

Asymmetric Synthesis of 3-amino-4-hydroxy-2-(hydroxymethyl)pyrrolidines as potential glycosidase inhibitors

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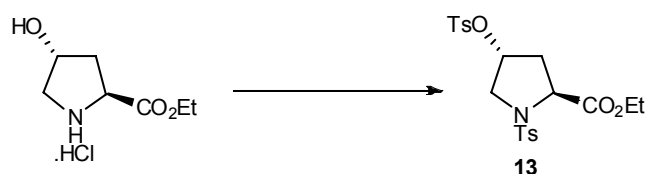
Supplementary Information:

(2*S*,4*R*)-2-Ethoxycarbonyl-4-hydroxypyrrolidinium chloride



Thionyl chloride (11.18 g, 6.82 ml, 94.0 mmol) was added to a 0 °C solution of *trans*-hydroxy-L-proline **12** (11.20 g, 85 mmol) dissolved in ethanol (100 ml). The solution was refluxed for 5 h before being cooled and the solvent was removed under reduced pressure. The resulting crude material was purified by recrystallisation (from ethanol) to give the title compound (16.20 g, 98%) as a white solid; mp 150-153 °C (from ethanol) (Lit.¹ 153-153.5 °C); $[\alpha]_D^{20}$ -26.4 (c=2.5 MeOH); ν_{\max} (solid)/cm⁻¹ 3311br (OH), 2954m, 2699s, 1698 (C=O), 1593m, 1401m, 1273s, 1236s, 1076s, 956s; δ_H (300 MHz; D₂O) 1.41 (3H, t, *J* 7.1, CH₃CH₂O), 2.40 (1H, ddd, *J* 14.2, 10.4, 4.4, *HH*-3), 2.61 (1H, ddt, *J* 14.2, 7.8, 1.8, *HH*-3), 3.52 (1H, dt, *J* 12.6, 1.8, *HH*-5), 3.64 (1H, dd, *J* 12.6, 3.7, *HH*-5), 4.42 (2H, q, *J* 7.1, CH₃CH₂O), 4.74-4.84 (2H, m, H-2, H-4); δ_C (75 MHz; D₂O) 13.1 (CH₃), 36.7 (CH₂), 53.4 (CH₂), 58.2 (CH), 63.9 (CH₂), 69.4 (CH), 169.8 (C); (ES) *m/z* (%) 160 (M+H⁺, 100%); Accurate mass (FAB) Found 160.0974 (M+H⁺ C₇H₁₄NO₃ requires 160.0974); Anal. Found C, 43.18; H, 7.13; N, 7.24; C₇H₁₄NO₃Cl requires C, 42.97; H, 7.21; N, 7.13.

(2*S*,4*R*)-2-Ethoxycarbonyl-1-(toluene-4-sulfonyl)-4-(toluene-4-sulfonyloxy)pyrrolidine (**13**)



Triethylamine (16.30 g, 22.5 ml, 161 mmol) and DMAP (0.05 g, 0.06 mmol) were added to a stirring solution of (2*S*,4*R*)-2-ethoxycarbonyl-4-hydroxypyrrolidinium chloride (6.30 g, 32.2 mmol) in dichloromethane (41 ml). The reaction mixture was stirred for 1 h before a solution of *p*-toluenesulfonyl chloride (15.40 g, 80.7 mmol) dissolved in dichloromethane (80 ml) was added dropwise. The reaction mixture was stirred for a further 48 h before being diluted with dichloromethane (50 ml). The organic layer was washed with water (3 × 50 ml), dried (MgSO₄) and the solvent removed under reduced pressure. The resulting crude material was purified by recrystallisation from ethanol to give **13** (14.47 g, 96%) as a white solid; mp 76-78 °C (from ethanol) (Lit.² 75-76 °C); $[\alpha]_D^{20}$ -74.2 (c=2.43 MeOH); ν_{\max} (solid)/cm⁻¹ 2878s, 1737s (C=O), 1596m, 1358s, 1346s, 1196m, 1166s, 1159s, 953s; δ_H (300 MHz; CDCl₃) 1.20 (3H, t, *J* 7.2, CH₃CH₂O), 2.16 (1H, ddd, *J* 14.0, 7.5, 5.1, *HH*-3), 2.32

