Supporting Information

Design and Synthesis of the Stabilized Analogs of Belactosin A with the Unnatural *cis*-Cyclopropane Structure

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General methods and materials

^{1}H-NMR spectra were recorded in $CDCl_3$ at ambient temperature unless otherwise noted, at 400 or 500 MHz, with TMS as an internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ at ambient temperature unless otherwise noted, at 100 or 125 MHz. Silica gel column chromatography was performed with silica gel 60 N (spherical, neutral, 63-210 μm, Kanto Chemical Co., Inc.). Flash column chromatography was performed with silica gel 60 N (spherical, neutral, 40-50 μm, Kanto Chemical Co., Inc.). Combustion analysis was performed to confirm ≥95% sample purity (within ±0.4% of the calculated value).

Synthesis of 3a-6a and 3b-6b

(4*R*)-4-Benzyl-3-(4-methylpentanoyl)oxazolidin-2-one **8**

The title compound **8** (11.1 g, 40.3 mmol, quant., a colorless oil) was prepared from carboxylic acid **7** (6.54 ml, 52.0 mmol, 1.3 equiv) and (4*R*)-4-benzyl-2-oxazolidinone (7.09 g, 40.0 mmol, 1.0 equiv) as described for imide **13**. $[\alpha]^{23}_{D}$ -54.95 (*c* 1.55, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39-7.24 (m, 3H, aromatic), 7.24-7.17 (m, 2H, aromatic), 4.74-4.61 (m, 1H, NCH), 4.25-4.11 (m, 2H, OCH_2), 3.30 (dd, *J* = 13.5, 3.1 Hz, 1H, benzyl CH_2), 3.05-2.84 (m, 2H, $COCH_2$), 2.76 (dd, *J* = 13.5, 9.9 Hz, 1H, benzyl CH_2), 1.72-1.52 (m, 3H, $CHCH_2$), 0.94 (d, *J* = 6.3 Hz, 6H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.6, 153.4, 135.3, 129.4, 128.9, 127.3, 66.1, 55.1, 37.9, 33.6, 33.1, 27.6, 22.3; LRMS (ESI) *m/z* 298.14 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{16}H_{21}NO_3Na$: 298.1414 $[(M+Na)^+]$, found: 298.1411.

Imide **9**

Imide **9** (11.9 g, 30.6 mmol, 76%, a white solid) was prepared as a single isomer from imide **8** (11.1 g, 40.3 mmol) as described for imide **14**. $[\alpha]^{23}_{D}$ -32.93 (*c* 0.73, $CHCl_3$); mp 89-90 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39-7.31 (m, 2H, aromatic), 7.31-7.22 (m, 3H, aromatic), 4.73-4.59 (m, 1H, NCH), 4.32-4.19 (m, 1H, COCH), 4.20-4.09 (m, 2H, OCH_2), 3.35 (dd, *J* = 13.5, 3.1 Hz, 1H, benzyl CH_2), 2.83-2.67 (m, 2H, $COCH_2$ and benzyl CH_2), 2.49 (dd, *J* = 16.6, 4.5 Hz, 1H, $COCH_2$), 1.69-1.48 (m, 2H, $CHCH_2$), 1.43 (s, 9H, CCH_3), 1.38-1.29 (m, 1H, $CHCH_2$), 0.94 (d, *J* = 6.7 Hz, 3H, CH_3), 0.92 (d, *J* = 6.7 Hz, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.4, 171.3, 152.9, 135.8, 129.5, 128.9, 127.2, 80.7, 65.8, 55.6, 40.9, 37.6, 37.5, 37.2, 28.0, 25.7, 23.3, 21.7; LRMS (ESI) *m/z* 412.21 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{22}H_{31}NO_5Na$: 412.2094 $[(M+Na)^+]$, found: 412.2089.

2-Isobutylsuccinic acid 4-*t*-butyl ester **10**

The title compound **10** (7.39 g, 32.1 mmol, quant., a colorless oil) was prepared from imide **9** (11.9 g, 30.6 mmol) as described for carboxylic acid **15**. $[\alpha]^{23}_{D}$ -15.24 (*c* 0.49, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.90-2.81 (m, 1H, COCH), 2.59 (dd, *J* = 16.6, 9.2 Hz, 1H, $COCH_2$), 2.37 (dd, *J* = 16.6, 5.2 Hz, 1H, $COCH_2$), 1.71-1.54 (m, 2H, $CHCH_2$), 1.44 (s, 9H, CCH_3), 1.35-1.26 (m, 1H, $CHCH_2$), 0.94 (d, *J* = 6.3 Hz, 3H, CH_3), 0.91 (d, *J* = 6.3 Hz, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 181.3, 171.0, 81.0, 40.9, 39.5, 37.6, 28.0, 25.7, 22.5, 22.2; LRMS (ESI) *m/z* 253.14 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{12}H_{22}O_4Na$: 253.1410 $[(M+Na)^+]$, found: 253.1410.

(2*R*,3*S*)-3-Isobutyl-4-oxooxetane-2-carboxylic acid *t*-butyl ester **11**

The title compound **11** (3.95 g, 17.3 mmol, 2 steps 57%, a brown liquid) was prepared as a single isomer from carboxylic acid **10** (7.04 g, 30.6 mmol) as described for β-lactone **16**. $[\alpha]^{23}_{D}$ -9.34 (*c* 0.77, $CHCl_3$); ^{1}H-NMR (400 MHz,

$CDCl_3$) δ 4.48 (d, *J* = 4.0 Hz, 1H, OCH), 3.71 (ddd, *J* = 9.9, 9.9, 4.0 Hz, 1H, COCH), 1.89-1.70 (m, 3H, $CHCH_2$), 1.52 (s, 9H, CCH_3), 0.98 (d, *J* = 6.3 Hz, 3H, $CHCH_3$), 0.94 (d, *J* = 5.8 Hz, 3H, $CHCH_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.7, 167.1, 83.6, 72.5, 55.6, 36.7, 27.9, 26.4, 22.4, 21.7; LRMS (ESI) *m/z* 251.13 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{12}H_{20}O_4Na$: 251.1254 [$(M+Na)^+$], found: 251.1255.

(4*R*)-4-Benzyl-3-propionyloxazolidin-2-one **13**

To a solution of propionic acid **12** (5.84 ml, 78.0 mmol, 1.3 equiv) in THF (300 ml) was added triethylamine (20.9 ml, 150 mmol, 2.5 equiv) and PivCl (8.87 ml, 72.0 mmol, 1.2 equiv) at 0 °C. After 1 h at 0 °C, LiCl (2.80 g, 66.0 mmol, 1.1 equiv) and (4*R*)-4-benzyl-2-oxazolidinone (10.6 g, 60.0 mmol, 1.0 equiv) were added and the resulting mixture was warmed to rt. After 38 h at rt, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield the title compound **13** (12.3 g, 52.7 mmol, 88%) as a colorless oil [lit. (for *ent*-**13**) a white solid. mp 43-46 °C].[1] $[\alpha]^{22}_D$ -59.79 (*c* 1.42, $CHCl_3$) [lit. (for *ent*-**13**) $[\alpha]^{rt}_D$ 55.6 (*c* 1.27, $CHCl_3$)][1]; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.34 (dd, *J* = 7.4, 6.9 Hz, 2H, aromatic), 7.28 (t, *J* = 7.4 Hz, 1H, aromatic), 7.21 (d, *J* = 6.9 Hz, 2H, aromatic), 4.73-4.62 (m, 1H, NCH), 4.24-4.14 (m, 2H, OCH_2), 3.31 (dd, *J* = 13.2, 2.9 Hz, 1H, benzyl CH_2), 3.04-2.87 (m, 2H, $COCH_2$), 2.77 (dd, *J* = 13.2, 9.7 Hz, 1H, benzyl CH_2), 1.21 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 174.0, 153.5, 135.3, 129.4, 128.9, 127.3, 66.2, 55.1, 37.9, 29.2, 8.2; LRMS (ESI) *m/z* 256.09 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{13}H_{15}NO_3Na$: 256.0944 [$(M+Na)^+$], found: 256.0941. ^{1}H-NMR, ^{13}C-NMR, $[\alpha]^{22}_D$ data are in agreement with those reported for *ent*-**13** by May.[1]

