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 \textmu m, Kanto Chemical Co., Inc.). Flash column chromatography was performed with silica gel 60 N (spherical, neutral, 40-50 \textmu m, Kanto Chemical Co., Inc.). Combustion analysis was performed to confirm $\geq$95% sample purity (within $\pm0.4\%$ of the calculated value).

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

(4R)-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 (4R)-4-benzyl-2-oxazolidinone (7.09 g, 40.0 mmol, 1.0 equiv) as described for imide 13. [\alpha]$_D^{23}$ -54.95 (c 1.55, CHCl$_3$); $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 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$_3$) $\delta$ 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$_{18}$H$_{21}$NO$_2$Na: 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]$_D^{23}$ -32.93 (c 0.73, CHCl$_3$); mp 89-90 $^\circ$C; $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 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$_2$), 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, C(CH$_3$)$_3$), 1.38-1.29 (m, 1H, CHCH$_3$), 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$_3$) $\delta$ 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$_{23}$NO$_2$Na: 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]$_D^{23}$ -15.24 (c 0.49, CHCl$_3$); $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 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, C(CH$_3$)$_3$), 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$_3$) $\delta$ 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$_{23}$O$_2$Na: 253.1410 [(M+Na)$^+$], found: 253.1410.

(2R,3S)-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 $\beta$-lactone 16. [\alpha]$_D^{23}$ -9.34 (c 0.77, CHCl$_3$); $^1$H-NMR (400 MHz,
CDCl$_3$ δ 4.48 (d, $J = 4.0$ Hz, 1H, OCH$_3$), 3.71 (ddd, $J = 9.9$, 9.9, 4.0 Hz, 1H, COCH$_3$), 1.89-1.70 (m, 3H, CHCH$_2$), 1.52 (s, 9H, C(CH$_3$)$_3$), 0.98 (d, $J = 6.3$ Hz, 3H, CHCH$_3$)$_3$, 0.94 (d, $J = 5.8$ Hz, 3H, CH$_2$CH$_3$); $^{13}$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$_2$Na: 251.1254 [(M+Na)$^+$], found: 251.1255.

(4R)-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 (4R)-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$_2$SO$_4$ 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$^1$] $^{[2]D} -59.79$ (c 1.42, CHCl$_3$) [lit. (for ent-13) $[a]_D^0$ 55.6 (c 1.27, CHCl$_3$))]; $^{1}$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$_3$), 3.31 (dd, $J = 13.2$, 2.9 Hz, 1H, benzyl CH$_3$), 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$_2$CH$_2$)$_3$; $^{13}$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$_{22}$NO$_3$Na: 256.0944 [(M+Na)$^+$], found: 256.0941. $^{1}$H-NMR, $^{13}$C-NMR, $[a]^{22}_D$ 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$_2$CO-C$_7$H$_3$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$_2$SO$_4$ 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. $[a]^{24}_D$ -32.02 (c 1.22, CHCl$_3$); mp 103-104 °C$^1$; $^{1}$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, C(CH$_3$)$_3$), 1.20 (d, $J = 6.9$ Hz, 3H, CHCH$_3$)$_3$; $^{13}$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$_{22}$NO$_3$Na: 370.1625 [(M+Na)$^+$], found: 370.1618. $^{1}$H-NMR and $^{13}$C-NMR data are in agreement with those reported for ent-14 by Stončius.$^2$

(2S)-2-Methylsuccinic acid 4-tert-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·H$_2$O (821 mg, 19.6 mmol, 2.0 equiv) at 0 °C. After 5 min at 0 °C, the reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was cooled to 0 °C, quenched with sat. Na$_2$SO$_4$ and concentrated in vacuo to remove THF. The residual aqueous solution was diluted with 2 M NaOH, extracted with DCM
to remove (4R)-4-benzyl-2-oxazolidinone. The aqueous layer was acidified with citric acid and was extracted with DCM. The organic layer was dried over Na$_2$SO$_4$ 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.

To a solution of carboxylic acid 14 (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 and the resulting mixture was concentrated in vacuo. The residue was dissolved in AcOEt, washed with 1 M HCl 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 (AcOEt) to yield the corresponding chlorinated product as a brown oil.

