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Chemistry & Drug Discovery

Supporting Information

Cis-configured aziridines are new pseudo-irreversible dual-mode inhibitors of *Candida albicans* secreted aspartic protease 2

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Experimental part

Experimental part

General information

ESI mass spectra were recorded on an Agilent 1100 LC/MSD-Trap. For the characterization of the purity of the compounds LC/MS-analyses using the HPLC system 1100 from Agilent and a Phenomenex Jupiter 4 µ Proteo 90A RP C-18 column (4.5 x 150 mm) with a water-acetonitrile gradient was used (40% acetonitrile containing 0.1% formic acid for 5min, 40-95% for 25 min, 95% for 15 min). NMR spectra were recorded on an AVANCE 400 MHz spectrometer from Bruker Biospin GmbH, Germany (solvent: $CDCl_3$, ^{1}H-NMR: 400.13 MHz; ^{13}C-NMR: 100.61 MHz). IR spectra were recorded on a FT-IR spectrometer, type PharmalyzIR, from BioRad, USA. α values were detemined on a 241 polarimeter from PerkinElmer, USA. Melting points were determined in open capillary on a type 510 apparatus from Büchi, Switzerland. Column chromatography was performed with silica gel 60 from Merck (0.063-0.2 mm or 70-230 mesh). For TLC alumina sheets from Merck coated with silica gel 60 F_{254} were used. All solvents were purified and dried prior to use according to standard literature procedures. All reactions were performed in a N_2 atmosphere under strict exclusion of humidity.

Syntheses

The syntheses and analytical data of the following compounds were published previously: **1a-d**, **2**, **3a+b**, **5a**, **8a+b**, **8a**, **10d1**, **10d2**, **11a+b**.[1]

Syntheses and analytical data of the new compounds

The epoxide-based inhibitor **4a** was synthesized with the same methods as described in ref. [1] for compund **5a**: starting from Boc-*(S)*-phenylalaninal, obtained by DIBAL-H reduction of Boc-*(S)*-PheOMe, a Horner-Wadsworth-Emmons olefination[2] with ethyl(diphenylphosphono) acetate[3] was subsequently performed, and finally the obtained *Z*-configured olefin was epoxidized with mCPBA.

Ethyl-(*R*)-[(3*R*)-((S)-1-*tert*-Butoxycarbonylamino-2-phenylethyl)oxirane]-2-carboxylate (4a)

Column chromatography (silica gel 60, cyclohexane/EtOAc 2:1, R_f = 0.49,).

Yellowish solid.

Mp: 112-113 °C.

$[a]_D^{23}$ = + 3.40 ° (c = 1.00, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{18}H_{25}NO_5$, 335.40; found: $[M+Na]^+$ 358.4.

LC-MS: R_t = 21.1 min, k' = 22.44 (purity 100%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 1.20 (t, 3 H, C<u>*H*₃</u>, *J* =7.1 Hz), 1.34 (s, 9 H, C(C<u>*H*₃</u>)₃), 2.73-2.99 (m_c, 2 H, C<u>*H*₂</u>Ph, *J* = 6.8, 7.9 Hz), 3.30 (m, 1 H, Epox-C<u>*H*</u>-CH), 3.40 (d, 1 H, Epox-C<u>*H*</u>-CO, *J* = 4.3 Hz), 3.98 (m, 1 H, C<u>*H*</u>CH_2Ph, *J* = 6.8 Hz), 4.03 (q, 2 H, OC<u>*H*₂</u>, *J* = 7.1 Hz), 4.63 (br s, 1 H, N<u>*H*</u>), 7.07-7.24 (m, 5 H, Ar-<u>*H*</u>) ppm.

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 14.05 (<u>*C*</u>H_3), 28.31 (C(<u>*C*</u>H_3)$_3$), 41.27 (<u>*C*</u>H_2Ph), 52.87 (<u>*C*</u>HCH_2Ph), 55.78 (Epox-<u>*C*</u>H-CO), 58.29 (Epox-<u>*C*</u>H-CH), 61.74 (O<u>*C*</u>H_2), 79.69 (<u>*C*</u>$(CH_3)_3$), 126.67, 128.53, 129.40 (Ar-<u>*C*</u>H), 136.76 (Ar-<u>*C*</u>), 154.96 (<u>*C*</u>=O [Boc]), 167.33 (EtO-<u>*C*</u>=O) ppm.

IR (neat): $\tilde{\nu}$ = 3358 (m, CO<u>N-H</u>); 3026, 2954, 2924, 2854 (m, =C-H, C-H); 1743 (s, O-<u>C=O</u>); 1684 (s, N-<u>C=O</u>); 1527 (s, OC<u>N-H</u>); 1414, 1382 (w, aromat. C=C, C-H); 1250, 1201, 1177 (C-O-C); 912, 863 (m, Epox C-O-C); 752, 701, 639 (s, aromat. C-H) cm^{-1}.

The 2-methyl-2-phenyl-substituted epoxides **6a+b** and **6c+d** were synthesized by glycide ester synthesis as described in ref.[4], and by subsequent hydrolysis with KOH and DPPA-mediated peptide coupling. The four resulting diastereomers were separated by column chromatography yielding the *cis*-configured diastereomers **6a+b** and the *trans*-configured diastereomers **6c+d**.

Benzyl-(*S*)-2-[((2*R*,3*R*)+(2*S*,3*S*)-3-Methyl-3-phenyloxiranecarbonyl)amino]-3-phenylpropionate (6a+b)

Column chromatography (silica gel 60, cyclohexane/EtOAc 4:1, R_f = 0.16).

Yellowish solid.

$[a]_D^{22}$ = - 6.64 ° (c = 1.22, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{26}H_{25}NO_4$, 415.49; found: $[M+Na]^+$ 438.6.

LC-MS: R_t = 23.6 min, k' = 25.22 and R_t = 23.9 min, k' = 25.56 (purity 95%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 1.61 (s, 3 H, C<u>*H*₃</u>), 2.87 (m_c, 2 H, β-C<u>*H*₂</u>, *J* = 5.5, 7.3 Hz), 3.57 (s, 1 H, Epox-C<u>*H*</u>), 4.37 (dt, 1 H, α-C<u>*H*</u>, *J* = 5.5, 7.8 Hz), 4.77, 4.78 (2 x d, je 1 H, OC<u>*H*₂</u>Ph, *J* = 12.1 Hz), 6.22 (br d, 1 H, N<u>*H*</u>, *J* = 7.8 Hz), 7.00-7.25 (m, 15 H, Ar-<u>*H*</u>) ppm [(*R*,*R*,*S*)-Isomer].

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 1.62 (s, 3 H, C<u>*H*₃</u>), 1.97-2.02, 2.35-2.40 (2 x dd, 2 H, β-C<u>*H*₂</u>, *J* = 5.3, 7.9 Hz), 3.58 (s, 1 H, Epox-C<u>*H*</u>), 4.40 (dt, 1 H, α-C<u>*H*</u>, *J* = 5.3, 8.4 Hz), 4.88, 4.89 (2 x d, je 1 H, OC<u>*H*₂</u>Ph, *J* = 12.1 Hz), 6.22 (br d, 1 H, N<u>*H*</u>, *J* = 8.4 Hz), 7.10-7.34 (m, 15 H, Ar-<u>*H*</u>) ppm [(*S*,*S*,*S*)-Isomer].

