Supporting Information

Total Synthesis and Stereochemical Assignment of Burkholdac B, a Depsipeptide HDAC Inhibitor

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Total synthesis of Cheng's revised thailandepsin A 3a

(*R*)-Allyl 4-(*tert*-butoxycarbonylamino)-6-(methylthio)-3-oxohexanoate (**13**)¹

To a solution of Boc-D-Met-OH (1 g, 4.0 mmol, 1 equiv) in CH_2Cl_2 (20 mL), were added DMAP (98 mg, 0.80 mmol, 0.2 equiv), pentafluorophenol (812 mg, 4.4 mmol, 1.1 equiv) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (923 mg, 4.8 mmol, 1.2 equiv). The reaction mixture was stirred at rt for 4 h. The layers were separated after addition of 1 M HCl to the reaction mixture. The organic layer was washed with saturated aqueous NaHCO_3 solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting white solid was placed under high vacuum.

To a solution of diisopropylamine (1.85 mL, 12.9 mmol, 3.3 equiv) in THF (10 mL), were added dropwise at $-78\text{ }^{\circ}\text{C}$ a solution of BuLi in hexanes (1.6 M) (9 mL, 14.1 mmol, 3.6 equiv) followed by allylacetate (1.37 mL, 12.4 mmol, 3.2 equiv). The reaction was stirred for 30 min at $-78\text{ }^{\circ}\text{C}$ and a solution in THF (15 mL) of the intermediate ester of Boc-D-Met-OH was added. After stirring at $-78\text{ }^{\circ}\text{C}$ for 3 h, the reaction mixture was quenched with 1 M HCl solution (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with saturated aqueous NaHCO_3 solution (25 mL) and then with saturated brine (25 mL), dried over MgSO_4 , and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **13** as a yellow oil (918.5 mg, 71%): ^1H NMR (300 MHz, CDCl_3) δ ppm 6.08–5.77 (m, 1H), 5.46–5.10 (m, 3H), 4.76–4.56 (m, 2H), 4.48 (br s, 1H), 3.63 (app dd, $J=16.2$, 3.0 Hz, 2H), 2.54 (t, $J=7.2$ Hz, 2H), 2.32–2.19 (m, 1H), 2.10 (s, 3H), 1.87 (dt, $J=14.2$, 7.0 Hz, 1H), 1.45 (s, 9H).

(3S,4R)-Allyl 4-(tert-butoxycarbonylamino)-3-hydroxy-6-(methylthio)hexanoate (14)¹

To a solution of **13** (887.7 mg, 2.68 mmol, 1 equiv) in anhydrous MeOH (10 mL) at $-78\text{ }^{\circ}\text{C}$, was added KBH_4 in portions (506 mg, 9.37 mmol, 3.5 equiv). The reaction was stirred for 10 min at $-78\text{ }^{\circ}\text{C}$, warmed to $-20\text{ }^{\circ}\text{C}$ for 30 min and to $0\text{ }^{\circ}\text{C}$ for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was concentrated *in vacuo* and EtOAc/water (1:1, 20 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over MgSO_4 , and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (2:8) to provide **14** as a white solid (718.5 mg, 81%): ^1H NMR (300 MHz, CDCl_3) δ ppm 6.08–5.77 (m, 1H), 5.52–5.19 (m, 2H), 4.77 (d, $J=7.7$ Hz, 1H), 4.68–4.59 (m, 2H), 4.17–3.97 (m, 1H), 3.69 (br s, 1H), 3.39 (br s, 1H), 2.74–2.44 (m, 4H), 2.11 (s, 3H), 1.91 (m, 1H), 1.80–1.57 (m, 1H), 1.45 (s, 9H).

(3*S*,4*R*)-Allyl 4-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tritylthio)-propanamido)-3-hydroxy-6-(methylthio)hexanoate (15)

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14 (150 mg, 0.24 mmol, 1 equiv) was treated with 3.9 mL of TFA in 10 mL of CH₂Cl₂ for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine (495 mg, 100%).

To a solution of Fmoc-D-Cys(Tr)-OH (726 mg, 2.12 mmol, 1 equiv) in CH₂Cl₂ (30 mL) cooled at 0 °C, PyBOP (1.3 g, 2.5 mmol, 1.2 equiv), and Hünig's base (1.3 mL, 7.43 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine (495 mg, 2.12 mmol, 1 equiv) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (3:7) as eluent to give **15** as a colourless oil (830 mg, 50%): [α]_D²⁸ –2 (*c* 0.1, CH₃OH); IR 3318, 3060, 2925, 2901, 1712, 1654, 1524, 1446, 1250, 1176, 1037, 747, 706 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ ppm 7.87–7.67 (m, 2H), 7.57 (d, *J*=7.2 Hz, 2H), 7.50–7.37 (m, 8H), 7.36–7.14 (m, 14H), 6.14 (d, *J*=8.5 Hz, 1H), 5.98–5.73 (m, 1H), 5.38–5.09 (m, 2H), 4.95 (d, *J*=7.0 Hz, 1H), 4.58 (d, *J*=5.6 Hz, 2H), 4.41 (d, *J*=6.0 Hz, 2H), 4.26–4.16 (m, 1H), 4.05–3.89 (m, 2H), 3.80 (d, *J*=7.2 Hz, 1H), 3.32 (d, *J*=3.9 Hz, 1H), 2.82–2.68 (m, 1H), 2.65–2.36 (m, 5H), 2.01 (s, 3H), 1.97–1.80 (m, 1H), 1.78–1.61 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ ppm 172.2, 170.1, 144.2 (3C), 143.7, 143.6, 141.3 (2C), 131.7, 129.5 (6CH), 128.1 (6CH), 127.8 (2CH), 127.1 (2CH), 127.0 (3CH), 124.9 (2CH), 120.0 (2CH), 118.7, 70.0, 67.4, 66.0, 65.5, 54.3, 52.7, 47.1, 38.1, 33.6, 30.6, 28.6, 15.5; ES⁺ MS *m/z* 823 ([M+Na]⁺ 100%); HRMS (ESI) *m/z* calcd. for C₂₉H₄₄N₄NaO₇ (M+Na)⁺ 823.2846, found 823.2849.

(5*R*,8*S*,11*R*,12*S*)-Allyl 5-((*R*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-11-(2-(methylthio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetradecan-14-oate (8)

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15 (325 mg, 0.41 mmol, 1 equiv) was treated with 1 mL of diethylamine in 10 mL of CH₃CN for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine (235 mg, 100%).

To a solution of Fmoc-D-Ile-OH (158 mg, 0.45 mmol, 1.1 equiv) in CH₂Cl₂ (30 mL) cooled at 0 °C, PyBOP (254 g, 0.49 mmol, 1.2 equiv), and Hünig's base (213 μL, 1.22 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine (235 mg, 0.41 mmol, 1 equiv) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:4 to 3:7) as eluent to afford **8** as a white solid (265 mg, 99%): $[\alpha]_D^{23} +33.9$ (*c* 0.20, CH₃OH); **mp** 60–62 °C; **IR** 3288, 3056, 3036, 2966, 2962, 2921, 1707, 1646, 1536, 1446, 1234, 1172, 1033, 841, 743, 690 cm⁻¹; **¹H NMR (400 MHz, CDCl₃/CD₃OD) δ ppm** 7.79 (d, *J*=7.5 Hz, 2H), 7.59 (d, *J*=7.5 Hz, 1H), 7.56 (d, *J*=7.5 Hz, 1H), 7.47–7.17 (m, 23H), 6.04–5.79 (m, 1H), 5.38–5.12 (m, 2H), 4.60 (dd, *J*=5.5, 1.0 Hz, 2H), 4.52 (dd, *J*=10.5, 6.5 Hz, 1H), 4.30 (dd, *J*=10.0, 6.5 Hz, 1H), 4.20–3.88 (m, 5H), 2.78 (dd, *J*=12.3, 6.3 Hz, 1H), 2.66–2.38 (m, 5H), 2.05 (s, 3H), 2.01–1.92 (m, 1H), 1.88–1.83 (m, 1H), 1.53–1.32 (m, 1H), 1.17–1.00 (m, 1H), 0.98–0.81 (m, 6H); **¹³C NMR (100 MHz, CDCl₃/CD₃OD) δ ppm** 172.2, 171.5, 170.0, 156.9, 144.1, 143.7, 143.4, 141.2 (2C), 131.7, 129.3 (6CH), 128.0 (6CH), 127.7 (2CH), 127.0 (2CH), 126.8 (3CH), 124.9; 124.8 (2CH), 119.9 (2CH), 118.4, 77.2, 70.0, 67.0, 65.4, 60.0, 52.9, 52.4, 47.0, 38.1, 36.9, 32.9, 30.5, 29.0, 24.7, 15.4, 15.3, 11.3; **ES⁺ MS *m/z*** 936 ([M+Na]⁺ 100%); **HRMS (ESI) *m/z*** calcd. for C₂₉H₄₄N₄NaO₇ (M+Na)⁺ 936.3687, found 936.3704.