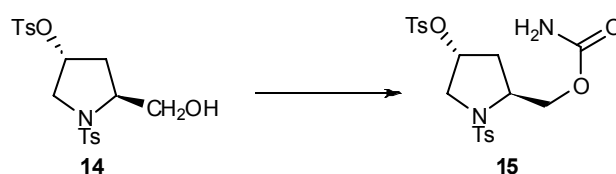
(1H, dddd, J 14.0, 7.5, 3.0, 1.5, HH -3), 2.39 (3H, s, Ar 4-Me), 2.41 (3H, s, Ar 4-Me), 3.54 (1H, dapp t, J 12.3, 1.5, HH -5), 3.66 (1H, dd, J 12.3, 4.2, HH -5), 4.07-4.21 (3H, m, $\text{CH}_3\text{CH}_2\text{O}$, H-2), 4.92-4.97 (1H, m, H-4), 7.27 (2H, d, J 8.1, $2 \times$ Ar H-3), 7.29 (2H, d, J 8.1, $2 \times$ Ar H-3), 7.58 (2H, d, J 8.1, $2 \times$ Ar H-2), 7.67 (2H, d, J 8.1, $2 \times$ Ar H-2); δ_{C} (75 MHz; CDCl_3) 14.0 (CH_3), 21.2 (CH_3), 21.6 (CH_3), 37.2 (CH_2), 53.8 (CH_2), 59.2 (CH), 61.8 (CH_2), 78.2 (CH), 127.6₀ ($2 \times$ CH), 127.6₄ ($2 \times$ CH), 129.7 ($2 \times$ CH), 129.9 ($2 \times$ CH), 133.0 (C), 134.2 (C), 144.1 (C), 145.3 (C), 171.1 (C); (ES) m/z (%) 468 ($\text{M}+\text{H}^+$, 100%), 485 (58, $\text{M}+\text{NH}_4^+$); Accurate mass (FAB) Found 468.1150 ($\text{M}+\text{H}^+$ $\text{C}_{21}\text{H}_{26}\text{NO}_7\text{S}_2$ requires 468.1151); Anal. Found C, 54.17; H, 5.55; N, 2.94; $\text{C}_{21}\text{H}_{25}\text{NO}_7\text{S}_2$ requires C, 53.95; H, 5.39; N, 2.97.

(2*S*,4*R*)-2-(Hydroxymethyl)-1-(toluene-4-sulfonyl)-4-(toluene-4-sulfonyloxy)pyrrolidine (14)



LiCl (2.07 g, 42.0 mmol) and NaBH₄ (1.86 g, 42.0 mmol) were added to a 0 °C solution of **13** (6.53 g, 14.0 mmol) dissolved in a 1:1 mixture of ethanol (80 ml) and THF (80 ml). The solution was stirred for 3.5 h before being quenched with water (50 ml). Ethanol was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate (3×50 ml). The organic layers were combined, dried (MgSO_4) and the solvent was removed under reduced pressure to give **14** (5.41 g, 91%) as a white solid; mp 82-84 °C (Lit.³ 86-87 °C); $[\alpha]_{\text{D}}^{20} -21.5$ ($c=1.03$ MeOH); ν_{max} (solid)/ cm^{-1} 3532br (OH), 2963m, 1597m, 1335s, 1173s, 1090m, 889s, 812s; δ_{H} (300 MHz; CDCl_3) 1.88-1.94 (1H, br dd, J 14.1, 7.8, HH -3), 2.03 (1H, ddd, J 14.1, 8.4, 4.8, HH -3), 2.33 (3H, s, Ar 4-Me), 2.34 (3H, s, Ar 4-Me), 3.28 (1H, br s, OH), 3.51-3.67 (4H, m, CHHO , $2 \times$ H-5, H-2), 3.81 (1H, dd, J 11.4, 3.3, CHHO), 4.86-4.92 (1H, m, H-4), 7.22 (4H, d, J 8.1, $4 \times$ Ar H-3), 7.48 (2H, d, J 8.1, $2 \times$ Ar H-2), 7.62 (2H, d, J 8.1, $2 \times$ Ar H-2); δ_{C} (75 MHz; CDCl_3) 21.2 (CH_3), 21.2 (CH_3), 34.8 (CH_2), 55.0 (CH_2), 59.9 (CH), 63.7 (CH_2), 78.6 (CH), 127.2 ($2 \times$ CH), 127.4 ($2 \times$ CH), 129.4 ($2 \times$ CH), 129.6 ($2 \times$ CH), 132.8 (C), 133.0 (C), 143.8 (C), 144.8 (C); (ES) m/z (%) 254 ($(\text{M}+\text{H}^+)-\text{HOSO}_2\text{C}_6\text{H}_4\text{CH}_3$, 100%), 443 (97, $\text{M}+\text{NH}_4^+$), 426 (72, $\text{M}+\text{H}^+$); Accurate mass (FAB) Found 426.1045 ($\text{M}+\text{H}^+$ $\text{C}_{19}\text{H}_{24}\text{NO}_6\text{S}_2$ requires 426.1045); Anal. Found C, 53.64; H, 5.54; N, 3.06; $\text{C}_{21}\text{H}_{25}\text{NO}_7\text{S}_2$ requires C, 53.63; H, 5.45; N, 3.29.