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 25.67 (<u>*C*</u>H_3), 38.28 (β-<u>*C*</u>H_2), 52.43 (α-<u>*C*</u>H), 61.95 (Epox-<u>*C*</u>H-CO), 65.17 (Epox-<u>*C*</u>), 66.76 (O<u>*C*</u>H_2Ph), 126.10, 126.87, 127.79, 128.25, 128.34, 128.44,

128.60, 128.87, 129.24 (Ar-*C*H), 134.87, 135.30, 136.95 (Ar-*C*), 166.30 (N-*C*=O), 170.68 (BnO-*C*=O) ppm [(*R*,*R*,*S*)-Isomer].

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 25.35 (*C*H_3), 38.00 (β-*C*H_2), 52.43 (α-*C*H), 61.80 (Epox-*C*H-CO), 65.23 (Epox-*C*), 66.97 (O*C*H_2Ph), 126.40, 126.97, 127.79, 128.30, 128.39, 128.49, 128.60, 128.87, 129.24 (Ar-*C*H), 134.98, 135.33, 137.64 (Ar-*C*), 166.48 (N-*C*=O), 169.76 (BnO-*C*=O) ppm [(*S*,*S*,*S*)-Isomer].

IR (neat): $\tilde{\nu}$ = 3410 (w, br, OCN-H); 3063, 2982 (w, =C-H, C-H); 1738 (s, O-C=O); 1673 (s, N-C=O); 1518 (m, OCN-H); 1445, 1383 (w, aromat. C=C, C-H); 1190, 1124 (s, C-O-C); 957, 888 (m, Epox C-O-C); 742, 697 (s, aromat. C-H) cm^{-1}.

Benzyl-(*S*)-2-[((2*S*,3*R*)+(2*R*,3*S*)-3-Methyl-3-phenyloxiranecarbonyl)amino]-3-phenylpropionate (6c+d)

Column chromatography (silica gel 60, cyclohexane/EtOAc 4:1, R_f = 0.21).

SRS : *RSS* = 1 : 1. – Colourless solid.

Mp.: 93-94 °C.

$[a]_D^{22}$ = + 2.68 ° (c = 1.12, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{26}H_{25}NO_4$, 415.49; found: $[M+Na]^+$ 438.5.

LC-MS: R_t = 24.1 min, k' = 25.78 (purity 99%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 1.35 (s, C*H*$_3$, Dia1) and 1.65 (s, C*H*$_3$, Dia2) (together 3 H), 3.06-3.11 (dd, β-C*H*$_2$, *J* = 5.8, 8.1 Hz, Dia1) and 3.12-3.22 (m_c, β-C*H*$_2$, *J* = 6.3, 7.5 Hz, Dia2) and 3.23-3.30 (dd, β-C*H*$_2$, *J* = 5.8, 8.3 Hz, Dia1) (together 2 H), 3.40 (s, Epox-C*H*, Dia1) and 3.42 (s, Epox-C*H*, Dia2) (together 1 H), 4.95-5.06 (m, together 1 H, α-C*H*, *J* = 5.8, 8.4 Hz), 5.14-5.22 (m, together 2 H, OC*H*$_2$Ph, *J* = 12.1 Hz), 6.66 (br d, together 1 H, N*H*, *J* = 8.4 Hz), 7.06-7.37 (m, 15 H, Ar-*H*) ppm.

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 16.97, 17.19 (*C*H_3), 37.83, 37.91 (β-*C*H_2), 52.35, 52.79 (α-*C*H), 62.89 (Epox-*C*H-CO), 63.33, 63.53 (Epox-*C*), 67.37 (O*C*H_2Ph), 125.12, 125.16, 127.21, 127.25, 128.03, 128.39, 128.44, 128.60, 128.65, 128.74, 129.03, 129.13 (Ar-*C*H), 134.90, 135.03, 135.27, 135.59 (Ar-*C*), 166.48, 166.74 (N-*C*=O), 170.82, 170.93 (BnO-*C*=O) ppm.

IR (neat): $\tilde{\nu}$ = 3258 (w, br, OCN-H); 3063, 3033 (w, =C-H, C-H); 1730 (s, O-C=O); 1653 (s, N-C=O); 1545 (s, OCN-H); 1445, 1383 (w, aromat. C=C, C-H); 1275, 1225, 1180 (s, C-O-C); 911, 856 (m, Epox C-O-C); 751, 695 (s, aromat. C-H) cm^{-1}.

The oxirane-2,3-dicarboxylate derivatives **7a+b** were synthesized as described for **3a+b** in ref. [1]: starting from diethyl maleate a Weitz-Schäffer epoxidation[5] was performed. Subsequent hydrolysis with pig liver esterase (PLE) [6] lead to the *(R,S)*-configured half ester (ee = 18%)

which was coupled with *(S)*-Phe-*(S)*-LeuOBn using PyBOP (1-benzotriazole oxytrispyrrolidino phosphonium hexafluoro phosphate) as coupling reagent.

Ethyl-(2*R*,3*S*)+(2*S*,3*R*)-3-[(*S*)-1-((*S*)-1-Benzyloxycarbonyl-3-methylbutylcarbamoyl)-2-phenylethylcarbamoyl]oxirane-2-carboxylate (7a+b)

Column chromatography (silica gel 60, cyclohexane/EtOAc 2:1, R_f = 0.15)

RSSS : *SRSS* = 1.44 : 1. – Yellowish solid.

$[a]_D^{22}$ = - 20.08 ° (c = 1.21, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{26}H_{34}N_2O_7$, 510.59; found: $[M+Na]^+$ 533.7.

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 0.84-0.88 (2 x d, together 6 H, δ-C*H*$_3$ [Leu], *J* = 6.6 Hz), 1.24-1.33 (2 x t, together 3 H, C*H*$_3$, *J* = 7.1 Hz), 1.46-1.61 (m, together 3 H, γ-C*H* [Leu], β-C*H*$_2$ [Leu]), 2.99-3.05 (dd, β-C*H*$_2$ [Phe], *J* = 6.6, 8.3 Hz, *RSSS*) and 3.10-3.16 (m, β-C*H*$_2$ [Phe], *J* = 6.6, 8.1 Hz, *SRSS*) and 3.24-3.30 (dd, β-C*H*$_2$ [Phe], *J* = 6.6, 7.9 Hz, *RSSS*) (together 2 H), 3.66-3.72 (2 x d, together 2 H, Epox-C*H*, *J* = 4.8, 5.3 Hz), 4.09-4.24 (m, together 2 H, OC*H*$_2$, *J* = 7.1 Hz), 4.53-4.64 (m, α-C*H* [Phe], *RSSS*, α-C*H* [Leu]) and 4.84-4.93 (m, α-C*H* [Phe], *SRSS*) (together 2 H), 5.06-5.16 (m, together 2 H, OC*H*$_2$Ph, *J* = 12.4 Hz), 6.05 (d, N*H* [Phe], *J* = 8.1 Hz, *RSSS*) and 6.59 (br d, N*H* [Phe], *J* = 9.6 Hz, *SRSS*) (together 1 H), 7.05 (br d, together 1 H, N*H* [Leu], *J* = 7.9 Hz), 7.18-7.36 (m, together 10 H, Ar-*H*) ppm.