(7*S*,11*R*,14*R*,17*R*,18*R*,*E*)-Allyl 11-((*R*)-*sec*-butyl)-7,18-dihydroxy-17-(2-(methylthio)-ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-((tritylthio)methyl)-2-thia-10,13,16-triazanonadec-5-en-19-oate (10)

8 (264 mg, 0.29 mmol, 1 equiv) was treated with 1 mL of diethylamine in 10 mL of CH₃CN for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine (200 mg, 100%).

To a solution of the β -hydroxy acid **9** (130 mg, 0.32 mmol, 1.1 equiv) in CH₂Cl₂ (10 mL) cooled at 0 °C, PyBOP (180 mg, 0.35 mmol, 1.2 equiv) and Hünig's base (151 μ L, 0.87 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine (200 mg, 0.29 mmol, 1 equiv) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:1) as eluent to provide **10** as a white solid (206 mg, 65%): $[\alpha]_D^{23}$ +40.5 (*c* 0.127, CH₃OH). **mp** 58–60 °C; **IR** 3273, 3052, 2958, 2929, 1740, 1630, 1531, 1487, 1446, 1180, 854, 747, 694 cm⁻¹; **¹H NMR (400 MHz, CDCl₃/CD₃OD) δ ppm** 7.47–7.17 (m, 30H), 5.97–5.74 (m, 1H), 5.59–5.45 (m, 1H), 5.44–5.34 (m, 1H), 5.34–5.26 (m, 1H), 5.21 (dd, *J*=10.3, 1.3 Hz, 1H), 4.58 (d, *J*=5.5 Hz, 2H), 4.35 (q, *J*=6.2 Hz, 1H), 4.14 (dd, *J*=7.5, 5.5 Hz, 1H), 4.11–4.07 (m, 1H), 4.07–3.97 (m, 1H), 3.89 (ddd, *J*=10.5, 5.0, 3.0 Hz, 1H), 2.66–2.36 (m, 7H), 2.33 (d, *J*=6.5 Hz, 2H), 2.26–2.16 (m, 2H), 2.09 (q, *J*=7.4 Hz, 2H), 2.00 (s, 3H), 1.97–1.83 (m, 2H), 1.71 (ddd, *J*=14.7, 9.7, 4.8 Hz, 1H), 1.51–1.32 (m, 1H), 1.15 (ddd, *J*=13.6, 9.5, 7.0 Hz, 1H), 0.98–0.82 (m, 6H); **¹³C NMR (100 MHz, CDCl₃/CD₃OD) δ ppm** 173.1, 172.1, 171.6, 170.4, 144.7 (3C), 144.1 (3C), 132.4, 131.7, 130.0, 129.4 (6CH), 129.3 (6CH), 127.9 (6CH), 127.7 (6CH), 126.8 (3CH), 126.5 (3CH), 118.2, 70.1, 69.3, 66.8, 66.5, 65.4, 59.2, 52.9, 52.6, 43.2, 38.2, 35.9, 32.9, 31.3, 31.1, 30.4, 28.9, 24.6, 15.6, 15.1, 11.4; **ES⁺ MS *m/z*** 1115 ([M+Na]⁺ 100%).

Thailandepsin A 3a

To a solution of **10** in methanol, were added morpholine (32 μ L, 0.36 mmol, 2.1 equiv) and $\text{Pd}(\text{PPh}_3)_4$ (19.7 mg, 17 μ mol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH_2Cl_2 followed by a gradient of 5% then 10% MeOH in CH_2Cl_2) to afford the carboxylic acid as a yellow solid (162 mg, 90%).

A solution of the previous carboxylic acid (161.8 mg, 0.154 mmol, 1 equiv) in CH_2Cl_2 (115 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (63.5 mg, 0.18 mmol, 1.2 equiv) in CH_2Cl_2 (30 mL) containing DMAP (45 mg, 0.37 mmol, 2.4 equiv) at room temperature over 3.5 h. The reaction was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:0 to 98:2) to give the protected dithiol (37.9 mg, 24%) as a white amorphous solid.

To a vigorously stirring solution of I_2 (93 mg, 0.37 mmol, 10 equiv) in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (70 mL:40 mL) was added the protected dithiol (38 mg, 0.037 mmol, 1 equiv) in $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (7 mL:4 mL) dropwise over 2.5 h and stirred for a further 30 min. 0.01 N of $\text{Na}_2\text{S}_2\text{O}_3$ (20 mL) was added, followed by brine (8 mL) and the aqueous phase extracted twice with EtOAc (30 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/ CH_2Cl_2 1:1 to $\text{MeOH}/\text{CH}_2\text{Cl}_2$ 1:99 then 2:98) to give **3a** as a white solid (7.7 mg, 38%): $[\alpha]_D^{32} -30.7$ (*c* 0.29, CH_3OH); mp 86–88 $^\circ\text{C}$; IR 3383, 3322, 2966, 2925, 1728, 1654, 1536, 1515, 1270, 1160 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ ppm 7.57 (d, $J=7.0$ Hz, 1H), 6.74 (d, $J=9.0$ Hz, 1H), 6.51 (t, $J=13.1$ Hz, 1H), 5.88 (d, $J=4.1$ Hz, 1H), 5.66 (dt, $J=15.3, 1.6$ Hz, 1H), 5.48 (dd, $J=3.8, 1.9$ Hz, 1H), 4.91 (td, $J=8.4, 3.3$ Hz, 1H), 4.55–4.31 (m, 1H), 4.18 (dd, $J=5.6, 4.5$ Hz, 1H), 3.60–3.38 (m, 2H), 3.25–3.05 (m, 3H), 2.85 (d, $J=10.7$ Hz, 1H), 2.70 (d, $J=3.9$ Hz, 2H),

2.66–2.56 (m, 2H), 2.54–2.42 (m, 2H), 2.42–2.31 (m, 1H), 2.10 (s, 3H), 2.09–1.96 (m, 1H), 1.70–1.56 (m, 3H), 1.36–1.16 (m, 1H), 1.06 (d, $J=6.9$ Hz, 3H), 0.99 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ ppm 171.4, 171.2, 169.9, 169.0, 133.9, 128.1, 70.6, 70.5, 61.2, 56.9, 54.5, 42.3, 40.8, 40.4, 38.8, 35.7, 33.6, 31.2, 29.3, 26.1, 16.1, 15.4, 11.4; ES^+ MS m/z 570 ($[\text{M}+\text{Na}]^+$ 100%); HRMS (ESI) m/z calcd. for $\text{C}_{23}\text{H}_{37}\text{N}_3\text{NaO}_6\text{S}_3$ ($\text{M}+\text{Na}$) $^+$ 570.1737, found 570.1745.

Total synthesis of burkholdac diastereomer 6a

(4S,5S)-Allyl 4-(*tert*-butoxycarbonylamino)-5-methyl-3-oxoheptanoate (16)

To a solution of Boc-L-Ile-OH (949 mg, 3.95 mmol, 1 equiv) in CH₂Cl₂ (15 mL), were added DMAP (96.5 mg, 0.79 mmol, 0.2 equiv), pentafluorophenol (800 mg, 4.35 mmol, 1.1 equiv) and EDCI (909 mg, 4.74 mmol, 1.2 equiv). The reaction mixture was stirred at rt for 4 h. The layers were separated after addition of 1 M HCl to the reaction mixture. The organic layer was washed with saturated aqueous NaHCO₃ solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting white solid was placed under high vacuum.

To a solution of diisopropylamine (1.83 mL, 13.0 mmol, 3.3 equiv) in THF (4.5 mL), was added dropwise a solution of BuLi in hexanes (2.5 M) (5.7 mL, 14.2 mmol, 3.6 equiv) followed by allylacetate (1.35 mL, 12.5 mmol, 3.2 equiv) at –78 °C. The reaction was stirred at –78 °C for 30 min and a solution in THF (13 mL) of the intermediate ester of Boc-L-Ile-OH was added. After stirring at –78 °C for 3 h, the reaction mixture was quenched with 1 M HCl solution. The layers were separated and the aqueous layer was extracted with EtOAc (3 times). The combined organic layers were washed with saturated aqueous NaHCO₃ solution and then with saturated brine, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **16** as a colourless oil (875 mg, 70%): [α]_D²³ –31.9 (*c* 0.42, CH₃OH); IR 3347, 2967, 2933, 1751, 1702, 1517, 1457, 1363, 1318, 1246, 1160, 1013, 990 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ ppm 6.06–5.84 (m, 1H), 5.42–5.20 (m, 2H), 5.05 (d, *J*=8.1 Hz, 1H), 4.64 (d, *J*=5.6 Hz, 2H), 4.33 (dd, *J*=8.6, 4.5 Hz, 1H), 3.52–3.68 (m, 2H), 1.97 (br s, 1H), 1.45 (s, 9H), 1.41–1.24 (m, 1H), 1.17–1.03 (m, 1H), 0.99 (d, *J*=7.1 Hz, 3H), 0.94–0.87 (m, 3H).