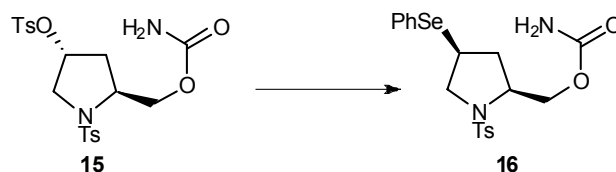
(2*S*,4*R*)-2-(Carbamoyloxymethyl)-1-(toluene-4-sulfonyl)-4-(toluene-4-sulfonyloxy)pyrrolidine (15)



Trichloroacetylisocyanate (0.38 g, 0.24 ml, 3.8 mmol) was added to a 0 °C solution of **14** (1.38 g, 3.2 mmol) in dichloromethane (5 ml). After 2 h TLC analysis confirmed

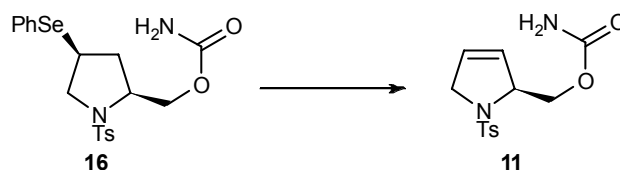
all starting material had been consumed. Dichloromethane was removed under reduced pressure and the resulting crude material was dissolved in methanol (6.5 ml). The solution was cooled to 0 °C and aqueous potassium carbonate (19.4ml, 0.5 moldm⁻³, 9.7 mmol) was added. The solution was stirred for 4 h before the solvent was removed under reduced pressure. Water (10 ml) was added and the resulting solution extracted with ethyl acetate (3 × 20 ml), the organic layers were combined, dried (MgSO₄) and the solvent removed under reduced pressure to give **15** (1.37 g, 91%) as a white solid; mp 94-97 °C; [α]_D²⁰ -36.1 (c=1.95 MeOH); ν_{\max} (solid)/cm⁻¹ 2957s, 1723s (C=O), 1597s, 1401m, 1331s, 1346s, 1174s, 1089m, 956s, 889s; δ_{H} (300 MHz; CDCl₃) 1.95-2.05 (2H, m, 2 × H-3), 2.44 (3H, s, Ar 4-Me), 2.46 (3H, s, Ar 4-Me), 3.55 (1H, ddd, *J* 12.6, 3.3, 0.9, *HH*-5), 3.61 (1H, dd, *J* 12.6, 4.2, *HH*-5), 3.84-3.91 (1H, m, H-2), 4.19 (1H, dd *J* 11.4, 5.7, *CHHO*), 4.38 (1H, dd, *J* 11.4, 3.3, *CHHO*), 4.81-4.88 (3H, m, H-4, NH₂), 7.30 (2H, d, *J* 8.1, 2 × Ar H-3), 7.32 (2H, d, *J* 8.1, 2 × Ar H-3), 7.59 (2H, d, *J* 8.1, 2 × Ar H-2), 7.70 (2H, d, *J* 8.1, 2 × Ar H-2); δ_{C} (75 MHz; CDCl₃) 21.6 (CH₃), 21.6 (CH₃), 35.7 (CH₂), 54.5 (CH₂), 57.1 (CH), 66.4 (CH₂), 78.2 (CH), 127.6 (2 × CH), 127.7 (2 × CH), 129.8 (2 × CH), 129.9 (2 × CH), 133.1 (C), 133.6 (C), 144.1 (C), 145.2 (C), 156.3 (C); (ES) *m/z* (%) 469 (M+H⁺, 100%), 486 (51, M+NH₄⁺); Accurate mass (FAB) Found 469.1103 (M+H⁺ C₂₀H₂₅N₂O₇S₂ requires 469.1103).

