Electronic Supplementary Information

"Polar Patch" Proteases as Glycopeptiligases

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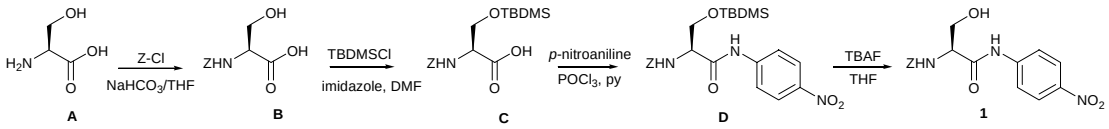
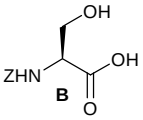


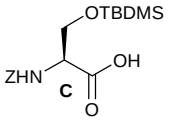
Scheme 1: Synthesis of compound 1.

N-Benzyloxycarbonyl-L-serine B



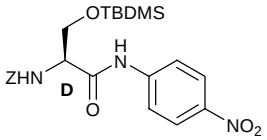
L-Serine **A** (5.00 g, 47.6 mmol) was dissolved in sodium hydrogencarbonate (200 mL of a saturated aqueous solution). Benzyl chloroformate (12.2 g, 71.4 mmol) was added. After 18 h, the reaction mixture was extracted with diethyl ether (3 x 100 mL). The aqueous layer was acidified to pH 3 with concentrated hydrochloric acid then extracted with ethyl acetate (4 x 100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Recrystallisation (ethyl acetate/petrol) afforded *N*-benzyloxycarbonyl-L-serine **B** (7.10 g, 62%) as a white crystalline solid, m.p 112-113 ^oC [Lit. 116-118 ^oC (ethyl acetate/petrol)];[1] $[\alpha]_D^{24}$ +8.2 (*c*, 1.0 in acetic acid) [Lit. $[\alpha]_D^{20}$ +5.95 (*c*, 1.0 in acetic acid)];[1] δ_H (400MHz, $CDCl_3$) 3.66 (2H, bs, C<u>H</u>$_2$OH), 4.05 (1H, m, αH), 4.88 (1H, bs, CH$_2$O<u>H</u>), 5.04 (2H, s, C<u>H</u>$_2$-Ph), 7.32 (1H, d, $J_{NH,\alpha H}$ 8.7 Hz, NH), 7.30-7.37 (5H, m, Ar-H), 12.63 (1H, br s, COOH).

_N_-Benzyloxycarbonyl-_O-tert_-butyldimethylsilyl-L-serine C



_N_-Benzyloxycarbonyl-L-serine **B** (2.00 g, 8.37 mmol), imidazole (recrystallised from ethanol, 2.24 g, 33.5 mmol) and *tert*-butyldimethylsilyl chloride (1.90 g, 12.6 mmol) were dissolved in DMF (80 mL) and heated to 60 ^{o}C under argon. After 72 h, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a product (R_f 0.6) with complete consumption of the starting material (R_f 0.1). The reaction mixture was concentrated *in vacuo*. The residue was suspended in petrol, and extracted with sodium hydrogen carbonate (5% w/v, 3 x 35 mL). The phases were separated and the aqueous layer was acidified to pH 3 using potassium hydrogensulfate (1 M solution). The resulting solution was extracted with ethyl acetate (3 x 40 mL). The combined organic layers were washed with brine (40 mL) and dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was recrystallised (ethyl acetate/petrol) to afford _N_-benzyloxycarbonyl-_O-tert_-butyldimethylsilyl-L-serine **C** (2.26 g, 77%) as a white crystalline solid, m.p. 77-78 ^{o}C; $[\alpha]_D^{24}$ + 24.8 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr) 3337 (br, N-H), 3068 (br, O-H), 1759, 1739 (s, NC(O)O), 1690 (s, C(O)OH) cm^{-1}; δ_H (400 MHz, d_6-DMSO) 0.01, 0.02 (6H, 2 x s, Si-CH_3), 0.86 (9H, s, Si-C$(CH_3)_3$), 3.81 (2H, d, $J_{CH2,CH}$ 1.2 Hz, Si-O-C$\underline{H}_2$), 4.13 (1H, m, αH), 5.04 (2H, s, C$\underline{H}_2$-Ph), 7.29-7.37 (6H, m, Ar, NH); δ_C (100 MHz, d_6-DMSO) -4.7, -4.6 (2 x q, 2 x Si-CH_3), 18.8 (s, Si-$\underline{C}(CH_3)_3$), 26.7, 26.6 (2 x q, 3 x Si-C($\underline{C}H_3)_3$), 57.0 (d, αC), 63.6 (t, CH_2Ph), 66.3 (t, CH_2OH), 128.6, 128.7, 129.2 (3 x d, 5 x Ar-C), 137.8 (s, Ar-C), 156.8 (s, NC(O)O), 172.5 (s, CO_2H); *m/z* (ESI+) 354 (M+H$^+$, 100), (M+Na$^+$, 55%); HRMS (ESI+) Calcd. for $C_{17}H_{28}NO_5Si$ (M+H$^+$) 354.1737. Found 354.1741.