Imide **14**

To a solution of imide **13** (12.3 g, 52.7 mmol) in THF (500 ml) was added NaHMDS (42.0 ml, 79.1 mmol, 1.5 equiv, 1.9 M in THF) at -78 °C. After 30 min at -78 °C, $BrCH_2CO_2t$-Bu (15.5 ml, 106 mmol, 2.0 equiv) was added. After 25 h at -78 °C, the reaction was quenched with AcOH (6.04 ml, 106 mmol, 2.0 equiv) and the resulting mixture was concentrated *in vacuo* to remove THF. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was separated by silica gel column chromatography (*n*-hexane/ AcOEt 5:1) and purified by recrystallization from *n*-hexane to yield imide **14** (12.0 g, 34.5 mmol, 66%, single isomer) as a colorless needle. $[\alpha]^{24}_D$ -32.02 (*c* 1.22, $CHCl_3$); mp 103-104 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.30 (m, 2H, aromatic), 7.30-7.23 (m, 3H, aromatic), 4.75-4.61 (m, 1H, NCH), 4.27-4.09 (m, 3H, OCH_2 and COCH), 3.33 (dd, *J* = 13.7, 2.9 Hz, 1H, benzyl CH_2), 2.85 (dd, *J* = 16.6, 9.7 Hz, 1H, $COCH_2$), 2.76 (dd, *J* = 13.7, 9.7 Hz, 1H, benzyl CH_2), 2.39 (dd, *J* = 16.6, 4.6 Hz, 1H, $COCH_2$), 1.43 (s, 9H, CCH_3), 1.20 (d, *J* = 6.9 Hz, 3H, $CHCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 176.3, 171.1, 152.9, 135.6, 129.4, 128.8, 127.1, 80.6, 65.9, 55.3, 38.9, 37.5, 34.5, 28.0, 17.0; LRMS (ESI) *m/z* 370.16 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{19}H_{25}NO_5Na$: 370.1625 [$(M+Na)^+$], found: 370.1618. ^{1}H-NMR and ^{13}C-NMR data are in agreement with those reporeted for *ent*-**14** by Stončius.[2]

(2*S*)-2-Methylsuccinic acid 4-*t*-butyl ester **15**

To a solution of imide **14** (3.40 g, 9.79 mmol) in 75% aqueous THF (100 ml) was added hydrogen peroxide (4.76 g, 48.9 mmol, 5.0 equiv, 35%) and $LiOH \cdot H_2O$ (821 mg, 19.6 mmol, 2.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mxiture was warmed to rt and stirred for 1 h. The reaction mixture was cooled to 0 °C, quenched with sat. $Na_2S_2O_3$ and concentrated *in vacuo* to remove THF. The residual aqueous solution was diluted with 2 M NaOH, extracted with DCM

to remove (4*R*)-4-benzyl-2-oxazolidinone. The aqueous layer was acidified with citric acid and was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt) to yield the title compound **15** (1.60 g, 8.50 mmol, 87%) as a white solid. $[\alpha]^{23}_D$ -6.93 (*c* 1.04, $CHCl_3$) [lit. $[\alpha]^{23}_D$ -7.0 (*c* 0.86, $CHCl_3$)][3]; mp 56-57 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 2.95-2.85 (m, 1H, COCH), 2.64 (dd, *J* = 16.6, 8.0 Hz, 1H, $COCH_2$), 2.37 (dd, *J* = 16.6, 5.7 Hz, 1H, $COCH_2$), 1.44 (s, 9H, CCH_3), 1.24 (d, *J* = 7.4 Hz, 3H, $CHCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 181.6, 171.0, 81.0, 38.7, 35.8, 28.0, 16.7; LRMS (ESI) *m/z* 211.09 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_9H_{16}O_4Na$: 211.0941 [$(M+Na)^+$], found: 211.0938. ^{1}H-NMR, ^{13}C-NMR and $[\alpha]^{23}_D$ data are in agreement with those reported by Davies.[3]

(2*R*,3*S*)-3-Methyl-4-oxooxetane-2-carboxylic acid *t*-butyl ester **16**

To a solution of carboxylic acid **15** (3.20 g, 17.0 mmol) in THF (170 ml) was added LiHMDS (23.4 ml, 37.4 mmol, 2.2 equiv, 1.6 M in THF) at -78 °C. After 45 min at -78 °C, CCl_4 (1.80 ml, 18.7 mmol, 1.1 equiv) was added. After 1 h at -78 °C, the reaction was quenched with AcOH (2.43 ml, 42.5 mmol, 2.5 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with 1 M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (AcOEt) to yield the corresponding chlorinated product as a brown oil.

To a solution of the oil in Et_2O (100 ml) was added 5% $NaHCO_3$ (100 ml). After 21 h at rt, the reaction mixture was diluted with AcOEt, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column dhromatography (*n*-hexane/ AcOEt 10:1) to yield the title compound **16** (1.33 g, 7.16 mmol, 2 steps 42%, single isomer) as a white solid. $[\alpha]^{23}_D$ -31.12 (*c* 0.85, $CHCl_3$); mp 42-43 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.45 (d, *J* = 4.0 Hz, 1H, lactone CH), 3.73 (qd, *J* = 7.4, 4.0 Hz, 1H, lactone CH), 1.52 (s, 9H, CCH_3), 1.50 (d, *J* = 7.4 Hz, 3H, $CHCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.9, 167.0, 83.5, 73.1, 51.6, 27.8, 12.5; LRMS (ESI) *m/z* 209.1 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_9H_{14}O_4Na$: 209.0790 [$(M+Na)^+$], found: 209.0789.

Alcohol **17**

To a solution of β-lactone **16** (1.32 g, 7.08 mmol) in MeOH (71 ml) was added triethylamine (3.94 ml, 28.3 mmol, 4.0 equiv). After 3 h at rt, the reaction mixture was concentrated *in vacuo* and the crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 5:1) to yield alcohol **17** (1.55 g, 7.11 mmol, quant.) as a pale yellow liquid. $[\alpha]^{23}_D$ 8.96 (*c* 0.48, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.25 (dd, *J* = 5.7, 3.4 Hz, 1H, $CHOH$), 3.69 (s, 3H, CO_2CH_3), 3.14 (d, *J* = 5.7 Hz, 1H, OH), 2.98 (qd, *J* = 7.4, 3.4 Hz, 1H, $CHCH_3$), 1.49 (s, 9H, CCH_3), 1.25 (d, *J* = 7.4 Hz, 3H, $CHCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.1, 172.3, 83.0, 72.2, 51.7, 43.2, 27.8, 12.1; LRMS (ESI) *m/z* 241.10 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{10}H_{18}O_5Na$: 241.1046 [$(M+Na)^+$], found: 241.1048.

Alcohol **18**

To a solution of alcohol **17** (31.5 mg, 0.144 mmol) in THF (2.0 ml) was added LiHMDS (361 μl, 0.577 mmol, 4.0 equiv) at -78 °C. After 5 min at -78 °C, 3-bromo-2-methylpropene (291 μl, 2.89 mmol, 20 equiv) was added and the resulting solution was warmed to 0 °C. After 100 min at 0 °C, the reaction was quenched with AcOH and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column

chromatography (*n*-hexane/ AcOEt 15:1) to yield alcohol **18** (16.9 mg, 0.0621 mmol, 43%, single isomer) as a colorless oil. $[\alpha]^{23}_{D}$ -15.63 (*c* 0.93, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.86 (br, 1H, alkenyl CH_2), 4.72 (br, 1H, alkenyl CH_2), 4.12 (d, *J* = 7.6 Hz, 1H, C<u>H</u>OH), 3.72 (s, 3H, CO_2CH_3), 3.40 (d, *J* = 7.6 Hz, 1H, OH), 2.67 (d, *J* = 13.5 Hz, 1H, CCH_2), 2.35 (d, *J* = 13.5 Hz, 1H, CCH_2), 1.69 (s, 3H, allyl CH_3), 1.51 (s, 9H, CCH_3), 1.15 (s, 3H, CCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.0, 171.8, 141.3, 115.4, 83.3, 76.2, 52.0, 50.1, 43.0, 28.0, 23.7, 16.9; LRMS (ESI) *m/z* 295.15 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{14}H_{24}O_5Na$: 295.1516 $[(M+Na)^+]$, found: 295.1520.