(2*S*,4*S*)-2-(Carbamoyloxymethyl)-4-phenylselanyl-1-(toluene-4-sulfonyl)pyrrolidine (16)



Sodium borohydride (0.19 g, 5.13 mmol) was added to a solution of diphenyldiselenide (0.84 g, 2.70 mmol) in ethanol (10 ml). The solution was degassed (3 cycles freeze-pump-thaw) and to this mixture a degassed (3 cycles freeze-pump-thaw) solution of **15** (1.93 g, 4.28 mmol) in THF (10 ml) was added. The reaction mixture was refluxed for 2 h before solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (40:60, ethyl acetate:petroleum-ether) to give **16** (1.76 g, 94%) as a white solid; mp 125-128 °C; [α]_D²⁰ +27.0 (c=1.15 MeOH); ν_{\max} (solid)/cm⁻¹ 3460br (NH), 2920s, 1698s (C=O), 1613s, 1406m, 1336s, 1152s, 1084m, 733s; δ_{H} (300 MHz; CDCl₃) 1.71 (1H, ddd, *J* 13.2, 10.2, 7.5, *HH*-3), 2.29 (1H, dapp t, *J* 13.2, 7.5, *HH*-3), 2.43 (3H, s, Ar 4-Me), 2.80 (1H, app tdd, *J* 10.2, 7.5, 6.6, H-4), 3.26 (1H, dd, *J* 12.3, 10.2, *HH*-5), 3.78 (1H, dd *J* 12.3, 6.6, *HH*-5), 3.90-3.98 (1H, m, H-2), 4.17 (1H, dd, *J* 11.1, 5.7, *CHHO*), 4.26 (1H, dd, *J* 11.1, 5.1, *CHHO*), 4.76 (2H, s br, NH₂), 7.25-7.37 (5H, m, 3 × Ph-H, 2 × Ar H-3), 7.40-7.44 (2H, m, 2 × Ph-H), 7.67 (2H, d, *J* 8.4, 2 × Ar H-2); δ_{C} (75 MHz; CDCl₃) 21.5 (CH₃), 33.7 (CH₂), 36.3 (CH), 55.7 (CH₂), 58.7 (CH), 66.7 (CH₂), 127.3₄ (2 × CH), 127.2₉ (C), 128.2 (CH), 129.2 (2 × CH), 129.9 (2 × CH), 134.4 (C), 134.8 (2 × CH), 143.9 (2 × C), 156.4 (C); (ES) *m/z* (%) 394 (M-OCONH₂, 100%), 455 (67, M+H⁺); Accurate mass (FAB) Found 455.0543 (M+H⁺ C₁₉H₂₃N₂O₄SSe requires 455.0544); Anal. Found C, 49.99; H, 4.89; N, 6.32; C₁₉H₂₂N₂O₄S⁸⁰Se requires C, 50.33; H, 4.89; N, 6.18.

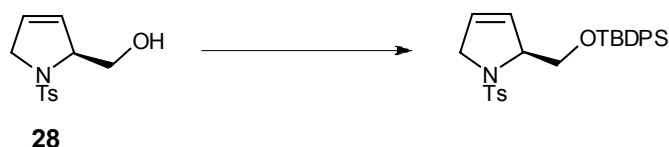
(S)-2-Carbamoyloxymethyl-1-(toluene-4-sulfonyl)-2,5-dihydro-1H-pyrrole (11)



Pyridine (0.32 ml, 3.9 mmol) and hydrogen peroxide (0.75 ml, 30% solution) were added to a 0 °C solution of **16** (1.33 g, 2.9 mmol) dissolved in dichloromethane (16 ml). The reaction mixture was stirred for 2 h before being diluted with dichloromethane (20 ml). The organic layer was washed with 5% aqueous KHSO₄ (2 × 10 ml), saturated aqueous NaHCO₃ (2 × 10 ml) and water (2 × 10 ml) before being dried (MgSO₄). The solvent was removed under reduced pressure and the resulting crude material was purified by flash column chromatography on silica (2:98, methanol:dichloromethane) to give **11** (0.45 g, 52%) as an off-white solid; mp 149-152 °C; $[\alpha]_D^{20}$ -208.8 (c=1.02 MeOH); ν_{\max} (solid)/cm⁻¹ 3461br (NH), 2921s, 1730s (C=O), 1706s, 1600m, 1402m, 1311m, 1160s, 1049s, 817s; δ_H (300 MHz; CDCl₃) 2.41 (3H, s, Ar 4-Me), 4.06-4.14 (2H, m, 2 × H-5), 4.15 (1H, dd, *J* 11.1, 5.4, CHHO), 4.40 (1H, dd, *J* 11.1, 3.9, CHHO), 4.61-4.65 (1H, m, H-2), 4.92 (2H, brs, NH₂), 5.50 (1H, dapp q, *J* 6.3, 2.1, H-3), 5.70 (1H, ddd, *J* 6.3, 5.4, 2.1, H-4), 7.30 (2H, d, *J* 8.1, Ar H-3), 7.70 (2H, d, *J* 8.1, Ar H-2); δ_C (75 MHz; CDCl₃) 21.5 (CH₃), 55.7 (CH₂), 66.1 (CH), 66.7 (CH₂), 126.7 (CH), 126.8 (CH), 127.4 (2 × CH), 129.8 (2 × CH), 134.2 (C), 143.7 (C), 156.6 (C); (ES) *m/z* (%) 236 (M-OCONH₂, 100%), 297 (22, M+H⁺); Accurate mass (FAB) Found 297.0908 (M+H⁺ C₁₃H₁₇N₂O₄S requires 297.0909).