(3*R*,4*S*,5*S*)-Allyl 4-(*tert*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (17)¹

To a solution of **16** (467 mg, 1.49 mmol, 1 equiv) in anhydrous MeOH (5 mL) at –78 °C, was added KBH₄ in portions (281 mg, 5.2 mmol, 3.5 equiv). The reaction was stirred for 10 min at –78 °C, warmed to –20 °C for 30 min and to 0 °C for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was

concentrated *in vacuo* and EtOAc/water (1:1, 20 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to provide **17** as a colourless oil (295 mg, 63%): ¹H NMR (400 MHz, CDCl₃) δ ppm 6.07–5.81 (m, 1H), 5.43–5.19 (m, 2H), 4.63 (d, *J*=6.1 Hz, 2H), 4.40 (d, *J*=9.6 Hz, 1H), 4.03 (br s, 1H), 3.58 (ddd, *J*=9.3, 7.1, 5.3 Hz, 1H), 3.24 (d, *J*=4.5 Hz, 1H), 2.71–2.56 (m, 1H), 2.56–2.41 (m, 1H), 1.93–1.68 (m, 1H), 1.68–1.51 (m, 1H), 1.45 (s, 9H), 1.12–0.84 (m, 7H).

(3*R*,4*S*,5*S*)-Allyl 4-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-(tritylthio)-propanamido)-3-hydroxy-5-methylheptanoate (18)

18

17 (282 mg, 0.89 mmol, 1 equiv) was treated with 2 mL of TFA in 5 mL of CH₂Cl₂ for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine.

To a solution of Fmoc-D-Cys(Tr)-OH (428 mg, 1.25 mmol, 1.4 equiv) in CH₂Cl₂ (6 mL) cooled at 0 °C, PyBOP (744 mg, 1.43 mmol, 1.6 equiv), and Hünig's base (467 μL, 2.68 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine in CH₂Cl₂ (2 mL) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) as eluent to give **18** as a colourless oil (477 mg, 68%): [α]_D²³ +8.6 (*c* 0.14, CH₃OH); mp 48–50 °C; IR 3407, 3326, 3056, 2962, 2929, 2868, 1716, 1659, 1524, 1446, 1315, 1250, 1172, 1042, 988, 903 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ ppm 7.78 (dd, *J*=7.3, 5.3 Hz, 2H), 7.58 (t, *J*=6.1 Hz, 2H), 7.52–7.37 (m, 8H), 7.35–7.16 (m, 11H), 5.98–5.73 (m, 2H), 5.25 (dd, *J*=17.4, 1.3 Hz, 1H), 5.16 (dd, *J*=10.1, 1.0 Hz, 1H), 5.03 (d, *J*=7.1 Hz, 1H), 4.54 (dd, *J*=5.8, 1.3 Hz, 2H), 4.40 (t, *J*=5.8 Hz, 2H), 4.21 (t, *J*=6.6 Hz, 1H),

4.09–3.97 (m, 1H), 3.94–3.71 (m, 2H), 2.80 (dd, $J=13.1, 7.1$ Hz, 1H), 2.64–2.48 (m, 2H), 2.43 (dd, $J=9.1$ Hz, 1H), 1.88–1.68 (m, 1H), 1.63–1.41 (m, 1H), 1.00–0.73 (m, 7H); ^{13}C NMR (100 MHz, CDCl_3) δ ppm 172.6, 170.7, 156.1, 144.3 (3C), 143.7; 143.6, 141.3 (2C), 131.8, 129.5 (6CH), 128.1 (6CH), 127.8 (2CH), 127.1 (2CH), 127.0 (3CH), 125.0 (2CH), 120.0 (2CH), 118.5, 68.6, 67.4, 67.2, 65.4, 57.7, 54.4, 47.0, 38.0, 34.5, 33.5, 23.5, 16.3, 11.5; ES^+ MS m/z 805 ($[\text{M}+\text{Na}]^+$ 100%); HRMS (ESI) m/z calcd. for $\text{C}_{48}\text{H}_{51}\text{N}_2\text{O}_6\text{S}_1$ ($\text{M}+\text{H}$) $^+$ 783.3462, found 783.3461.

(5*S*,8*S*,11*S*,12*R*)-Allyl 11-((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-5-(2-(methylthio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetradecan-14-oate (19)

19

18 (470 mg, 0.6 mmol, 1 equiv) was treated with 2.3 mL of diethylamine in 11.6 mL of CH_3CN for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and CHCl_3 was performed to afford the free amine.

To a solution of Fmoc-L-Met-OH (290 mg, 0.78 mmol, 1.3 equiv) in CH_2Cl_2 (5 mL) cooled at 0 °C, PyBOP (500 mg, 0.96 mmol, 1.6 equiv), and Hünig's base (314 μL , 1.8 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added in CH_2Cl_2 (1 mL). The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (2:8 then 3:7 then 4:6) as eluent to afford **19** as a colorless oil (495 mg, 90%): $[\alpha]_{\text{D}}^{23}$ -1.2 (c 0.35, CH_3OH); IR 3305, 3060, 2958, 2921, 1712, 1650, 1520, 1438, 1250, 1172, 1050 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ ppm 7.77 (d, $J=7.2$ Hz, 2H), 7.62–7.49 (m, 2H), 7.49–7.12 (m, 19H), 6.53 (d, $J=7.5$ Hz, 1H), 6.23 (d, $J=9.8$ Hz, 1H), 5.82 (tdd, $J=16.9, 10.9, 5.5$ Hz, 1H), 5.55 (d, $J=7.2$ Hz, 1H), 5.36–5.06 (m, 2H), 4.52 (d, $J=5.7$ Hz, 2H), 4.47–4.23 (m, 2H), 4.20–4.02 (m, 3H), 4.02–3.91 (m, 1H), 3.91–3.77 (m, 1H), 3.26 (d, $J=5.3$ Hz, 1H), 2.81 (dd, $J=12.8, 7.5$ Hz, 1H), 2.69–2.31 (m, 5H), 2.17–1.98 (m, 4H), 1.97–1.81 (m, 1H), 1.75–1.59

(m, 1H), 1.59–1.39 (m, 1H), 1.04–0.72 (m, 7H); ^{13}C NMR (100 MHz, CDCl_3) δ ppm 172.4, 171.4, 170.3, 156.3, 144.3 (3C), 143.6; 143.5, 141.3 (2C), 131.9, 129.5 (6CH), 128.1 (6CH), 127.8 (2CH), 127.1 (2CH), 126.9 (3CH), 125.0, 124.9, 120.0 (2CH), 118.5, 68.7, 67.2, 67.1, 65.4, 57.8, 54.0, 52.6, 47.1, 37.7, 34.7, 32.9, 30.4, 30.1, 24.0, 16.1, 15.2, 11.3; ES^+ MS m/z 936 ($[\text{M}+\text{Na}]^+$ 100%); HRMS (ESI) m/z calcd. for $\text{C}_{53}\text{H}_{60}\text{N}_3\text{O}_7\text{S}_2$ ($\text{M}+\text{H}$) $^+$ 914.3867, found 914.3867.

(7*S*,11*S*,14*R*,17*S*,18*R*,*E*)-Allyl 17-((*S*)-*sec*-butyl)-7,18-dihydroxy-11-(2-(methylthio)-ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-((tritylthio)methyl)-2-thia-10,13,16-triazanonadec-5-en-19-oate (12a)

12a

19 (478 mg, 0.53 mmol, 1 equiv) was treated with 2 mL of diethylamine in 10 mL of CH_3CN for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and CHCl_3 was performed to afford the free amine.