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 14.02 (*C*H$_3$), 21.94, 22.59 (2 x δ-*C*H$_3$ [Leu]), 24.60 (γ-*C*H [Leu]), 37.56 (β-*C*H$_2$ [Phe]), 41.45 (β-*C*H$_2$ [Leu]), 50.98 (α-*C*H [Leu]), 52.95 (Epox-*C*H-C(O)N), 53.49 (α-*C*H [Phe]), 54.43 (Epox-*C*H-CO$_2$Et), 62.26 (O*C*H$_2$), 66.96 (O*C*H$_2$Ph), 126.97, 127.92, 128.20, 128.46, 128.63, 129.36 (Ar-*C*H), 135.28, 136.29 (Ar-*C*), 164.81 (N-*C*=O [Epox]), 165.89 (EtO-*C*=O), 169.44 (N-*C*=O [Phe-Leu]), 171.91 (BnO-*C*=O) ppm.

IR (neat): $\tilde{\nu}$ = 3369, 3308 (w, br, CON-H); 3065, 3031, 2957, 2928 (w, =C-H, C-H); 1740 (s, O-C=O); 1656 (s, N-C=O); 1532 (s, OCN-H); 1452, 1381 (w, aromat. C=C, C-H); 1209, 1150 (s, C-O-C); 957, 858 (m, Epox C-O-C); 745, 698 (s, aromat. C-H) cm^{-1}.

The synthesis of the aziridine-2,3-dicarboxylate derived inhibitor **9a+b** started from *(R,R)*-tartrate. The *(S,S)*-bromo alcohol was obtained by a literature-known two-step procedure.[7] From this intermediate the azido alcohol[8] was obtained through reaction with sodium azide as mixture of *syn(R,R)*- and *anti(R,S)*-diastereomers (7.3:1). These were subjected to ring closure by Staudinger reaction[9] to give the *cis*- and *trans*-aziridine-2,3-dicarboxylates, which were separated by column chromatography. Benzylation of the *cis*-isomer with benzyl bromide yielded the *N*-benzylated aziridine-2,3-dicarboxylate which was hydrolyzed and coupled with *(S)*-PheOBn to give the inhibitor **9a+b** as diastereomeric mixture.

Ethyl-(2*S*,3*R*)+(2*R*,3*S*)-1-Benzyl-3-((*S*)-1-benzyloxycarbonyl-2-phenylethylcarbamoyl)aziridine-2-carboxylate (9a+b)

Column chromatography (silica gel 60, cyclohexane/EtOAc 2:1, R_f = 0.21).

Dia1 : Dia2 = 1 : 1.7. – Yellowish solid.

$[a]_D^{22}$ = + 4.27 ° (c = 1.03, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{29}H_{30}N_2O_5$, 486.57; found: $[M+H]^+$ 487.6.

LC-MS: R_t = 24.6 min, k' = 26.33 (purity 94%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 1.21 (t, C*H*$_3$, *J* = 7.1 Hz, Dia1) and 1.25 (t, C*H*$_3$, *J* = 7.1 Hz, Dia2) (together 3 H), 2.55-2.63 (m, together 2 H, Azi-C*H*, *J* = 7.0 Hz), 2.95-3.04, 3.08-3.18 (2 x m, together 2 H, β-C*H*$_2$, *J* = 5.6, 6.3, 7.4 Hz), 3.47, 3.82 (2 x d, NC*H*$_2$Ph, *J* = 13.4 Hz, Dia1) and 3.65, 3.69 (2 x d, NC*H*$_2$Ph, *J* = 13.6 Hz, Dia2) (together2 H), 4.03-4.16 (m, together 2 H, OC*H*$_2$, *J* = 7.1 Hz), 4.76 (dt, α-C*H*, *J* = 6.3, 7.6 Hz, Dia1) and 4.85 (dt, α-C*H*, *J* = 5.6, 7.1 Hz, Dia2) (together 1 H), 5.05, 5.06 (2 x d, OC*H*$_2$Ph, *J* = 12.1 Hz, Dia2) and 5.11, 5.12 (2 x d, OC*H*$_2$Ph, *J* = 12.4 Hz, Dia1) (together 2 H), 6.97-7.36 (m, together 16 H, N*H*, Ar-*H*) ppm.

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 13.94, 14.06 (*C*H$_3$), 38.00, 38.12 (β-*C*H$_2$), 43.85, 44.18 (Azi-*C*H-C(O)N), 45.13, 45.22 (Azi-*C*H-CO$_2$Et), 52.92, 53.32 (α-*C*H), 61.44, 61.54 (O*C*H$_2$), 62.12, 62.43 (N*C*H$_2$Ph), 66.84, 66.91 (O*C*H$_2$Ph), 126.84, 126.89, 127.57, 127.99, 128.21, 128.26, 128.34, 128.48, 128.54, 128.59, 129.20, 129.33 (Ar-*C*H), 135.21, 135.28, 135.85, 135.88, 136.51 (Ar-*C*), 165.98, 166.37 (N-*C*=O), 167.18, 167.28 (EtO-*C*=O), 170.43, 170.81 (BnO-*C*=O) ppm.

IR (neat): $\tilde{\nu}$ = 3379 (w, br, CON-H); 3063, 3031, 2927 (w, =C-H, C-H); 1740 (s, O-C=O); 1678 (s, N-C=O); 1518, 1497 (m, OCN-H); 1452, 1381 (m, aromat. C=C, C-H); 1191, 1024 (s, C-O-C); 738, 697 (s, aromat. C-H) cm^{-1}.

The *(S)*-phenylalaninol derivative **12** was obtained by coupling of the racemic *N*-benzylated 2-phenyl aziridine-2-carboxylate with TBDMS-protected phenylalaninol[10] and subsequent deprotection with TBAF. Only one diastereomer could be detected with unknown absolute configuration at the ring carbons.

(2*R*,3*R*) or (2*S*,3*S*)-(1-Benzyl-3-phenylaziridine-2-carboxylic acid-((*S*)-1-benzyl-2-hydroxyethyl)amide (12)

Column chromatography (silica gel 60, cyclohexane/EtOAc 1:2, R_f = 0.23).

Yellowish solid.

$[a]_D^{22}$ = - 8.70 ° (c = 0.23, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{25}H_{26}N_2O_2$, 386.50; found: $[M+Na]^+$ 409.7.