Alcohol **19**

To a solution of alcohol **18** (165 mg, 0.604 mmol) in MeOH (10 ml) was added Pd/C (160 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 15 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield alcohol **19** (174 mg, 0.634 mmol, quant.) as a colorless oil. $[\alpha]^{23}_{D}$ 1.18 (*c* 0.16, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.10 (br, 1H, C<u>H</u>OH), 3.71 (s, 3H, CO_2CH_3), 3.33 (br, 1H, OH), 1.79 (dd, *J* = 13.9, 7.2 Hz, 1H, CH_2), 1.74-1.59 (m, 1H, C<u>H</u>CH_3), 1.58-1.49 (m, 1H, CH_2), 1.49 (s, 9H, CCH_3), 1.17 (s, 3H, CCH_3), 0.92 (d, *J* = 6.3 Hz, 3H, CHC<u>H</u>$_3$), 0.84 (d, *J* = 6.3 Hz, 3H, CHC<u>H</u>$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.6, 171.9, 83.1, 76.6, 51.8, 50.1, 43.6, 28.0, 24.9, 24.5, 23.0, 16.8; LRMS (ESI) *m/z* 297.17 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{14}H_{26}O_5Na$: 297.1673 $[(M+Na)^+]$, found: 297.1668.

(2*R*,3*S*)-3-Isobutyl-3-methyl-4-oxooxetane-2-carboxylic acid *t*-butyl ester **20**

To a solution of alcohol **19** (132 mg, 0.482 mmol) in 50% aqueous THF (10 ml) was added $LiOH \cdot H_2O$ (202 mg, 4.82 mmol, 10 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt. After 18 h at rt, the reaction mixture was diluted with 33% aqueous THF (15 ml). After 47 h at rt, the reaction mixture was concentrated *in vacuo* to remove THF and the residual aqueous solution was diluted with 0.1 M NaOH, extracted with DCM. The aqueous layer was acidified with 1 M HCl and was extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a colorless viscous oil.

To a solution of the viscous oil in DCM (10 ml) was added triethylamine (277 µl, 1.99 mmol, 5.0 equiv) and PyBOP (311 mg, 0.598 mmol, 1.5 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 75 min. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 49:1) to yield the title compound **20** (79.3 mg, 0.327 mmol, 2 steps 68%) as a colorless oil. $[\alpha]^{24}_{D}$ -24.89 (*c* 0.58, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.65 (s, 1H, COCH), 1.90-1.78 (m, 2H, C<u>H</u>$_2$ and C<u>H</u>CH_3), 1.71-1.62 (m, 1H, CH_2), 1.52 (s, 9H, CCH_3), 1.31 (s, 3H, CCH_3), 1.00 (d, *J* = 6.3 Hz, 6H, CHC<u>H</u>$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.3, 166.5, 83.8, 60.7, 43.8, 28.1, 24.5, 24.0, 22.1, 15.0; LRMS (ESI) *m/z* 265.14 $[(M+Na)^+]$; HRMS (ESI) calcd for $C_{13}H_{22}O_4Na$: 265.1410 $[(M+Na)^+]$, found: 265.1410.

(*S*)-4-benzyl-3-((*S*)-3-methylpentanoyl)oxazolidin-2-one **23**

To a solution of NaOH (40.0 g, 1.00 mol) in H_2O (250 ml) was added L-isoleucine **21** (20.0 g, 153 mmol) at 0 °C. After 10 min at 0 °C, H_2NOSO_3H (20.0 g, 177 mmol, 1.2 equiv) was added. After 30 min at 0 °C, 2.5 M NaOH (150 ml) and H_2NOSO_3H (20.0 g, 177 mmol, 1.2 equiv) was added and the resulting mixture was warmed to rt. After 25 h at rt, the reaction mixture was refluxed for 3 h. The reaction mixture was cooled to 0 °C, H_2SO_4 (64 ml) was added at

a rate to keep the internal temperature below 20 °C. The resulting mixture was extracted with DCM, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid **22** as a colorless oil.

To a solution of the oil in THF (590 ml) was added triethylamine (50.0 ml, 359 mmol, 3.1 equiv) and PivCl (17.3 ml, 141 mmol, 1.2 equiv) at 0 °C. After 1 h at 0 °C, LiCl (5.47 g, 129 mmol, 1.1 equiv) and (4*S*)-4-benzyl-2-oxazolidinone (20.4 g, 115 mmol, 1.0 equiv) were added and the resulting mixture was warmed to rt. After 13 h at rt, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 3:1) to yield imide **23** (23.0 g, 83.6 mmol, 2 steps 74%) as a yellow oil. $[\alpha]^{21}_D$ 57.70 (*c* 1.28, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40-7.18 (m, 5H, aromatic), 4.74-4.65 (m, 1H, BnCH), 4.23-4.12 (m, 2H, OCH_2), 3.32 (dd, *J* = 13.0, 3.6 Hz, 1H, benzyl CH_2), 2.99 (dd, *J* = 16.2, 5.8 Hz, 1H, $COCH_2$), 2.78-2.68 (m, 2H, $COCH_2$ and benzyl CH_2), 2.09-1.95 (m, 1H, $C\underline{H}CH_3$), 1.52-1.39 (m, 1H, $C\underline{H}_2CH_3$), 1.36-1.21 (m, 1H, $C\underline{H}_2CH_3$), 0.98 (d, *J* = 6.7 Hz, 3H, $CHC\underline{H}_3$), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, $CH_2C\underline{H}_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.9, 153.4, 135.3, 129.4, 128.9, 127.3, 66.0, 55.1, 42.1, 37.9, 31.1, 29.4, 19.2, 11.3; LRMS (ESI) *m/z* 298.14 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{16}H_{21}NO_3Na$: 298.1414 [$(M+Na)^+$], found: 298.1419.

(2*S*,3*R*)-*tert*-butyl 3-((*S*)-*sec*-butyl)-4-oxooxetane-2-carboxylate **24**

To a solution of imide **23** (16.4 g, 59.5 mmol) in THF (800 ml) was added NaHMDS (47.0 ml, 89.3 mmol, 1.5 equiv, 1.9 M in THF) at -78 °C. After 30 min at -78 °C, $BrCH_2CO_2t$-Bu (17.5 ml, 119 mmol, 2.0 equiv) was added. After 30 min at -78 °C, the reaction was quenched with AcOH (6.98 ml, 119 mmol, 2.0 equiv) and the resulting mixture was concentrated *in vacuo* to remove THF. The residue was dissolved in AcOEt, washed with water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1-5:1) to give the corresponding imide as a white solid.

To a solution of the solid in THF (380 ml) was added H_2O (120 ml), hydrogen peroxide (22.9 ml, 235 mmol, 5.0 equiv, 35%) and $LiOH\cdot H_2O$ (3.95 g, 94.1 mmol, 2.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was cooled to 0 °C, quenched with sat. $Na_2S_2O_3$ and concentrated *in vacuo* to remove THF. The residual aqueous solution was diluted with 0.1 M NaOH, extracted with DCM to remove (4*S*)-4-benzyl-2-oxazolidinone. The aqueous layer was acidified with citric acid and was extracted with DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding carboxylic acid as a colorless oil.

To a solution of the oil in THF (500 ml) was added LiHMDS (63.1 ml, 101 mmol, 2.2 equiv, 1.6 M in THF) at -78 °C. After 35 min at -78 °C, CCl_4 (4.87 ml, 50.5 mmol, 1.1 equiv) was added. After 1 h at -78 °C, the reaction was quenched with AcOH (12.0 ml, 200 mmol, 3.0 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with 1 M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding chlorinated product as a brown oil.