To a solution of alcohol 13 (31.5 mg, 0.144 mmol) in THF (2.0 ml) was added LiHMDS (23.4 ml, 37.4 mmol, 2.2 equiv, 1.6 M in THF) 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$_2$SO$_4$ 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. [α]$_D^{23}$ -6.93 (c 1.04, CHCl$_3$) [lit. [α]$_D^{23}$ -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$_9$H$_{16}$O$_2$Na: 211.0941 [(M+Na)$^+$], found: 211.0938. $^1$H-NMR, $^{13}$C-NMR and [α]$_D^{23}$ data are in agreement with those reported by Davies.$^3$

(2R,3S)-3-Methyl-4-oxooxetane-2-carboxylic acid t-buty l 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$_2$SO$_4$ 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, 2 steps 42%) as a white solid.

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 10:1) to yield alcohol 17 (1.55 g, 7.11 mmol, quant.) as a pale yellow liquid. [α]$_D^{23}$ 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$_2$CH$_3$), 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, 28.9, 12.5; LRMS (ESI) m/z 209.1 [(M+Na)$^+$]; HRMS (ESI) calcd for C$_{9}$H$_{16}$O$_{2}$Na: 209.0790 [(M+Na)$^+$], found: 209.0789.

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$_2$SO$_4$ 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. [α]$_D^{23}$ 6.93 (c 1.04, CHCl$_3$) [lit. [α]$_D^{23}$ -6.93 (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$_9$H$_{16}$O$_2$Na: 211.0941 [(M+Na)$^+$], found: 211.0938. $^1$H-NMR, $^{13}$C-NMR and [α]$_D^{23}$ data are in agreement with those reported by Davies.$^3$
chromatography (n-hexane/ AcOEt 15:1) to yield alcohol 18 (16.9 mg, 0.0621 mmol, 43%, single isomer) as a colorless oil. [α]_D^23 -15.63 (c 0.93, CHCl₃); 1H-NMR (400 MHz, CDCl₃) δ 4.86 (br, 1H, alkenyl CH₂), 4.72 (br, 1H, alkenyl CH₂), 4.12 (d, J = 7.6 Hz, 1H, CH(OH)), 3.72 (s, 3H, CO₂CH₃), 3.40 (d, J = 7.6 Hz, 1H, OH), 2.67 (d, J = 13.5 Hz, 1H, CH₂), 2.35 (d, J = 13.5 Hz, 1H, CCH₂), 1.69 (s, 3H, allyl CH₂), 1.51 (s, 9H, CCH₃), 1.15 (s, 3H, CCH₃); 13C-NMR (100 MHz, CDCl₃) 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 C₁₄H₂₄O₃Na: 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. [α]_D^23 1.18 (c 0.16, CHCl₃); 1H-NMR (400 MHz, CDCl₃) δ 4.10 (br, 1H, CH(OH)), 3.71 (s, 3H, CO₂CH₃), 3.33 (br, 1H, OH), 1.79 (dd, J = 13.9, 7.2 Hz, 1H, CH₂), 1.74-1.59 (m, 1H, CH₂CH₃), 1.58-1.49 (m, 1H, CH₂), 1.49 (s, 9H, CCH₃), 1.17 (s, 3H, CCH₃), 0.92 (d, J = 6.3 Hz, 3H, CHCH₃), 0.84 (d, J = 6.3 Hz, 3H, CHCH₃); 13C-NMR (100 MHz, CDCl₃) 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 C₁₄H₂₆O₃Na: 297.1673 [(M+Na)⁺], found: 297.1668.