To a solution of the β -hydroxy acid **9** (298 mg, 0.73 mmol, 1.4 equiv) in CH_2Cl_2 (10 mL) cooled at 0 °C, PyBOP (435 mg, 0.84 mmol, 1.6 equiv), and Hünig's base (274 μL , 1.57 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (4:6 then 1:1) as eluent to provide **12a** as a white solid (347 mg, 61%): $[\alpha]_{\text{D}}^{23}$ +2.1 (c 0.26, CH_3OH); mp 130–132 °C; IR 3395, 3056, 2966, 2933, 1728, 1654, 1536, 1487, 1438, 1270, 1180, 1025, 976, 849 cm^{-1} ; ^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ ppm 7.61–7.04 (m, 30H), 5.97–5.76 (m, 1H), 5.60–5.45 (m, 1H), 5.39 (t, $J=6.1$ Hz, 1H), 5.28 (d, $J=17.2$ Hz, 1H), 5.19 (d, $J=10.6$ Hz, 1H), 4.56 (d, $J=5.6$ Hz, 2H), 4.39–4.29 (m, 1H), 4.24 (t, $J=7.1$ Hz, 1H), 4.18–4.02 (m, 2H), 3.87–3.70 (m, 1H), 2.64–2.16 (m, 10H), 2.16–1.98 (m, 7H), 1.92 (td, $J=14.4, 7.5$ Hz, 1H), 1.59 (q, $J=7.6$ Hz, 1H), 1.54–1.40 (m, 1H), 1.02–0.73 (m, 7H); ^{13}C NMR (100 MHz,

CDCl₃/CD₃OD) δ ppm 172.4 (2C), 171.9, 171.0, 144.7 (3C), 144.2 (3C), 132.4, 131.7, 129.5, 129.4 (6CH), 129.3 (6CH), 127.8 (6CH), 127.7 (6CH), 126.7 (3CH), 126.4 (3CH), 118.1, 68.6, 68.4, 66.8, 66.4, 65.3, 57.7, 52.8, 52.5, 42.6, 40.0, 37.3, 34.5, 32.9, 31.3, 31.1, 29.9, 24.1, 15.8, 14.9, 10.8; **ES⁺ MS m/z** 1115 ([M+Na]⁺ 100%).

(1*S*,5*S*,6*S*,9*R*,20*S*,*E*)-6-((*S*)-*sec*-Butyl)-5-hydroxy-20-(2-(methylthio)ethyl)-2-oxa-11,12-dithia-7,19,22-triazabicyclo[7.7.6]docos-15-ene-3,8,18,21-tetraone (6a)

To a solution of **12a** (326 mg, 0.3 mmol, 1 equiv) in methanol (10 mL), were added morpholine (55 μ L, 0.63 mmol, 2.1 equiv) and Pd(PPh₃)₄ (35 mg, 30 μ mol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH₂Cl₂ then 5% of MeOH in CH₂Cl₂ with formic acid) to afford the carboxylic acid as a yellow solid (315 mg, 100%).

A solution of the previous carboxylic acid (315 mg, 0.3 mmol, 1 equiv) in CH₂Cl₂ (250 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (124 mg, 0.36 mmol, 1.2 equiv) in CH₂Cl₂ (60 mL) containing DMAP (88 mg, 0.72 mmol, 2.4 equiv) at room temperature over 4 h. The reaction was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (CH₂Cl₂ then CH₂Cl₂/MeOH 99:1) to give the protected dithiol (224 mg, 68%) as a yellow solid.

To a vigorously stirring solution of I₂ (528 mg, 2.1 mmol, 10 equiv) in CH₂Cl₂/MeOH (390 mL:39 mL) was added the protected dithiol (215 mg, 0.21 mmol, 1 equiv) in MeOH/CH₂Cl₂ (220 mL:22 mL) dropwise over 2.5 h and stirred for a further 30 min. Na₂S₂O₃ (50 mL) was added, followed by brine (5 mL) and the aqueous phase extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/CH₂Cl₂ 1:1 then CH₂Cl₂ then

MeOH/CH₂Cl₂ 1:99 to 2:98) to give **6a** as a white solid (74 mg, 65%): [α]_D³² -139.1 (*c* 0.40, CH₃OH); mp 148–150 °C; IR 3375, 3305, 2962, 2917, 1736, 1663, 1552, 1438, 1295, 1238, 1168 cm⁻¹; ¹H NMR (400 MHz, CD₂Cl₂) δ ppm 7.07–6.94 (m, 2H), 6.65 (br s, 1H), 5.94 (t, *J*=11.1 Hz, 1H), 5.67 (d, *J*=15.2 Hz, 1H), 5.67–5.64 (m, 1H), 4.85 (ddd, *J*=10.9, 7.8, 3.5 Hz, 1H), 4.19 (td, *J*= 8.6, 4.3 Hz, 1H), 4.04–3.91 (m, 1H), 3.80 (dt, *J*= 9.5, 4.3 Hz, 1H), 3.49–3.30 (m, 2H), 3.15–3.03 (m, 1H), 2.98 (dd, *J*=13.1, 7.1 Hz, 1H), 2.81–2.57 (m, 4H), 2.57–2.40 (m, 5H), 2.40–2.22 (m, 2H), 2.09 (s, 3H), 1.92–1.77 (m, 1H), 1.61 (ddd, *J*=13.3, 7.5, 3.0 Hz, 1H), 1.02 (ddd, *J*=13.8, 6.7, 3.3 Hz, 1H), 0.95–0.77 (m, 6H); ¹³C NMR (100 MHz, CD₂Cl₂) δ ppm 171.6, 170.6, 170.4, 170.2, 132.1, 130.4, 69.7 (2CH), 60.3, 56.8, 54.8, 42.9 (2CH₂), 41.4, 41.0, 36.0, 32.5, 31.5, 27.1, 23.4, 16.7, 15.2, 12.1; HRMS (ESI) *m/z* calcd. for C₂₃H₃₈N₃O₆S₃ (M+H)⁺ 548.1917, found 548.1901.

Total synthesis of burkholdac diastereomer **6b**

(4*R*,5*R*)-Allyl 4-(*tert*-butoxycarbonylamino)-5-methyl-3-oxoheptanoate (20)

To a solution of Boc-D-Ile-OH (527 mg, 2.5 mmol, 1 equiv) in CH₂Cl₂ (10 mL), were added DMAP (61 mg, 0.50 mmol, 0.2 equiv), pentafluorophenol (507 mg, 2.75 mmol, 1.1 equiv) and EDCI (577 mg, 3.0 mmol, 1.2 equiv). The reaction mixture was stirred at rt for 4 h. The layers were separated after addition of 1 M HCl to the reaction mixture. The organic layer was washed with saturated aqueous NaHCO₃ solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting white solid was placed under high vacuum.

To a solution of diisopropylamine (1.06 mL, 7.5 mmol, 3.3 equiv) in THF (2.5 mL), were added dropwise a solution of BuLi in hexanes (2.5 M) (3.3 mL, 8.2 mmol, 3.6 equiv) followed by allylacetate (779 μ L, 7.2 mmol, 3.2 equiv) at -78°C . The reaction was stirred at -78°C for 30 min and a solution in THF (7.5 mL) of the intermediate ester of Boc-D-Ile-OH was added. After stirring at -78°C for 3 h, the reaction mixture was quenched with 1 M HCl solution. The layers were separated and the aqueous layer was extracted with EtOAc (3 times). The combined organic layers were washed with saturated aqueous NaHCO₃ solution and then with saturated brine, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **20** as a colourless oil (480.3 mg, 67%): $[\alpha]_{\text{D}}^{28} +42.7$ (*c* 0.08, CH₃OH); IR 3342, 2966, 2925, 1753, 1704, 1503, 1458, 1368, 1307, 1246, 1160, 984, 935 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ ppm 6.06–5.81 (m, 1H), 5.43–5.19 (m, 2H), 5.03 (d, *J*=8.1 Hz, 1H), 4.64 (d, *J*=6.1 Hz, 2H), 4.33 (dd, *J*=8.6, 4.5 Hz, 1H), 3.67–3.52 (m, 2H), 2.07–1.88 (m, 1H), 1.45 (s, 9H), 1.41–1.27 (m, 1H), 1.16–1.04 (m, 1H), 1.00 (d, *J*=6.6 Hz, 3H), 0.96–0.85 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δ ppm 202.2, 166.4, 155.7, 131.5, 118.8, 80.0, 66.0, 64.3, 47.2, 36.3, 28.3 (3CH₃), 24.1, 16.0, 11.6; ES⁺ MS *m/z* 336 ([M+Na]⁺ 100%), 377 ([M+Na+CH₃CN]⁺ 100%); HRMS (ESI) *m/z* calcd. for C₁₆H₂₇NNaO₅ (M+Na)⁺ 336.1781, found 336.1781.

