1H), 6.24 (s, 1H), 6.16 (s, 1H), 4.72 – 4.54 (m, *J* = 3.8 Hz, 1H), 4.22 (s, 2H), 3.91 (s, 1H), 3.85 – 3.74 (m, 1H), 3.71 (s, 3H), 2.71 (dd, *J* = 16.3, 10.2 Hz, 1H), 2.53 (ddd, *J* = 16.3, 2.7, 1.4 Hz, 1H), 1.67 (d, *J* = 1.2 Hz,

3H), 1.39 – 1.15 (m, 4H), 1.10 (s, 9H), 1.09 (s, 9H), 0.89 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). HRMS (ESI) m/z Calc. $C_{29}H_{52}N_2O_4Si_2$ 548.35384, found 548.35315. FT-IR (ATR, cm^{-1}) 2928, 2865, 1621, 1590, 1509, 1461, 1405, 1374, 1281, 1198, 1115, 1015, 882, 836, 776, 680.

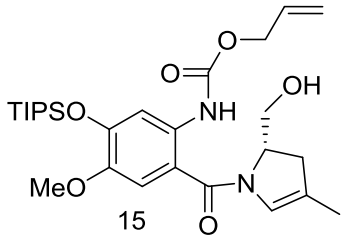


13

(*S*)-allyl-(2-(2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (13)

Allyl chloroformate (0.30 mL, 3.00 mmol, 1.1 eq) was added to a solution of amine **12** (1.5 g, 2.73 mmol) in the presence of dry pyridine (0.48 mL, 6.00 mmol, 2.2 eq) in dry dichloromethane (20 mL) at -78 °C (acetone/dry ice bath). After 30 min, the bath was removed and the reaction mixture was allowed to warm to room temperature. The reaction mixture was diluted with dichloromethane and saturated aqueous copper sulphate was added. The organic layer was then washed sequentially with saturated aqueous sodium bicarbonate and brine. The organic phase was dried over magnesium sulphate, filtered and the solvent was removed by rotary evaporation under reduced pressure to afford the product **13,** which was used in the next step without further purification. LC/MS, method 2, 4.45 min (ES+) *m/z* (relative intensity) 632.91 ([*M* + H]$^{+\cdot}$, 100); $[\alpha]^{21}_D$ = -76° (*c* = 2.14, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 7.83 – 7.66 (m, 1H), 6.77 (s, 1H), 6.17 (s, 1H), 5.94 (ddt, *J* = 17.2, 10.4, 5.7 Hz, 1H), 5.43 – 5.13 (m, 2H), 4.77 – 4.51 (m, 3H), 4.05 – 3.64 (m, 5H), 2.72 (dd, *J* = 16.5,

10.1 Hz, 1H), 2.56 (ddt, *J* = 16.2, 4.4, 1.6 Hz, 1H), 1.78 – 1.59 (m, 3H), 1.43 – 1.19 (m, 3H), 1.11 (d, *J* = 7.3 Hz, 18H), 0.88 (s, 9H), 0.17 – 0.02 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.3, 153.3, 148.2, 145.7, 136.4, 132.7, 132.2, 125.2, 122.2, 117.8, 112.8, 65.6, 62.5, 59.3, 56.4, 36.3, 29.7, 25.8, 18.2, 17.9, 13.8, 12.9, -5.4. HRMS (ESI) m/z Calc. $C_{33}H_{56}N_2O_6Si_2$ 632.37497, found 632.37396. FT-IR (ATR, cm^{-1}) 2928, 2865, 1732, 1621, 1588, 1518, 1462, 1406, 1375, 1320, 1251, 1226, 1196, 1112, 1026, 882, 835, 775, 680.

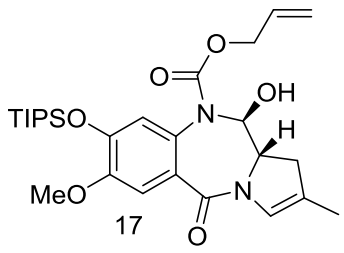


15

(*S*)-allyl-(2-(2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamate (15).

Crude **13** was dissolved in a 7/1/1/2 mixture of acetic acid/methanol/tetrahydrofuran/water (44 mL) and allowed to stir at room temperature. After 3 h, complete disappearance of starting material was observed by LC/MS. The reaction mixture was diluted with ethyl acetate and washed sequentially with water (2 x 500 mL), saturated aqueous sodium bicarbonate (200 mL) and brine. The organic phase was dried over magnesium sulphate, filtered, and excess ethyl acetate removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 25/75 *v/v* ethyl acetate/hexane). The pure fractions were

combined and the eluent was removed by rotary evaporation under reduced pressure to afford the desired product **15** (1 g, 71 %). LC/MS, method 2, 3.70 min (ES+) *m/z* (relative intensity) 519.13 ([*M* + H]$^{+\cdot}$, 95); $[\alpha]^{28}_{D}$ = -84.9° (*c* = 0.20, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.34 (s, 1H), 7.69 (s, 1H), 6.78 (s, 1H), 6.15 (s, 1H), 5.95 (ddt, *J* = 17.2, 10.5, 5.7 Hz, 1H), 5.33 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.23 (ddd, *J* = 10.4, 2.6, 1.3 Hz, 1H), 4.73 (tt, *J* = 7.8, 4.8 Hz, 1H), 4.63 (dt, *J* = 5.7, 1.4 Hz, 2H), 4.54 (s, 1H), 3.89 – 3.70 (m, 5H), 2.87 (dd, *J* = 16.5, 10.5 Hz, 1H), 2.19 (dd, *J* = 16.8, 4.6 Hz, 1H), 1.70 (d, *J* = 1.3 Hz, 3H), 1.38 – 1.23 (m, 3H), 1.12 (s, 10H), 1.10 (s, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 153.3, 148.6, 146.0, 132.6, 132.0, 125.0, 122.6, 118.0, 113.6, 112.7, 66.9, 65.7, 62.0, 56.4, 37.3, 17.9, 13.7, 12.8. HRMS (ESI) m/z Calc. $C_{27}H_{42}N_2O_6Si$ 518.28849 found 518.28784. FT-IR (ATR, cm^{-1}) 2943, 2866, 1731, 1587, 1518, 1461, 1406, 1376, 1323, 1197, 1174, 1112, 1024, 882, 839, 766, 683.

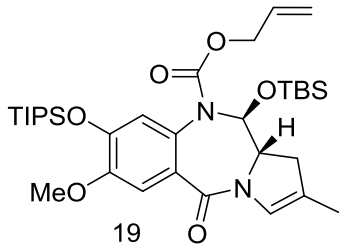


17

(*11S,11aS*)-allyl-11-hydroxy-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (17).

Dimethyl sulphoxide (0.35 mL, 4.83 mmol, 2.5 eq) was added dropwise to a solution of oxalyl chloride (0.2 mL, 2.32 mmol, 1.2 eq) in dry dichloromethane (10 mL) at -78 °C (dry ice /acetone bath) under an atmosphere of argon. After 10 min, a solution of **15** (1 g, 1.93 mmol) in dry dichloromethane (8 mL) was added slowly whilst maintaining the

temperature at -78°C. After 15 min, triethylamine (1.35 mL, dried over 4 Å molecular sieves, 9.65 mmol, 5 eq) was added dropwise and the dry ice/acetone bath was removed. The reaction mixture was allowed to reach room temperature and was washed with cold hydrochloric acid (0.1 M), saturated aqueous sodium bicarbonate and brine. The organic phase was dried over magnesium sulphate, filtered and excess dichloromethane was removed by rotary evaporation under reduced pressure to afford product **17** (658 mg, 66%). LC/MS, method 2, 3.52 min (ES+) *m/z* (relative intensity) 517.14 ($[M + H]^{+\cdot}$, 100); $[\alpha]^{20}_{D}$ = 91.1° (*c* = 0.77, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.20 (s, 1H), 6.75 – 6.63 (m, *J* = 8.8, 4.0 Hz, 2H), 5.89 – 5.64 (m, *J* = 9.6, 4.1 Hz, 2H), 5.23 – 5.03 (m, 2H), 4.68 – 4.38 (m, 2H), 3.84 (s, 3H), 3.83 – 3.77 (m, 1H), 3.40 (s, 1H), 3.05 – 2.83 (m, 1H), 2.59 (d, *J* = 17.1 Hz, 1H), 1.78 (d, *J* = 1.3 Hz, 3H), 1.33 – 1.16 (m, 3H), 1.09 (d, *J* = 2.2 Hz, 9H), 1.07 (d, *J* = 2.1 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0, 156.1, 150.6, 147.8, 131.8, 127.7, 125.7, 123.3, 121.8, 121.5, 118.4, 111.2, 86.1, 67.0, 59.4, 55.5, 38.8, 17.9, 17.8, 13.7, 12.8. HRMS (ESI) m/z Calc. $C_{33}H_{54}N_2O_6Si_2$ 516.27284 found 516.27246. FT-IR (ATR, cm^{-1}) 2943, 2866, 1707, 1618, 1599, 1510, 1462, 1434, 1408, 1304, 1287, 1212, 1036, 881, 812, 730, 682.

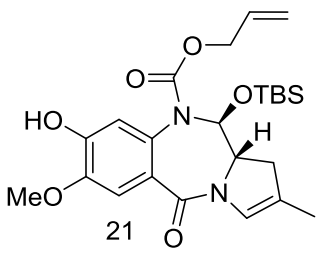


19

(*11S,11aS*)-allyl-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (19)

Tert-butyldimethylsilyltriflate (0.70 mL, 3.00 mmol, 3 eq) was added to a solution of compound **17** (520 mg, 1.00 mmol) and 2,6-lutidine (0.46 mL, 4.00 mmol, 4 eq) in dry dichloromethane (40 mL) at 0 °C under argon. After 10 min, the cold bath was removed and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was washed with water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over magnesium sulphate, filtered and the solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; gradient, 10/90 to 20/80 *v/v* ethyl acetate/hexane). Pure fractions were collected and combined and excess eluent was removed by rotary evaporation under reduced pressure to give the product **19** (540 mg, 85 %). LC/MS, method 2, 4.42 min (ES+) *m/z* (relative intensity) 653.14 ($[M + Na]^{+\cdot}$, 100); $[\alpha]^{22}_{D}$ = 23.8° (*c* = 0.21, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.20 (s, 1H), 6.71 – 6.64 (m, *J* = 5.5 Hz, 2H), 5.83 (d, *J* = 9.0 Hz, 1H), 5.80 – 5.68 (m, *J* = 5.9 Hz, 1H), 5.14 – 5.06 (m, 2H), 4.58 (dd, *J* = 13.2, 5.2 Hz, 1H), 4.36 (dd, *J* = 13.3, 5.5 Hz, 1H), 3.84 (s, 3H), 3.71 (td, *J* = 10.1, 3.8 Hz, 1H), 2.91 (dd, *J* = 16.9, 10.3 Hz, 1H), 2.36 (d, *J* = 16.8 Hz, 1H), 1.75 (s, 3H), 1.31 – 1.16 (m, 3H), 1.12 – 1.01 (m, *J* = 7.4, 2.1 Hz, 18H), 0.89 – 0.81 (m, 9H), 0.25 (s, 3H), 0.19 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 157.6, 155.2, 150.9, 147.6, 136.6, 132.2, 128.1, 126.5, 123.7, 122.0, 120.9, 120.2, 117.7, 111.2, 86.7, 66.5, 61.4, 55.6, 39.1, 25.7, 25.5, 24.4, 17.8, 13.8, 12.7, -4.4, -5.5. HRMS (ESI) m/z Calc. $C_{33}H_{54}N_2O_6Si_2$ 630.35932 found 630.35883. FT-IR (ATR, cm^{-1}) 2946, 2865 1710, 1647,

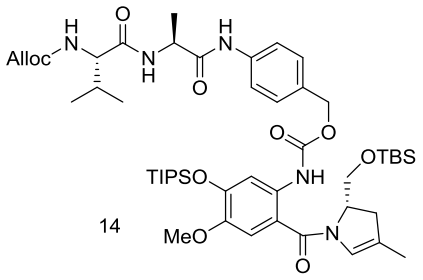
1601, 1512, 1460, 1431, 1406, 1370, 1308, 1244, 1214, 1189, 1116, 1069, 1047, 1019, 881, 837, 815, 778, 768, 685.