LC-MS: R_t = 19.8 min, k' = 21.00 (purity 100%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 2.30 (br s, 1H, O*H*), 2.49-2.56, 2.67-2.73 (2 x dd, je 1 H, ß-C*H*$_2$, *J* = 6.3, 6.8, 7.3 Hz), 2.61 (d, 1 H, Azi-C*H*-CO, *J* = 7.1 Hz), 2.83-2.93 (m_c, 2 H, C*H*$_2$OH, *J* = 3.8, 4.5, 7.1 Hz), 3.17 (d, 1 H, Azi-C*H*-Ph, *J* = 7.1 Hz), 3.71, 3.73 (2 x d, je 1 H, NC*H*$_2$Ph, *J* = 13.2 Hz), 3.82-3.92 (m, 1 H, α-C*H*, *J* = 4.5, 7.3, 7.8 Hz), 6.31 (d, 1 H, N*H*, *J* = 7.8 Hz), 7.05-7.42 (m, 15 H, Ar-*H*) ppm.

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 36.61 (β-*C*H$_2$), 45.88 (Azi-*C*H-CO), 47.36 (Azi-*C*H-Ph), 51.91 (α-*C*H), 63.01 (N*C*H$_2$Ph), 63.50 (*C*H$_2$OH), 126.55, 127.67, 127.86, 128.23, 128.27, 128.50, 128.62, 128.68, 129.16 (Ar-*C*H), 135.29, 137.22, 137.56 (Ar-*C*), 167.76 (N-*C*=O) ppm.

IR (neat): $\tilde{\nu}$ = 3571 (w, br, O-H), 3331 (w, br, CON-H); 3032, 2920, 2851 (m, =C-H, C-H); 1632 (s, N-C=O); 1531 (s, OCN-H); 1452, 1352 (w, aromat. C=C, C-H); 749, 696 (s, aro-mat. C-H) cm^{-1}.

The *N*-benzylated aziridine-2-carboxylic acid derived inhibitors (**13, 14, 15, 17**) were synthesized as described previously in ref. [1]: first, a Cromwell synthesis[11] starting with cinnamic acid ester was performed, followed by bromination and ring closure with benzyl amine. *Trans*- and *cis*-racemates of the aziridine-2-carboxylates (ratio *cis:trans* = 4:1) were separated by column chromatography. Hydrolysis of the *cis*-configured esters and DPPA-mediated peptide coupling yielded the inhibitors as diastereomeric mixtures.

Benzyl-(*S*)-2-{[(*S*)-1-((2*R*,3*R*)+(2*S*,3*S*)-1-Benzyl-3-phenylaziridine-2-carbonyl)pyrrolidine-2-carbonyl]amino}-4-methylpentanoate (13a+b)

Column chromatography (silica gel 60, cyclohexane/EtOAc 1:1, R_f = 0.23).

Dia1 : Dia2 = 1 : 2.7. – Yellowish viscous liquid.

$[a]_D^{22}$ = - 76.01 ° (c = 1.38, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{34}H_{39}N_3O_4$, 553.71; found: $[M+H]^+$ 554.4

LC-MS: R_t = 23.5 min, k' = 25.11 (Dia2); R_t = 23.9 min, k' = 25.56 (Dia1) (purity 96%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 0.85 (d, δ-C*H*$_3$ [Leu], *J* = 5.8 Hz, Dia1) and 0.88 (d, δ-C*H*$_3$ [Leu], *J* = 5.8 Hz, Dia2) (together 6 H), 1.38-1.67 (m, together 3 H, γ-C*H* [Leu], β-C*H*$_2$ [Leu]), 1.84-1.95 (br m, together 2 H, γ-C*H*$_2$ [Pro]), 2.09-2.30 (br m, together 2 H, β-C*H*$_2$ [Pro]), 2.67 (d, Azi-C*H*-CO, *J* = 7.1 Hz, Dia2) and 2.70 (d, Azi-C*H*-CO, *J* = 7.1 Hz, Dia1) (together 1 H), 3.01 (d, Azi-C*H*-Ph, *J* = 7.1 Hz, Dia2) and 3.03 (d, Azi-C*H*-Ph, *J* = 7.1 Hz, Dia1) (together 1 H), 3.25-3.33, 3.45-3.57 (2 x br m, together 2 H, δ- C*H*$_2$ [Pro]), 3.68, 4.03 (2 x d, NC*H*$_2$Ph,

J = 13.1 Hz, Dia2) and 3.72, 3.99 (2 x d, NC*H*$_2$Ph, *J* = 13.1 Hz, Dia1) (together 2 H), 4.25-4.31 (m, together 1 H, α-C*H* [Pro]), 4.38-4.45 (m, together 1 H, α-C*H* [Leu]), 5.09-5.19 (m, together 2 H, OC*H*$_2$Ph, *J* = 12.4 Hz), 6.20 (br d, N*H* [Leu], *J* = 8.4 Hz, Dia1) and 7.11 (br d, N*H* [Leu], *J* = 8.0 Hz, Dia2) (together 1 H), 7.17-7.48 (m, together 15 H, Ar-*H*) ppm.

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 22.04, 22.12, 22.40, 22.56 (δ-*C*H$_3$ [Leu]), 24.75, 24.81 (γ-*C*H [Leu]), 24.87, 24.95 (γ-*C*H$_2$ [Pro]), 26.28, 26.76 (β-*C*H$_2$ [Pro]), 40.58, 40.75 (β-*C*H$_2$ [Leu]), 46.49, 46.55 (δ-*C*H$_2$ [Pro]), 46.84, 46.89 (Azi-*C*H-CO), 48.15, 48.20 (Azi-*C*H-Ph), 51.30, 51.36 (α-*C*H [Leu]), 59.29, 59.97 (α-*C*H [Pro]), 63.55, 63.77 (N*C*H$_2$Ph), 66.70, 67.09 (O*C*H$_2$Ph), 126.39, 126.90, 127.02, 128.13, 128.24, 128.31, 128.47, 128.49, 128.52, 128.55, 129.27, 129.67 (Ar-*C*H), 135.54, 135.61, 135.66, 137.62, 137.67, 138.07 (Ar-*C*), 166.72, 166.97 (N-*C*=O [Azi-Pro]), 170.43, 170.60 (N-*C*=O [Pro-Leu]), 172.13, 172.34 (BnO-*C*=O) ppm.

IR (neat): $\tilde{\nu}$ = 3276 (w, br, CON-H); 3062, 2958, 2872 (w, =C-H, C-H); 1740 (s, O-C=O); 1682, 1646, 1617 (s, N-C=O); 1541, 1497 (w, OCN-H); 1451 (m, aromat. C=C, C-H); 1242, 1187, 1151 (s, C-O-C); 735, 697 (s, aromat. C-H) cm^{-1}.

Benzyl-(*S*)-2-((*S*)-2-{(*S*)-2-[((2*R*,3*R*)+(2*S*,3*S*)-1-Benzyl-3-phenylaziridine-2-carbonyl)-amino]-3-phenylpropionylamino}propionylamino)-4-methylpentanoate(14d1, 14d2)

Column chromatography (silica gel 60, cyclohexane/EtOAc 1:2, R_f = 0.17 / 0.30).

[**d1**, 3.3 : 1]. – Yellowish solid.

[**d2**, 1 : 10.4]. – Yellowish solid.

Mp.: 135-138 °C (**d1**).

$[a]_D^{22}$ = - 18.42 ° (c = 0.38, $CHCl_3$) (**d1**); $[a]_D^{22}$ = - 13.93 ° (c = 0.28, $CHCl_3$) (**d2**).