(2R,3S)-3-isobutyl-3-methyl-4-oxooxetane-2-carboxylic acid r-buty ester 20
To a solution of alcohol 19 (132 mg, 0.482 mmol) in 50% aqueous THF (10 ml) was added LiOH·H₂O (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 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 Na₂SO₄ 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. NaHCO₃ and brine, dried over Na₂SO₄ 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. [α]_D^24 -24.89 (c 0.58, CHCl₃); 1H-NMR (500 MHz, CDCl₃) δ 4.65 (s, 1H, COCH), 1.90-1.78 (m, 2H, CH₂ and CHCH₃), 1.71-1.62 (m, 1H, CH₂), 1.52 (s, 9H, CCH₃), 1.31 (s, 3H, CCH₃), 1.00 (d, J = 6.3 Hz, 6H, CHCH₃); 13C-NMR (125 MHz, CDCl₃) 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 C₁₃H₂₂O₃Na: 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 H₂O (250 ml) was added L-isoleucine 21 (20.0 g, 153 mmol) at 0 °C. After 10 min at 0 °C, H₂NOSO₂H (20.0 g, 177 mmol, 1.2 equiv) was added. After 30 min at 0 °C, 2.5 M NaOH (150 ml) and H₂NOSO₂H (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, H₂SO₄ (64 ml) was added at
a rate to keep the internal temperature below 20 °C. The resulting mixture was extracted with DCM, dried over Na₂SO₄ 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 30 min at 0 °C, the reaction mixture was quenched with AcOH (6.98 ml, 119 mmol, 2.0 equiv) and the resulting mixture was concentrated in vacuo and the residue was dissolved in AcOEt, washed with 0.1 M NaOH, extracted with DCM, dried over Na₂SO₄ 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. [α]²¹_0D = 57.70 (c 1.28, CHCl₃); ¹H-NMR (400 MHz, CDCl₃) δ 7.40-7.18 (m, 5H, aromatic), 4.74-4.65 (m, 1H, BnCH), 4.23 (m, 2H, OCH₂), 3.32 (dd, J = 13.0, 3.6 Hz, 1H, benzyl CH₂), 2.99 (dd, J = 16.2, 5.8 Hz, 1H, COCH₂), 2.78-2.68 (m, 2H, COCH₂ and benzyl CH₂), 2.09-1.95 (m, 1H, CHCH₂), 1.52-1.39 (m, 1H, CH₂CH₃), 1.36-1.21 (m, 1H, CH₂CH₃), 0.98 (d, J = 6.7 Hz, 3H, CH₃CH₂), 0.94 (dd, J = 7.4, 7.4 Hz, 3H, CH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃) 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 C₁₉H₂₃NO₃Na: 298.1414 [(M+Na)]⁺; found: 298.1419.

(2S,3R)-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, BrCH₂CO₂t-Bu (17.5 ml, 119 mmol, 2.0 equiv) was added. After 30 min at -78 °C, the reaction mixture 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 Na₂SO₄ 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 H₂O (120 ml), hydrogen peroxide (22.9 ml, 235 mmol, 5.0 equiv, 35%) and LiOH·H₂O (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. Na₂S₂O₃ and concentrated in vacuo to remove THF. The residual aqueous solution was diluted with 0.1 M NaOH, extracted with DCM to remove (4S)-4-benzyl-2-oxazolidinone. The aqueous layer was acidified with citric acid and was extracted with DCM. The organic layer was dried over Na₂SO₄ 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, CCl₄ (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 Na₂SO₄ 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% NaHCO₃ (250 ml). After 43 h at rt, the reaction mixture was diluted with AcOEt, washed with sat. NaHCO₃ and brine, dried over Na₂SO₄ 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. [α]²⁰_D = 5.39 (c 0.76,
CHCl₃); mp 55-56 °C; ¹H-NMR (400 MHz, CDCl₃) 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, CH₂CH₃), 1.63-1.44 (m, 1H, CH₂CH₃), 1.51 (s, 9H, C(CH₃)₃), 1.37-1.23 (m, 1H, CH₂CH₃), 1.10 (d, J = 6.7 Hz, 3H, CH(CH₃)₃), 0.95 (dd, J = 7.4, 7.4 Hz, 3H, CH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃) 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 C₁₃H₂₀O₃Na: 251.1259 [(M+Na)⁺], found: 251.1258.

To a solution of the oil in DCM (120 ml) was added THF (10 ml), MS 4 Å (2.10 g) and Ag₂CO₃ (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.

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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. NaHCO₃ and brine, dried over Na₂SO₄ 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. [α]²⁵D < 19.25 (c 0.30, CHCl₃); mp 52-54 °C. ¹H-NMR (400 MHz, CDCl₃) δ 4.53 (d, J = 4.7 Hz, 1H, CHOH), 3.67 (s, 3H, OCH₃), 3.09 (d, J = 4.7 Hz, 1H, OH), 1.78-1.65 (m, 2H, CH₂CH₃ and CH₂CH₂CH₃), 1.47 (s, 9H, t-butyl CH₃), 1.29-1.13 (m, 1H, CH₂CH₃), 1.07 (s, 3H, CCH₃), 0.90 (dd, J = 7.4, 7.4 Hz, 3H, CH₂CH₃), 0.86 (d, J = 7.2 Hz, 3H, CHCH₂); ¹³C-NMR (100 MHz, CDCl₃) 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 C₁₄H₂₆O₂Na: 297.1673 [(M+Na)⁺], found: 297.1668.