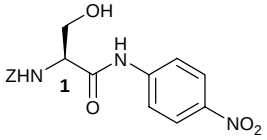
_N_-Benzyloxycarbonyl-_O-tert_-butyldimethylsilyl-L-serine-_para_-nitroanilide D



_N_-Benzyloxycarbonyl-_O-tert_-butyldimethylsilyl-L-serine **C** (600 mg, 1.70 mmol) and *para*-nitroaniline (356 mg, 2.55 mmol) were dissolved in anhydrous pyridine (10 mL) and cooled to –15 ^{o}C. Phosphorous oxychloride (0.30 mL, 2.21 mmol) was added and the mixture stirred at –15 ^{o}C. After 2 h, t.l.c. (petrol:ethyl acetate, 3:1) showed the formation of a product (R_f 0.3) with complete consumption of the starting material (R_f 0.1). The reaction was quenched with ice water (50 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic layers were washed with sodium hydrogencarbonate (30 mL of a saturated aqueous solution) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (petrol:ethyl acetate, 3:1) to afford _N_-benzyloxycarbonyl-_O-tert_-butyldimethylsilyl-L-serine-*para*-nitroanilide **D** (820 mg, 80%) as a yellow crystalline solid; m.p. 113-114 ^{o}C (ethyl acetate/petrol); $[\alpha]_D^{23}$ – 6.4 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr) 3328 (br, N-H), 1717, 1682 (s, NC(O)O), 1616, 1600 (s, C(O)NH), 1502, 1341 (s, NO_2) cm^{-1}; δ_H (400 MHz, d_6-DMSO) -0.3 (6H, 2 x s, Si$(CH_3)_2$), 0.77 (9H, s, C$(CH_3)_3$), 3.79 (2H, m, CH_2O-Si), 4.38 (1H, m, αH), 5.04 (2H, 2 x s, C$\underline{H}_2$Ph), 7.33

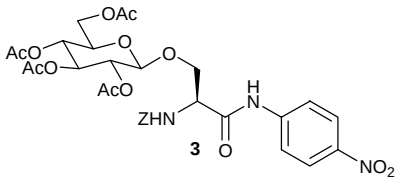
(5H, m, Ar-H), 7.60 (1H, d, $J_{NH,\alpha H}$ 7.7 Hz, N<u>H</u>-αH), 7.87 (2H, d, *J* 9.3 Hz, *o*-Ar-H *p*NA), 8.22 (2H, d, *m*-Ar-H *p*NA), 10.73 (1H, s, N<u>H</u>-Ar-C); δ_C (100 MHz, d_6-DMSO) -5.5 (2 x q, 2 x Si-$(CH_3)_2$), 18.1 (s, Si-<u>C</u>$(CH_3)_3$), 25.8 (q, 3 x Si-C(<u>C</u>$H_3)_3$), 63.1 (t, CH_2OSi), 67.5 (t, <u>C</u>H_2Ar), 119.2 (d, 2 x *m*-Ar-C *p*NA), 125.1 (d, 2 x *o*-Ar-C *p*NA), 128.2, 128.5, 128.6 (3 x d, 5 x Ar-C), 137.7, 143.16, 145.8 (3 x s, 3 x Ar-C), 156.8 (s, NC(O)O), 171.1 (s, C(O)N); *m/z* (ESI+) 969 ($2M+Na^+$, 100), 496 ($M+Na^+$, 55 %); HRMS (ESI+) Calcd. for $C_{23}H_{31}N_3O_6NaSi$ ($M+Na^+$) 496.1880. Found 496.1889.