LOOP-ESI-MS: calc. f. $C_{41}H_{46}N_4O_5$, 674.85; found: $[M+Na]^+$ 697.8 (**d1, d2**).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 0.88, 0.90 (2 x d, je 3 H, δ-C*H*$_3$ [Leu], *J* = 6.1 Hz), 0.95 (d, 3 H, β-C*H*$_3$ [Ala], *J* = 7.3 Hz), 1.49-1.66 (m, together 3 H, γ-C*H* [Leu], β-C*H*$_2$ [Leu]), 2.60 (d, 1 H, Azi-C*H*-CO, *J* = 7.1 Hz), 2.86-3.02 (m_c, 2 H, β-C*H*$_2$, *J* = 5.8, 7.1 Hz), 3.14 (d, 1 H, Azi-C*H*-Ph, *J* = 7.1 Hz), 3.68, 3.69 (2 x d, je 1 H, NC*H*$_2$Ph, *J* = 13.1 Hz), 4.13-4.26 (m, together 2 H, α-C*H* [Phe], α-C*H* [Ala], *J* = 5.8, 7.3, 8.1 Hz), 4.39-4.46 (dt, 1 H, α-C*H* [Leu], *J* = 6.5, 7.8 Hz), 4.96 (d, 1 H, N*H* [Ala], *J* = 8.1 Hz), 5.12, 5.13 (2 x d, je 1 H, OC*H*$_2$Ph, *J* = 12.4 Hz), 6.63 (d, 1 H, N*H* [Leu], *J* = 7.8 Hz), 6.69 (d, 1 H, N*H* [Phe], *J* = 5.8 Hz), 7.03-7.40 (m, 20 H, Ar-*H*) ppm [**d1**].

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 0.89, 0.92 (2 x d, je 3 H, δ-C*H*$_3$ [Leu], *J* = 6.3 Hz), 1.16 (d, 3 H, β-C*H*$_3$ [Ala], *J* = 7.1 Hz), 1.42-1.61 (m, together 3 H, γ-C*H* [Leu], β-C*H*$_2$ [Leu]), 2.21-2.29, 2.44-2.50 (2 x dd, je 1 H, β-C*H*$_2$, *J* = 5.8, 8.0 Hz), 2.61 (d, 1 H, Azi-C*H*-CO, *J* = 7.1 Hz), 3.19 (d, 1 H, Azi-C*H*-Ph, *J* = 7.1 Hz), 3.65, 3.84 (2 x d, je 1 H, NC*H*$_2$Ph, *J* = 13.1 Hz), 4.02-4.10

(m, 1 H, α-C*H* [Phe], *J* = 5.8, 7.3 Hz), 4.22-4.33 (dt, 1 H, α-C*H* [Ala], *J* = 7.1, 7.6 Hz), 4.48-4.56 (dt, 1 H, α-C*H* [Leu], *J* = 6.5, 8.0 Hz), 5.14, 5.15 (2 x d, je 1 H, OCH_2Ph, *J* = 12.1 Hz), 5.89 (d, 1 H, N*H* [Ala], *J* = 7.6 Hz), 6.37 (d, 1 H, N*H* [Leu], *J* = 8.0 Hz), 6.78 (d, 1 H, N*H* [Phe], *J* = 7.3 Hz), 7.03-7.41 (m, 20 H, Ar-*H*) ppm [**d2**].

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 16.81 (β-CH_3 [Ala]), 21.79, 22.87 (δ-CH_3 [Leu]), 24.74 (γ-CH [Leu]), 36.98 (β-CH_2 [Phe]), 40.61 (β-CH_2 [Leu]), 46.65 (Azi-CH-CO), 48.17 (Azi-CH-Ph), 48.34 (α-CH [Ala]), 51.08 (α-CH [Leu]), 54.44 (α-CH [Phe]), 63.07 (NCH_2Ph), 66.74 (OCH_2Ph), 126.84, 127.28, 127.58, 127.63, 127.99, 128.21, 128.28, 128.35, 128.44, 128.59, 128.97, 129.27 (Ar-CH), 134.30, 135.65, 135.86, 137.28 (Ar-C), 168.86 (N-C=O [Azi-Phe]), 169.73 (N-C=O [Phe-Ala]), 171.39 (N-C=O [Ala-Leu]), 172.23 (BnO-C=O) ppm [**d1**].

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 17.54 (β-CH_3 [Ala]), 21.82, 22.81 (δ-CH_3 [Leu]), 24.78 (γ-CH [Leu]), 37.08 (β-CH_2 [Phe]), 40.98 (β-CH_2 [Leu]), 45.67 (Azi-CH-CO), 47.71 (Azi-CH-Ph), 48.79 (α-CH [Ala]), 50.90 (α-CH [Leu]), 54.07 (α-CH [Phe]), 62.99 (NCH_2Ph), 66.94 (OCH_2Ph), 127.01, 127.29, 127.73, 127.85, 128.07, 128.16, 128.30, 128.53, 128.62, 128.76, 129.16, 129.54 (Ar-CH), 134.92, 135.43, 136.12, 137.56 (Ar-C), 168.14 (N-C=O [Azi-Phe]), 170.04 (N-C=O [Phe-Ala]), 171.15 (N-C=O [Ala-Leu]), 172.30 (BnO-C=O) ppm [**d2**].

IR (neat): $\tilde{\nu}$ = 3271 (w, br, CON-H); 3065, 2926 (w, =C-H, C-H); 1744 (m, O-C=O); 1637 (s, N-C=O); 1519, 1493 (w, OCN-H); 1451 (m, aromat. C=C, C-H); 1252, 1215, 1160 (s, C-O-C); 737, 694 (s, aromat. C-H) cm^{-1} (**d1**).

Methyl-(*S*)-1-[(*S*)-2-((*S*)-2-{(*S*)-2-[((2*R*,3*R*)+(2*S*,3*S*)-1-Benzyl-3-phenylaziridine-2-carbonyl)amino]-3-phenylpropionylamino}-4-methylpentanoylamino)propionyl]pyrrolidine-2-carboxylate (15a+b)

Column chromatography (silica gel 60, cyclohexane/EtOAc 1:5, R_f = 0.09).

Dia1 : Dia2 = 5.3 : 1. – Yellowish solid.

Mp.: 141-143 °C.

$[a]_D^{22}$ = - 49.68 ° (c = 0.62, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{40}H_{49}N_5O_6$, 695.87; found: $[M+Na]^+$ 718.9.