(2S,3R)-4-t-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. Na₂S₂O₃ and sat. NaHCO₃ (1:3), extracted with CHCl₃, the organic layer was washed with water, dried over Na₂SO₄ 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 BH₃·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. NH₄Cl and brine, dried over Na₂SO₄ 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. [α]²⁵D < 4.07 (c 1.01, CHCl₃); ¹H-NMR (400 MHz, CDCl₃) δ 4.04 (d, J = 10.8 Hz, 1H, CHOH), 3.71 (s, 3H, OCH₃), 3.62 (d, J = 10.8 Hz, 1H, OH), 2.19-2.07 (m, 1H, CH₂CH₃), 1.47 (s, 9H, t-butyl CH₃), 1.24-1.01 (m, 2H, CH₂CH₂CH₃), 1.06 (s, 3H, CCH₃), 0.94-0.87 (m, 6H, CH₂CH₃ and CHCH₂); ¹³C-NMR (100 MHz, CDCl₃) 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 C₁₄H₂₆O₂Na: 297.1673 [(M+Na)⁺], found: 297.1668.

(2S,3S)-2-((R)-2-(benzylxy)-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 BrOH (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/ CHCl₃/ MeOH 5:1:0:0-0.1:0.0:100:5) to yield carboxylic acid 31 (128 mg, 0.436 mmol, 3 steps 82%) as a colorless viscous oil. [α]²⁴D < 4.55 (c 0.24, CHCl₃); ¹H-NMR (500 MHz, CD₃OD) δ 7.43-7.29 (m, 5H, aromatic), 5.19 (d, J = 12.0 Hz, 1H, benzyl CH₂), 5.14 (d, J = 12.0 Hz, 1H, benzyl CH₂), 4.41 (s, 1H, OCH), 1.90-1.81 (m, 1H, CH₂CH₃), 1.58-1.46 (m, 1H, CH₃CH₃), 1.29-1.13 (m, 1H, CH₂CH₃).
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. NaHCO₃ and brine, dried over Na₂SO₄ 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. [α]²⁴D 0.86 (c 1.17, CHCl₃); mp 38-39 °C; ¹H-NMR (400 MHz, CDCl₃) δ 7.41-7.33 (m, 5H, aromatic), 5.29 (d, J = 11.9 Hz, 1H, benzyl CH₂), 5.25 (d, J = 11.9 Hz, 1H, benzyl CH₂), 4.70 (s, 1H, OCH), 1.89-1.75 (m, 1H, CH₃CH₂), 1.77-1.66 (m, 1H, CH₂CH₃), 1.12 (s, 3H, CCH₃), 1.01 (d, J = 6.7 Hz, 3H, CH₂CH₃), 0.94 (dd, J = 7.4, 7.4 Hz, 3H, CH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃) 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) caleld for C₁₆H₂₀O₄Na: 299.1254 [(M+Na)+], found: 299.1250.