Alternative synthesis of 25:

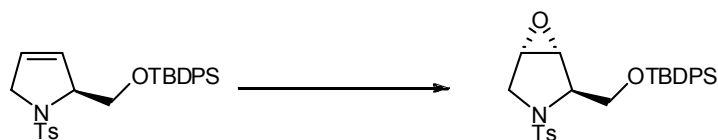
(S)-2-(tert-butyl diphenylsilyloxymethyl)-1-(Toluene-4-sulfonyl)-2,5-dihydro-1H-pyrrole



Imidazole (0.045 g, 0.67 mmol) and DMAP (0.0081 g, 0.066 mmol) were added to a 0 °C solution of **28** (0.14 g, 0.556 mmol) dissolved in dichloromethane (5 ml) and the solution was stirred for 15 min. ^tButyldiphenylsilyl chloride (0.18 g, 0.17 ml, 0.67 mmol) was added and the mixture stirred for a further 4 h. The reaction mixture was diluted with dichloromethane (10 ml) and was washed with water (10 ml). The organic layer was dried (MgSO₄), and the solvent removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (10:90, ethyl acetate:petroleum-ether) to give the title compound (0.22 g, 81%) as a white solid; mp 69-71 °C; $[\alpha]_D^{20}$ -172.6 (c= 1.0 MeOH); ν_{\max} (CH₂Cl₂)/cm⁻¹; 3055m,

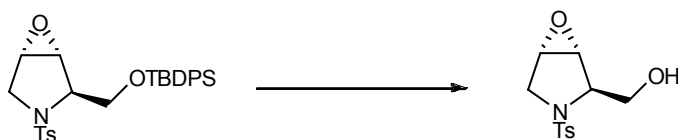
2928m, 1593m, 1464m, 1428m, 1346s, 1264s, 1093s, 1029w, 823m, 765s; δ_{H} (300 MHz, CDCl_3) 1.07 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.42 (3H, s, Ar 4-Me), 3.77 (1H, dd, J 9.9, 7.2, CHHO), 4.02 (1H, dd, J 9.9, 3.9, CHHO), 4.10-4.14 (2H, m, $2 \times \text{H-5}$), 4.43-4.46 (1H, m, H-2), 5.69 (1H, ddd, J 6.3, 3.6, 1.8, H-3), 5.76 (1H, dapp q, J 6.3, 2.4, H-4), 7.27 (2H, d, J 8.1, $2 \times \text{Ar H-3}$), 7.37-7.48 (6H, m, $6 \times \text{Ph-H}$), 7.62 (2H, d, J 8.1, $2 \times \text{Ar H-2}$), 7.63-7.69 (4H, m, $4 \times \text{Ph-H}$); δ_{C} (75 MHz; 300 MHz, CDCl_3) 19.2 (C), 21.5 (CH_3), 26.8 ($3 \times \text{CH}_3$), 55.9 (CH_2), 67.1 (CH_2), 68.0 (CH), 125.7 (CH), 127.4 ($2 \times \text{CH}$), 127.7 ($4 \times \text{CH}$), 128.6 (CH), 128.6 ($2 \times \text{CH}$), 129.7 ($2 \times \text{CH}$), 135.6 ($4 \times \text{CH}$), 133.3 (C), 137.1 ($2 \times \text{C}$), 143.2 (C); (ES) m/z (%) 414 (M-Ph, 100%), 509 (23, $\text{M}+\text{NH}_4^+$), 492 (16 $\text{M}+\text{H}^+$); Accurate mass (FAB) 492.2029 Found ($\text{M}+\text{H}^+$ $\text{C}_{28}\text{H}_{34}\text{NO}_3\text{SSi}$ requires 492.2029).