(3*S*,4*R*,5*R*)-Allyl 4-(*tert*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (21)¹

21

To a solution of **20** (465 mg, 1.49 mmol, 1 equiv) in anhydrous MeOH (5 mL) at $-78\text{ }^{\circ}\text{C}$, was added KBH_4 in portions (281 mg, 5.22 mmol, 3.5 equiv). The reaction was stirred for 10 min at $-78\text{ }^{\circ}\text{C}$, warmed to $-20\text{ }^{\circ}\text{C}$ for 30 min and to $0\text{ }^{\circ}\text{C}$ for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was concentrated *in vacuo* and EtOAc/water (1:1, 20 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over MgSO_4 , and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) to provide **21** as a white solid (342 mg, 73%): $^1\text{H NMR}$ (300 MHz, CDCl_3) δ ppm 6.05–5.78 (m, 1H), 5.42–5.20 (m, 2H), 4.63 (d, $J=5.7$ Hz, 2H), 4.41 (d, $J=10.2$ Hz, 1H), 4.12–3.95 (m, 1H), 3.69–3.48 (ddd, $J=9.4, 7.2, 5.3$ Hz, 1H), 3.26 (d, $J=4.9$ Hz, 1H), 2.62 (dd, $J=3.0$ Hz, 1H), 2.49 (dd, $J=9.0$ Hz, 1H), 1.88–1.67 (m, 1H), 1.61–1.50 (m, 1H), 1.45 (s, 9H), 1.11–0.83 (m, 7H); ES^+ MS m/z 338 ($[\text{M}+\text{Na}]^+$ 100%), 379 ($[\text{M}+\text{Na}+\text{CH}_3\text{CN}]^+$ 100%).

(3S,4R,5R)-Allyl 4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(tritylthio)propanamido)-3-hydroxy-5-methylheptanoate (22)

22

21 (336 mg, 1.07 mmol, 1 equiv) was treated with 2 mL of TFA in 5 mL of CH_2Cl_2 for 1 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and CHCl_3 was performed to afford the free amine.

To a solution of Fmoc-D-Cys(Tr)-OH (401 mg, 1.17 mmol, 1 equiv) in CH₂Cl₂ (4 mL) at 0 °C, PyBOP (665 mg, 1.28 mmol, 1.2 equiv), and Hünig's base (557 µL, 3.19 mmol, 3 equiv) were added. After 10 min, the previous deprotected amine in CH₂Cl₂ (2 mL) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) as eluent to give **22** as a white solid (432.3 mg, 52%): $[\alpha]_D^{28} +0.6$ (*c* 0.47, CH₃OH); mp 60–62 °C; IR 3314, 3052, 2966, 1708, 1659, 1520, 1446, 1258, 1172, 1033 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ ppm 7.76 (t, *J*=7.3 Hz, 2H), 7.56 (t, *J*=7.6 Hz, 2H), 7.53–7.36 (m, 8H), 7.36–7.10 (m, 11H), 5.82 (d, *J*=9.6 Hz, 2H), 5.40–5.10 (m, 2H), 4.93 (d, *J*=7.6 Hz, 1H), 4.56 (d, *J*=5.1 Hz, 2H), 4.49–4.30 (m, 2H), 4.30–4.14 (m, 1H), 4.05–3.90 (m, 1H), 3.83 (ddd, *J*=9.7, 7.5, 5.1 Hz, 1H), 3.73 (q, *J*=6.4 Hz, 1H), 3.25 (br s, 1H), 2.77–2.59 (m, 2H), 2.53 (d, *J*=16.2 Hz, 1H), 2.40 (dd, *J*=16.4, 9.3 Hz, 1H), 1.87–1.70 (m, 1H), 1.58–1.45 (m, 1H), 1.00–0.75 (m, 7H); ¹³C NMR (100 MHz, CDCl₃) δ ppm 172.6, 170.6, 156.1, 144.3 (3C), 143.58, 143.56, 141.3 (2C), 131.8, 129.5 (6CH), 128.1 (6CH), 127.8 (2CH), 127.1 (2CH), 126.9 (3CH), 124.92, 124.86, 120.0 (2CH), 118.5, 68.5, 67.4, 67.0, 65.4, 57.5, 54.2, 47.0, 38.1, 34.3, 33.2, 23.3, 16.2, 11.6; ES⁺ MS *m/z* 805 ([M+Na]⁺ 100%); HRMS (ESI) *m/z* calcd. for C₄₈H₅₀N₂NaO₆S (M+Na)⁺ 805.3282, found 805.3280.

(5*R*,8*S*,11*R*,12*S*)-Allyl 11-((*R*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-5-(2-(methylthio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetradecan-14-oate (23)

22 (405 mg, 0.52 mmol, 1 equiv) was treated with 2 mL of diethylamine in 10 mL of CH₃CN for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine.

To a solution of Fmoc-D-Met-OH (250 mg, 0.67 mmol, 1.3 equiv) in CH₂Cl₂ (5 mL) cooled at 0 °C, PyBOP (431 g, 0.83 mmol, 1.6 equiv), and Hünig's base (271 µL, 1.55 mmol, 3 equiv)

were added. The reaction mixture was stirred for 10 min before the previous free amine was added in CH₂Cl₂ (1 mL). The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/Petroleum ether (2:8 then 3:7 then 4:6) as eluent to afford **23** as a white foam (398 mg, 84%): [α]_D²³ +22.9 (*c* 0.29, CH₃OH); **mp** 144–146 °C; **IR** 3293, 3060, 2962, 2925, 1708, 1650, 1524, 1438, 1266, 1221, 1172, 1025 cm⁻¹; **¹H NMR (400 MHz, CDCl₃) δ ppm** 7.78 (d, *J*=7.6 Hz, 2H), 7.54 (dd, *J*=7.6, 3.5 Hz, 2H), 7.49–7.36 (m, 8H), 7.36–7.06 (m, 11H), 6.49 (d, *J*=6.1 Hz, 1H), 6.09 (d, *J*=9.1 Hz, 1H), 5.97–5.77 (m, 1H), 5.58 (d, *J*=6.1 Hz, 1H), 5.30 (dd, *J*=17.2, 1.5 Hz, 1H), 5.21 (dd, *J*=10.4, 1.3 Hz, 1H), 4.70–4.50 (m, 2H), 4.43 (dd, *J*=10.4, 7.3 Hz, 1H), 4.37–3.79 (m, 6H), 3.93–3.79 (m, 1H), 3.24 (br s, 1H), 2.86 (dd, *J*=12.6, 6.1 Hz, 1H), 2.61–2.34 (m, 5H), 2.18–1.94 (m, 4H), 1.94–1.73 (m, 2H), 1.47–1.63 (m, 1H), 1.06–0.77 (m, 7H); **¹³C NMR (100 MHz, CDCl₃) δ ppm** 172.7, 171.0, 170.1, 156.2, 144.2 (3C), 143.7, 143.4, 141.33, 141.29, 131.9, 129.4 (6CH), 128.1 (6CH), 127.8 (2CH), 127.1 (2CH), 126.9 (3CH), 124.9 (2CH), 120.0 (2CH), 118.3, 68.5, 67.22, 67.19, 65.3, 57.7, 54.6, 52.8, 47.0, 38.0, 34.4, 32.9, 31.0, 30.2, 23.6, 16.2, 15.3, 11.6; **ES⁺ MS *m/z*** 936 ([M+Na]⁺ 100%); **HRMS (ESI) *m/z*** calcd. for C₅₃H₅₉N₃NaO₇S₂ (M+Na)⁺ 936.3687, found 936.3679.

(7*R*,11*R*,14*S*,17*R*,18*S*,*Z*)-Allyl 17-*sec*-butyl-7,18-dihydroxy-11-(2-(methylthio)ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-(tritylthiomethyl)-2-thia-10,13,16-triazaicos-5-en-20-oate (12b)

12b

23 (374 mg, 0.41 mmol, 1 equiv) was treated with 2 mL of diethylamine in 10 mL of CH₃CN for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine.