(*11S,11aS*)-allyl-11-((tert-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (21)

Lithium acetate (87 mg, 0.85 mmol, 1 eq) was added to a solution of compound **19** (540 mg, 0.85 mmol) in wet dimethylformamide (6 mL, 50:1 DMF/water). After 4 hours, the reaction was complete and the reaction mixture was diluted with ethyl acetate (25 mL) and washed with aqueous citric acid (0.1 M, pH 3), water and brine. The organic layer was dried over magnesium sulphate, filtered and excess ethyl acetate removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; gradient, 25/75 to 75/25 *v/v* ethyl acetate/hexane). Pure fractions were collected and combined and the eluent was removed by rotary evaporation under reduced pressure to give **21** (400 mg, quantitative). LC/MS, method 2, (3.33 min (ES+) *m/z* (relative intensity) 475.26 ($[M+H]^+$, 100). $[\alpha]^{25}_D$ = +60.7° (*c* = 0.201, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 (s, 1H), 6.74 (s, 1H), 6.71 – 6.65 (m, 1H), 6.13 (s, 1H), 5.85 (d, *J* = 9.0 Hz, 1H), 5.76 (ddd, *J* = 12.1, 10.1, 5.2 Hz, 1H), 5.17 – 5.03 (m, 2H), 4.60 (dd, *J* = 13.8, 5.0 Hz, 1H), 4.41 (dd, *J* = 13.7, 5.3 Hz, 1H), 3.92 (s, 3H),

3.75 (td, *J* = 9.6, 3.7 Hz, 1H), 2.91 (ddt, *J* = 16.7, 10.2, 1.9 Hz, 1H), 2.47 – 2.32 (m, 1H), 1.77 (d, *J* = 1.7 Hz, 3H), 0.87 (s, 9H), 0.23 (d, *J* = 17.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 155.3, 148.1, 146.4, 132.2, 129.4, 125.7, 123.8, 121.2, 117.5, 116.4, 110.5, 87.0, 77.5, 77.2, 76.8, 66.5, 61.6, 56.3, 39.2, 25.8, 18.0, 13.9, -4.2, -5.3. HRMS (ESI) m/z Calc. $C_{24}H_{34}N_2O_6Si$ 474.22589 found 474.22674. FT-IR (ATR, cm^{-1}) 2930, 1707, 1631, 1513, 1413, 1277, 1198, 1070, 836, 748, 666.

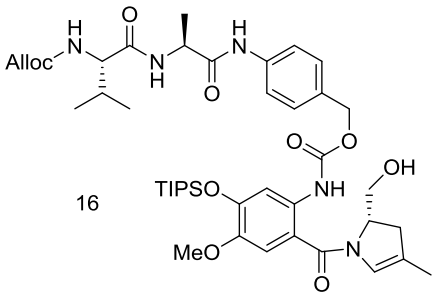


14

Allyl-3-(2-(2-(4-((((2-((S)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl) carbamoyl)oxy)methyl)phenyl)hydrazinyl)propanamido)-4-methyl-2-oxopentanoate (14).

Triethylamine (2.23 mL, 18.04 mmol, 2.2 eq) was added to a stirred solution of the amine **12** (4 g, 8.20 mmol) and triphosgene (778 mg, 2.95 mmol, 0.36 eq) in dry tetrahydrofuran (40 mL) at 5 °C (ice bath). The progress of the isocyanate reaction was monitored by LC/MS analysis by periodically quenching aliquots with methanol. A solution of the alloc-Val-Ala-PAB-OH (4.12 g, 12.30 mmol, 1.5 eq) and triethylamine (1.52 mL, 12.30 mmol, 1.5 eq) in dry tetrahydrofuran (40 mL) was rapidly added to the freshly prepared

isocyanate. The reaction mixture was allowed to stir at 40 °C for 4 h. The solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; gradient, 1/99 to 5/95 *v/v* methanol/dichloromethane).The pure fractions were combined and the eluent was removed by rotary evaporation under reduced pressure to give the product **14** (3.9 g, 50%). LC/MS, method 2, 4.23 min (ES+) *m/z* (relative intensity) 952.36 ($[M + H]^{+\cdot}$, 100); $[\alpha]^{26}_{D}$ = -83.5° (*c* = 0.20, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.62 (br s, 1H), 8.46 (s, 1H), 7.77 (br s, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.5 Hz, 2H), 6.76 (s, 1H), 6.57 (d, *J* = 7.6 Hz, 1H), 6.17 (s, 1H), 6.03 – 5.83 (m, 1H), 5.26 (dd, *J* = 33.8, 13.5 Hz, 3H), 5.10 (s, 2H), 4.70 – 4.60 (m, 2H), 4.58 (dd, *J* = 5.7, 1.3 Hz, 2H), 4.06 – 3.99 (m, 1H), 3.92 (s, 1H), 3.82 – 3.71 (m, 1H), 3.75 (s, 3H), 2.79 – 2.64 (m, 1H), 2.54 (d, *J* = 12.9 Hz, 1H), 2.16 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.67 (s, 3H), 1.46 (d, *J* = 7.0 Hz, 3H), 1.35 – 1.24 (m, 3H), 1.12 (s, 9H), 1.10 (s, 9H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.87 (s, 9H), 0.07 – -0.02 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 170.0, 165.3, 156.5, 153.4, 148.2, 145.7, 137.7, 132.4, 132.2, 129.0, 125.2, 122.3, 119.8, 118.1, 115.7, 112.8, 66.3, 66.1, 62.5, 60.5, 59.2, 56.3, 49.6, 39.0, 36.3, 31.1, 29.7, 29.5, 25.8, 22.7, 19.2, 18.1, 17.9, 13.8, 12.9, -5.4. HRMS (ESI) m/z Calc. $C_{49}H_{77}N_5O_{10}Si_2$ 951.52817 found 951.52502. FT-IR (ATR, cm^{-1}) 3293, 2948, 2930, 2866, 1730, 1703, 1646, 1602, 1518, 1463, 1408, 1376, 1319, 1287, 1248, 1227, 1197, 1113, 1027, 883, 836, 777, 682.

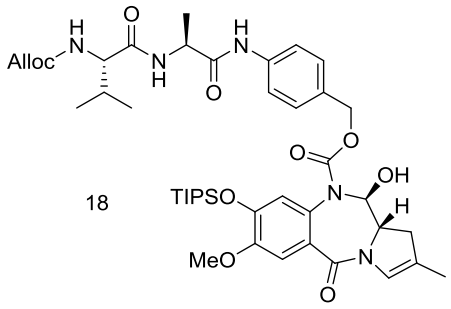


16

Allyl-3-(2-(2-(4-((((2-((*S*)-2-(hydroxymethyl)-4-methyl-2,3-dihydro-1H-pyrrole-1-carbonyl)-4-methoxy-5-((triisopropylsilyl)oxy)phenyl)carbamoyl)oxy)methyl) phenyl)hydrazinyl)propanamido)-4-methyl-2-oxopentanoate (16).

The TBS ether **14** (1.32 g, 1.38 mmol) was dissolved in a 7/1/1/2 mixture of acetic acid/methanol/tetrahydrofuran/water (22 mL) and allowed to stir at room temperature. After 3 h, no more starting material was observed by LC/MS analysis. The reaction mixture was diluted with ethyl acetate (25 mL) and washed sequentially with water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over magnesium sulphate, filtered, and excess ethyl acetate removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 2/98 *v/v* methanol/dichloromethane). Pure fractions were collected and combined and the eluent was removed by rotary evaporation under reduced pressure to afford **16** (920 mg, 80%). LC/MS, method 2, 3.60 min (ES+) *m/z* (relative intensity) 838.18 ($[M+H]^{+\cdot}$, 100). $[\alpha]^{19}_D$ = -83.8° (*c* = 0.207, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.87 (s, 1H), 8.39 (s, 1H), 7.65 (s, 1H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.29 (s, 1H), 7.24 (d, *J* = 7.6 Hz, 1H), 6.78 (s, 1H), 6.12 (s, 1H), 5.88 (ddd, *J* = 16.3, 10.8, 5.4 Hz, 1H), 5.67 (d, *J* = 8.4 Hz, 1H), 5.29 (d, *J* = 17.1 Hz, 1H), 5.19 (d, *J* = 10.5 Hz, 1H), 5.15 – 5.01 (m, 2H), 4.69 (p, *J* = 6.4 Hz, 2H), 4.63 – 4.49 (m, 3H), 4.12 (dt, *J* = 7.7, 3.8 Hz, 1H),

3.90 – 3.65 (m, 5H), 2.92 – 2.76 (m, 1H), 2.30 – 2.17 (m, 1H), 2.17 – 2.02 (m, 1H), 1.67 (d, *J* = 1.6 Hz, 3H), 1.41 (d, *J* = 7.0 Hz, 3H), 1.36 – 1.21 (m, 3H), 1.10 (d, *J* = 7.4 Hz, 18H), 0.93 (dd, *J* = 10.5, 6.8 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.90, 170.34, 166.80, 153.54, 148.46, 132.48, 132.01, 131.66, 129.02, 124.97, 122.78, 119.92, 117.97, 113.82, 112.56, 66.43, 66.22, 66.04, 61.62, 60.41, 56.35, 49.63, 37.25, 31.21, 26.99, 19.21, 17.90, 17.71, 13.70, 12.84. HRMS (ESI) m/z Calc. $C_{43}H_{63}N_5O_{10}Si$ 837.44170 found 837.44050. FT-IR (ATR, cm^{-1}) 3492, 3291, 2962, 2865, 1732, 1713, 1637, 1522, 1410, 1375, 1321, 1173, 1139, 1110, 1049, 1026, 988, 919, 882, 832, 764, 709, 682, 520.