_N_-Benzyloxycarbonyl-L-serine-_para_-nitroanilide 1



N-Benzyloxycarbonyl-*O*-*tert*-butyldimethylsilyl-L-serine-*para*-nitroanilide **D** (2.10 g, 4.44 mmol) was dissolved in anhydrous THF (50 mL). Tetrabutylammonium fluoride (35.0 mL of a 1 M solution in THF, 35.5 mmol) was added and the reaction was stirred under an atmosphere of argon at RT. After a 24 h period, t.l.c. (ethyl acetate) showed formation of a product (R_f 0.4) with complete consumption of the starting material (R_f 0.7). The reaction mixture was concentrated *in vacuo*, re-suspended in ethyl acetate (100 mL) and washed with water (2 x 100 mL). The aqueous layers were re-extracted with ethyl acetate (2 x 50 mL) and the combined organic layers dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate) to afford *N*-benzyloxycarbonyl-L-serine-*para*-nitroanilide **1** (1.30 g, 85%) as a yellow crystalline solid; m.p. 154-155 ^oC (ethyl acetate/petrol); $[\alpha]_D^{22}$ – 49.5 (*c*, 1.0 in MeOH); ν_{max} (KBr) 3333 (br, N-H, O-H), 1683 (s, NC(O)O), 1616, 1597 (s, C(O)N), 1511, 1340 (s, NO_2) cm^{-1}; δ_H (400 MHz, CD_3OD) 3.87 (2H, d, $J_{CH2,\alpha H}$ 5.2 Hz, C<u>H</u>$_2$OH), 4.40 (1H, t, *J* 5.1 Hz, αH), 5.13, 5.16 (2H, 2 x s, PhC<u>H</u>$_2$), 7.32-7.35 (5H, m, 5 x Ar-H), 7.85 (2H, d, *J* 8.8 Hz, *m*-Ar-H *p*NA), 8.21 (2H, d, *o*-Ar-H *p*NA); δ_C (100 MHz, CD_3OD) 58.2 (d, αCH), 62.2 (t, CH_2-OH), 66.9 (t, <u>C</u>H_2-Ar), 119.7 (d, 2 x *m*-Ar-C *p*NA), 124.7 (d, 2 x *o*-Ar-C *p*NA), 127.9, 128.1, 128.4 (3 x d, 5 x Ar-C), 137.2, 143.8, 144.9 (3 x s, 3 x Ar-C), 157.7 (s, NC(O)O), 171.0 (s, C(O)N); *m/z* (ESI+) 360 ($M+H^+$, 70), 377 ($M+NH_4^+$, 100%); HRMS (ESI+) Calcd. for $C_{17}H_{17}N_3O_6Na$ ($M+Na^+$) 382.1015. Found 382.1008.