To a solution of the oil in ether (250 ml) was added 5% $NaHCO_3$ (250 ml). After 43 h at rt, the reaction mixture was diluted with AcOEt, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield β-lactone **24** (9.93 g, 43.5 mmol, 4 steps 73%, single isomer) as a pale yellow solid. $[\alpha]^{20}_D$ 5.39 (*c* 0.76,

$CHCl_3$); mp 55-56 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.54 (d, *J* = 4.5 Hz, 1H, OCH), 3.51 (dd, *J* = 9.0, 4.5 Hz, 1H, COCH), 2.03-1.91 (m, 1H, C<u>H</u>CH_3), 1.63-1.44 (m, 1H, C<u>H$_2$</u>CH_3), 1.51 (s, 9H, CCH_3), 1.37-1.23 (m, 1H, C<u>H$_2$</u>CH_3), 1.10 (d, *J* = 6.7 Hz, 3H, CHC<u>H$_3$</u>), 0.95 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.0, 167.4, 83.5, 70.6, 62.7, 34.0, 27.8, 27.1, 16.0, 10.6; LRMS (ESI) *m/z* 251.73 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{12}H_{20}O_4Na$: 251.1259 [$(M+Na)^+$], found: 251.1258.

<u>(2*S*, 3*R*)-1-*tert*-butyl 4-methyl 3-((*S*)-*sec*-butyl)-2-hydroxysuccinate **25**</u>

To a solution of β-lactone **24** (1.01 g, 4.44 mmol) in MeOH (45.0 ml) was added triethylamine (2.47 ml, 17.8 mmol, 4.0 equiv). After 10 h at rt, the reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 10:1) to yield alcohol **25** (1.09 g, 4.19 mmol, 94%) as a pale yellow oil. $[\alpha]^{20}_D$ -11.93 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.26 (dd, *J* = 9.2, 3.4 Hz, 1H, C<u>H</u>OH), 3.67 (s, 3H, OCH_3), 3.21 (d, *J* = 9.2 Hz, 1H, OH), 2.65 (dd, *J* = 8.6, 3.4 Hz, 1H, COCH), 2.07-1.97 (m, 1H, C<u>H</u>CH_3), 1.68-1.56 (m, 1H, C<u>H$_2$</u>CH_3), 1.48 (s, 9H, CCH_3), 1.35-1.23 (m, 1H, C<u>H$_2$</u>CH_3), 0.96 (d, *J* = 6.9 Hz, 3H, CHC<u>H$_3$</u>), 0.92 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.4, 172.8, 82.5, 69.8, 53.6, 51.4, 33.3, 27.9, 26.2, 16.9, 10.7; LRMS (ESI) *m/z* 283.16 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{13}H_{24}O_5Na$: 283.1521 [$(M+Na)^+$], found: 283.1530.

<u>(2*S*,3*R*)-3-((*S*)-*sec*-butyl)-4-oxooxetane-2-carboxylic acid **27**</u>

To a solution of β-lactone **24** (557 mg, 2.44 mmol) in DCM (10.0 ml) was added TFA (10.0 ml) at -5 °C. After 21 h at -5 °C, the reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 4:1-0:1) to yield β-lactone **27** (399 mg, 2.32 mmol, 95%) as a colorless oil. $[\alpha]^{24}_D$ 1.74 (*c* 0.69, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.37 (br, 1H, COOH), 4.72 (d, *J* = 4.5 Hz, 1H, OCH), 3.69 (dd, *J* = 9.0, 4.5 Hz, 1H, COCH), 2.07-1.94 (m, 1H, C<u>H</u>CH_3), 1.66-1.52 (m, 1H, C<u>H$_2$</u>CH_3), 1.39-1.24 (m, 1H, C<u>H$_2$</u>CH_3), 1.12 (d, *J* = 6.7 Hz, 3H, CHC<u>H$_3$</u>), 0.97 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.0, 168.2, 69.5, 63.5, 34.2, 27.2, 16.0, 10.8; LRMS (ESI) *m/z* 171.07 [$(M-H)^-$]; HRMS (ESI) calcd for $C_8H_{11}O_4$: 171.0663 [$(M-H)^-$], found: 171.0660.

<u>(2*S*,3*S*)-4-*tert*-butyl 1-methyl 2-((*S*)-*sec*-butyl)-3-hydroxy-2-methylsuccinate **26**</u>

To a solution of diisopropylamine (8.78 ml, 62.2 mmol, 5.1 equiv) in THF (80 ml) was added *n*-BuLi (22.9 ml, 61.0 mmol, 5.0 equiv, 2.66 M in Hexane) at 0 °C. After 30 min at 0 °C, the reaction mixture was cooled to -78 °C and β-lactone **27** (2.10 g, 12.2 mmol) in THF (40 ml) was added *via cannula*. After 30 min at -78 °C, CH_3I (11.4 ml, 183 mmol, 15 equiv) was added and the reaction mixture was warmed to -40 °C. After 5 min at -40 °C, the reaction was quenched with AcOH (4.29 ml, 74.9 mmol, 6.1 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with 1M HCl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding methylated product **28** (dr 3:1) as a yellow oil.

To a solution of the oil in DCM (120 ml) was added THF (10 ml), MS 4 Å (2.10 g) and Ag_2CO_3 (15.1 g, 54.9 mmol, 4.5 equiv). The resulting mixture was cooled to 0 °C and *t*-butyl bromide (8.22 ml, 73.2 mmol, 6.0 equiv) was added. After 5 min at 0 °C, the reaction mixture was warmed to rt and was stirred for 56 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 99:1) to give the corresponding *t*-butyl ester (dr 3:1) as a colorless oil.

To a solution of the oil in MeOH (31 ml) was added NaOMe (617 μl, 3.10 mmol, 1.0 equiv, 28% in MeOH) at 0 °C. After 1 h at 0 °C, the reaction was quenched with AcOH (273 μl, 4.77 mmol, 1.5 equiv) and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in AcOEt, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 99:1) to yield alcohol **26** (502 mg, 1.83 mmol, 3 steps 15%, single isomer) as a white solid. $[\alpha]^{25}_D$ 19.25 (*c* 0.30, $CHCl_3$); mp 52-54 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.53 (d, *J* = 4.7 Hz, 1H, C<u>H</u>OH), 3.67 (s, 3H, OCH_3), 3.09 (d, *J* = 4.7 Hz, 1H, OH), 1.78-1.65 (m, 2H, C<u>H</u>CH_3 and C<u>H$_2$</u>CH_3), 1.47 (s, 9H, *t*-butyl CH_3), 1.29-1.13 (m, 1H, C<u>H$_2$</u>CH_3), 1.07 (s, 3H, CCH_3), 0.90 (dd, *J* = 7.4, 7.4 Hz, 3H, CH_2C<u>H$_3$</u>), 0.86 (d, *J* = 7.2 Hz, 3H, CHC<u>H$_3$</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.1, 173.5, 83.4, 72.7, 54.0, 51.2, 40.3, 27.9, 24.3, 14.9, 13.6, 12.8; LRMS (ESI) *m/z* 297.17 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{14}H_{26}O_5Na$: 297.1673 [$(M+Na)^+$], found: 297.1668.

(2*S*,3*R*)-4-*tert*-butyl 1-methyl 2-((*S*)-*sec*-butyl)-3-hydroxy-2-methylsuccinate **29**

To a solution of alcohol **26** (20.6 mg, 0.0751 mmol) in DCM (1.0 ml) was added DMP (41.4 mg, 0.0977 mmol, 1.3 equiv) and the reaction mixture was stirred for 3 h. The reaction was quenched with a solution of sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ (1:3), extracted with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to give the corresponding glyoxylate ester as a colorless oil.