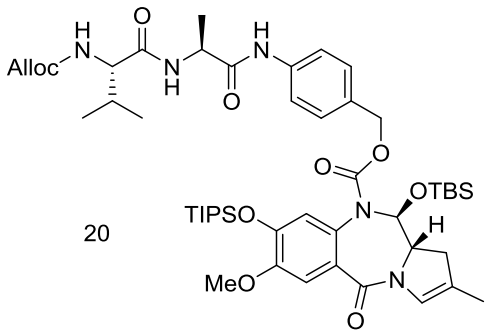


18

(*11S,11aS*)-4-(2-(1-((1-(allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl-11-hydroxy-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (18).

Dimethyl sulphoxide (0.2 mL, 2.75 mmol, 2.5 eq) was added dropwise to a solution of oxalyl chloride (0.11 mL, 1.32 mmol, 1.2 eq) in dry dichloromethane (7 mL) at -78°C (dry ice /acetone bath) under an atmosphere of argon. After 10 min, a solution of **16** (920

mg, 1.10 mmol) in dry dichloromethane (5 mL) was added slowly whilst maintaining the temperature at -78°C. After 15 min, triethylamine (0.77 mL, dried over 4 Å molecular sieves, 5.50 mmol, 5 eq) was added dropwise and the dry ice/acetone bath was removed. The reaction mixture was allowed to reach room temperature and washed with cold hydrochloric acid (0.1 M), saturated aqueous sodium bicarbonate and brine. The organic phase was dried over magnesium sulphate, filtered, and excess dichloromethane was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to column flash chromatography (silica gel; gradient, 2/98 to 5/95 *v/v* methanol/dichloromethane). The pure fractions were combined. Eluent removal by rotary evaporation under reduced pressure afforded **18** (550 mg, 60%). LC/MS, method 2, 3.43 min (ES+) *m/z* (relative intensity) 836.01 ($[M]^{+\cdot}$, 100). $[\alpha]^{21}_{D}$ = +35.4° (*c* = 3.05, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 (s, 1H), 7.52 – 7.40 (m, 2H), 7.21 – 7.08 (m, *J* = 11.5 Hz, 2H), 6.67 (s, 1H), 6.60 – 6.47 (m, *J* = 7.4 Hz, 1H), 5.97 – 5.83 (m, 1H), 5.79 – 5.66 (m, 1H), 5.38 – 4.90 (m, 6H), 4.68 – 4.52 (m, *J* = 18.4, 5.5 Hz, 4H), 4.04 – 3.94 (m, *J* = 6.5 Hz, 1H), 3.87 – 3.76 (m, 5H), 3.00 – 2.88 (m, 1H), 2.66 – 2.49 (m, 2H), 2.21 – 2.08 (m, 2H), 1.76 (s, 3H), 1.45 (d, *J* = 7.0 Hz, 3H), 1.09 – 0.98 (m, *J* = 8.9 Hz, 18H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 170.2, 163.0, 156.5, 156.3, 150.6, 147.9, 137.8, 132.4, 131.6, 128.5, 127.7, 125.7, 123.2, 121.8, 121.6, 119.8, 118.1, 111.2, 86.1, 67.6, 66.1, 60.4, 59.6, 55.5, 49.6, 38.7, 31.1, 29.7, 19.2, 17.8, 17.8, 17.5, 13.7, 12.8. HRMS (ESI) m/z Calc. $C_{43}H_{61}N_5O_{10}Si$ 835.42605 found 835.42340. FT-IR (ATR, cm^{-1}) 3305, 2942, 2866, 1693, 1647, 1601, 1511, 1462, 1410, 1302, 1214, 1117, 1036, 909, 881, 811, 729, 682, 646, 501.



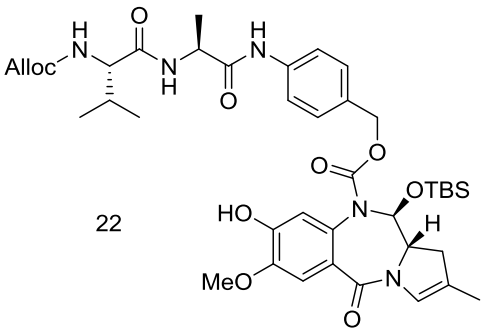
20

(*11S,11aS*)-4-(2-(1-((1-(allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-8-((triisopropylsilyl)oxy)-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (20)

Tert-butyldimethylsilyltriflate (0.38 mL, 1.62 mmol, 3 eq) was added to a solution of compound **18** (450 mg, 0.54 mmol) and 2,6-lutidine (0.25 mL, 2.16 mmol, 4 eq) in dry dichloromethane (5 mL) at 0°C under argon. After 10 min, the cold bath was removed and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was washed with water, saturated aqueous sodium bicarbonate and brine. The organic phase was dried over magnesium sulphate, filtered and excess solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to column flash chromatography (silica gel; 50/50 *v/v* hexane/ethyl acetate). The pure fractions were combined. Eluent removal by rotary evaporation under reduced pressure afforded **20** (334 mg, 65%).

LC/MS, method 2, 4.18 min (ES+) *m/z* (relative intensity) 950.50 ($[M]^{+\cdot}$, 100). $[\alpha]^{21}_{D}$ = 24.7° (*c* = 1.05, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.53 (s, 1H), 8.02 (s, 1H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.21 (s, 1H), 7.08 (d, *J* = 8.2 Hz, 2H), 6.72 – 6.61 (m, *J* = 8.9 Hz, 2H), 6.16 (s, 1H), 5.97 – 5.79 (m, *J* = 24.4, 7.5 Hz, 2H), 5.41 – 5.08 (m, 5H), 4.86 (d,

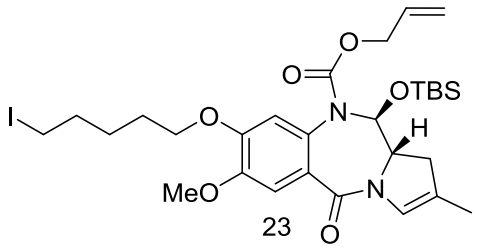
J = 12.5 Hz, 1H), 4.69 – 4.60 (m, 1H), 4.57 (s, 1H), 4.03 (t, *J* = 6.7 Hz, 1H), 3.87 (s, 3H), 3.74 (td, *J* = 9.6, 3.6 Hz, 1H), 2.43 – 2.09 (m, *J* = 34.8, 19.4, 11.7 Hz, 3H), 1.76 (s, 3H), 1.43 (d, *J* = 6.9 Hz, 3H), 1.30 – 1.21 (m, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 8.4 Hz, 3H), 0.84 (s, 9H), 0.23 (s, 3H), 0.12 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.7, 169.7, 163.5, 155.4, 150.9, 147.7, 137.6, 132.4, 132.1, 128.4, 128.2, 126.6, 123.7, 122.0, 120.9, 119.6, 118.2, 111.2, 86.9, 77.3, 77.1, 77.0, 76.8, 76.8, 67.1, 66.2, 61.5, 60.7, 55.6, 49.6, 45.0, 39.1, 31.9, 30.9, 29.4, 25.6, 22.7, 19.2, 17.8, 17.8, 17.8, 17.4, 14.2, 14.1, 13.8, 12.7, 0.0, -4.4, -5.5. HRMS (ESI) m/z Calc. $C_{49}H_{75}N_5O_{10}Si_2$ 949.51252 found 949.50922. FT-IR (ATR, cm^{-1}) 3293, 2948, 2866, 1708, 1648, 1603, 1513, 1462, 1409, 1305, 1279, 1244, 1216, 1072, 882, 838, 820, 781, 683.



22

(*11S,11aS*)-4-(2-(1-((1-(allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl-11-((tert-butyldimethylsilyl)oxy)-8-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (22).

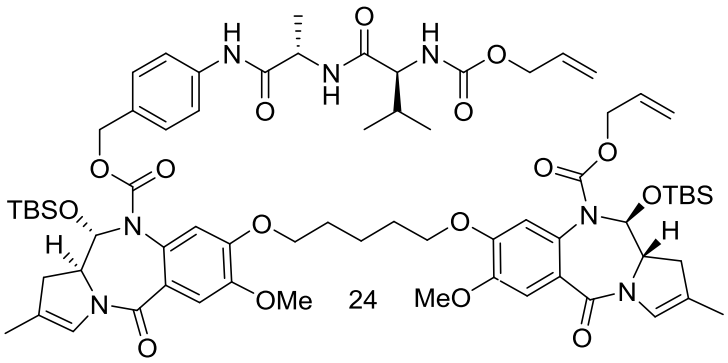
Lithium acetate (50 mg, 0.49 mmol, 1 eq) was added to a solution of **20** (470 mg, 0.49 mmol) in wet dimethylformamide (4 mL, 50:1 DMF/water). After 4 h, the reaction was complete and the reaction mixture was diluted with ethyl acetate and washed with aqueous citric acid (0.1 M, pH 3), water and brine. The organic layer was dried over magnesium sulphate, filtered and excess ethyl acetate was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to column flash chromatography (silica gel; gradient, 50/50 to 25/75 *v/v* hexane/ethyl acetate). The pure fractions were combined. Eluent removal by rotary evaporation under reduced pressure afforded **22** (400 mg, quantitative). LC/MS, method 2, 3.32 min (ES+) *m/z* (relative intensity) 794.18 ($[M+H]^{+\cdot}$, 100). $[\alpha]^{21}_{D}$ = +45° (*c* = 0.79, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.82 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.19 (s, 1H), 7.15 – 6.99 (m, 3H), 6.72 – 6.62 (m, 2H), 6.53 (s, 1H), 5.98 – 5.77 (m, 2H), 5.63 (d, *J* = 8.3 Hz, 1H), 5.29 (d, *J* = 17.1 Hz, 1H), 5.19 (d, *J* = 10.4 Hz, 1H), 5.12 (d, *J* = 12.6 Hz, 1H), 4.86 (d, *J* = 12.6 Hz, 1H), 4.74 – 4.63 (m, 1H), 4.63 – 4.49 (m, 2H), 4.16 – 4.04 (m, 1H), 3.80 (s, 3H), 3.75 (ddd, *J* = 10.2, 8.9, 3.7 Hz, 1H), 2.99 – 2.83 (m, 1H), 2.46 – 2.28 (m, 1H), 2.12 (q, *J* = 6.7 Hz, 1H), 1.77 (d, *J* = 1.5 Hz, 3H), 1.40 (d, *J* = 7.0 Hz, 3H), 1.01 – 0.88 (m, 6H), 0.84 (s, 9H), 0.24 (s, 3H), 0.13 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.79 , 170.28 , 163.44 , 156.57 , 148.15 , 146.49 , 132.47 , 131.91 , 129.20 , 128.15 , 123.64 , 121.27 , 119.81 , 118.00 , 116.46 , 110.41 , 86.91 , 67.14 , 66.05 , 61.46 , 60.48 , 56.08 , 49.65 , 39.06 , 31.88 , 31.12 , 25.62 , 22.69 , 19.25 , 17.85 , 17.77 , 14.12 , 13.79 , -4.33 , -5.41. HRMS (ESI) m/z Calc. $C_{40}H_{55}N_5O_{10}Si$ 793.37910 found 793.37891. FT-IR (ATR, cm^{-1}) 3296, 2957, 1707, 1647, 1515, 1467, 1416, 1300, 1074, 838, 782, 732.