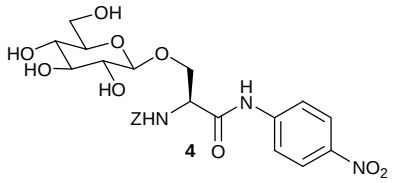
_O_-(2,3,4,6-_O_-Acetyl)-β-D-glucopyranosyl-_N_-benzyloxycarbonyl-L-serine-_para_-nitroanilide 3



2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **2** (186 mg, 0.38 mmol) was added to a solution of *N*-benzyloxycarbonyl-L-serine-*para*-nitroanilide **1** (200 mg, 0.57 mmol) in anhydrous DCM (4 mL) with 4Å molecular sieves. Trimethylsilyltriflate (15 μL, 0.06 mmol) was added to the solution and left to stir under argon. After 16 h, t.l.c. (ethyl acetate:petrol, 2:1) indicated formation of a product (R_f 0.3) with consumption of the starting material (R_f 0.7). The reaction mixture was filtered through celite, washed with water (10 mL) and the aqueous layer

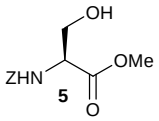
extracted with DCM (3 x 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue purified by flash column chromatography (2:1, ethyl acetate:petrol) to afford *O*-(2,3,4,6-*O*-acetyl)-β-D-glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-*para*-nitroanilide **3** (90 mg, 40%) as a yellow oil; $[\alpha]_D^{25}$ -3.90 (*c*, 1.0 in $CHCl_3$); ν_{max} (thin film) 3354 (br, O-H, N-H), 1755 (s, C=O), 1521, 1340 (s, NO_2) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.99, 2.01, 2.03, 2.04 (12H, 4 x s, 4 x CH_3), 3.71-3.76 (1H, m, H-5), 3.92 (1H, at, *J* 8.4 Hz, C<u>H</u>H'O), 4.15-4.23 (3H, m, CH<u>H</u>'O, H-6, H-6'), 4.56-4.60 (1H, $J_{1,2}$ 5.4 Hz , H-1), 4.62-4.68 (1H, bs, αH), 4.99 (1H, at, *J* 9.2 Hz, H-2), 5.09 (1H, at, *J* 9.1 Hz, H-4), 5.15, 5.17 (2H, 2 x s, C<u>H</u>$_2$Ar), 5.19 (1H, at, *J* 2.5 Hz, H-3), 5.69 (1H, bs, N<u>H</u>CH), 7.34-7.39 (5H, m, Ar), 7.35 (2H, d, *J* 9.0 Hz, *m*-H), 8.22 (2H, d, *o*-H); δ_C (125 MHz, $CDCl_3$) 20.4, 20.4, 20.5, 20.6 (4 x q, 4 x CH_3), 56.9 (d, αH), 61.2 (t, C-6), 67.5 (t, <u>C</u>H$_2$Ar), 67.9 (d, C-4), 69.8 (t, CH_2O), 70.9 (d, C-2), 71.2 (d, C-5), 72.2 (d, C-3), 101.9 (d, C-1), 119.3 (d, 2 x *m*-C), 124.9 (d, 2 x *o*-C), 128.1, 128.2, 128.4 (3 x d, 5 x Ar), 135.7 (s, Ar-C), 143.0 (s, Ar-C-NH), 143.8 (s, Ar-C-NO_2), 168.2 (s, NC(O)O), 169.2, 169.3, 169.4, 169.9 (4 x s, 4 x C(O)Me), 170.7 (s, C(O)N); *m/z* (ESI+) 712 ($M+Na^+$, 100), 1401 ($2M+Na^+$, 30%); HRMS (ESI+) calcd. for $C_{31}H_{35}N_3O_{15}Na$ ($M+Na^+$) 712.1966. Found 712.1969.