To a solution of the oil and (*R*)-2-methyl-CBS-1,3,2-oxazaborolidine (23.2 mg, 0.0837 mmol, 1.1 equiv) in THF (840 μl) was added BH_3-THF (441 μl, 0.419 mmol, 5.0 equiv, 0.95 M in THF) at -78 °C. After 1 h at -78 °C, the reaction was quenched with MeOH and the resulting mixture was warmed to -40 °C. After 30 min at -40 °C, the solvent was removed under reduced pressure and the residue was dissolved in AcOEt, washed with 1 M HCl, 1M NaOH, sat. NH_4Cl and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product (dr 99:1) was purified by flash column chromatography (*n*-hexane/ AcOEt 98:2-15:1) to yield alcohol **29** (20.2 mg, 0.0736 mmol, 2 steps 98%, single isomer) as a colorless oil. $[\alpha]^{25}_D$ 4.07 (*c* 1.01, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.04 (d, *J* = 10.8 Hz, 1H, C<u>H</u>OH), 3.71 (s, 3H, OCH_3), 3.62 (d, *J* = 10.8 Hz, 1H, OH), 2.19-2.07 (m, 1H, C<u>H</u>CH_3), 1.47 (s, 9H, *t*-butyl CH_3), 1.24-1.01 (m, 2H, C<u>H$_2$</u>CH_3), 1.06 (s, 3H, CCH_3), 0.94-0.87 (m, 6H, CH_2C<u>H$_3$</u> and CHC<u>H$_3$</u>); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.8, 172.1, 82.5, 75.0, 53,2, 51.8, 37.9, 27.9, 25.0, 13.3, 12.6, 12.5; LRMS (ESI) *m/z* 297.17 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{14}H_{26}O_5Na$: 297.1673 [$(M+Na)^+$], found: 297.1668.

(2*S*,3*S*)-2-((*R*)-2-(benzyloxy)-1-hydroxy-2-oxoethyl)-2,3-dimethylpentanoic acid **31**

To a solution of alcohol **29** (144 mg, 0.530 mmol) in 1,4-dioxane (5.0 ml) was added 1 M NaOH (5.0 ml) and the reaction mixture was warmed to 80 °C. After 17 h at 80 °C, the reaction mixture was neutralized with weak acid resin (DIAION WK10) and the solvent was removed under reduced pressure to give the corresponding dicarboxylic acid as a colorless viscous oil.

The oil was dissolved in TFAA (2.0 ml) at 0 °C and the reaction mixture was stirred for 2 h at 0 °C. The solvent was removed under reduced pressure at 0 °C to give the corresponding acid anhydride as a white solid.

A solution of the solid in BnOH (1.0 ml) was stirred for 13 h at rt and then the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt/ $CHCl_3$/ MeOH 5:1:0:0-0:0:100:5) to yield carboxylic acid **31** (128 mg, 0.436 mmol, 3 steps 82%) as a colorless viscous oil. $[\alpha]^{24}_D$ 4.55 (*c* 0.24, $CHCl_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ 7.43-7.29 (m, 5H, aromatic), 5.19 (d, *J* = 12.0 Hz, 1H, benzyl CH_2), 5.14 (d, *J* = 12.0 Hz, 1H, benzyl CH_2), 4.41 (s, 1H, OCH), 1.90-1.81 (m, 1H, C<u>H</u>CH_3), 1.58-1.46 (m, 1H, C<u>H$_2$</u>CH_3),

1.12-1.04 (m, 1H, C$\underline{H}_2$CH$_3$), 1.04 (s, 3H, CCH$_3$), 0.87 (d, *J* = 6.9 Hz, 3H, CHC$\underline{H}_3$), 0.85 (dd, *J* = 7.4, 7.4 Hz, 3H, CH$_2$C$\underline{H}_3$); ^{13}C-NMR (125 MHz, CD$_3$OD) δ 178.3, 173.9, 137.0, 129.7, 129.6, 129.4, 75.3, 67.9, 54.4, 39.7, 26.0, 14.7, 13.4, 13.0; LRMS (ESI) *m/z* 293.14 [(M-H)$^-$]; HRMS (ESI) calcd for $C_{16}H_{21}O_5$: 293.1395 [(M-H)$^-$], found: 293.1391.

(2*R*,3*S*)-benzyl 3-((*S*)-*sec*-butyl)-3-methyl-4-oxooxetane-2-carboxylate **32**

To a solution of carboxylic acid **31** (124 mg, 0.420 mmol) in DCM (42 ml) was added triethylamine (175 µl, 1.26 mmol, 3.0 equiv) and PyBOP (328 mg, 0.629 mmol, 1.5 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and was stirred for 25 min. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. NaHCO$_3$ and brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane/ AcOEt 15:1) to yield β-lactone **32** (87.3 mg, 0.316 mmol, 75%) as a white solid. $[\alpha]^{24}_D$ 0.86 (*c* 1.17, CHCl$_3$); mp 38-39 °C; ^{1}H-NMR (400 MHz, CDCl$_3$) δ 7.41-7.33 (m, 5H, aromatic), 5.29 (d, *J* = 11.9 Hz, 1H, benzyl CH$_2$), 5.25 (d, *J* = 11.9 Hz, 1H, benzyl CH$_2$), 4.70 (s, 1H, OCH), 1.89-1.75 (m, 1H, C$\underline{H}$CH$_3$), 1.77-1.66 (m, 1H, C$\underline{H}_2$CH$_3$), 1.12 (s, 3H, CCH$_3$), 1.12-1.04 (m, 1H, C$\underline{H}_2$CH$_3$), 1.01 (d, *J* = 6.7 Hz, 3H, CHC$\underline{H}_3$), 0.94 (dd, *J* = 7.4, 7.4 Hz, 3H, CH$_2$C$\underline{H}_3$); ^{13}C-NMR (100 MHz, CDCl$_3$) δ 172.3, 167.5, 134.7, 128.8, 128.7, 128.7, 74.7, 67.4, 66.1, 39.1, 24.0, 14.2, 11.9, 11.8; LRMS (ESI) *m/z* 299.13 [(M+Na)$^+$]; HRMS (ESI) calcd for $C_{16}H_{20}O_4Na$: 299.1254 [(M+Na)$^+$], found: 299.1250.

(2*R*,3*S*)-3-((*S*)-*sec*-butyl)-3-methyl-4-oxooxetane-2-carboxylic acid **35**

To a solution of β-lactone **32** (23.3 mg, 0.0843 mmol) in THF (1.5 ml) was added Pd/C (23 mg). The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred under an atmosphere of hydrogen for 2 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield carboxylic acid **35** (16.3 mg, 0.0875 mmol, quant) as a white solid. $[\alpha]^{24}_D$ -4.42 (*c* 0.82, CHCl$_3$); mp 85-87 °C; ^{1}H-NMR (500 MHz, CDCl$_3$) δ 8.81 (br, 1H, COOH), 4.74 (s, 1H, OCH), 1.93-1.83 (m, 1H, C$\underline{H}$CH$_3$), 1.82-1.72 (m, 1H, C$\underline{H}_2$CH$_3$), 1.33 (s, 3H, CCH$_3$), 1.20-1.09 (m, 1H, C$\underline{H}_2$CH$_3$), 1.06 (d, *J* = 6.9 Hz, 3H, CHC$\underline{H}_3$), 0.97 (dd, *J* = 7.4, 7.4 Hz, 3H, CH$_2$C$\underline{H}_3$); ^{13}C-NMR (125 MHz, CDCl$_3$) δ 172.9, 172.0, 74.4, 66.4, 39.1, 24.0, 14.2, 12.2, 11.8; LRMS (ESI) *m/z* 185.08 [(M-H)$^-$]; HRMS (ESI) calcd for $C_9H_{13}O_4$: 185.0819 [(M-H)$^-$], found: 185.0814.

Target compound **3a**

To a solution of carbamate **A** (61.3 mg, 0.120 mmol) in DCM (600 µl) was added TFA (600 µl). After 15 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow oil.

To a solution of β-lactone **11** (41.3 mg, 0.181 mmol, 1.5 equiv) in DCM (1.0 ml) was added TFA (1.0 ml) at -5 °C. After 20 h at -5 °C, the reaction mixture was concentrated *in vacuo* to give the corresponding carboxylic acid **33** as a brown oil.