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 0.77-0.85 (m, together 6 H, δ-CH_3 [Leu], *J* = 6.4, 6.8 Hz), 0.97-1.07, 1.17-1.25, 1.40-1.48 (3 x m, together 3 H, β-CH_2 [Leu], γ-CH [Leu]), 1.33 (d, together 3 H, β-CH_3 [Ala], *J* = 6.9 Hz), 1.88-2.03, 2.12-2.23 (2 x br m, together 4 H, γ-CH_2 [Pro], β-CH_2 [Pro]), 2.61 (d, Azi-CH-CO, *J* = 7.1 Hz, Dia1) and 2.63 (d, Azi-CH-CO, *J* = 7.1 Hz, Dia2) (together 1 H), 2.86-2.94 (m, together 2 H, β-CH_2 [Phe], *J* = 6.3, 7.1 Hz), 3.13 (d, Azi-CH-Ph, *J* = 7.1 Hz, Dia1) and 3.16 (d, Azi-CH-Ph, *J* = 7.1 Hz, Dia2) (together 1 H), 3.50-3.58, 3.70-3.77 (2 x m,together 2 H, δ-CH_2 [Pro]), 3.60-3.68 (m, together 2 H, NCH_2Ph, *J* = 12.9 Hz), 3.69 (s, together 3 H, OCH_3), 4.11-4.18 (m, together 1 H, α-CH [Leu], *J* = 5.3,

8.6 Hz), 4.20-4.26 (m, together 1 H, α-C*H* [Phe], *J* = 5.8 Hz), 4.47-4.57 (m, together 1 H, α-C*H* [Pro], *J* = 4.0, 8.5 Hz), 4.57-4.66 (m, together 1 H, α-C*H* [Ala], *J* = 6.8, 7.5 Hz), 5.08 ((br d, together 1 H, N*H* [Leu], *J* = 8.6 Hz), 6.72 (br d, together 1 H, N*H* [Phe], *J* = 5.8 Hz), 6.81 (d, 1 H, N*H* [Ala], *J* = 7.5 Hz), 7.04-7.38 (m, together 15 H, Ar-*H*) ppm.

^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 17.53 (β-*C*H_3 [Ala]), 21.58, 22.86 (δ-*C*H_3 [Leu]), 24.36 (γ-*C*H [Leu]), 24.87 (γ-*C*H_2 [Pro]), 28.89 (β-*C*H_2 [Pro]), 37.53 (β-*C*H_2 [Phe]), 40.01 (β-*C*H_2 [Leu]), 46.55 (Azi-*C*H-CO), 46.58 (δ-*C*H_2 [Pro]), 46.73 (α-*C*H [Ala]), 48.11 (Azi-*C*H-Ph), 51.32 (α-*C*H [Leu]), 52.14 (O*C*H_3), 54.50 (α-*C*H [Phe]), 58.77 (α-*C*H [Pro]), 63.11(N*C*H_2Ph), 126.91, 127.13, 127.47, 127.85, 128.13, 128.47, 128.74, 129.12, 129.26 (Ar-*C*H), 135.54, 136.01, 137.39 (Ar-*C*), 168.44 (N-*C*=O [Azi-Pro]), 169.81 (N-*C*=O [Phe-Leu]), 170.59 (N-*C*=O [Ala-Pro]), 170.67 (N-*C*=O [Leu_Ala]), 172.48 (MeO-*C*=O) ppm.

IR (neat): $\tilde{\nu}$ = 3285 (w, br, CON-H); 3061, 2925, 2853 (w, =C-H, C-H); 1744 (s, O-C=O); 1631 (s, N-C=O); 1512 (w, OCN-H); 1452 (m, aromat. C=C, C-H); 1197, 1173 (s, C-O-C); 739, 698 (s, aromat. C-H) cm^{-1}.

(*R*)-2-[((2*R*,3*R*)+(2*S*,3*S*)-1-Benzyl-3-phenyl-aziridine-2-carbonyl)-amino]-3-methyl-butyric acid benzyl ester (17a+b)

Column chromatography (silica gel, cyclohexane/ethylacetate 5:1.

Dia1 : Dia2 = 3 : 2. – yellowish viscous liquid.

$[a]_D^{20}$ = 13.4° (c = 0.88, $CHCl_3$).

LOOP-ESI-MS: calc. f. $C_{28}H_{30}N_2O_3$, 442.56; found: 443.3 $[M+H]^+$.

LC-MS: Dia 1: R_t = 22.2 min; Dia 2: R_t = 22.6 min (purity 100%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = dia 1: ($CDCl_3$, 400.13 MHz): δ [ppm] = 0.70, 0.73 (2 x d, 2 x 3H, Val-?-C*H*$_3$, *J* = 6.9 Hz), 1.95 (m, 1H, Val-ß-C*H*), 2.64 (d, 1H, Azi-C*H*-CO, *J* = 7.1 Hz), 3.17 (d, 1H, Azi-C*H*-Ph, *J* = 7.4 Hz), 3.57, 3.97 (2 x d, 2H, *N*-C*H*$_2$-Ph), 4.18 (m, 1H, Val-a-C*H*), 4.96 (m, 2H, CO_2C*H*$_2$Ph), 6.88 (d, 1H, N*H*, *J* = 9.1 Hz), 7.11-7.43 (m, 15H, Ar-C*H*).

dia 2: ($CDCl_3$, 400.13 MHz): δ [ppm] = 0.22, 0.36 (2 x d, 2 x 3H, Val-?-C*H*$_3$, *J* = 6.8 Hz), 1.72 (m, 1H, Val-ß-C*H*), 2.64 (d, 1H, Azi-C*H*-CO, *J* = 7.1 Hz), 3.19 (d, 1H, Azi-C*H*-Ph, *J* = 8.5 Hz), 3.65, 3.92 (2 x d, 2H, *N*-C*H*$_2$-Ph), 4.34 (m, 1H, Val-a-C*H*), 5.13 (m, 2H, CO_2C*H*$_2$Ph), 6.78 (d, 1H, N*H*, *J* = 9.1 Hz), 7.11-7.43 (m, 15H, Ar-C*H*).

^{13}C-NMR: dia 1: ($CDCl_3$, 100.62 MHz): δ [ppm] = 17.69, 18.72 (Val-?-*C*H_3), 31.86 (Val-ß-*C*H), 46.97 (Azi-*C*H-CO-NH), 48.18 (Azi-*C*H-Ph), 56.48 (Val-a-*C*H), 63.41 (*N*-*C*H_2), 66.63 (OBn-*C*H_2), 127.50-128.75 (Ar-*C*H), 134.78 (Ar-*C*$_q$-Azi), 135.59 (Ar-*C*$_q$-OBn), 137.82, 137.97 (Ar-*C*$_q$-*N*-CH_2-Ph), 167.28 (*C*O-NH), 170.79 (*C*O_2Bn).