(2R,3R,4S)-3,4-Epoxy-2-(tert-butylidiphenylsilyloxymethyl)-1-(toluene-4-sulfonyl)pyrrolidine



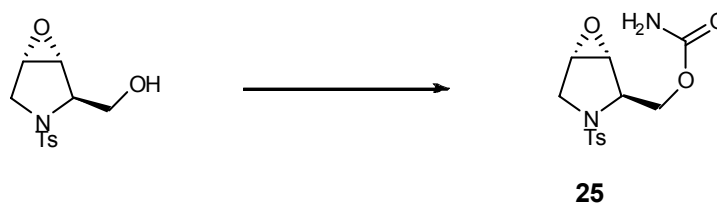
Na_2EDTA (0.75 ml, 4×10^{-4} mol dm^{-3} , 0.00032 mmol) and trifluoroacetone (0.19 g, 0.16 ml, 1.77 mmol) were added to a 0 °C solution of the alkene from the previous reaction **11** (0.231 g, 0.78 mmol) dissolved in acetonitrile (12 ml). Solid NaHCO_3 (0.11 g, 1.29 mmol) mixed with oxone® (0.50g, 0.81 mmol) was then added to the solution portion-wise over 1 h. The reaction mixture was stirred a further 2 hr before sodium sulphate (0.5 g) was added. The solution was diluted with dichloromethane (10 ml), filtered and the filtrate was concentrated under vacuum. The resulting material was purified by flash column chromatography on silica (10:90, ethyl acetate:petroleum-ether) to afford the title compound (0.048 g, 60%) as a white solid; mp 118-119 °C; $[\alpha]_{\text{D}}^{20}$ -86.0 ($c = 1.18$ MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1} 1748w, 1430w, 1343s, 1235m, 1156s, 1090s, 1045s, 1010m, 928w, 847s; δ_{H} (300 MHz, CDCl_3) 1.07 (9H, s, $\text{C}(\text{CH}_3)_3$), 2.43 (3H, s, Ar 4-Me), 3.55 (1H, d, J 3.0, H-3 or H-4), 3.57 (1H, d, J 12.6, HH-5), 3.58 (1H, d J 3.0, H-3 or H-4), 3.67 (1H, d, J 12.6, HH-5), 3.91 (1H, dd, J 10.5, 5.4, CHHO), 3.93 (1H, dd, J 10.5, 6.6, CHHO), 3.88-3.98 (1H, m overlapping, H-2), 7.28 (2H, d, J 8.1, $2 \times \text{Ar H-3}$), 7.39-7.48 (6H, m, $6 \times \text{Ph-H}$), 7.60-7.69 (6H, m, $4 \times \text{Ph-H}$, $2 \times \text{Ar H-2}$); δ_{C} (75 MHz; 300 MHz, CDCl_3) 19.1 (C), 21.6 (CH_3), 26.8 ($3 \times \text{CH}_3$), 49.6 (CH_2), 55.1 (CH), 57.7 (CH), 61.7 (CH), 64.9 (CH_2), 127.5 ($2 \times \text{CH}$), 127.9 ($4 \times \text{CH}$), 129.5 ($2 \times \text{CH}$), 129.9 (CH), 129.9 ($2 \times \text{CH}$), 132.7 (C), 132.9 (C), 135.2 (C), 135.6 ($3 \times \text{CH}$), 143.4 (C); (ES) m/z (%) 509 (23, $\text{M}+\text{NH}_4^+$), 430 (M-Ph, 100%), 525 (22, $\text{M}+\text{NH}_4^+$), 508 (7, $\text{M}+\text{H}^+$); Accurate mass (FAB) 508.1977 Found ($\text{M}+\text{H}^+$ $\text{C}_{28}\text{H}_{34}\text{NO}_4\text{SSi}$ requires 508.1978); Anal. Found C, 66.14; H, 6.56; N, 2.60; $\text{C}_{28}\text{H}_{33}\text{NO}_4\text{SSi}$ requires: C, 66.29; H, 6.55; N, 2.75.