To a solution of the oil in DCM (2.0 ml) was added triethylamine (25 µl, 0.181 mmol, 1.5 equiv) and PivCl (22 µl, 0.181 mmol, 1.5 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.5 ml) was added triethylamine (50 µl, 0.361 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM (2.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. NaHCO$_3$ and brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash

column chromatography (*n*-hexane/ AcOEt 2:1-3:2) to yield target compound **3a** (42.0 mg, 0.0745 mmol, 2 steps 62%) as a white amorphous solid. $[\alpha]^{20}_{D}$ -2.32 (*c* 0.34, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41-7.23 (m, 7H, aromatic), 7.23-7.11 (m, 3H, aromatic), 7.08 (br, 1H, amide NH), 6.67 (d, *J* = 8.1 Hz, 1H, amide NH), 5.61 (d, *J* = 6.3 Hz, 1H, carbamate NH), 5.10 (s, 2H, benzyl CH_2), 4.58 (d, *J* = 4.5 Hz, 1H, NCOCH), 4.33-4.19 (m, 1H, Ala CH), 4.00-3.87 (m, 1H, NCH), 3.81-3.69 (m, 1H, NCOCHC<u>H</u>), 2.77 (br, 1H, cyclopropyl CH), 2.63 (br, 2H, benzyl CH_2), 1.97-1.58 (m, 6H, $BnCH_2$, NCHC<u>H</u>$_2$ (1H), isobutyl CH_2 and isobutyl CH), 1.39 (d, *J* = 7.2 Hz, 3H, Ala CH_3), 1.24-1.10 (m, 1H, NCHC<u>H</u>$_2$), 1.07-0.84 (m, 8H, cyclopropyl CH_2 (1H), cyclopropyl CH, isobutyl CH_3), 0.27-0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.7, 170.0, 155.9, 141.3, 136.4, 128.5, 128.5, 128.2, 128.1, 127.9, 126.1, 73.7, 66.8, 56.2, 50.6, 49.8, 37.0, 36.9, 32.5, 32.4, 26.9, 26.4, 22.3, 21.9, 19.0, 14.5, 11.6; LRMS (ESI) *m/z* 564.29 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{32}H_{42}N_3O_6$: 564.3074 $[(M+H)^+]$, found: 564.3065; Anal. calcd for $C_{32}H_{41}N_3O_6 \cdot 0.1H_2O$: C, 67.97; H, 7.34; N, 7.43. Found: C, 67.82; H, 7.46; N, 7.14.

Target compound **4a**

To a solution of carbamate **A** (40.2 mg, 0.0789 mmol) in DCM (800 µl) was added TFA (800 µl). After 15 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a yellow oil.

To a solution of β-lactone **20** (28.7 mg, 0.118 mmol, 1.5 equiv) in DCM (1.0 ml) was added TFA (1.0 ml) at -5 °C. After 20 h at -5 °C, the reaction mixture was concentrated *in vacuo* to give the corresponding carboxylic acid **34** as a colorless oil.

To a solution of the oil in DCM (1.0 ml) was added triethylamine (16 µl, 0.118 mmol, 1.5 equiv) and PivCl (15 µl, 0.118 mmol, 1.5 equiv) at 0 °C. After 30 min at 0 °C, the reaction mixture was used as a solution of the corresponding acid anhydride in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (33 µl, 0.237 mmol, 3.0 equiv) and a solution of the acid anhydride in DCM (1.0 ml) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 2:1) to yield target compound **4a** (45.6 mg, 0.0789 mmol, 2 steps 100%) as a colorless viscous oil. $[\alpha]^{20}_{D}$ -15.09 (*c* 0.41, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.22 (m, 7H, aromatic), 7.22-7.09 (m, 3H, aromatic), 6.79 (br, 1H, amide NH), 6.77 (br, 1H, amide NH), 5.77 (br, 1H, carbamate NH), 5.13 (d, *J* = 12.6 Hz, 1H, benzyl CH_2), 5.07 (d, *J* = 12.6 Hz, 1H, benzyl CH_2), 4.73 (s, 1H, NCOCH), 4.35 (dq, *J* = 6.9, 6.9 Hz, 1H, Ala CH), 4.11-3.97 (m, 1H, NCH), 2.75 (br, 1H, cyclopropyl CH), 2.61 (br, 2H, benzyl CH_2), 1.92-1.56 (m, 5H, NCHC<u>H</u>$_2$ (1H), $BnCH_2$, isobutyl CH_2 (1H) and isobutyl CH), 1.43 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.36-1.08 (m, 5H, isobutyl CH_2 (1H), NCHC<u>H</u>$_2$ (1H) and CCH_3), 1.08-0.78 (m, 8H, cyclopropyl CH_2 (1H), cyclopropyl CH and isobutyl CH_3), 0.28-0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.2, 172.8, 169.2, 155.9, 141.5, 136.5, 128.5, 128.3, 128.1, 128.0, 126.0, 77.5, 66.7, 60.4, 50.8, 49.5, 43.7, 37.6, 32.3, 32.1, 27.6, 24.5, 24.0, 22.2, 19.1, 15.9, 15.1, 10.8; LRMS (ESI) *m/z* 578.32 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{33}H_{44}N_3O_6$: 578.3230 $[(M+H)^+]$, found: 578.3224; Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.1H_2O$: C, 68.39; H, 7.51; N, 7.25. Found: C, 68.45; H, 7.60; N, 7.00.

Target compound **5a**

To a solution of carbamate **A** (35.7 mg, 0.0700 mmol) in DCM (350 µl) was added TFA (350 µl). After 15 min at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a white solid.

To a solution of carboxylic acid **35** (16.3 mg, 0.0875 mmol, 1.3 equiv) in DCM (1.0 ml) was added EDC·HCl (14.6 mg, 0.0761 mmol, 1.1 equiv) and HOAt (10.4 mg, 0.0761 mmol, 1.1 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was used as a solution of the corresponding HOAt ester in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (29.2 µl, 0.210 mmol, 3.0 equiv) and a solution of the HOAt ester in DCM (1.0 ml) at 0 °C. After 2 h at 0 °C, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **5a** (41.2 mg, 0.0713 mmol, 2 steps quant.) as a colorless viscous oil. $[\alpha]^{24}_D$ -8.24 (*c* 0.53, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.39-7.22 (m, 7H, aromatic), 7.22-7.10 (m, 3H, aromatic), 6.82 (br, 2H, amide NH), 5.85 (d, *J* = 7.4 Hz, 1H, carbamate NH), 5.14 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 5.06 (d, *J* = 12.3 Hz, 1H, benzyl CH_2), 4.60 (s, 1H, OCH), 4.37 (dq, *J* = 7.4, 6.9 Hz, 1H, Ala CH), 4.09-3.99 (m, 1H, NCH), 2.75 (br, 1H, cyclopropyl CH), 2.67-2.54 (m, 2H, PhC$\underline{H}_2$$CH_2$), 1.83-1.63 (m, 5H, NCHC$\underline{H}_2$ (1H), *sec*-butyl CH_2 (1H), $PhCH_2C\underline{H}_2$ (2H) and *sec*-butyl CH), 1.43 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.28-1.15 (m, 1H, NCHC$\underline{H}_2$), 1.20 (s, 3H, CCH_3), 1.14-0.86 (m, 9H, *sec*-butyl CH_2 (1H), cyclopropyl CH, cyclopropyl CH_2 (1H) and *sec*-butyl CH_3), 0.29-0.18 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 172.7, 169.2, 155.8, 141.5, 136.4, 128.4, 128.2, 128.0, 128.0, 126.0, 76.2, 66.7, 65.2, 50.8, 49.4, 39.1, 37.6, 32.3, 32.1, 27.6, 23.9, 19.1, 15.0, 14.1, 12.6, 11.8, 10.7; LRMS (ESI) *m/z* 600.30 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{33}H_{43}N_3O_6Na$: 600.3044 [$(M+Na)^+$], found: 600.3046; Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.2H_2O$: C, 68.18; H, 7.52; N, 7.23. Found: C, 68.20; H, 7.61; N, 7.10.

Target compound **6a**

To a solution of carbamate **B** (44.3 mg, 0.0846 mmol) in DCM (500 µl) was added TFA (500 µl). After 1 h at rt, the reaction mixture was concentrated *in vacuo* to give the corresponding amine as a colorless viscous oil.

To a solution of carboxylic acid **35** (24.6 mg, 0.132 mmol, 1.6 equiv) in DCM (1.0 ml) was added HOAt (15.6 mg, 0.115 mmol, 1.4 equiv) and EDC·HCl (22.0 mg, 0.115 mmol, 1.4 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was used as a solution of the corresponding HOAt ester in DCM.