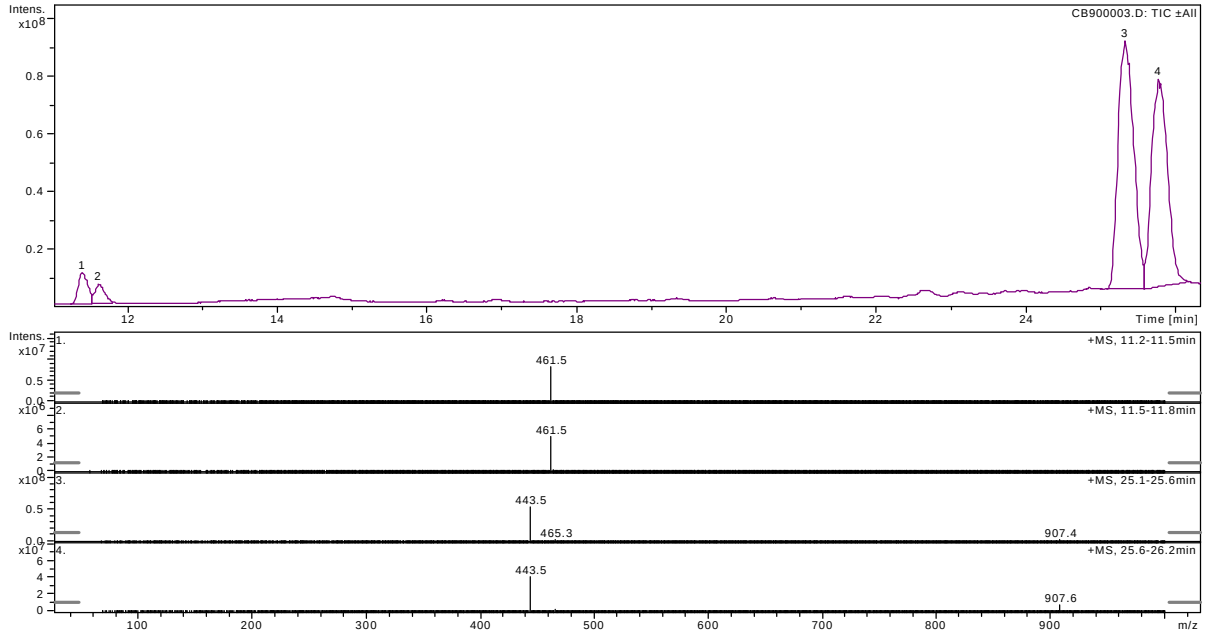
dia 2: ($CDCl_3$, 100.62 MHz): δ [ppm] = 17.08, 18.28 (Val-?-$\underline{C}H_3$), 31.27 (Val-ß-$\underline{C}H$), 46.66 (Azi-$\underline{C}H$-CO-NH), 48.03 (Azi-$\underline{C}H$-Ph), 56.76 (Val-a-$\underline{C}H$), 63.41 (*N*-$\underline{C}H_2$), 66.96 (OBn-$\underline{C}H_2$), 127.50-128.75 (Ar-$\underline{C}H$), 134.78 (Ar-$\underline{C}_q$-Azi), 135.59 (Ar-$\underline{C}_q$-OBn), 137.82, 137.97 (Ar-$\underline{C}_q$-*N*-CH_2-Ph), 167.72 ($\underline{C}$O-NH), 171.61 ($\underline{C}O_2$Bn).
IR (neat): $\tilde{n}$ [cm^{-1}] = 3391 (w, CO-NH), 2964 (w, C-H), 1737 (m, CO_2Bn), 1674 (m, CO-NH), 1511 (w, Ph), 1186 (m, CO_2Bn), 1147 (m, CO_2Bn), 738 (m, Ph), 697 (s, Ph).

Separation of diastereomers: *via* preparative HPLC on an Aglient 1100 with a G1365B MWD detector and a G1361A PrepPump; solid phase: Agilent RP Zorbax SB C18, 21.2 x 150 mm, 7 µm; mobile phase: water-acetonitrile gradient (50% acetonitrile containing 0.1% formic acid for 25 min, 50-80% for 15 min).

The succinic acid derivative **16** was obtained by hydrolysis of diethyl succinate with 1 eq. KOH and subsequent DPPA-mediated coupling with *(S)*-PheOBn.

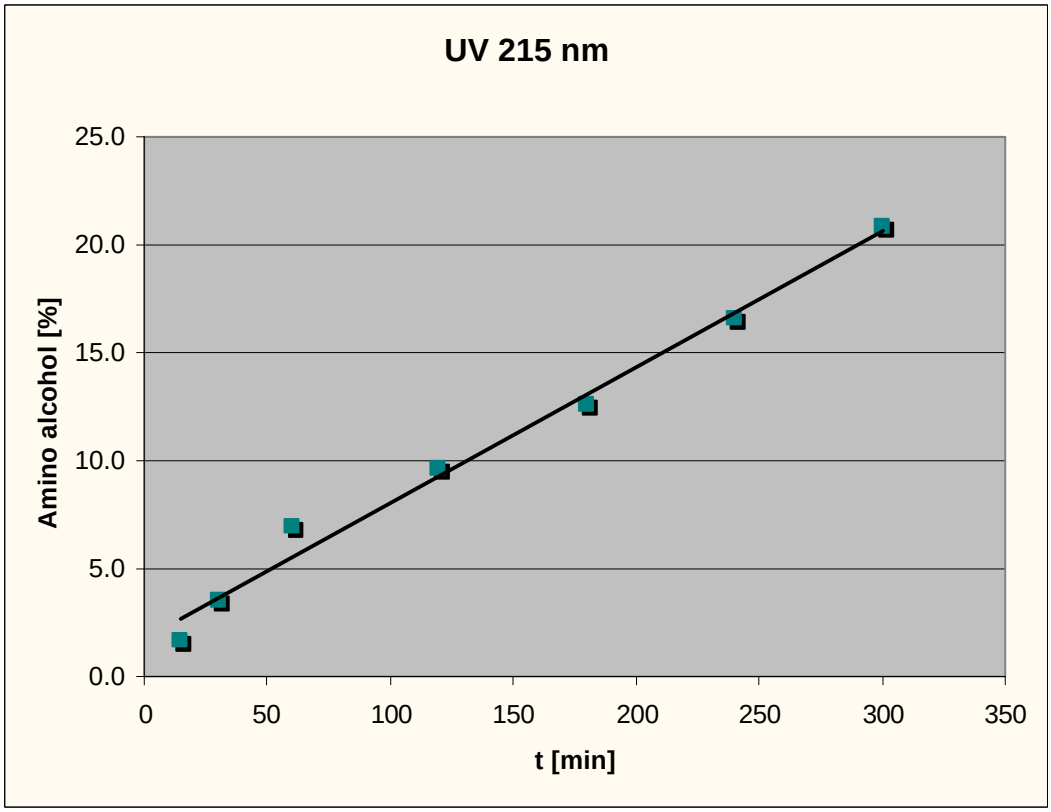
Ethyl-*N*-((*S*)-1-Benzyloxycarbonyl-2-phenylethyl)succinate (16)
Column chromatography (silica gel 60, cyclohexane/EtOAc 2:1, R_f = 0.33).
Yellowish solid.
Mp.: 77-79 °C.
LOOP-ESI-MS: calc. f. $C_{22}H_{25}NO_5$, 383.45; found: $[M+Na]^+$ 406.4.
^{1}H-NMR ($CDCl_3$, 400.13 MHz): δ = 1.16 (t, 3 H, C$\underline{H_3}$, *J* = 7.1 Hz), 2.40 (t, 2 H, C$\underline{H_2}$-C(O)N, *J* = 6.8 Hz), 2.54 (t, 2 H, C$\underline{H_2}$-CO_2Et, *J* = 6.8 Hz),3.01-3.10 (m_c, 2 H, β-C$\underline{H_2}$, *J* = 5.8, 8.1 Hz), 4.04 (q, 2 H, OC$\underline{H_2}$, *J* = 7.1 Hz), 4.81-4.87 (dt, 1 H, α-C$\underline{H}$, *J* = 5.8, 7.8 Hz), 5.05, 5.07 (2 x d, je 1 H, OC$\underline{H_2}$Ph, *J* = 12.1 Hz), 6.05 (d, 1 H, N$\underline{H}$, *J* = 7.8 Hz), 7.12-7.31 (m, 10 H, Ar-$\underline{H}$) ppm.
^{13}C-NMR ($CDCl_3$, 100.61 MHz): δ = 14.12 ($\underline{C}H_3$), 29.34 ($\underline{C}H_2$-CO_2Et), 30.81 ($\underline{C}H_2$-C(O)N), 37.80 (β-$\underline{C}H_2$), 53.14 (α-$\underline{C}$H), 60.66 (O$\underline{C}H_2$), 67.20 (O$\underline{C}H_2$Ph), 126.99, 127.85, 128.39, 128.47, 128.54, 129.29 (Ar-$\underline{C}$H), 135.01, 135.64 (Ar-$\underline{C}$), 170.91 (BnO-$\underline{C}$=O), 171.29 (N-$\underline{C}$=O), 172.67 (EtO-$\underline{C}$=O) ppm.
IR (neat): $\tilde{\nu}$ = 3356 (m, CO$\underline{N\text{-}H}$); 3056, 2973 (w, =C-H, C-H); 1716 (s, O-$\underline{C=O}$); 1638 (m, N-$\underline{C=O}$); 1512 (s, OC$\underline{N\text{-}H}$); 1437, 1367 (w, aromat. C=C, C-H); 1243, 1187, 1032 (s, C-O-C); 732, 698 (s, aromat. C-H) cm^{-1}.