(2R,3R,4S)-3,4-Epoxy-2-hydroxymethyl-1-(toluene-4-sulfonyl)pyrrolidine



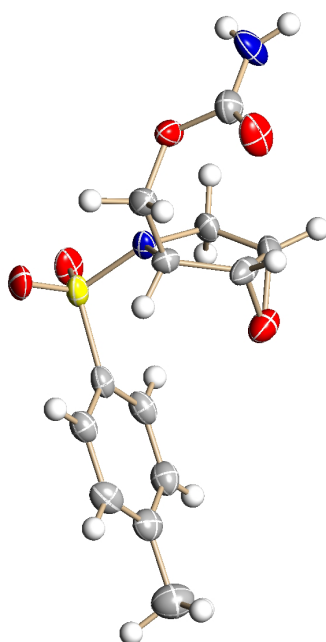
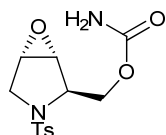
Tertiary butyl ammonium fluoride (0.081 ml of a 1 mol dm⁻³ solution in THF, 0.081 mmol) was added to a 0 °C solution of the epoxide from the previous step (0.037 g, 0.073 mmol) dissolved in THF (1.9 ml). The reaction mixture was stirred for 2 h before the solvent was removed under vacuum. The resulting material was purified by flash column chromatography on silica (50:50, ethyl acetate:petroleum-ether) to give the title compound (0.016 g, 84%) as a white solid; Mp: 127-129 °C (Lit.⁴ 131-133 °C); $[\alpha]_D^{20}$ -91.0 (c= 1.53 MeOH); ν_{\max} (CH₂Cl₂)/cm⁻¹ 3503br (OH), 1332s, 1317s, 1219m, 1155s, 1092m, 1008m, 848s, 816m, 670s; δ_H (300 MHz, CDCl₃) 2.43 (3H, s, Ar 4-Me), 3.51 (1H, d, *J* 3.0, H-3 or H-4), 3.57 (1H, d, *J* 3.0, H-3 or H-4), 3.58 (1H, d overlapping, *J* 12.6, *HH*-5), 3.68 (1H, d, *J* 12.6, *HH*-5), 3.80 (1H, dd, *J* 12.6, 6.0, *CHHO*), 3.88 (1H, dd, *J* 12.6, 4.2, *CHHO*), 3.90 (1H, dd, *J* 6.0, 4.2, H-2), 7.31 (2H, d, *J* 8.1, 2 × Ar H-3), 7.66 (2H, d, *J* 8.1, 2 × Ar H-2); δ_C (75 MHz; 300 MHz, CDCl₃; Me₄Si): 21.6 (CH₃), 49.5 (CH₂), 55.0 (CH), 57.2 (CH), 62.5 (CH), 63.2 (CH₂), 127.6 (2 × CH), 129.6 (2 × CH), 134.5 (C), 143.9 (C); (ES) *m/z* (%) 252 (M+H⁺-H₂O, 100%), 270 (7, M+H⁺); Accurate mass (FAB) 270.0800 Found (M+H⁺ C₁₂H₁₆NO₄S requires 270.0800); Anal. Found C, 53.34; H, 5.40; N, 5.03; C₁₂H₁₅NO₄S requires: C, 53.50; H, 5.61; N, 5.20.

(2*R*,3*R*,4*S*)-2-(Carbamoyloxymethyl)-3,4-epoxy-1-(toluene-4-sulfonyl)pyrrolidine



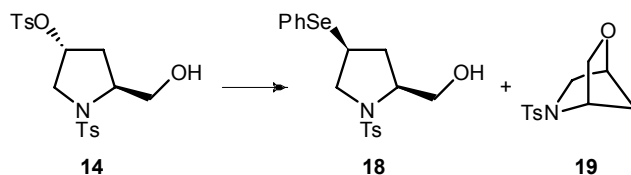
Trichloroacetylisocyanate (0.036 g, 0.0023 ml, 0.031 mmol) was added to a 0 °C solution of the alcohol from the previous step (0.0056 g, 0.021 mmol) in dichloromethane (1 ml). After 8 h TLC analysis confirmed all starting material had been consumed. The crude material was dissolved in methanol (1 ml) and hydrolysis was carried out using potassium carbonate (1ml, 0.5 mol dm⁻³, 0.5 mmol). After 18 h the reaction was worked up to yield **25** (0.0054 g, 78%) as a white solid. Analytical data reported in the main paper.

A single crystal of **25** was obtained by recrystallisation from CHCl₃. Crystal data: C₁₃H₁₆N₂O₅S, *M* = 312.34, orthorhombic, *a* = 7.7577 (8), *b* = 17.7377 (19), *c* = 20.872 (2) Å, *U* = 2872.0 (5) Å³, *T* = 150(2) K, space group *P*2₁2₁2₁, *Z* = 8, absorption coefficient = 0.249 mm⁻¹, 20984 reflections measured, 5053 unique (*R*_{int} = 0.0491), absolute structure parameter = 0.12(6). The final *wR*(*F*²) was 0.0699 for all data. CCDC deposition number 656329.



X-Ray structure for **25** (ellipsoids are shown at 50% probability). There are two unique molecules in the asymmetric unit of which only one is shown.