(*11S,11aS*)-allyl 11-((*tert*-butyldimethylsilyl)oxy)-8-((5-iodopentyl)oxy)-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (21).

Diiodopentane (0.63 mL, 4.21 mmol, 5 eq) and potassium carbonate (116 mg, 0.84 mmol, 1 eq) were added to a solution of phenol **21** (400 mg, 0.84 mmol) in acetone (4 mL, dried over 4 Å molecular sieves). The reaction mixture was then warmed to 60 °C and stirred for 6 h. The acetone was removed by rotary evaporation under reduced pressure and the resulting residue was subjected to flash column chromatography (silica gel; 50/50, v/v, hexane/ethyl acetate,). Pure fractions were combined and the eluent was removed to provide **23** (500 mg, 90%). LC/MS, method 2, 3.90 min (ES+) *m/z* (relative intensity) 670.91 ($[M]^+$, 100). $[\alpha]^{28}_D$ = +60.2° (*c* = 0.204, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.23 (s, 1H), 6.69 (s, 1H), 6.60 (s, 1H), 5.87 (d, *J* = 8.8 Hz, 1H), 5.83 – 5.68 (m, *J* = 5.6 Hz, 1H), 5.15 – 5.01 (m, 2H), 4.67 – 4.58 (m, 1H), 4.45 – 4.35 (m, 1H), 4.04 – 3.93 (m, 2H), 3.91 (s, 3H), 3.73 (td, *J* = 10.0, 3.8 Hz, 1H), 3.25 – 3.14 (m, *J* = 8.5, 7.0 Hz, 2H), 2.92 (dd, *J* = 16.8, 10.3 Hz, 1H), 2.38 (d, *J* = 16.8 Hz, 1H), 1.95 – 1.81 (m, 4H), 1.77 (s, 3H), 1.64 – 1.49 (m, 2H), 0.88 (s, 9H), 0.25 (s, 3H), 0.23 (s, 3H). ^{13}C NMR (126 MHz, Chloroform-d) δ 163.4, 155.1, 150.2, 149.1, 132.2, 128.6, 126.1, 123.7, 120.9, 117.3, 114.3, 110.9, 105.0, 87.0, 68.7, 66.3, 61.5, 60.4, 56.2, 39.1, 33.2, 27.9, 27.0, 25.6, 17.9, 13.8, 6.3, -4.2, -5.3. HRMS (ESI) m/z Calc. $C_{29}H_{43}IN_2O_6Si$ 670.20078 found

670.19897. FT-IR (ATR, cm^{-1}) 2951, 2929, 2855, 1710, 1643, 1603, 1513, 1454, 1431, 1407, 1371, 1310, 1271, 1070, 1046, 837, 781.

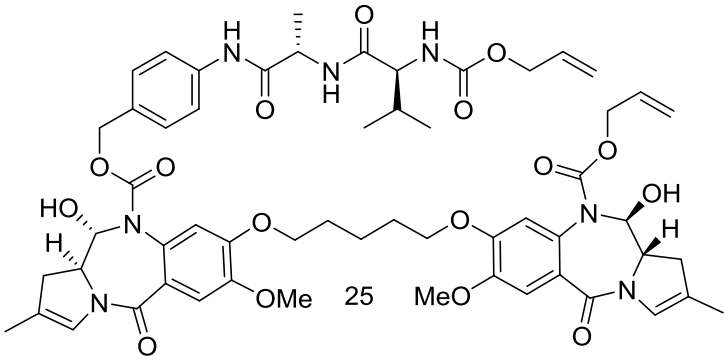


24

(*11S*)-allyl 8-((5-(((11S)-10-(((4-(2-(1-((1-(allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl)oxy)carbonyl)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-((tert-butyldimethylsilyl)oxy)-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (24).

Potassium carbonate (96 mg, 0.693 mmol, 1 eq) was added to a solution of **23** (697 mg, 1.039 mmol, 1.5 eq) and phenol **22** (500 mg, 0.693 mmol) in dry acetone (40 mL). The reaction was degassed with argon and warmed to 65 °C (argon balloon). The reaction was stirred for 6 h, at which point LC/MS analysis showed starting material **22** consumption. The acetone was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 80% ethyl acetate in hexane to 100% ethyl acetate). Pure fractions were combined and the eluent was removed by rotary evaporation under reduced pressure to give the product **24** (800 mg, 86%) and

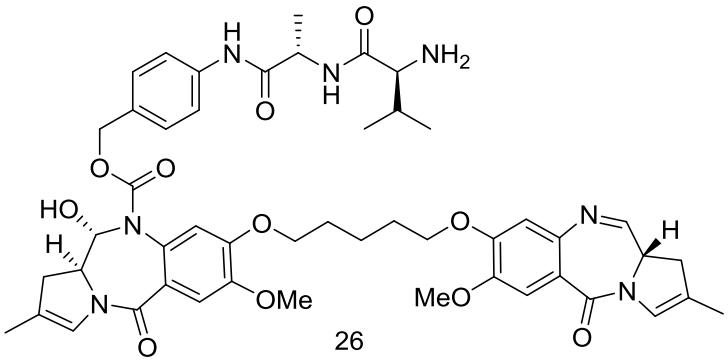
the recovered starting material **23** (250 mg). LC/MS, method 2, 4.07 min (ES+) *m/z* (relative intensity) 1336.55 ($[M+H]^{+\cdot}$, 50). $[\alpha]^{23}_{D}$ = +72° (*c* = 1.096, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.77 (s, 1H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.25 (s, 1H), 7.19 (s, 1H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.81 – 6.65 (m, 3H), 6.63 (s, 1H), 6.42 (s, 1H), 5.99 – 5.69 (m, 4H), 5.47 – 5.15 (m, 4H), 5.15 – 4.97 (m, 2H), 4.81 – 4.49 (m, 5H), 4.41 (dd, *J* = 13.8, 5.2 Hz, 1H), 4.08 – 3.94 (m, 3H), 3.92 – 3.67 (m, 9H), 3.62 (d, *J* = 7.6 Hz, 1H), 2.93 (dd, *J* = 16.8, 10.1 Hz, 2H), 2.38 (d, *J* = 17.1 Hz, 2H), 2.15 (d, *J* = 5.9 Hz, 1H), 1.78 (dd, *J* = 3.1, 1.6 Hz, 10H), 1.62 (d, *J* = 25.4 Hz, 2H), 1.42 (d, *J* = 7.2 Hz, 3H), 1.02 – 0.75 (m, 24H), 0.30 – 0.14 (m, 12H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.4, 170.1, 163.4, 163.4, 156.5, 155.1, 150.3, 148.9, 148.8, 138.0, 132.5, 132.2, 131.9, 128.8, 128.6, 126.0, 126.0, 125.7, 123.7, 121.1, 121.0, 119.6, 118.1, 117.4, 114.2, 114.1, 110.9, 110.9, 110.7, 87.1, 69.0, 68.6, 67.1, 66.4, 66.1, 61.5, 60.6, 60.4, 56.1, 56.0, 49.6, 39.1, 31.0, 28.7, 25.7, 25.6, 22.7, 19.2, 17.9, 17.9, 17.7, 13.8, -4.2, -4.3, -5.2. HRMS (ESI) m/z Calc. $C_{69}H_{97}N_7O_{16}Si_2$ 1335.66031 found 1335.65796. FT-IR (ATR, cm^{-1}) 3295, 2930, 2856, 1710, 1645, 1604, 1513, 1462, 1433, 1410, 1374, 1309, 1272, 1246, 1212, 1073, 1047, 838, 781, 755.



(11S)-allyl-8-((5-(((11S)-10-(((4-(2-(1-((1-(allyloxy)-4-methyl-1,2-dioxopentan-3-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl)oxy)carbonyl)-11-hydroxy-7-methoxy-2-methyl-5-oxo-5,10,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-11-hydroxy-7-methoxy-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (25)

A buffered solution of tetra-*n*-butylammonium fluoride (5.98 mL, 5.98 mmol, 3.5 eq) and acetic acid (391 µL, 6.84 mmol, 4 eq) was added to a solution of **24** (2.285g, 1.71 mmol, 1 eq) in dry tetrahydrofuran (15 mL). The reaction was degassed three times with argon. The starting material was totally consumed after 4 hours according to LC/MS analysis. The reaction mixture was diluted with ethyl acetate (50 mL) and washed sequentially with water, aqueous sodium hydrogen carbonate, and brine. The organic phase was dried over magnesium sulphate and filtered. The solvent was removed by rotary evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; 1% methanol in ethyl acetate). Pure fractions were combined and the eluent was removed by rotary evaporation under reduced pressure to give the product **25** (1.51g, 80%). LC/MS, method 2, 2.87 min (ES+) *m/z* (relative intensity) 1108.11 ($[M+H]^{+\cdot}$, 100). $[\alpha]^{20}_{D}$ = +161° (*c* = 0.075, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.14 (d, *J* = 7.0 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.19 (dd, *J* = 17.4, 8.4 Hz, 3H), 7.07 (s, 1H), 7.05 (s, 1H), 6.80 (s, 1H), 6.73 (s, 1H), 6.71 – 6.57 (m, 4H), 6.01 – 5.71 (m, 2H), 5.58 (s, 2H), 5.29 (dd, *J* = 17.2, 1.9 Hz, 1H), 5.22 – 4.97 (m, 4H), 4.83 (d, *J* = 12.4 Hz, 1H), 4.58 (d, *J* = 13.6 Hz, 1H), 4.52 – 4.33 (m, 4H), 4.03 – 3.85 (m, 4H), 3.79 (d, *J* = 8.0 Hz, 6H), 3.66 (td, *J* = 9.8, 3.3 Hz, 2H), 2.91 (dd, *J* = 17.3, 10.4 Hz, 2H), 2.50 (p, *J* = 1.8 Hz, 2H), 1.90 – 1.67 (m, 10H), 1.56 (d, *J* = 7.8 Hz, 2H),

1.29 (d, *J* = 7.0 Hz, 3H), 0.85 (dd, *J* = 18.5, 6.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.97, 162.05, 155.97, 154.34, 149.80, 148.24, 133.61, 132.88, 128.39, 122.66, 121.35, 118.82, 116.91, 114.36, 110.43, 85.55, 68.45, 64.41, 60.02, 59.86, 55.72, 48.99, 38.38, 30.35, 28.20, 22.25, 19.14, 18.05, 13.42. HRMS (ESI) m/z Calc. $C_{57}H_{69}N_7O_{16}$ 1107.48736 found 1107.48733. FT-IR (ATR, cm^{-1}) 3314, 3013, 2959, 2937, 1698, 1601, 1513, 1464, 1435, 1412, 1305, 1243, 1212, 1115, 1037, 1017, 983, 745, 665, 612.