O–β-D-Glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-*para*-nitroanilide 4



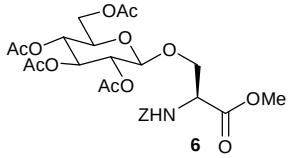
O-(2,3,4,6-*O*-Acetyl)-β-D-glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-*para*-nitroanilide **3** (128 mg, 0.22 mmol) was dissolved in methanol (1.5 mL). Hydrazine monohydrate (150 μL, 1.74 mmol) was added and the reaction stirred under an atmosphere of argon. After 16 h, t.l.c. (ethyl acetate:methanol, 9:1) indicated the formation of a product (R_f 0.4) with complete consumption of the starting material (R_f 0.7). The reaction mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (ethyl acetate:methanol, 9:1) to afford *O*-β-D-glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-*para*-nitroanilide **4** (90 mg, 79 %) as a yellow oil; $[\alpha]_D^{22}$ –7.5 (*c*, 1.0 in MeOH); ν_{max} (thin film) 3305 (br, OH), 1749 (s, C=O), 1506, 1343 (NO_2) cm^{-1}; δ_H (CD_3OD, 500 MHz) 3.20 (1H, dd, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.0 Hz, H-2), 3.27-3.34 (2H, m, H-4, H-5), 3.37 (1H, dd, $J_{3,4}$ 10.8 Hz, H-3), 3.66 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6,6'}$ 11.8 Hz, H-6), 3.88 (1H, d, H-6'), 3.92 (1H, dd, $J_{CH,CH'}$ 10.5 Hz, $J_{CH,\alpha H}$ 5.9 Hz, C<u>H</u>H'O), 4.23-4.25 (1H, m, CH<u>H</u>'O), 4.35 (1H, d, H-1), 4.55-4.59 (1H, m, αH), 5.15 (2H, d, *J* 6.0 Hz, C<u>H</u>$_2$Ph), 7.31-7.40 (5H, m, Ar), 7.87 (2H, d, *J* 8.9 Hz, *m*-Ar-H *p*NA), 8.23 (2H, d, *o*-Ar-H *p*NA); δ_C (CD_3OD, 125 MHz) 56.3 (d, αH), 61.5 (t, C-6), 67.0 (t, <u>C</u>H$_2$Ph), 69.6 (t, CH_2O), 70.5 (d, C-4), 74.0 (d, C-2), 76.9, 77.0 (2 x d, C-3, C-5), 103.7 (d, C-1), 119.8 (d, *m*-Ar-C *p*NA), 124.7 (d, *o*-Ar-C *p*NA), 127.9, 128.0, 128.1, (3 x d, 5 x Ar), 143.9, 144.6, 157.7 (3 x s, 3 x Ar-C), 170.4 (s, NC(O)O), 172.1 (s, C(O)N); *m/z* (ESI-) 520 ($M-H^+$, 100%); HRMS (ESI-) Calcd for $C_{23}H_{26}N_3O_{11}$ 520.1567. Found 520.1567.

N-Benzyloxycarbonyl-L-serine methyl ester 5



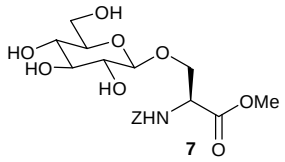
Thionyl chloride (1.18 mL, 16.3 mmol) was added dropwise to a solution of *N*-benzyloxycarbonyl-L-serine **B** (2.6 g, 10.9 mmol) in anhydrous methanol (20 mL) at 0 ^{o}C. The reaction mixture was stirred under nitrogen. After a period of 3 h, t.l.c. (9:1, ethyl acetate:methanol) indicated formation of a product (R_f 0.6) with complete consumption of the starting material (R_f 0.0). The reaction mixture was concentrated *in vacuo* and resuspended in diethyl ether (20 mL). The solution was filtered and the filtrate was washed with sodium hydrogen carbonate (20 mL of a saturated aqueous solution), dried ($MgSO_4$) and triturated with petrol to yield *N*-benzyloxycarbonyl-L-serine methyl ester **5** (2.0 g, 75 %) as a waxy solid; m.p. 31-32 ^{o}C [Lit. 33-35 ^{o}C];[2] $[\alpha]_D^{25}$ – 17.3 (*c*, 1.0 in MeOH), [Lit. $[\alpha]_D^{25}$ – 13.2 (*c*, 1.0 in MeOH)];[2] δ_H (200 MHz, $CDCl_3$) 3.73 (3H, s, CH_3), 3.85 (1H, dd, $J_{CH,NH}$ 3.5 Hz, $J_{CH,CH'}$ 11.3 Hz, C<u>H</u>H'O), 3.96 (1H, dd, $J_{CH',NH}$ 3.9 Hz, CH<u>H</u>'O), 4.40-4.45 (1H, m, αH), 5.10 (2H, s, C<u>H</u>$_2$Ph), 5.98 (1H, d, *J* 7.9 Hz, NH), 7.39 (5H, s, Ar-H).