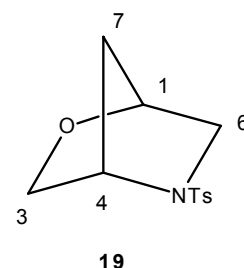
Attempted selanylation of **14**:



Sodium borohydride (0.012 g, 0.32 mmol) was added to a solution of diphenyldiselenide (0.054 g, 0.17 mmol) in ethanol (2 ml). The solution was degassed (3 cycles freeze-pump-thaw) and to this mixture a degassed (3 cycles freeze-pump-thaw) solution of **14** (0.116 g, 0.273 mmol) in THF (2 ml) was added. The reaction mixture was refluxed for 3 h before solvent was removed under reduced pressure. The resulting crude material was purified by flash column chromatography on silica (40:60, ethyl acetate:petroleum-ether) to give **18** (0.028 g, 25%) and **19** (0.029 g, 42%) as colourless oils:

18: δ_{H} (250 MHz; CDCl_3) 1.64-1.76 (1H, m, *HH*-3), 2.18-2.28 (1H, m, *HH*-3), 2.44 (3H, s, Ar 4-Me), 2.68-2.82 (1H, m, H-4), 3.31 (1H, dd, *J* 12.0, 10.3, *HH*-5), 3.62-3.82 (4H, m, *HH*-5, H-2, CH_2O), 7.24-7.33 (5H, m, $3 \times \text{Ph-H}$, $2 \times \text{Ar H-3}$), 7.39-7.42 (2H, m, $2 \times \text{Ph-H}$), 7.65 (2H, d, *J* 8.3, $2 \times \text{Ar H-2}$).

19: δ_{H} (250 MHz; CDCl_3) 1.32 (1H, dd, *J* 9.9, 2.5, *HH*-7), 1.70 (1H, brd, *J* 9.9, *HH*-7), 2.42 (3H, s, Ar 4-Me), 3.21 (1H, dd, *J* 9.8, 1.5, H-6*exo*), 3.40 (1H, d, *J* 9.8, H-6*endo*), 3.65 (1H, dd, *J* 7.8, 1.6, H-3*exo*), 3.88 (1H, d, *J* 7.8, H-3*endo*), 4.45 (1H, brs, H-1 or H-4), 4.50 (1H, brs, H-1 or H-4), 7.32 (2H, d, *J* 8.2, $2 \times \text{Ar H-3}$), 7.72 (2H, d, *J* 8.4, $2 \times \text{Ar H-2}$); (ES) *m/z* (%) 254 ($\text{M}+\text{H}^+$, 100%)



Glycosidase inhibition

All enzymes and substrates were purchased from Sigma. Enzyme and substrate solutions were made using 0.2 M sodium phosphate buffers at suitable pH and protein concentrations (pH 6.5 and 0.2 U mL⁻¹ for α -galactosidase (green coffee beans), pH 7.3 and 2.0 U mL⁻¹ for β -galactosidase (bovine liver), pH 6.0 and 1.5 U mL⁻¹ for α -glucosidase (*Saccharomyces cerevisiae*), pH 5.0 and 0.2 U mL⁻¹ for β -glucosidase (almond, *Prunus* sp.), pH 4.5 and 0.2 U mL⁻¹ for α -mannosidase (jack bean, *Canavalia ensiformis*), pH 4.0 and 1.0 U mL⁻¹ for naringinase (*Penicillium decumbens*) and pH 4.25 and 0.2 U mL⁻¹ for *N*-acetyl- β -D-glucosaminidase (bovine kidney)). PNP-glycosides were used as substrates at a concentration of 5 mM.

Compounds tested for enzyme inhibition were dissolved in distilled water at a concentration of 1 mg mL⁻¹. Where required, compounds were dissolved in methanol (*ca.* 20 μ L) before dilution using distilled water. Inhibitor solutions were stored at -20 °C. All assays were carried out in triplicate using water as a blank in place of the inhibitor. Reaction time was determined based on the length of time needed to give a final absorbance of 0.3–1.5 units. Linearity of the reaction time course was checked using a series of incubation times. Rate of colour development after the addition of Trinder glucose reagent was determined using a linear time course.

Enzyme solution (10 μ L) inhibitor solution (10 μ L) and substrate solution (50 μ L) were combined in the well of a flat-bottomed 96-well (300 μ L) microtitre plate. The reaction mixture was incubated at 25 °C for 5–20 min and was stopped using glycine solution (70 μ L, 0.4 M, adjusted to pH 10.4 using NaOH). Absorbance at 405 nm was measured immediately in a microtitre plate reader (Molecular Devices VersaMax microplate reader). Percentage activity was calculated by reference to the control reaction for each assay and percentage inhibition determined by subtraction from 100%.

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