26

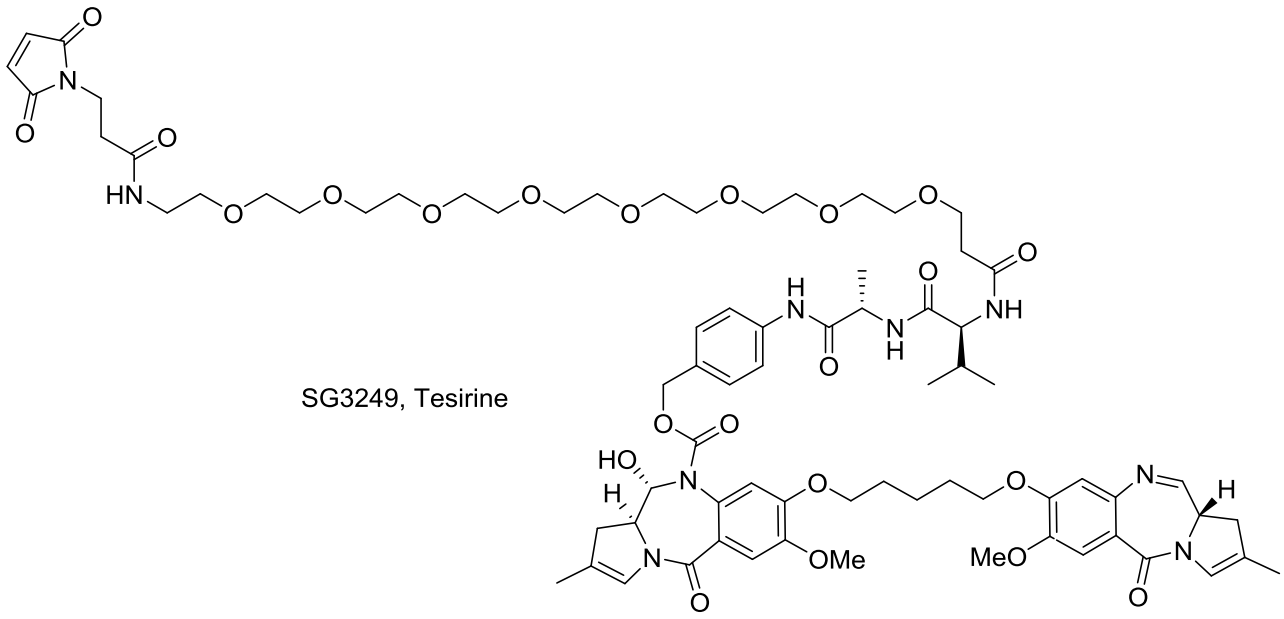
(*11S*)-4-(2-(1-((1-amino-3-methyl-1-oxobutan-2-yl)amino)-1-oxopropan-2-yl)hydrazinyl)benzyl 11-hydroxy-7-methoxy-8-((5-((7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (26).

*Tetrakis***(**triphenylphosphine**)**palladium(0) (55 mg, 0.047 mmol, 0.06 eq) was added to a solution of **25** (874 mg, 0.79 mmol) and pyrrolidine (0.16 mL, 1.975 mmol, 2.5 eq) in dry dichloromethane (50 mL). The reaction was flushed with argon and stirred for 20 min at room temperature, after which the reaction was diluted with dichloromethane and washed sequentially with saturated aqueous ammonium chloride and brine. The organic phase

was dried over magnesium sulphate, filtered and excess dichloromethane removed by rotary evaporation under reduced pressure. The resulting residue **26** was used as a crude mixture for the next reaction. LC/MS, method 2, 2.38 min (ES+) *m/z* (relative intensity) 922.16 ($[M+H]^{+\cdot}$, 40).

An NMR of macrocycle **27** was recorded by leaving a sample of **26** in solution in deuterated chloroform until fully cyclised. (see **Figure 6** below).

^{1}H NMR of **27** (400 MHz, Chloroform-*d*) δ 9.54 (s, 1H), 7.60 (d, *J* = 7.1 Hz, 1H), 7.56 (s, 1H), 7.47 – 7.39 (m, 2H), 7.19 (s, 1H), 7.14 – 7.03 (m, 3H), 6.98 (d, *J* = 5.5 Hz, 1H), 6.70 (s, 1H), 6.41 (s, 1H), 6.23 (s, 1H), 5.70 (d, *J* = 9.8 Hz, 1H), 5.56 (d, *J* = 12.1 Hz, 1H), 4.50 (t, *J* = 7.1 Hz, 1H), 4.42 (d, *J* = 12.1 Hz, 1H), 4.34 (dd, *J* = 11.3, 5.1 Hz, 1H), 4.21 (t, *J* = 5.2 Hz, 1H), 4.18 – 3.96 (m, 2H), 3.94 – 3.76 (m, 8H), 3.67 (s, 1H), 3.28 (dd, *J* = 5.0, 2.2 Hz, 1H), 3.19 – 3.04 (m, 2H), 3.03 – 2.89 (m, 1H), 2.58 (d, *J* = 17.2 Hz, 1H), 2.47 (dd, *J* = 17.3, 5.1 Hz, 1H), 2.17 – 2.01 (m, 2H), 1.92 – 1.68 (m, 9H), 1.55 (s, 2H), 1.35 – 1.14 (m, 4H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.8 Hz, 3H).



(*11S,11aS*)-4-((*2S,5S*)-37-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5-isopropyl-2-methyl-4,7,35-trioxo-10,13,16,19,22,25,28,31-octaoxa-3,6,34-triazaheptatriacontanamido)benzyl 11-hydroxy-7-methoxy-8-((5-(((S)-7-methoxy-2-methyl-5-oxo-5,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yl)oxy)pentyl)oxy)-2-methyl-5-oxo-11,11a-dihydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate SG3249, Tesirine.

1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDCI, 151 mg, 0.79 mmol, 1 eq) was added to a solution of crude **26** (0.79 mmol) and Mal-$(PEG)_8$-acid (468 mg, 0.79 mmol, 1eq) in dry dichloromethane (50 mL). The reaction mixture was degassed three times with argon and stirred for 2 h, at which time the starting material was completely consumed according to LC/MS analysis. The reaction mixture was diluted with dichloromethane and washed sequentially with water and brine. The organic phase was dried over magnesium sulphate, filtered, and excess dichloromethane removed by rotary

evaporation under reduced pressure. The resulting residue was subjected to flash column chromatography (silica gel; gradient, 0/100 to 10/90 *v/v* methanol/chloroform). The pure fractions were combined and the eluent was removed by rotary evaporation under reduced pressure to give **SG3249** (tesirine) (860 mg, 73% over 2 steps). LC/MS, method 2, 2.65 min (ES+) *m/z* (relative intensity) 1496.78 ($[M+H]^{+\cdot}$, 20). $[\alpha]^{24}_{D}$ = +262° (*c* = 0.056, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.20 (d, *J* = 7.0 Hz, 1H), 8.03 (t, *J* = 5.6 Hz, 1H), 7.97 – 7.84 (m, 2H), 7.55 (d, *J* = 8.1 Hz, 2H), 7.32 (s, 1H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.10 – 6.96 (m, 3H), 6.84 (s, 1H), 6.79 – 6.57 (m, 4H), 5.59 (d, *J* = 9.4 Hz, 1H), 5.16 (d, *J* = 12.7 Hz, 1H), 4.81 (d, *J* = 12.4 Hz, 1H), 4.38 (t, *J* = 7.1 Hz, 1H), 4.32 – 4.17 (m, 2H), 4.17 – 4.07 (m, 1H), 4.07 – 3.87 (m, 3H), 3.80 (d, *J* = 14.2 Hz, 6H), 3.74 – 3.62 (m, 1H), 3.59 (t, *J* = 7.2 Hz, 4H), 3.55 – 3.42 (m, 28H), 3.35 (d, *J* = 5.2 Hz, 2H), 3.21 – 3.11 (m, 2H), 3.11 – 2.98 (m, 2H), 2.98 – 2.83 (m, 1H), 2.49 – 2.28 (m, 5H), 2.03 – 1.88 (m, 1H), 1.87 – 1.65 (m, 10H), 1.64 – 1.47 (m, 2H), 1.29 (t, *J* = 5.9 Hz, 3H), 0.85 (dd, *J* = 17.1, 6.7 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.55, 171.29, 171.16, 170.78, 169.91, 164.80, 162.52, 155.03, 150.25, 139.27, 134.99, 128.84, 123.13, 122.67, 121.76, 119.28, 111.93, 110.93, 86.05, 70.21, 70.16, 70.02, 69.95, 69.46, 68.93, 68.79, 67.39, 57.94, 56.16, 54.03, 49.49, 38.97, 38.89, 36.39, 34.53, 34.40, 31.04, 28.69, 28.65, 22.72, 19.60, 18.55, 18.37, 13.88, 13.82. HRMS (ESI) m/z Calc. $C_{75}H_{101}N_9O_{23}$ 1495.70831 found 1495.70444. FT-IR (ATR, cm^{-1}) 3311, 2911, 2871, 1706, 1643, 1623, 1601, 1512, 1435, 1411, 1243, 1213, 1094, 1075, 946, 827, 747, 695, 664.

General information

Reaction progress was monitored by thin-layer chromatography (TLC) using Merck Kieselgel 60 F254 silica gel, with fluorescent indicator on aluminium plates. Visualisation of TLC was achieved with UV light or iodine vapour unless otherwise stated. Flash column chromatography was performed using Merck Kieselgel 60 F254 silica gel. Extraction and chromatography solvents were bought and used without further purification from Fisher Scientific, U.K. All chemicals were purchased from Aldrich, Lancaster or BDH.

^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 spectrometer. Coupling constants are quoted in hertz (Hz). Chemical shifts are recorded in parts per million (ppm) downfield from tetramethylsilane. Spin multiplicities are described as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentuplet) and m (multiplet). Optical rotations were measured at ambient temperature using a Bellingham and Stanley ADP 220 polarimeter. Mass spectrometry was performed on a ThermoQuest Navigator from Thermo Electron, Electrospray (ES) spectra were obtained at 20 to 30 V. All samples were run under electrospray ionisation mode using 50% acetonitrile in water and 0.1% formic acid as a solvent. The instrument was calibrated with [Glu]-Fibrinopeptide B immediately prior to measurement. Accurate mass measurements were performed using a Q-Exactive Orbitrap from ThermoFisher Scientific. Accurate mass calculation were performed using Xcalibur 3.1 software. FTIR was performed using a Bruker Alpha ATR.

A solution of the sample in chloroform was deposited on the crystal, allowed to dry, followed by measurement.