_O_-(2,3,4,6-_O_-Acetyl)-β-D-glucopyranosyl-_N_-benzyloxycarbonyl-L-serine-methyl ester 6



2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **2** (194 mg, 0.39 mmol) was added to a solution *N*-benzyloxycarbonyl-L-serine methyl ester **5** (150 mg, 0.59 mmol) in anhydrous DCM (3 mL) with 4Å molecular sieves. Trimethylsilyltriflate (13 μL, 0.06 mmol) was added to the solution and left to stir under argon. After 3 h, t.l.c. (ethyl acetate:petrol, 2:1) indicated formation of a product (R_f 0.5) with consumption of some of the starting material (R_f 0.7). The reaction mixture was filtered through celite, washed with water (10 mL) and the aqueous layer extracted with DCM (3 x 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue purified by flash column chromatography (2:1, ethyl acetate:petrol) to afford *O*-(2,3,4,6-*O*-acetyl)-β-D-glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-methyl ester **6** (104 mg, 46 %) as a clear oil; $[\alpha]_D^{25}$ + 7.5 (*c*, 1.0 $CHCl_3$); ν_{max} (thin film) 3360 (br, NH), 1750 (s, C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.00, 2.02, 2.03, 2.08 (12H, 4 x s, 4 x CH_3), 3.62 (1H, ddd, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 2.2 Hz, $J_{5,6'}$ 4.6 Hz, H-5), 3.76 (3H, s, OMe), 3.84-3.90 (1H, m, C<u>H</u>H'O), 4.11 (1H, dd, $J_{6,6'}$ 12.2 Hz, H-6), 4.22-4.26 (2H, m, H-6', CH<u>H</u>'O), 4.48-4.52 (2H, m, αH, H-1), 4.94 (1H, dd, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 9.4 Hz, H-2), 5.05 (1H, at, *J* 9.5 Hz, H-4), 5.13-5.18 (3H, m, H-3, C<u>H</u>$_2$Ph), 5.58 (1H, d, *J* 7.6 Hz, NH), 7.33-7.37 (5H, m, Ar-H); δ_C (100 MHz, $CDCl_3$) 20.5-20.7 (52.8 (q, OMe), 54.3 (d, α-C), 61.7 (t, C-6), 67.1 (t, <u>C</u>H$_2$Ph), 68.1 (d, C-4), 69.3 (t, CH_2O), 71.0 (d, C-2), 71.8 (d, C-5), 72.5 (d, C-3), 101.0 (d, C-1), 128.2, 128.3, 128.6 (3 x d, 5 x Ar-C), 136.1 (s, Ar-C), 156.0 (s, NC(O)O), 169.2, 169.3, 169.9, 170.2, 170.6 (5 x s, 5 x C=O); m/z (ESI+) 584 ($M+H^+$, 98), 606 ($M+Na^+$ 100 %); HRMS (ESI+) calcd. for $C_{26}H_{34}NO_{14}$ ($M-H^+$) 584.1979. Found 584.1969.