LC-MS studies, quantification of the amino alcohol



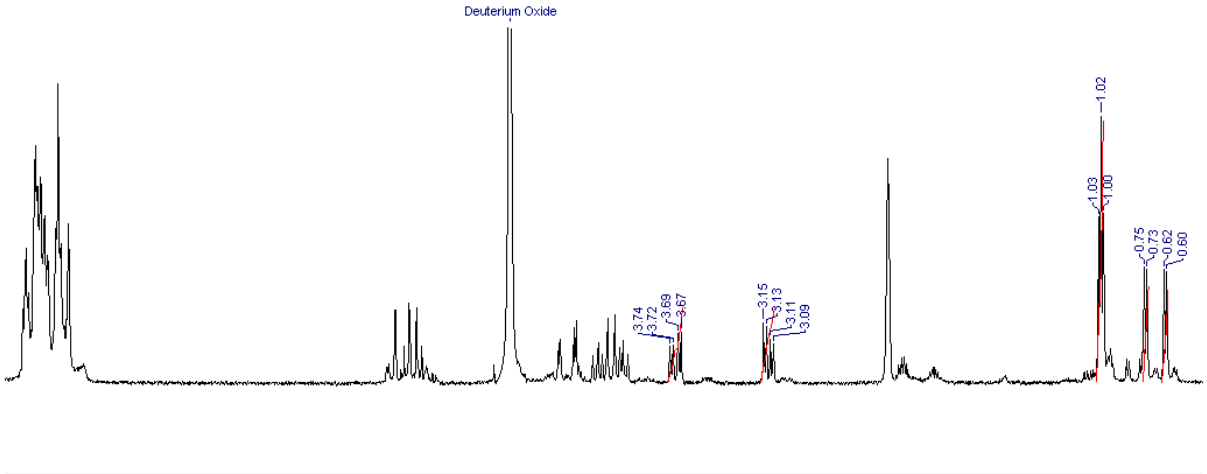
LC-MS run after 65 min incubation time of enzyme and inhibitor **17** (diastereomeric mixture).

The LC-MS studies to detect the hydrolysis product, i.e. the respective amino alcohol, were performed with inhibitor **17**. SAP2 and **17** (final concentration 290 µM) were incubated for 65 min under the standard assay conditions (total volume 700 µL, 50 µL enzyme solution) described above except that the inhibitor stock solution was prepared with acetonitrile (final concentration in the assay 5 %) instead of DMSO. The reaction mixture was then filtrated through a millipore filter and subjected to LC-MS: HPLC system 1100 from Agilent, Phenomenex Jupiter 4 µ Proteo 90A RP C-18 column (4.5 x 150 mm), water-acetonitrile gradient (40% acetonitrile containing 0.1% formic acid for 5 min, 40-95% for 25 min, 95% for 15 min), and detection at 215 nm. The peaks at a retention time of R_t = 10.5 and 10.9 min possessed a mass of 461.3 = $[M+H]^+$ corresponding to the amino alcohol with the molecular formula $C_{28}H_{32}N_2O_4$ (M_r = 460.58).



Quantification of the amino alcohols resulting from reaction of cpd. **17** (diastereomeric mixture) with SAP2 and subsequent hydrolysis. In order to quantify the amino alcohol aliquots were taken after 15, 30, 60, 120, 180, 240, and 300 min and subjected to LC-MS analysis (see above). The ratio amino alcohol / aziridine was determined by integrating the respective peaks (retention time of the aziridines **17** = 25.3 and 25.8 min).

NMR spectrum of inhibitor 17 after 5 h at pH 3.2



No.	(ppm)	(Hz)	Height
1	0.60	241.1	0.0194
2	0.62	247.9	0.0199
3	0.73	291.1	0.0199
4	0.75	298.2	0.0202
5	1.00	401.2	0.0296
6	1.02	407.3	0.0467
7	1.03	413.6	0.0290
8	3.09	1235.7	0.0068

No.	(ppm)	(Hz)	Height
9	3.11	1242.8	0.0077
10	3.13	1253.6	0.0097
11	3.15	1260.7	0.0105
12	3.67	1468.1	0.0089
13	3.69	1475.2	0.0086
14	3.72	1488.6	0.0065
15	3.74	1495.7	0.0063

No.	Annotation	(ppm)
1	Deuterium Oxide	4.75

No.	(ppm)	Value	Absolute Value
1	[0.59 .. 0.63]	2.137	4.63440e+4
2	[0.72 .. 0.75]	2.198	4.76620e+4
3	[1.00 .. 1.04]	6.000	1.30102e+5
4	[3.08 .. 3.16]	1.697	3.67870e+4
5	[3.65 .. 3.75]	1.721	3.73080e+4

In order to prove the stability of aziridine **17** (diastereomeric mixture) under the assay conditions additional ^{1}H-NMR studies were performed. A buffer pH 3.2, prepared with 50 mM deuterated sodium acetate and NaOD, was used. The inhibitor stock solution (4.5 mM) was prepared with deuterated acetonitrile. ^{1}H-NMR spectra were recorded after 10, 20, 30, 40, 50, 60, 75, and 90 min, as well as after 5 h. The integrals of the signals for the aziridine-ring protons (doublets at δ (ppm) 3.10, 3.14, 3.68, and 3.73) were measured. No changes in the NMR spectra were observed. The figure shows the NMR spectrum after 5 h.

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