To a solution of the aforementioned amine in DCM (1.0 ml) was added triethylamine (35.3 µl, 0.254 mmol, 3.0 equiv) and a solution of the HOAt ester in DCM (1.0 ml) at 0 °C. After 1 h at rt, the reaction mixture was diluted with AcOEt, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/ AcOEt 1:1) to yield target compound **6a** (45.4 mg, 0.0767 mmol, 2 steps 91%) as a colorless viscous oil. $[\alpha]^{24}_D$ -63.77 (*c* 0.81, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.40-7.24 (m, 7H, aromatic), 7.23-7.11 (m, 3H, aromatic), 6.31 (d, *J* = 3.6 Hz, 1H, amide NH), 6.07 (d, *J* = 8.1 Hz, 1H, amide NH), 5.33 (d, *J* = 6.7 Hz, 1H, carbamate NH), 5.11 (s, 2H, benzyl CH_2), 4.59 (s, 1H, OCH), 4.17 (dq, *J* = 7.2, 6.7 Hz, 1H, Ala CH), 4.14-4.04 (m, 1H, NCH), 2.95-2.83 (m, 1H, cyclopropyl CH), 2.70-2.51 (m, 2H, PhC$\underline{H}_2$$CH_2$), 1.90-1.60 (m, 4H, $PhCH_2C\underline{H}_2$ (2H), *sec*-butyl CH_2 (1H) and *sec*-butyl CH), 1.39 (d, *J* = 7.2 Hz, 3H, Ala CH_3), 1.23 (s, 3H, CCH_3), 1.17-1.05 (m, 2H, *sec*-butyl CH_2 and NCHC$\underline{H}$), 1.04 (d, *J* = 6.7 Hz, 3H, *sec*-butyl CHC$\underline{H}_3$), 1.01-0.88 (m, 7H, cyclopropyl CH_2 (1H), NCHCHC$\underline{H}_3$ and *sec*-butyl $CH_2C\underline{H}_3$), 0.81-0.67 (m, 1H, cyclopropyl CH), 0.49-0.36 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 171.8, 168.3, 156.1, 141.5, 136.0, 128.6, 128.5, 128.3, 128.3, 128.1, 126.0, 76.1, 67.1, 65.3, 53.1, 50.8, 39.0, 36.6, 34.6, 32.9, 26.8, 24.0, 21.5, 18.1, 15.3, 14.3, 12.5, 11.8, 11.0; LRMS (ESI) *m/z* 614.32 [$(M+Na)^+$]; HRMS (ESI) calcd for $C_{34}H_{45}N_3O_6Na$: 614.3201 [$(M+Na)^+$], found: 614.3203; Anal. calcd for $C_{34}H_{45}N_3O_6 \cdot 0.4H_2O$: C, 68.18; H, 7.71; N, 7.02. Found: C,

68.17; H, 7.73; N, 6.93.

General procedure for the preparation of the target compounds **3b-6b**

To a solution of the carbamate (1.0 equiv) in DCM (0.1 M) was added an equivalent amount of TFA and Pd/C (20 mg) at 0 °C. The flask was purged with hydrogen (balloon pressure) and the reaction mixture was stirred at 0 °C under an atmosphere of hydrogen until the starting material disappeared on TLC. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated *in vacuo* to yield the corresponding target compound.

Target compound **3b**

Target compound **3b** (9.60 mg, 0.0182 mmol, quant.) was obtained from **3a** as a colorless oil. $[\alpha]^{22}_{D}$ -14.02 (*c* 0.39, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.55 (br, 3H, NH_3^+), 8.48 (d, *J* = 8.6 Hz, 1H, amide NH), 8.25 (br, 1H, amide NH), 7.26-7.13 (m, 4H, aromatic), 7.13-7.03 (m, 1H, aromatic), 4.70 (d, *J* = 4.0 Hz, 1H, OCH), 4.30-4.13 (m, 1H, Ala CH), 4.08-4.39 (m, 1H, NCH), 3.84-3.74 (m, 1H, COCH), 2.79 (br, 1H, cyclopropyl CH), 2.72-2.60 (m, 1H, $PhCH_2$), 2.60-2.46 (m, 1H, $PhCH_2$), 1.85-1.62 (m, 5H, $PhCH_2C\underline{H}_2$, isobutyl CH and isobutyl CH_2), 1.54 (d, *J* = 6.3 Hz, 3H, Ala CH_3), 1.53-1.40 (m, 2H, $NCHC\underline{H}_2$), 1.15-1.04 (m, 1H, cyclopropyl CH), 0.95 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.91 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.91-0.79 (m, 1H, cyclopropyl CH_2), 0.41-0.31 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (500 MHz, THF-d8) δ 171.8, 170.9, 170.7, 143.3, 129.4, 129.2, 126.6, 74.4, 57.0, 50.6, 50.5, 38.5, 37.9, 34.5, 33.7, 28.5, 27.5, 23.1, 22.5, 18.3, 16.0, 11.0; LRMS (ESI) *m/z* 430.27 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{24}H_{36}N_3O_4$: 430.2700 $[(M+H)^+]$, found: 430.2705; Anal. calcd for $C_{26}H_{35}F_3N_3O_5 \cdot 2.5TFA \cdot 4.0H_2O$: C, 42.13; H, 5.19; N, 4.76. Found: C, 42.22; H, 4.96; N, 4.64.

Target compound **4b**

Target compound **4b** (14.1 mg, 0.0261 mmol, quant.) was obtained from **4a** as a colorless oil. $[\alpha]^{23}_{D}$ -32.25 (*c* 0.48, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.61 (d, *J* = 8.6 Hz, 1H, amide NH), 8.60 (br, 3H, NH_3^+), 8.19 (d, *J* = 2.9 Hz, 1H, amide NH), 7.24-7.13 (m, 4H, aromatic), 7.13-7.06 (m, 1H, aromatic), 4.86 (s, 1H, OCH), 4.34-4.25 (m, 1H, Ala CH), 4.12-4.01 (m, 1H, NCH), 2.77-2.70 (m, 1H, cyclopropyl CH), 2.70-2.62 (m, 1H, $PhCH_2$), 2.57-2.48 (m, 1H, $PhCH_2$), 1.90-1.62 (m, 5H, $PhCH_2C\underline{H}_2$, isobutyl CH_2 and isobutyl CH), 1.62-1.52 (m, 1H, $NCHC\underline{H}_2$), 1.56 (d, *J* = 6.9 Hz, 3H, Ala CH_3), 1.37-1.14 (m, 2H, $NCHC\underline{H}_2$ and cyclopropyl CH), 1.26 (s, 3H, CCH_3), 1.02 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.97 (d, *J* = 6.3 Hz, 3H, isobutyl CH_3), 0.89-0.79 (m, 1H, cyclopropyl CH_2), 0.46-0.38 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, THF-d8) δ 173.9, 171.3, 170.8, 143.4, 129.5, 129.1, 126.6, 78.6, 61.3, 50.4, 44.7, 39.0, 35.8, 33.7, 28.9, 24.6, 22.8, 18.3, 16.2, 16.1, 11.2; LRMS (ESI) *m/z* 444.29 $[(M+H)^+]$; HRMS (ESI) calcd for $C_{25}H_{38}N_3O_4$: 444.2857 $[(M+H)^+]$, found: 444.2860; Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 2.0TFA \cdot 3.0H_2O$: C, 45.26; H, 5.51; N, 5.11. Found: C, 45.49; H, 5.43; N, 5.20.