The LC/MS conditions were as follows:

Method 1 (default when not specified)

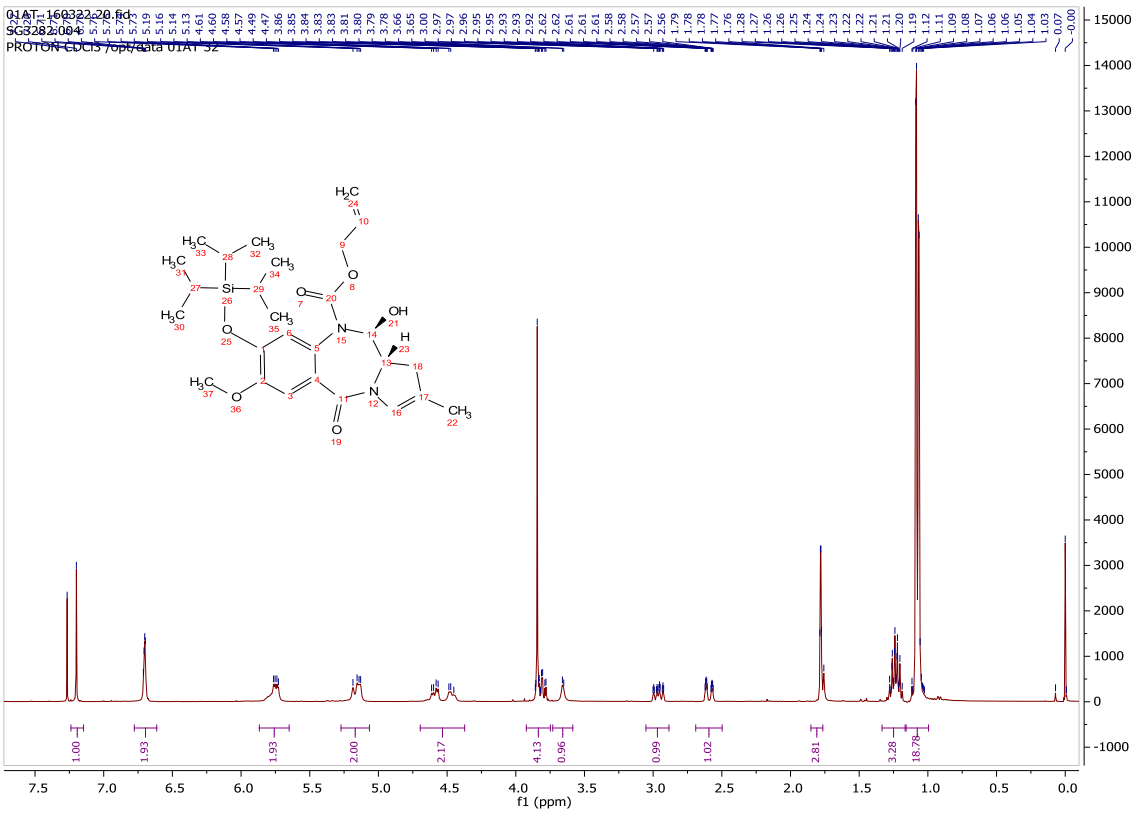
The HPLC (Waters Alliance 2695) was run using a mobile phase of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). Gradient: 5% B held over 1.0 min, then increased from 5% B to 95% B over 3 min. The composition was held for 0.1 min at 95% B, then returned to 5% B in 0.03 min and held for 0.87 min. Total gradient run time of 5 min.

Method 2 (suitable for more lipophilic compounds)

The HPLC (Waters Alliance 2695) was run using a mobile phase of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). Gradient: 5% B held over 1.0 min, then increased from 5% B to 95% B over 2.5 min. The composition was held for 0.5 min at 95% B, then returned to 5% B in 0.1 min and held for 0.9 min. Total gradient run time of 5 min.

Flow rate 3.0 mL/min, 400µL was split *via* a zero dead volume tee piece into the mass spectrometer. Wavelength detection range: 220 to 400 nm. Function type: diode array (535 scans). Column: Phenomenex Onyx Monolithic C18 50 mm x 4.60 mm.

Figure 1: ^{1}H and ^{13}C NMRs of key compound **17**:



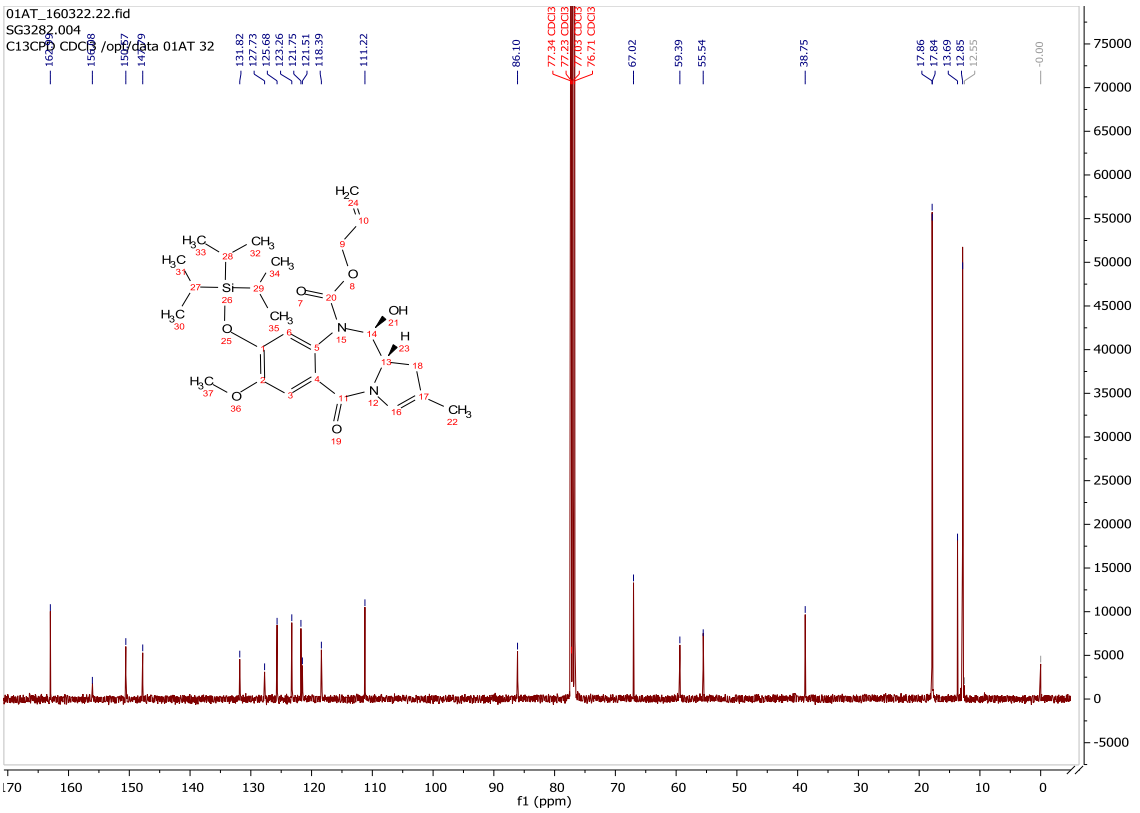


Figure 2: ^{1}H and ^{13}C NMRs of key compound **21**:

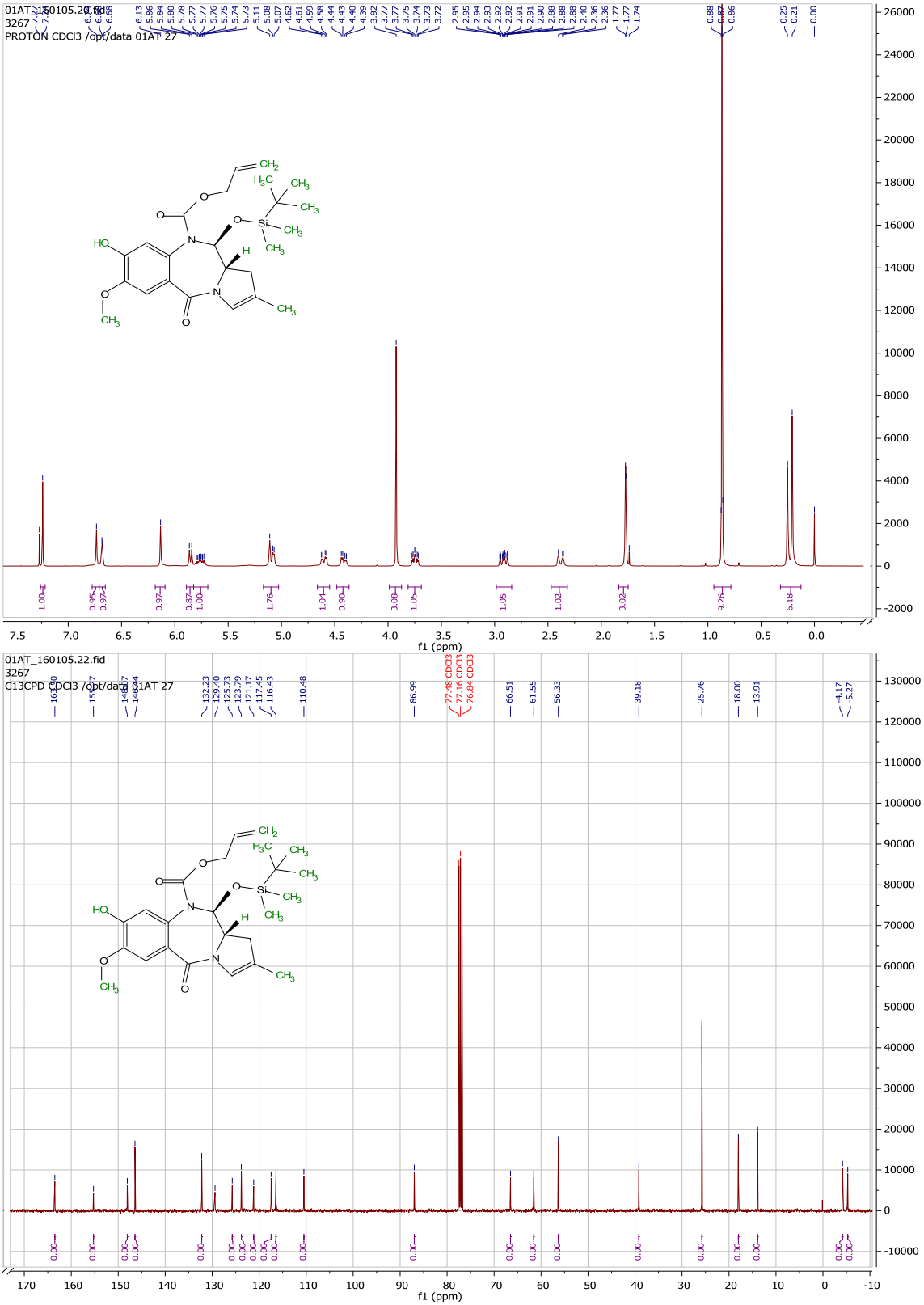


Figure 3: ^{1}H and ^{13}C NMRs of key compound **18**:

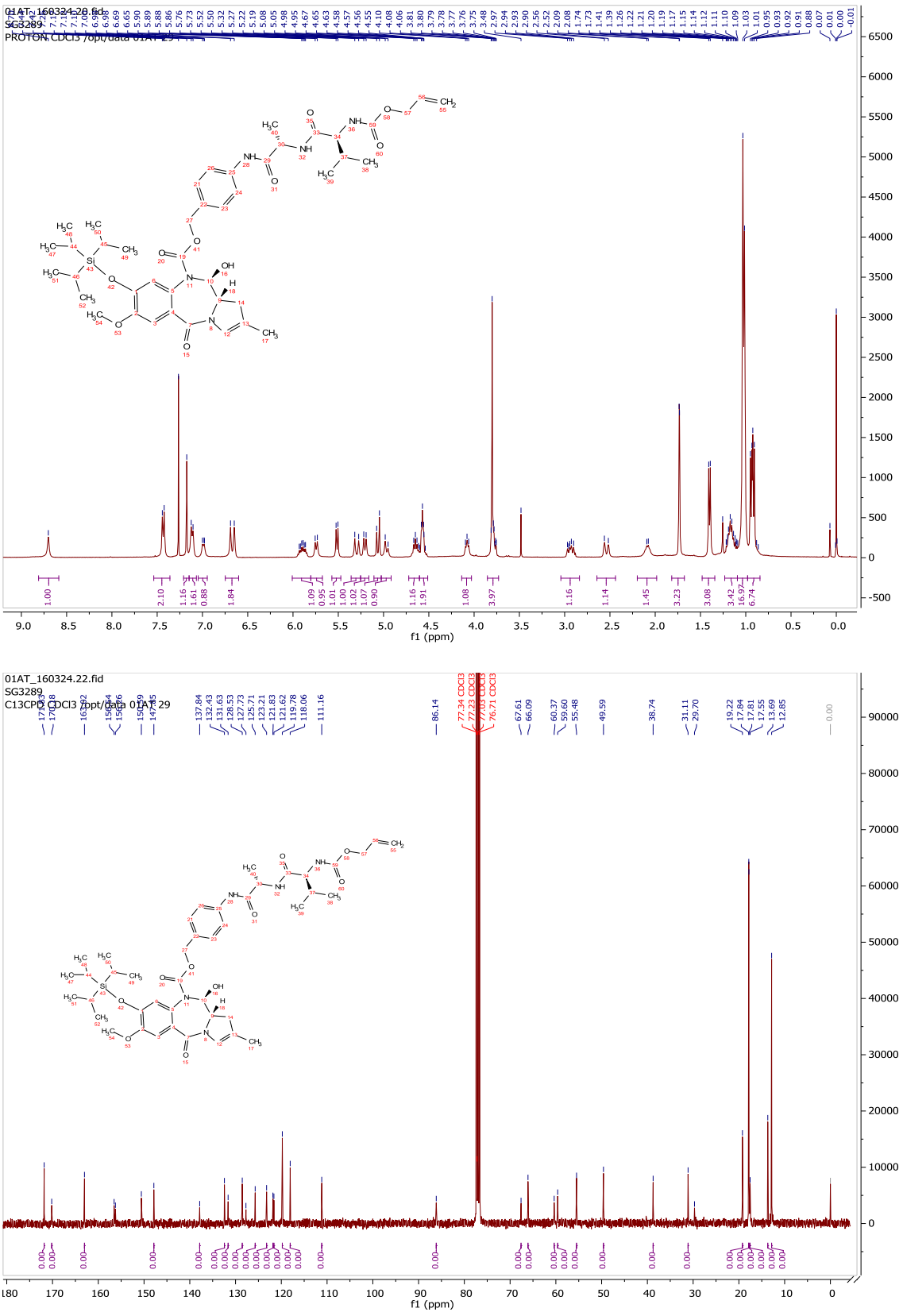


Figure 4: ^{1}H and ^{13}C NMRs of key compound **22**:

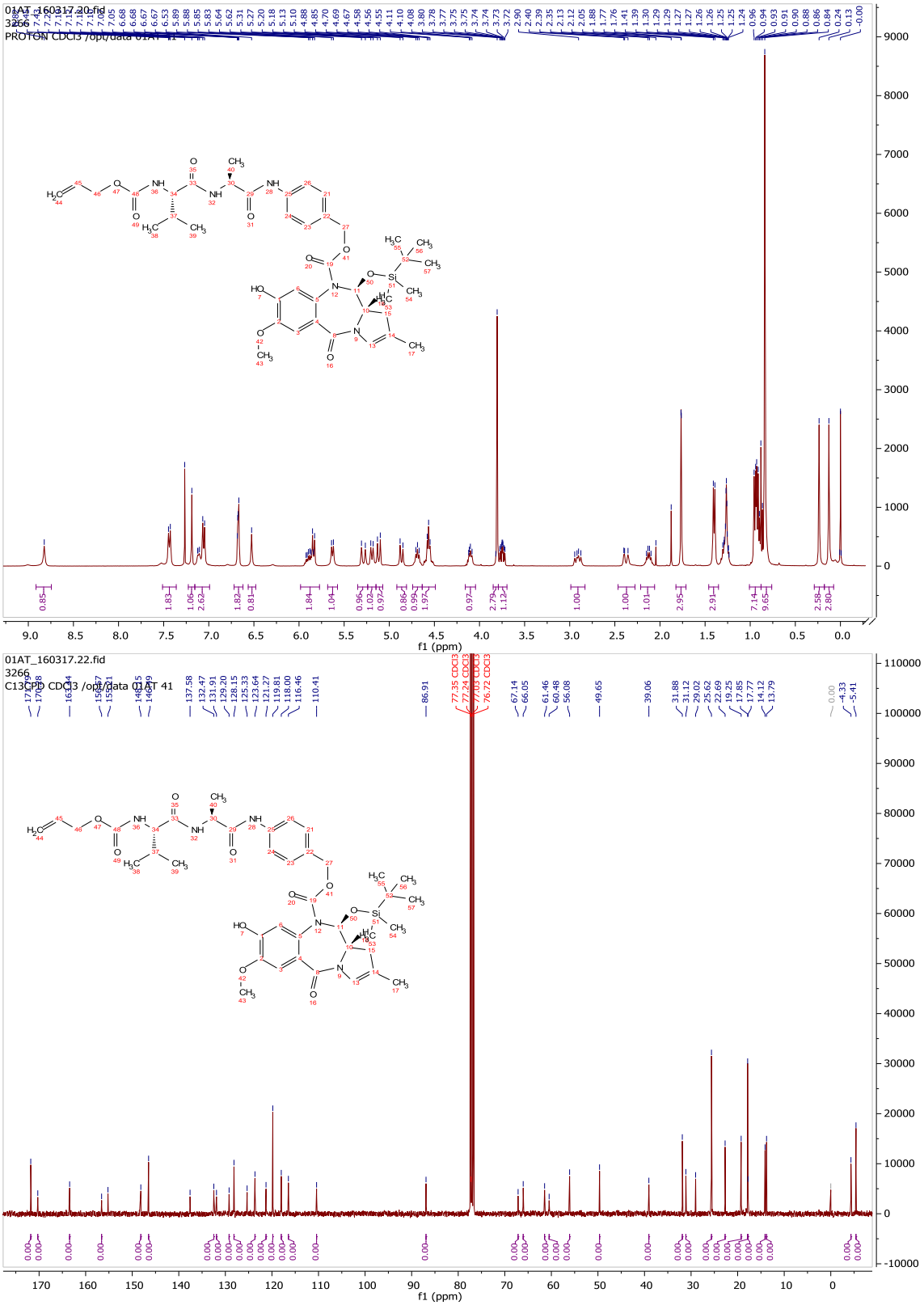


Figure 5: ^{1}H and ^{13}C NMRs of key compound **24**:

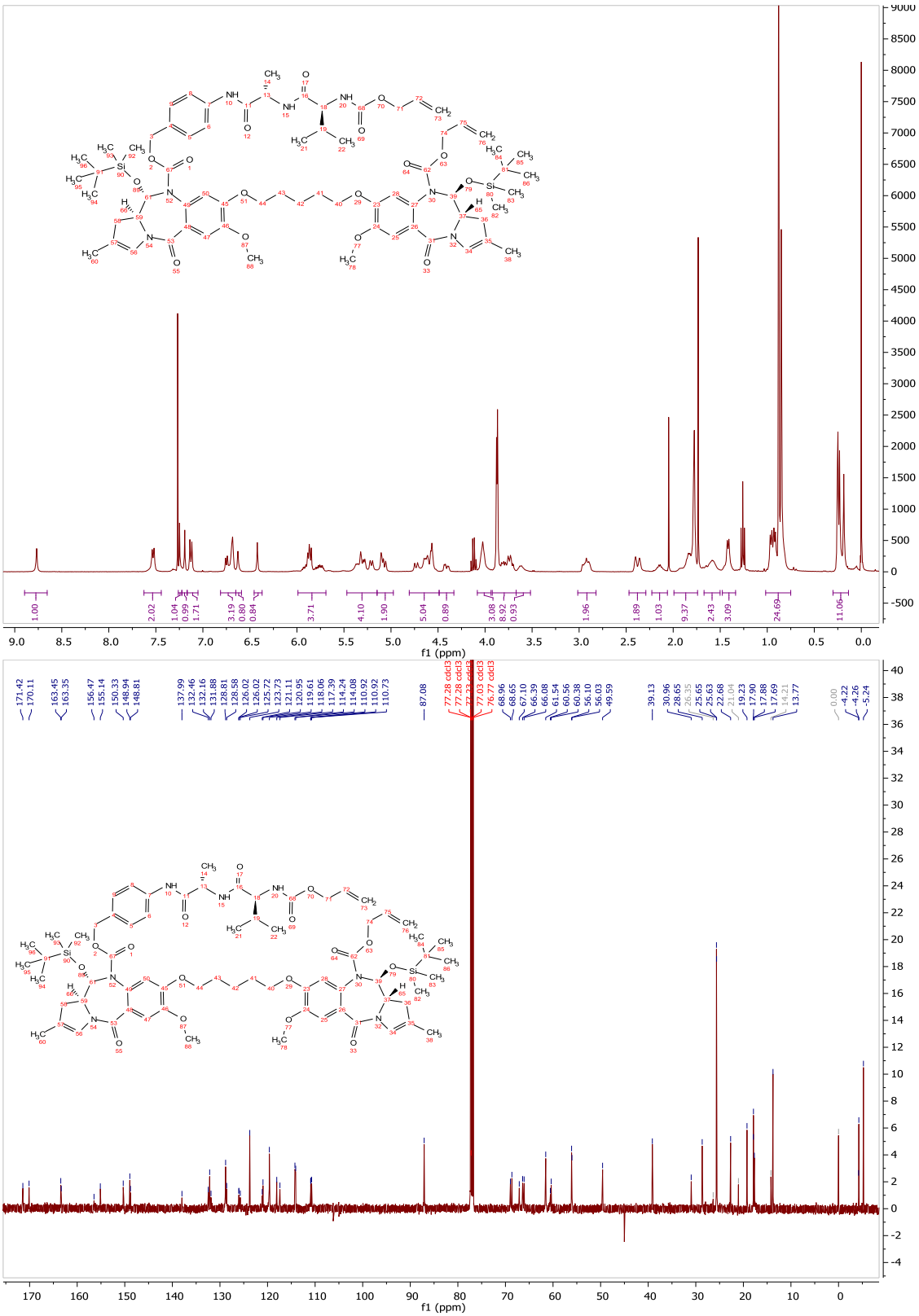


Figure 6: ^{1}H NMR of macrocycle **27**

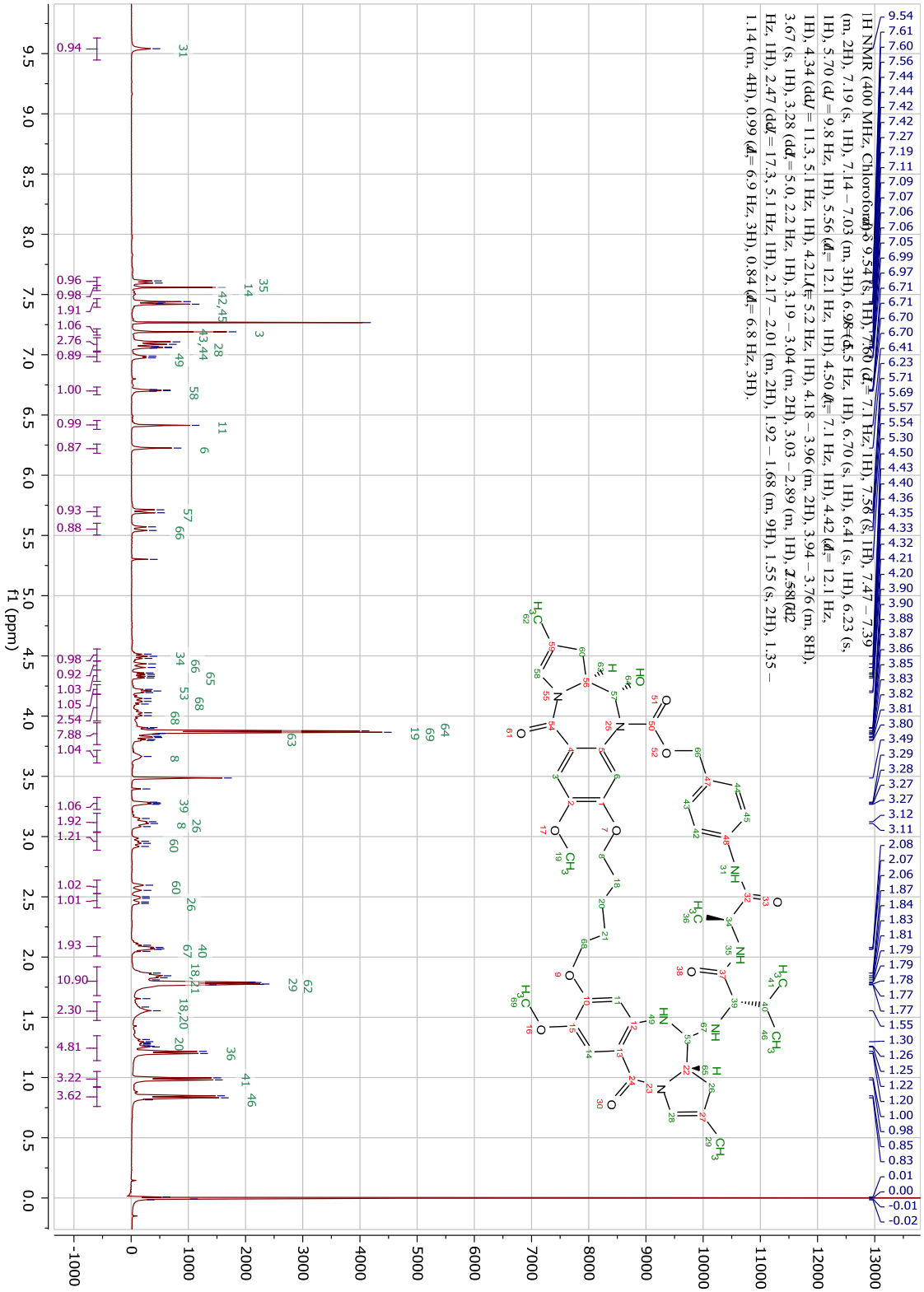


Figure 7: ^{1}H NMR of macrocycle **SG3249** (tesirine)

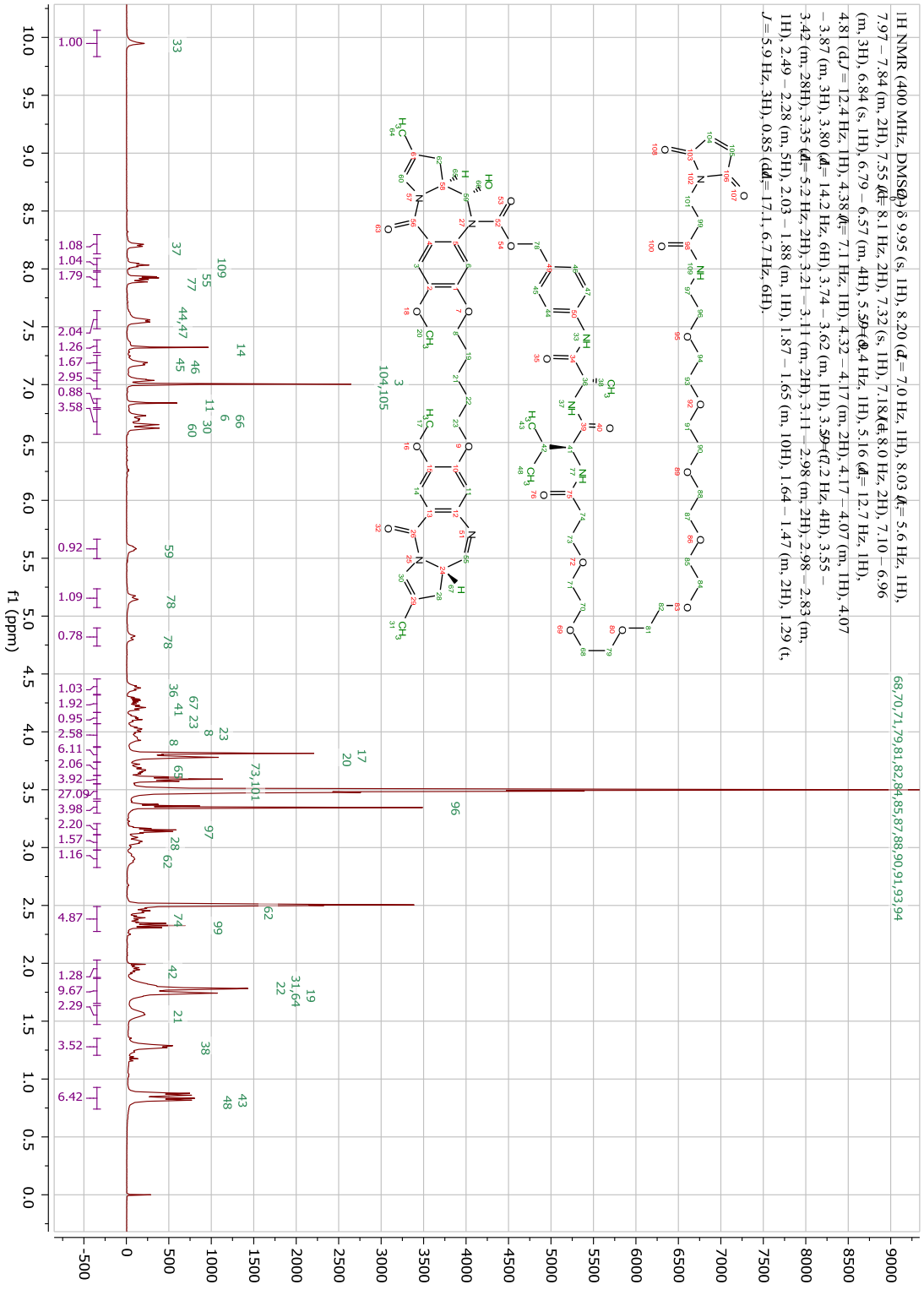


Figure 8: ^{13}C NMR of SG3249

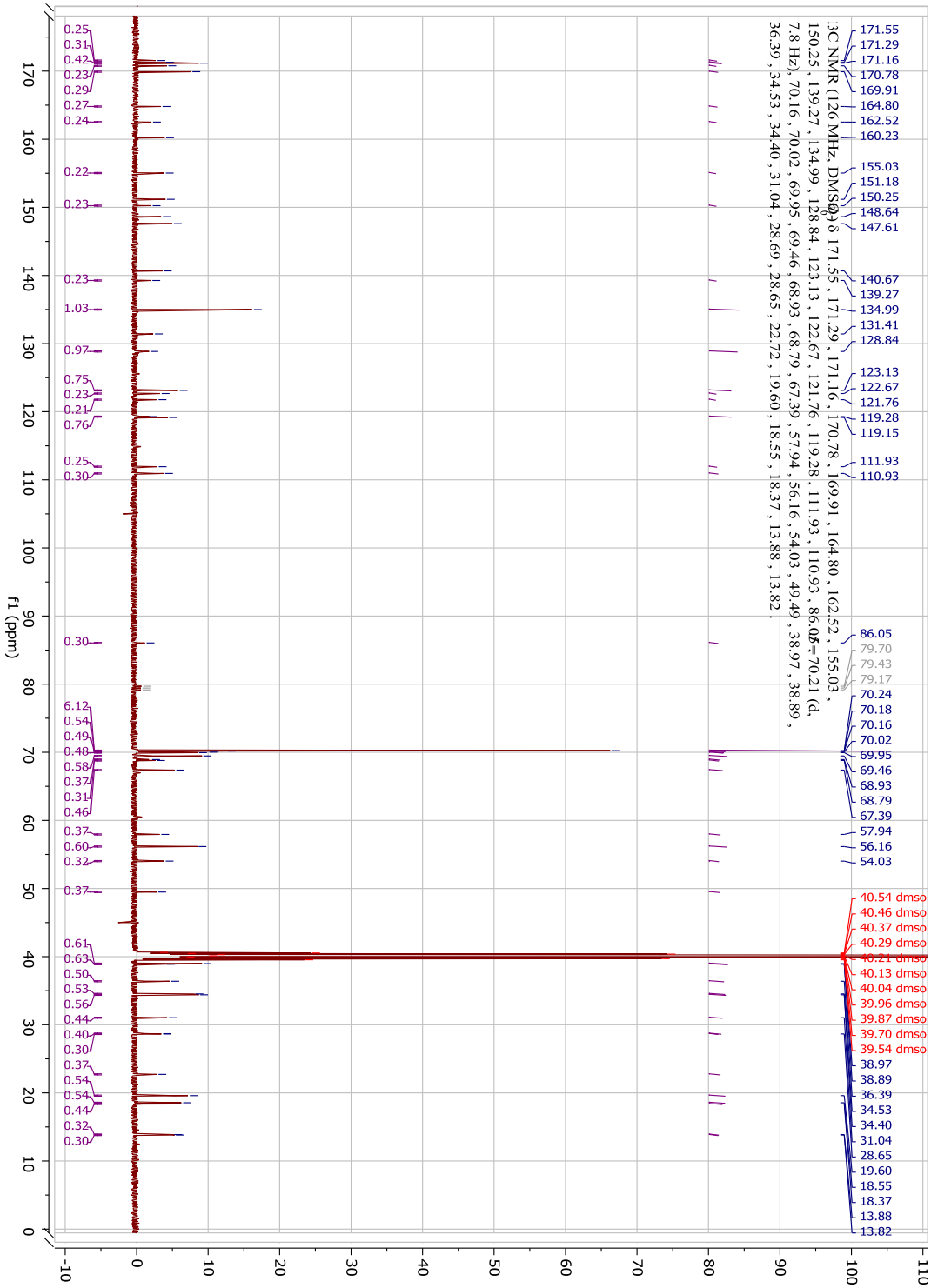


Figure 9: SG3249 accurate mass measurement