To a solution of the β -hydroxy acid **9** (233 mg, 0.57 mmol, 1.4 equiv) in CH₂Cl₂ (10 mL) cooled at 0 °C, PyBOP (340 mg, 0.65 mmol, 1.6 equiv), and Hünig's base (214 μ L, 1.23

mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (4:6 then 1:1) as eluent to provide **12b** as a white solid (375 mg, 84%): $[\alpha]_D^{23} +15.3$ (*c* 0.32, CH₃OH); mp 84–86 °C; IR 3286, 3011, 2957, 2931, 1726, 1639, 1527, 1489, 1438, 1214, 1176, 1035, 974, 914 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ ppm 7.55–7.12 (m, 31H), 6.68 (d, *J*=7.1 Hz, 1H), 6.14 (d, *J*=9.6 Hz, 1H), 6.01–5.77 (m, 1H), 5.53–5.42 (m, 1H), 5.42–5.25 (m, 2H), 5.25–5.13 (m, 1H), 4.57 (dd, *J*=5.6, 1.5 Hz, 2H), 4.49–4.23 (m, 2H), 4.20–3.99 (m, 2H), 3.85 (dt, *J*=9.6, 6.3 Hz, 1H), 3.29 (d, *J*=4.5 Hz, 1H), 2.92 (br s, 1H), 2.74–2.62 (m, 1H), 2.60–2.29 (m, 6H), 2.28–2.16 (m, 3H), 2.15–2.02 (m, 5H), 2.02–1.89 (m, 1H), 1.87–1.68 (m, 2H), 1.64–1.46 (m, 1H), 1.05–0.79 (m, 7H); ¹³C NMR (100 MHz, CDCl₃) δ ppm 172.6, 172.0, 171.0, 170.5, 144.8 (3C), 144.2 (3C), 132.3, 132.0, 130.4, 129.5 (6CH), 129.4 (6CH), 128.1 (6CH), 127.9 (6CH), 126.9 (3CH), 126.6 (3CH), 118.3, 69.9, 68.7, 66.9, 66.6, 65.3, 57.8, 53.9, 52.9, 44.1, 37.9, 34.5, 33.1, 31.3, 31.2, 30.5, 30.1, 23.9, 16.2, 15.4, 11.5; ES⁺ MS *m/z* 1115 ([M+Na]⁺ 100%).

5''-Epi-Burkholdac B (6b)

6b

To a solution of **12b** (345.7 mg, 0.316 mmol, 1 equiv) in methanol (10 mL), were added morpholine (58 μ L, 0.66 mmol, 2.1 equiv) and Pd(PPh₃)₄ (37 mg, 31.6 μ mol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH₂Cl₂ then 5% of MeOH in CH₂Cl₂ with formic acid) to afford the carboxylic acid as a yellow solid (333 mg, 100%).

A solution of the previous carboxylic acid (333 mg, 0.316 mmol, 1 equiv) in CH₂Cl₂ (250 mL) was added very slowly to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (131 mg, 0.38 mmol, 1.2 equiv) in CH₂Cl₂ (60 mL) containing DMAP (93 mg, 0.76 mmol, 2.4 equiv) at room temperature over 5 h. The reaction was stirred at rt overnight. The

reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (CH₂Cl₂ then CH₂Cl₂/MeOH 99:1) to give the protected dithiol (224 mg, 68%) as a yellow solid.

To a vigorously stirring solution of I₂ (471 mg, 1.9 mmol, 10 equiv) in CH₂Cl₂/MeOH (350 mL: 35 mL) was added the protected dithiol (192 mg, 0.19 mmol, 1 equiv) in MeOH/CH₂Cl₂ (200 mL:20 mL) dropwise over 2.5 h and stirred for a further 30 min. Na₂S₂O₃ (50 mL) was added, followed by brine (5 mL) and the aqueous phase extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/ CH₂Cl₂ 1:1 then CH₂Cl₂ then MeOH/CH₂Cl₂ 1:99 to 2:98) to give **6b** as a white solid (60 mg, 59%): [α]_D²³ -45 (*c* 0.26, CH₃OH); **mp** 108–110 °C; **IR** 3324, 2960, 2918, 1724, 1653, 1529, 1431, 1261, 1160, 1047, 1020, 979 cm⁻¹; **¹H NMR (400 MHz, CD₂Cl₂) δ ppm** 7.41 (br s, 2H), 6.74 (d, *J*=9.1 Hz, 1H), 6.31 (t, *J*=13.1 Hz, 1H), 5.71 (d, *J*=15.7 Hz, 1H), 5.59–5.43 (m, 1H), 4.81 (dt, *J*=9.1, 3.5 Hz, 1H), 4.57–4.40 (m, 1H), 4.30 (td, *J*=8.5, 4.6 Hz, 1H), 3.44–3.21 (m, 3H), 3.14 (d, *J*=15.2 Hz, 1H), 2.99 (d, *J*=10.1 Hz, 1H), 2.86–2.56 (m, 8H), 2.56–2.37 (m, 1H), 2.32–2.14 (m, 5H), 2.10–1.96 (m, 1H), 1.68–1.49 (m, 1H), 1.12–1.03 (m, 1H), 1.00 (d, *J*=6.6 Hz, 3H), 0.85 (t, *J*=7.6 Hz, 3H); **¹³C NMR (100 MHz, CD₂Cl₂) δ ppm** 172.5, 171.7, 170.6, 169.4 (C), 133.7, 129.8, 71.4, 70.1, 62.9, 58.0, 55.3, 41.9 (2CH₂), 41.4, 40.3, 36.3, 33.9, 31.7, 28.8, 26.5, 16.9, 15.8, 11.1; **ES⁺ MS *m/z*** 570 ([M+Na]⁺ 100%); **HRMS (ESI) *m/z*** calcd. for C₂₃H₃₇N₃NaO₆S (M+Na)⁺ 570.1737, found 570.1738.

Total synthesis of burkholdac B 6c

(4*R*,5*S*)-Allyl 4-(*tert*-butoxycarbonylamino)-5-methyl-3-oxoheptanoate (**24**)

To a solution of Boc-D-*allo*-Ile-OH (870 mg, 3.8 mmol, 1 equiv) in anhydrous CH_2Cl_2 (15 mL), were added DMAP (92 mg, 0.75 mmol, 0.2 equiv), pentafluorophenol (762 mg, 4.14 mmol, 1.1 equiv) and EDCI (865 mg, 4.5 mmol, 1.2 equiv). The reaction mixture was stirred at rt overnight. The layers were separated after addition of 1 M HCl to the reaction mixture. The organic layer was washed with saturated aqueous NaHCO_3 solution followed by saturated brine, then dried and concentrated *in vacuo*. The resulting colourless oil was placed under high vacuum.

To a solution of diisopropylamine (1.75 mL, 12.4 mmol, 3.3 equiv) in anhydrous THF (4.5 mL), were added dropwise a solution of BuLi in hexanes (2.5 M) (5.4 mL, 9.4 mmol, 3.6

equiv) followed by allylacetate (1.3 mL, 12.0 mmol, 3.2 equiv) at $-78\text{ }^{\circ}\text{C}$. The reaction was stirred at $-78\text{ }^{\circ}\text{C}$ for 30 min and a solution in anhydrous THF (13 mL) of the intermediate ester of Boc-D-*allo*-Ile-OH was added. After stirring at $-78\text{ }^{\circ}\text{C}$ for 3 h, the reaction mixture was quenched with 1 M HCl solution. The layers were separated and the aqueous layer was extracted with EtOAc (3 times). The combined organic layers were washed with saturated aqueous NaHCO_3 solution and then with saturated brine, dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9) to afford **24** as a colourless oil (851 mg, 72%): $[\alpha]_{\text{D}}^{23} +13.5$ (c 0.28, CH_3OH); IR 3363, 2970, 2921, 2876, 1753, 1704, 1511, 1462, 1368, 1238, 1156, 993 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ ppm 6.06–5.80 (m, 1H), 5.41–5.21 (m, 2H), 5.05 (d, $J=9.1$ Hz, 1H), 4.64 (d, $J=5.6$ Hz, 2H), 4.48 (dd, $J=9.1, 3.0$ Hz, 1H), 3.57 (s, 2H), 2.07–1.89 (m, 1H), 1.45 (s, 9H), 1.28 (td, $J=13.9, 7.2$ Hz, 1H), 0.97 (t, $J=7.3$ Hz, 3H), 0.94–0.82 (m, 1H), 0.79 (d, $J=7.1$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ ppm 202.2, 166.4, 155.9, 131.5, 118.9, 80.1, 66.1, 62.7, 46.7, 35.9, 28.3 (3 CH_3), 26.8, 13.8, 11.8.