_O_-β-D-Glucopyranosyl-_N_-benzyloxycarbonyl-L-serine-methyl ester 7

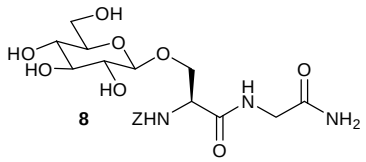


O-(2,3,4,6-*O*-Acetyl)-β-D-glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-methyl ester **6** (92 mg, 0.16 mmol) was dissolved in methanol (2 mL). Hydrazine monohydrate (63 μL, 1.26 mmol) was added and the reaction stirred under an atmosphere of argon. After 40 h, t.l.c. (ethyl acetate:methanol, 4:1) indicated the formation of a product (R_f 0.5) with complete consumption of the starting material (R_f 0.7). The reaction mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (ethyl acetate:methanol, 4:1) to afford *O*-β-D-glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-methyl ester **7** (22 mg, 33 %) as a clear oil; $[\alpha]_D^{25}$ – 6.1 (*c*, 1.0 MeOH); ν_{max} (thin film) 3426 (br, N-H, O-H), 1651 (s, C=O) cm^{-1}; δ_H (400 MHz, CD_3OD) 3.17 (1H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.1 Hz, H-2), 3.26-3.28 (1H, m, H-5), 3.31-3.33 (2H, m, H-3, H-4), 3.65-3.70 (1H, m, H-6), 3.76 (3H, s, OMe), 3.78-3.80 (1H, m, C<u>H</u>H'O), 3.86 (1H, dd, $J_{6,6'}$12.2 Hz, $J_{5,6'}$ 2.1 Hz, H-6'), 4.26 (1H, d, H-1), 4.38 (1H, dd, $J_{\alpha H,CH'}$ 3.8 Hz, $J_{CH,CH'}$ 9.8 Hz, CH<u>H</u>'O), 4.49 (1H, at, *J* 3.5 Hz, αH, 5.13 (2H, s, C<u>H</u>$_2$Ph), 7.28-7.31 (5H, m, Ar-H); δ_C (100 MHz, CD_3OD) 50.0 (q, CH_3), 56.2 (d, α-C), 63.0 (t, C-6), 68.2 (t, CH_2O), 71.0 (t, <u>C</u>H_2Ph), 71.8 (d, C-5), 75.4 (d, C-2), 78.2 (d, C-3), 78.5 (d, C-4), 105.0 (d, C-1), 129.3, 129.5, 129.9 (3 x d, 5 x Ar-C), 141.1 (s, Ar-C), 154.0 (s, NC(O)O), 172.8 (s, C=O); *m/z* (ESI+) 438 ($M+Na^+$, 90), 853 ($2M+Na^+$, 100 %); HRMS (ESI-) calcd. for $C_{18}H_{25}NO_{10}Cl$ ($M+Cl^-$) 450.1167. Found 450.1177.

General Method for Peptide ligation

O-β-D-Glucopyranosyl-*N*-benzyloxycarbonyl-L-serine-methyl ester **7** (10.0 mg, 0.024 mmol) and hydrochloride of acyl acceptor amine (0.072 mmol) were suspended in DMF (200 μL). Triethylamine (6.9 μL, 0.048 mmol) and appropriate CMM (200 μl of 1.4 mg/mL solution in water) were added. A further 4 aliquots of enzyme were added (100 μL of 1.4 mg/mL solution in water) at 24 h intervals. The reaction mixture was concentrated *in vacuo* and purified by column chromatography (water:isopropanol:ethyl acetate, 2:4:4) to yield ligated glycopeptide.