(2R,3S)-3-((S)-sec-butyl)-3-methyl-4-oxooxetane-2-carboxylate 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. [α]²⁴D -4.42 (c 0.82, CHCl₃); mp 85-87 °C; ¹H-NMR (500 MHz, CDCl₃) δ 8.81 (br, 1H, COOH), 4.74 (s, 1H, OCH), 1.93-1.83 (m, 1H, CH₂CH₂), 1.78-1.70 (m, 1H, CH₃CH₂), 1.25-1.09 (m, 1H, CH₂CH₃), 1.06 (d, J = 6.9 Hz, 3H, CH₂CH₃), 0.97 (dd, J = 7.4, 7.4 Hz, 3H, CH₂CH₃); ¹³C-NMR (125 MHz, CDCl₃) 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) caleld for C₉H₁₃O₂: 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. NaHCO₃ and brine, dried over Na₂SO₄ 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. [α]D20 -2.32 (c 0.34, CHCl3); 1H-NMR (400 MHz, CDCl3) 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 CH2), 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, NCOCH), 2.77 (br, 1H, cyclopropyl CH), 2.63 (br, 2H, benzyl CH2), 1.97-1.58 (m, 6H, BnCH2, NCH2CH2 (1H), isobutyl CH2 and isobutyl CH), 1.39 (d, J = 7.2 Hz, 3H, Ala CH3), 1.24-1.10 (m, 1H, NCHCH2), 1.07-0.84 (m, 8H, cyclopropyl CH2 (1H), cyclopropyl CH, isobutyl CH3), 0.27-0.18 (m, 1H, cyclopropyl CH3); 13C-NMR (100 MHz, CDCl3) 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 C32H42N3O6·0.1H2O: 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. NaHCO3 and brine, dried over Na2SO4 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. [α]D20 -15.09 (c 0.41, CHCl3); 1H-NMR (500 MHz, CDCl3) 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 CH2), 5.07 (d, J = 12.6 Hz, 1H, benzyl CH2), 4.73 (s, 1H, NCOCH), 4.35 (d, 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 CH2), 1.92-1.56 (m, 5H, NCHCH2 (1H), BnCH2, isobutyl CH2 (1H) and isobutyl CH), 1.43 (d, J = 6.9 Hz, 3H, Ala CH3), 1.36-1.08 (m, 5H, isobutyl CH2 (1H), NCHCH2 (1H) and CCH3), 1.08-0.78 (m, 8H, cyclopropyl CH2 (1H), cyclopropyl CH and isobutyl CH3), 0.28-0.18 (m, 1H, cyclopropyl CH2); 13C-NMR (100 MHz, CDCl3) δ 173.2, 172.8, 169.2, 155.9, 141.5, 136.5, 128.5, 128.3, 128.1, 128.0, 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 C33H44N3O6·0.1H2O: 578.3230 [(M+H)+], found: 578.3224; Anal. calcd for C33H44N3O6·0.1H2O: 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. NaHCO₃ and brine, dried over Na₂SO₄ 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. [α]D₂⁰ = -8.24 (c 0.53, CHCl₃);