(3*S*,4*R*,5*S*)-Allyl 4-(*tert*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (25)

To a solution of **24** (819 mg, 2.6 mmol, 1 equiv) in anhydrous MeOH (9 mL) at $-78\text{ }^{\circ}\text{C}$, was added KBH_4 in portions (493 mg, 9.1 mmol, 3.5 equiv). The reaction was stirred for 10 min at $-78\text{ }^{\circ}\text{C}$, warmed to $-20\text{ }^{\circ}\text{C}$ for 30 min and to $0\text{ }^{\circ}\text{C}$ for 10 min. The reaction mixture was quenched by dropwise addition of glacial acetic acid (not pH < 6). The mixture was concentrated *in vacuo* and EtOAc/water (1:1, 40 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over MgSO_4 , and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) to provide **25** as a colourless oil (571.6 mg, 69%): $[\alpha]_{\text{D}}^{23} -1.2$ (c 0.52, CH_3OH); IR 3436, 3367, 2966, 2933, 2872, 1691, 1516, 1458, 1393, 1360, 1242, 1156, 1070, 980, 931 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ ppm 6.05–5.82 (m, 1H), 5.41–5.15 (m, 2H), 4.62 (d, $J=5.6$ Hz, 2H), 4.43 (d, $J=10.1$ Hz, 1H), 3.99–3.83 (m,

1H), 3.71–3.54 (m, 1H), 3.23 (br s, 1H), 2.66 (dd, $J=3.0$ Hz, 1H), 2.59–2.36 (m, 1H), 2.02–1.80 (m, 1H), 1.44 (s, 9H), 1.41–1.31 (m, 1H), 1.29–1.11 (m, 1H), 1.00–0.74 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ ppm 173.0, 156.2, 131.8, 118.6, 79.5, 69.1, 65.4, 56.6, 38.5, 33.9, 28.3 (3 CH_3), 27.0, 13.2, 11.7; ES^+ MS m/z 338 ($[\text{M}+\text{Na}]^+$ 100%), 379 ($[\text{M}+\text{Na}+\text{CH}_3\text{CN}]^+$ 100%); HRMS (ESI) m/z calcd. for $\text{C}_{16}\text{H}_{30}\text{NO}_5$ ($\text{M}+\text{H}$) $^+$ 316.2118 found 316.2122.

(3*S*,4*R*,5*S*)-Allyl 4-((*S*)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(tritylthio)-propanamido)-3-hydroxy-5-methylheptanoate (26)

25 (547.6 mg, 1.74 mmol, 1 equiv) was treated with 4 mL of TFA in 10 mL of CH_2Cl_2 for 3 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH_3CN and CHCl_3 was performed to afford the free amine.

To a solution of Fmoc-D-Cys(Tr)-OH (832 mg, 2.4 mmol, 1 equiv) in CH_2Cl_2 (8 mL) cooled at 0 °C, PyBOP (1.45 g, 2.78 mmol, 1.2 equiv), and Hünig's base (908 μL , 5.2 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine in CH_2Cl_2 (4 mL) was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (1:9 then 2:8) as eluent to give **26** as a white foam (907 mg, 67%): $[\alpha]_{\text{D}}^{24} +8.3$ (c 0.45, CH_3OH); mp 44–46 °C; IR 3403, 3322, 3060, 2958, 1708, 1667, 1511, 1450, 1258, 1172, 1033, 988 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ ppm 7.75 (t, $J=6.8$ Hz, 2H), 7.54 (t, $J=8.3$ Hz, 2H), 7.49–7.34 (m, 8H), 7.33–7.13 (m, 11H), 5.97–5.72 (m, 2H), 5.36–5.12 (m, 2H), 4.93 (d, $J=7.6$ Hz, 1H), 4.64–4.48 (m, 2H), 4.48–4.30 (m, 2H), 4.18 (t, $J=6.6$ Hz, 1H), 4.00–3.81 (m, 2H), 3.70 (q, $J=6.4$ Hz, 1H), 3.22 (d, $J=4.5$ Hz, 1H), 2.76–2.51 (m, 3H), 2.43 (dd, $J=8.6$ Hz, 1H), 1.89 (qd, $J=6.9, 3.0$ Hz, 1H), 1.19 (dq, $J=14.1, 6.9$ Hz, 1H), 1.07 (dquin, $J=14.1, 7.2$ Hz, 1H), 0.91–0.66 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ ppm 172.9, 170.3,

156.1, 144.3 (3C), 143.6, 143.5, 141.3 (2C), 131.7, 129.5 (6CH), 128.1 (6CH), 127.8 (2CH), 127.1 (2CH), 126.9 (3CH), 124.91, 124.86, 120.0 (2CH), 118.5, 68.5, 67.4, 67.0, 65.4, 55.1, 54.2, 47.0, 38.3, 33.6, 33.2, 27.0, 13.2, 11.6; **ES⁺ MS** *m/z* 805 ([M+Na]⁺ 100%); **HRMS (ESI)** *m/z* calcd. for C₄₈H₅₁N₂O₆S₁ (M+H)⁺ 783.3462, found 783.3457.

(5R,8S,11R,12S)-Allyl 11-((S)-sec-butyl)-1-(9H-fluoren-9-yl)-12-hydroxy-5-(2-(methylthio)ethyl)-3,6,9-trioxo-8-((tritylthio)methyl)-2-oxa-4,7,10-triazatetradecan-14-oate (27)

26 (617.5 mg, 0.79 mmol, 1 equiv) was treated with 3 mL of diethylamine in 15 mL of CH₃CN for 2.5 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine.

To a solution of Fmoc-D-Met-OH (381 mg, 1.03 mmol, 1.3 equiv) in CH₂Cl₂ (10 mL) cooled at 0 °C, PyBOP (657 mg, 1.26 mmol, 1.6 equiv), and Hünig's base (413 μL, 2.37 mmol, 3 equiv) were added. The reaction mixture was stirred for 10 min before the previous free amine was added in CH₂Cl₂ (5 mL). The reaction mixture was stirred overnight and then concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/Petroleum ether (2:8 then 3:7 then 4:6) as eluent to afford **27** as a white foam (661.7 mg, 92%): [α]_D²³ +28.1 (c 0.42, CH₃OH); **mp** 66–68 °C; **IR** 3309, 3060, 2962, 2925, 2868, 1699, 1650, 1524, 1442, 1238, 1172, 1037, 907 cm⁻¹; **¹H NMR (400 MHz, CDCl₃) δ ppm** 7.87 (d, *J*=7.6 Hz, 2H), 7.64 (dd, *J*=7.3, 3.8 Hz, 2H), 7.58–7.45 (m, 8H), 7.45–7.19 (m, 11H), 6.56 (d, *J*=6.6 Hz, 1H), 6.22–6.06 (m, 1H), 6.06–5.86 (m, 1H), 5.63 (d, *J*=6.6 Hz, 1H), 5.47–5.24 (m, 2H), 4.76–4.58 (m, 2H), 4.55–4.45 (m, 1H), 4.45–4.36 (m, 1H), 4.36–4.19 (m, 2H), 4.11–3.96 (m, 3H), 3.34 (d, *J*=4.0 Hz, 1H), 2.92 (dd, *J*=12.6, 6.6 Hz, 1H), 2.75–2.45 (m, 5H), 2.24–2.06 (m, 4H), 2.06–1.86 (m, 2H), 1.46–1.29 (m, 1H), 1.29–1.14 (m, 1H), 1.04–0.84 (m, 6H); **¹³C NMR (100 MHz, CDCl₃) δ ppm** 173.0, 171.0, 169.8, 156.2, 144.2(3C), 143.7; 143.5, 141.33, 141.29, 131.8, 129.4 (6CH), 128.1 (6CH), 127.8 (2CH), 127.1 (2CH), 126.9 (3CH), 124.9 (2CH), 120.0 (2CH), 118.4, 68.5, 67.2, 67.2, 65.3, 55.4, 54.4, 52.8, 47.1, 38.3,

33.9, 32.9, 31.2, 30.2, 27.0, 15.3, 13.4, 11.7; **ES⁺ MS** *m/z* 936 ([M+Na]⁺ 100%); **HRMS (ESI)** *m/z* calcd. for C₅₃H₆₀N₃O₇S₂ (M+H)⁺ 914.3867, found 914.3863.

(7*S*,11*R*,14*R*,17*R*,18*R*,*E*)-Allyl 17-((*S*)-*sec*-butyl)-7,18-dihydroxy-11-(2-(methylthio)ethyl)-9,12,15-trioxo-1,1,1-triphenyl-14-((tritylthio)methyl)-2-thia-10,13,16-triazanona-dec-5-en-19-oate (12c)

27 (601 mg, 0.66 mmol, 1 equiv) was treated with 3.2 mL of diethylamine in 15 mL of CH₃CN for 2 h. The reaction mixture was concentrated *in vacuo*, and coevaporation with CH₃CN and CHCl₃ was performed to afford the free amine.