Target compound **5b**

Target compound **5b** (20.3 mg, 0.0375 mmol, quant.) was obtained from **5a** as a colorless oil. $[\alpha]^{23}_{D}$ -36.31 (*c* 0.77, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.67 (d, *J* = 8.6 Hz, 1H, amide NH), 8.58 (br, 3H, NH_3^+), 8.19 (br, 1H, amide NH), 7.26-7.13 (m, 4H, aromatic), 7.13-7.05 (m, 1H, aromatic), 4.77 (s, 1H, OCH), 4.38-4.26 (m, 1H, Ala CH), 4.14-4.01 (m, 1H, NCH), 2.77-2.61 (m, 2H, $PhCH_2$ and cyclopropyl CH), 2.58-2.46 (m, 1H, $PhCH_2$), 1.89-1.63 (m, 5H, *sec*-butyl CH_2, $PhCH_2C\underline{H}_2$ and *sec*-butyl CH), 1.63-1.52 (m, 1H, $NCHC\underline{H}_2$), 1.57 (d, *J* = 6.9 Hz, 3H, Ala CH_3),

1.27-1.08 (m, 2H, $NCHCH_2$ and cyclopropyl CH), 1.21 (s, 3H, CCH_3), 1.01 (d, J = 6.9 Hz, 3H, *sec*-butyl $CHCH_3$), 0.94 (dd, J = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_2CH_3), 0.89-0.78 (m, 1H, cyclopropyl CH_2), 0.48-0.38 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, THF-d8) δ 173.3, 171.5, 170.8, 143.4, 129.5, 129.1, 126.6, 77.1, 66.1, 50.5, 50.4, 40.3, 39.0, 36.1, 33.7, 28.9, 18.2, 16.1, 14.6, 13.1, 12.4, 11.2; LRMS (ESI) *m/z* 444.29 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{25}H_{38}N_3O_4$: 444.2857 [$(M+H)^+$], found: 444.2869; Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 1.5TFA \cdot 1.5H_2O$: C, 48.78; H, 5.66; N, 5.69. Found: C, 48.56; H, 5.81; N, 5.57.

Target compound **6b**

Target compound **6b** (22.0 mg, 0.0396 mmol, quant.) was obtained from **6a** as a colorless oil. $[\alpha]^{23}_D$ -51.07 (*c* 0.97, THF); ^{1}H-NMR (500 MHz, THF-d8) δ 8.58 (br, 3H, NH_3^+), 8.37 (d, J = 9.2 Hz, 1H, amide NH), 7.71 (d, J = 3.4 Hz, 1H, amide NH), 7.27-7.14 (m, 4H, aromatic), 7.14-7.06 (m, 1H, aromatic), 4.67 (s, 1H, OCH), 4.44-4.31 (m, 1H, Ala CH), 4.22-4.09 (m, 1H, NCH), 2.84-2.73 (m, 1H, cyclopropyl CH), 2.73-2.61 (m, 1H, $PhCH_2$), 2.58-2.44 (m, 1H, $PhCH_2$), 1.91-1.65 (m, 4H, $PhCH_2CH_2$ (2H), *sec*-butyl CH_2 (1H) and *sec*-butyl CH), 1.55 (d, J = 6.9 Hz, 3H, Ala CH_3), 1.19 (s, 3H, CCH_3), 1.16-1.05 (m, 2H, *sec*-butyl CH_2 and NCHCH), 1.05-0.92 (m, 1H, cyclopropyl CH), 1.01 (d, J = 6.9 Hz, 3H, *sec*-butyl $CHCH_3$), 0.98 (d, J = 6.3 Hz, 3H, $CHCH_3$), 0.93 (dd, J = 7.4, 7.4 Hz, 3H, *sec*-butyl CH_2CH_3), 0.84-0.75 (m, 1H, cyclopropyl CH_2), 0.65-0.56 (m, 1H, cyclopropyl CH_2); ^{13}C-NMR (125 MHz, THF-d8) δ 173.5, 170.8, 170.3, 143.3, 129.5, 129.2, 126.6, 77.2, 66.0, 54.0, 50.4, 40.2, 38.4, 36.1, 34.1, 29.1, 23.1, 18.4, 15.3, 14.6, 13.0, 12.4, 10.5; LRMS (ESI) *m/z* 458.30 [$(M+H)^+$]; HRMS (ESI) calcd for $C_{26}H_{40}N_3O_4$: 458.3013 [$(M+H)^+$], found: 458.3026; Anal. calcd for $C_{28}H_{39}F_3N_3O_5 \cdot 1.0TFA \cdot 1.8H_2O$: C, 51.40; H, 6.27; N, 5.99. Found: C, 51.44; H, 6.19; N, 5.87.

Stability testing of 2b-6b

0.1 M TEAA buffer

Solutions of **2b-6b** in DMSO (50 µl, 7.5 mM) were diluted with 0.1 M TEAA buffer (950 µl, pH 7.4) and the resulting mixtures were incubated at 37 °C. The time courses were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 µm), 0.8 ml/min, rt, 210 nm, eluents: **2b**, **3b**, MeOH/H_2O/TFA 60:40:0.1; **4b-6b**, MeOH/H_2O/TFA 65:35:0.1. retention times: **2b,** 15.1 min; **3b**, 15.3 min; **4b,** 14.2 min; **5b**, 13.9 min; **6b**, 16.4 min) at various time points from 0 to 32 h.

Human AB serum

Solutions of **2b-6b** in DMSO (50 µl, 23 mM) were diluted with human AB serum (950 µl, Sigma-Aldrich) and the resulting mixtures were incubated at 37 °C. At various time points from 0 to 60 min, the reaction mixtures (100 µl) were sampled, which were immediately quenched with CH_3CN (300 µl). The resulting mixtures were centrifuged (10,000 rpm) for 2 min at 4 °C and the supernatants were analyzed by RP-HPLC (Mightysil RP-18 GP 250-4.6 (5 µm), 0.8 ml/min, rt, 210 nm, eluents: **2b**, **3b**, MeOH/H_2O/TFA 60:40:0.1; **4b-6b**, MeOH/H_2O/TFA 65:35:0.1. retention times: **2b,** 15.1 min; **3b**, 15.3 min; **4b,** 14.2 min; **5b**, 13.9 min; **6b**, 16.4 min).

Proteasome assay

Inhibitory activity of the compound **6a** on the ChT-L activity of human 20S proteasome was measured as described previously.[4]

Cell proliferation assay

Inhibitory activity of the compound **6a** on the cell growth of HCT116 cells was measured as described previously.[4]

Inhibitory effect of 3a-5a and 2b-6b on proteasome CT-L activity and HCT116 cell growth

Table S1. Inhibitory Effect of **3a-5a** and **2b-6b** on Proteasome CT-L Activity and HCT116 Cell Growth

compound	IC_{50} [μM]	
	CT-L activity (proteasome)[a]	cell growth (HCT116)
3a	0.072	5.2
4a	1.3	3.8
5a	1.3	> 10
2b	0.080	1.0
3b	1.0	> 10
4b	7.3	4.0
5b	8.7	> 10
6b	4.5	> 10

[a]Based on three experiments.

Combustion analysis data for 3a-6a and 3b-6b

Table S2. Table listing combustion analysis data for **6a**

3a	Anal. calcd for $C_{32}H_{41}N_3O_6 \cdot 0.1H_2O$: C, 67.97; H, 7.34; N, 7.43.	Found: C, 67.82; H, 7.46; N, 7.14.
4a	Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.1H_2O$: C, 68.39; H, 7.51; N, 7.25.	Found: C, 68.45; H, 7.60; N, 7.00.
5a	Anal. calcd for $C_{33}H_{43}N_3O_6 \cdot 0.2H_2O$: C, 68.18; H, 7.52; N, 7.23.	Found: C, 68.20; H, 7.61; N, 7.10.
6a	Anal. calcd for $C_{34}H_{45}N_3O_6 \cdot 0.4H_2O$: C, 68.18; H, 7.71; N, 7.02.	Found: C, 68.17; H, 7.73; N, 6.93.
3b	Anal. calcd for $C_{26}H_{35}F_3N_3O_5 \cdot 2.5TFA \cdot 4.0H_2O$: C, 42.13; H, 5.19; N, 4.76.	Found: C, 42.22; H, 4.96; N, 4.64.
4b	Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 2.0TFA \cdot 3.0H_2O$: C, 45.26; H, 5.51; N, 5.11.	Found: C, 45.49; H, 5.43; N, 5.20.
5b	Anal. calcd for $C_{27}H_{37}F_3N_3O_5 \cdot 1.5TFA \cdot 1.5H_2O$: C, 48.78; H, 5.66; N, 5.69.	Found: C, 48.56; H, 5.81; N, 5.57.
6b	Anal. calcd for $C_{28}H_{39}F_3N_3O_5 \cdot 1.0TFA \cdot 1.8H_2O$: C, 51.40; H, 6.27; N, 5.99.	Found: C, 51.44; H, 6.19; N, 5.87.

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