1H-NMR (500 MHz, CDCl₃) 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 CH₂), 5.06 (d, J = 12.3 Hz, 1H, benzyl CH₂), 4.60 (s, 1H, OCH), 4.37 (dq, J = 7.4, 6.9 Hz, 1H, Ala CH), 4.09-3.99 (m, 1H, NCH₂), 2.75 (br, 1H, cyclopropyl CH), 2.67-2.54 (m, 2H, PhCH₂CH₂), 1.83-1.63 (m, 5H, NCHCH₂ (1H), sec-butyl CH₂ (1H), PhCH₂CH₂ (2H) and sec-butyl CH), 1.43 (d, J = 6.9 Hz, 3H, Ala CH₃), 1.28-1.15 (m, 1H, NCHCH₂), 1.20 (s, 3H, CCH₃), 1.14-0.86 (m, 9H, sec-butyl CH₂ (1H), cyclopropyl CH, cyclopropyl CH₂ (1H) and sec-butyl CH₃), 0.29-0.18 (m, 1H, cyclopropyl CH₃), 13C-NMR (125 MHz, CDCl₃) δ 172.8, 172.7, 169.2, 155.8, 141.5, 136.4, 128.4, 128.2, 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) calcld for C₃₃H₄₅N₃O₆Na: 600.3044 [(M+Na)⁺], found: 600.3046; Anal. calcld for C₃₃H₄₅N₃O₆·0.2H₂O: 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. NaHCO₃ and brine, dried over Na₂SO₄ 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. [α]D₂⁰ = -63.77 (c 0.81, CHCl₃); 1H-NMR (400 MHz, CDCl₃) 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 CH₂), 4.59 (s, 1H, OCH), 4.17 (dq, J = 7.2, 6.7 Hz, 1H, Ala CH), 4.14-4.04 (m, 1H, NCH₂), 2.95-2.83 (m, 1H, cyclopropyl CH), 2.70-2.51 (m, 2H, PhCH₂CH₂), 1.90-1.60 (m, 4H, PhCH₂CH₂ (2H), sec-butyl CH₂ (1H) and sec-butyl CH), 1.39 (d, J = 7.2 Hz, 3H, Ala CH₃), 1.23 (s, 3H, CCH₃), 1.17-1.05 (m, 2H, sec-butyl CH₂ and NCHCH₂), 1.04 (d, J = 6.7 Hz, 3H, sec-butyl CHCH₂), 1.01-0.88 (m, 7H, cyclopropyl CH₂ (1H), NCHCH₂ and sec-butyl CH₂CH₂), 0.81-0.67 (m, 1H, cyclopropyl CH), 0.49-0.36 (m, 1H, cyclopropyl CH₂); 13C-NMR (125 MHz, CDCl₃) δ 172.8, 171.8, 168.3, 156.1, 141.5, 136.0, 128.6, 128.5, 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) calcld for C₃₄H₄₃N₃O₇Na: 614.3201 [(M+Na)⁺], found: 614.3203; Anal. calcld for C₃₄H₄₃N₃O₇·0.4H₂O: C, 68.18; H, 7.71; N, 7.02. Found: C, S11
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 g, 0.0182 mmol, quant.) was obtained from 3a as a colorless oil. [α]22D -14.02 (c 0.39, THF); 1H-NMR (500 MHz, THF-d8) δ 8.55 (br, 3H, NH3+), 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, PhCH2), 2.60-2.46 (m, 1H, PhCH2), 1.85-1.62 (m, 5H, PhCH2CH2, isobutyl CH and isobutyl CH2), 1.54 (d, J = 6.3 Hz, 3H, Ala CH), 1.53-1.40 (m, 2H, NCHCH2), 1.15-1.04 (m, 1H, cyclopropyl CH), 0.95 (d, J = 6.3 Hz, 3H, isobutyl CH3), 0.91 (d, J = 6.3 Hz, 3H, isobutyl CH3), 0.91-0.79 (m, 1H, cyclopropyl CH2), 0.41-0.31 (m, 1H, cyclopropyl CH2); 13C-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 C26H33N2O4: 430.2700 [(M+H)+]; found: 430.2705; Anal. calcd for C26H33N2O4: 2.5TFA·4.0H2O: 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 g, 0.0261 mmol, quant.) was obtained from 4a as a colorless oil. [α]22D -32.25 (c 0.48, THF); 1H-NMR (500 MHz, THF-d8) δ 8.61 (d, J = 8.6 Hz, 1H, amide NH), 8.60 (br, 3H, NH3+), 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, PhCH2), 2.57-2.48 (m, 1H, PhCH2), 1.90-1.62 (m, 5H, PhCH2CH2, isobutyl CH2 and isobutyl CH), 1.62-1.52 (m, 1H, NCHCH2), 1.56 (d, J = 6.9 Hz, 3H, Ala CH3), 1.37-1.14 (m, 2H, NCHCH2 and cyclopropyl CH), 1.26 (s, 3H, CCH3), 1.02 (d, J = 6.3 Hz, 3H, isobutyl CH3), 0.97 (d, J = 6.3 Hz, 3H, isobutyl CH3), 0.89-0.79 (m, 1H, cyclopropyl CH2), 0.46-0.38 (m, 1H, cyclopropyl CH2); 13C-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 C27H35F3N2O4: 444.2857 [(M+H)+]; found: 444.2860; Anal. calcd for C27H35F3N2O4: 2.0TFA·3.0H2O: 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. [α]22D -36.31 (c 0.77, THF); 1H-NMR (500 MHz, THF-d8) δ 8.67 (d, J = 8.6 Hz, 1H, amide NH), 8.58 (br, 3H, NH3+), 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, PhCH2 and cyclopropyl CH), 2.58-2.46 (m, 1H, PhCH2), 1.89-1.63 (m, 5H, sec-butyl CH2, PhCH2CH2 and sec-butyl CH), 1.63-1.52 (m, 1H, NCHCH2), 1.57 (d, J = 6.9 Hz, 3H, Ala CH3),
1.27-1.08 (m, 2H, NCHCH₂ and cyclopropyl CH), 1.21 (s, 3H, CCH₃), 1.01 (d, J = 6.9 Hz, 3H, sec-butyl CHCH₃), 0.94 (dd, J = 7.4, 7.4 Hz, 3H, sec-butyl CH₂CH₂), 0.89-0.78 (m, 1H, cyclopropyl CH₂), 0.48-0.38 (m, 1H, cyclopropyl CH); ¹³C-NMR (125 MHz, THF-d₈) δ 173.3, 171.5, 170.8, 143.4, 129.5, 129.1, 126.6, 77.1, 66.1, 50.4, 50.5, 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 C₂₅H₃₈N₇O₇: 444.2857 [(M+H)⁺], found: 444.2869; Anal. calcd for C₂₅H₃₈F₃N₇O₇: 1.0TFA·1.5H₂O: 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. [α]²³D -51.07 (c 0.97, THF); ¹H-NMR (500 MHz, THF-d₈) δ 8.58 (br, 3H, NH₃⁺), 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, PhCH₂), 2.58-2.44 (m, 1H, PhCH₃), 1.91-1.65 (m, 4H, PhCH₂CH₂ (2H), sec-butyl CH₂ (1H) and sec-butyl CH), 1.55 (d, J = 6.9 Hz, 3H, Ala CH₃), 1.19 (s, 3H, CCH₃), 1.16-1.05 (m, 2H, sec-butyl CH₂ and NCHCH), 1.05-0.92 (m, 1H, cyclopropyl CH), 1.01 (d, J = 6.9 Hz, 3H, sec-butyl CHCH₃), 0.98 (d, J = 6.3 Hz, 3H, CHCH₃), 0.93 (dd, J = 7.4, 7.4 Hz, 3H, sec-butyl CH₂CH₃), 0.84-0.75 (m, 1H, cyclopropyl CH₂), 0.65-0.56 (m, 1H, cyclopropyl CH₂); ¹³C-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 C₂₆H₄₀N₇O₇: 458.3013 [(M+H)⁺], found: 458.3026; Anal. calcd for C₂₆H₄₀F₃N₇O₇·1.0TFA·1.8H₂O: 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/H₂O/TFA 60:40:0.1; 4b-6b, MeOH/H₂O/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 CH₃CN (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/H₂O/TFA 60:40:0.1; 4b-6b, MeOH/H₂O/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**