To a solution of the β-hydroxy acid **9** (374 mg, 0.92 mmol, 1.4 equiv) in anhydrous CH₂Cl₂ (18 mL) cooled at 0 °C, PyBOP (547 mg, 1.05 mmol, 1.6 equiv) and Hünig's base (344 μL, 1.97 mmol, 3 equiv) were added. After 10 min, the previous deprotected amine was added. The reaction mixture was stirred overnight and concentrated *in vacuo*. The crude material was purified by flash chromatography using EtOAc/hexane (4:6 then 1:1) as eluent to provide **12c** as a white solid (503 mg, 70%): [α]_D²³ +19.4 (*c* 0.41, CH₃OH); **mp** 134–136 °C; **IR** 3273, 3077, 3056, 2958, 2909, 1720, 1630, 1544, 1483, 1442, 1385, 1250, 1176, 1074, 1033, 980, 903 cm⁻¹; **¹H NMR (400 MHz, CDCl₃) δ ppm** 7.53–7.33 (m, 12H), 7.33–7.03 (m, 19H, NH), 6.57 (d, *J*=7.6 Hz, 1H), 5.98 (d, *J*=9.6 Hz, 1H), 5.91–5.75 (m, 1H), 5.51–5.39 (m, 1H), 5.39–5.22 (m, 2H), 5.18 (dd, *J*=10.4, 1.3 Hz, 1H), 4.54 (dd, *J*=5.3, 3.8 Hz, 2H), 4.47–4.26 (m, 2H), 4.05–3.77 (m, 3H), 3.27 (d, *J*=4.5 Hz, 1H), 2.95 (d, *J*=3.0 Hz, 1H), 2.71–2.12 (m, 10H), 2.12–1.97 (m, 6H), 1.97–1.79 (m, 2H), 1.23 (tt, *J*=13.9, 7.0 Hz, 1H), 1.10 (tt, *J*=14.3, 7.4 Hz, 1H), 0.93–0.63 (m, 6H); **¹³C NMR (100 MHz, CDCl₃) δ ppm** 172.9, 171.8, 171.0, 170.2, 144.8 (3C), 144.2 (3C), 132.3, 131.9, 130.4, 129.6 (6CH), 129.4 (6CH), 128.1 (6CH), 127.9 (6CH), 126.9 (3CH), 126.6 (3CH), 118.3, 69.9, 68.5, 67.0, 66.6, 65.3, 55.5, 53.6, 52.9, 44.0, 38.3, 33.9, 33.2, 31.3, 31.2, 30.4, 30.2, 27.0, 15.4, 13.6, 11.7; **ES⁺ MS** *m/z* 1115 ([M+Na]⁺ 100%).

Burkholdac B **6c**

6c

To a solution of **12c** (473 mg, 0.43 mmol, 1 equiv) in anhydrous methanol (12 mL), were added morpholine (80 μ L, 0.91 mmol, 2.1 equiv) and $\text{Pd}(\text{PPh}_3)_4$ (50 mg, 43 μ mol, 0.1 equiv). After 2.5 h of stirring, the solvent was removed and the residue was purified by flash chromatography (CH_2Cl_2 then 5% of MeOH in CH_2Cl_2 with formic acid) to afford the carboxylic acid as a yellow solid (456 mg, 100%).

A solution of the previous carboxylic acid (456 mg, 0.43 mmol, 1 equiv) in CH_2Cl_2 (343 mL) was added dropwise to a stirred solution of 2-methyl-6-nitrobenzoic anhydride (MNBA) (179 mg, 0.52 mmol, 1.2 equiv) in anhydrous CH_2Cl_2 (82 mL) containing DMAP (127 mg, 1.04 mmol, 2.4 equiv) at room temperature over 5 h. The reaction was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (CH_2Cl_2 then $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99:1 to 98:2) to give the protected dithiol (170 mg, 38%) as a yellow solid.

To a vigorously stirring solution of I_2 (417 mg, 1.64 mmol, 10 equiv) in anhydrous $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (310 mL:31 mL) was added the protected dithiol (170 mg, 0.16 mmol, 1 equiv) in anhydrous $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (177 mL:18 mL) dropwise over 2.5 h and stirred for a further 30 min. $\text{Na}_2\text{S}_2\text{O}_3$ (50 mL) was added, followed by brine (5 mL) and the aqueous phase extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexane/ CH_2Cl_2 1:1 then CH_2Cl_2 then MeOH/ CH_2Cl_2 1:99 to 2:98) to give burkholdac B (**6c**) as a white solid (36 mg, 39%): $[\alpha]_D^{32}$ -62.6 (c 0.34, CH_3OH); **mp** 110–112 $^\circ\text{C}$; **IR** 3334, 2962, 2925, 1728, 1650, 1520, 1430, 1274, 1160, 980 cm^{-1} ; **^1H NMR (400 MHz, CD_2Cl_2) δ ppm** 7.41 (br s, 1H), 7.24 (d, $J=7.1$ Hz, 1H), 6.74 (d, $J=9.6$ Hz, 1H), 6.45–6.26 (m, 1H), 5.71 (dt, $J=15.7, 2.0$ Hz, 1H), 5.55–5.44 (m, 1H), 4.84 (dt, $J=8.8, 3.5$ Hz, 1H), 4.64–4.47 (m, 1H),

4.30 (td, $J=8.7, 4.5$ Hz, 1H), 3.36 (br s, 1H), 3.26 (dd, $J=13.1, 7.1$ Hz, 2H), 3.10 (d, $J=12.6$ Hz, 1H), 2.94–2.82 (m, 2H), 2.81–2.72 (m, 2H), 2.82–2.59 (m, 5H), 2.56–2.40 (m, 1H), 2.34–2.20 (m, 1H), 2.17 (s, 3H), 2.10–1.97 (m, 2H), 1.59–1.42 (m, 1H), 1.29–1.15 (m, 1H), 0.91 (t, $J=7.6$ Hz, 3H), 0.88 (d, $J=6.6$ Hz, 3H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ ppm 172.4, 171.6, 170.6, 169.6, 133.6, 129.7, 71.3, 68.7, 62.1, 57.9, 55.1, 42.1, 41.6, 41.4, 40.1, 36.8, 34.0, 31.7, 28.8, 27.7, 15.8 (2CH₃), 11.9; HRMS (ESI) m/z calcd. for $\text{C}_{23}\text{H}_{41}\text{N}_4\text{O}_6\text{S}_3$ ($\text{M}+\text{NH}_4$)⁺ 565.2183, found 565.2170.

Reference

1. Preciado A.; Williams P. G. *J. Org. Chem.* **2008**, 73, 9228–9234

Table of $\Delta\delta$ values for ^1H and ^{13}C chemical shifts of diastereomers of 6 compared to burkholdac B

Atom numbering used for burkholdac B and diastereomers

#	^1H , 6c	^{13}C , 6c	^1H , 6b	^{13}C , 6b	^1H , 6a	^{13}C , 6a
1		0.2		0.1		0.1
2	-0.01	0	-0.01	0.2	-0.32	-3
3	0.01	0.2	0.02	0.2	0.28	-1.5
	-0.01		-0.04		0.21	
4	-0.01	0.1	0.04	0.1	0.01	-0.1
	-0.02		-0.08		-0.11	
5	-0.01	0.1	0.05	1.2	-0.09	-0.5
NH	0.02		0.02		-0.74	
6		0.2		0.3		-1.2
7	0.03	-0.1	0.08	-0.1	0.36	-0.5
	-0.02		0.01		-0.3	
8	0.01	0.2	0.01	0.3	0.11	-1.4
9		0.1		0.2		-0.7
10	-0.03	0.2	0.02	0.3	-0.2	1.4
11	-0.01	0.1	-0.07	1.5	-0.38	1.1
12	0.01	0	-0.18	0.8	0.92	-1.8
13	-0.02	0.1	0.17	-0.4	-0.22	-0.7
14	-0.01	0.2	-0.13	-1	-0.19	-4.1
	-0.01		0.04		0.09	
15	0	0.1	-0.06	-0.7	-0.02	0.3
16	-0.01	0.2	0.11	0.2	0	1.1
NH	0		0.17		-0.24	
OH						
17		0.2		0		1
18	0	0.2	-0.03	0.4	0.01	1.9
19	0	0.2	0.04	0	-0.04	-0.9
	-0.02		-0.09		0	
NH	0.01		0.01		0.27	
20	0	0.1	-0.06	-0.1	-0.09	1.4
	0.03		0.06		0.15	
21	-0.05	0.1	0	0	-0.39	-1.4
	0.01		0		0.01	
22	-0.04	0.1	-0.08	0.1	-0.45	-1.5
23	0.03	0.2	0.03	0.4	-0.01	1

Difference between observed ^1H and ^{13}C NMR data for the three diastereomers 6a, 6b and 6c in CD_2Cl_2 with that²⁹ reported in the literature for burkholdac B (Values are written in red if ^1H $\Delta\delta > 0.1$ ppm and if ^{13}C $\Delta\delta > 1$ ppm)

^1H and ^{13}C NMR spectra

15

3a

3a

19

12a

6a

6a

20

20

22

22

23

23

12b

6b

6b

24

24

25

25

12c

6c

6c