<table>
<thead>
<tr>
<th>compound</th>
<th>IC(_{50}) [(\mu)M]</th>
<th>CT-L activity (proteasome)(^a)</th>
<th>cell growth (HCT116)</th>
</tr>
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<tbody>
<tr>
<td>3a</td>
<td>0.072</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>1.3</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>1.3</td>
<td>&gt; 10</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>0.080</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>1.0</td>
<td>&gt; 10</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>7.3</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td>8.7</td>
<td>&gt; 10</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>4.5</td>
<td>&gt; 10</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Based on three experiments.

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

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

<table>
<thead>
<tr>
<th>3a</th>
<th>Anal. calcd for C(<em>{32})H(</em>{41})N(_3)O(_6)•0.1H(_2)O: C, 67.97; H, 7.34; N, 7.43.</th>
<th>Found: C, 67.82; H, 7.46; N, 7.14.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Anal. calcd for C(<em>{33})H(</em>{43})N(_3)O(_6)•0.1H(_2)O: C, 68.39; H, 7.51; N, 7.25.</td>
<td>Found: C, 68.45; H, 7.60; N, 7.00.</td>
</tr>
<tr>
<td>5a</td>
<td>Anal. calcd for C(<em>{33})H(</em>{43})N(_3)O(_6)•0.2H(_2)O: C, 68.18; H, 7.52; N, 7.23.</td>
<td>Found: C, 68.20; H, 7.61; N, 7.10.</td>
</tr>
<tr>
<td>6a</td>
<td>Anal. calcd for C(<em>{34})H(</em>{45})N(_3)O(_6)•0.4H(_2)O: C, 68.18; H, 7.71; N, 7.02.</td>
<td>Found: C, 68.17; H, 7.73; N, 6.93.</td>
</tr>
<tr>
<td>3b</td>
<td>Anal. calcd for C(<em>{26})H(</em>{35})F(_3)N(_3)O(_5)•2.5TFA•4.0H(_2)O: C, 42.13; H, 5.19; N, 4.76.</td>
<td>Found: C, 42.22; H, 4.96; N, 4.64.</td>
</tr>
<tr>
<td>4b</td>
<td>Anal. calcd for C(<em>{27})H(</em>{37})F(_3)N(_3)O(_5)•2.0TFA•3.0H(_2)O: C, 45.26; H, 5.51; N, 5.11.</td>
<td>Found: C, 45.49; H, 5.43; N, 5.20.</td>
</tr>
<tr>
<td>5b</td>
<td>Anal. calcd for C(<em>{27})H(</em>{37})F(_3)N(_3)O(_5)•1.5TFA•1.5H(_2)O: C, 48.78; H, 5.66; N, 5.69.</td>
<td>Found: C, 48.56; H, 5.81; N, 5.57.</td>
</tr>
<tr>
<td>6b</td>
<td>Anal. calcd for C(<em>{28})H(</em>{39})F(_3)N(_3)O(_5)•1.0TFA•1.8H(_2)O: C, 51.40; H, 6.27; N, 5.99.</td>
<td>Found: C, 51.44; H, 6.19; N, 5.87.</td>
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</tbody>
</table>
References