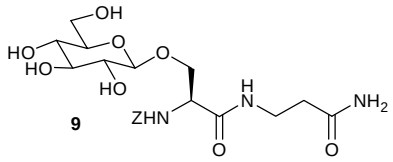
Z-(Glc)-Ser-Gly-NH_2 8



$[\alpha]_D^{19}$ - 2.6 (*c*, 0.5 in MeOH); δ_H (400 MHz, CD_3OD) 3.19 (1H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 1.2 Hz, H-2), 3.25-3.39 (3H, m, H-3, H-4, H-5), 3.64-3.70 (1H, m, H-6), 3.80-3.89 (4H, m, C<u>H</u>H'O, H-6', CH_2(Gly)), 4.25 (1H, dd, $J_{\alpha H,CH'}$ 3.0 Hz, $J_{CH,CH'}$ 10.2 Hz, CH<u>H</u>'O), 4.32 (1H, d, H-1), 4.39 (1H, at, *J* 4.6 Hz, αH), 5.13 (2H, s, C<u>H</u>$_2$Ph), 7.9-7.38 (5H, m, Ar-H); δ_C (100 MHz, CD_3OD) 42.4 (t, CH_2(Gly)), 55.8 (d, αC), 61.6 (t, C-6), 66.9 (d, <u>C</u>H_2Ph), 73.6 (d, CH_2O), 70.4 (d, C-5), 74.0 (d, C-2), 76.8 (d, C-3), 77.1 (d, C-4), 103.3 (d, C-1), 128.0, 128.4, 128.5 (3 x d, 5 x Ar-C), 140.9 (s, Ar-C), 163.0,

164.2 (2 x s, 3 x C=O); *m/z* (ESI+) 480 ($M+Na^+$, 100 %), HRMS (ESI+) calcd. for $C_{19}H_{27}N_3O_{10}Na$ ($M+Na^+$) 480.1594. Found 480.1578.

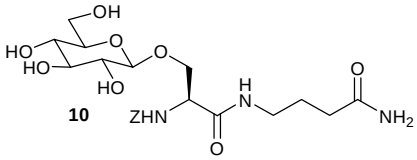
Z-(Glc)-Ser-β-Ala-NH_2 9



9

$[\alpha]_D^{19}$ +7.0 (c, 0.3 in MeOH); δ_H (500 MHz, CD_3OD) 1.71 (2H, q, *J* 6.0 Hz, CH_2), 3.18 (1H, at, *J* 8.1 Hz, H-2), 3.26-3.38 (3H, m, H-3, H-4, H-5), 3.67 (1H, dd, $J_{5,6}$ 5.2 Hz, $J_{6,6'}$ 11.6 Hz, H-6), 3.87 (1H, d, *J* 11.5 Hz, H-6'), 4.10 (1H, d, *J* 4.2 Hz, C<u>H</u>H'O), 4.23-4.25 (3H, m, C<u>H</u>$_2$NH, CH<u>H</u>'O), 4.27 (1H, d, $J_{1,2}$ 7.7 Hz, H-1), 4.49-4.54 (1H, m, αH), 5.13 (2H, s, C<u>H</u>$_2$Ph), 7.32-7.39 (5H, m, Ar-H); δ_C (100 MHz, CD_3OD) 38.3 (t, CH_2), 54.4 (d, αC), 61.3 (t, C-6), 66.3 (t, <u>C</u>H_2Ph), 67.0 (t, CH_2NH), 69.4 (t, CH_2O), 70.3, 76.6, 76.9 (3 x d, C-3, C-4, C-5), 73.6 (d, C-2), 104.1 (d, C-1), 127.3 (d, 5 x Ar-C), 136.9 (s, Ar-C), 157.4, 163.7, 171.0 (3 x s, 3 x C=O); *m/z* (ESI+) 494 ($M+Na^+$, 100 %).

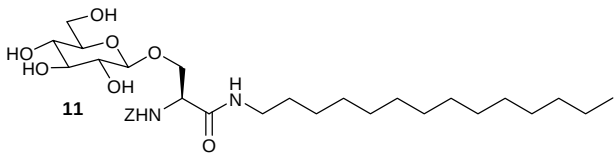
Glc-Z-Ser-GABA-NH_2 10



10

$[\alpha]_D^{21}$ -1.0 (c, 0.1 in MeOH); δ_H (500 MHz, CD_3OD) 1.30-1.35 (2H, m, CH_2), 2.03 (2H, bs, CH_2), 3.20 (1H, at, *J* 8.3 Hz, H-2), 3.29-3.33 (2H, m, H-4, H-5), 3.39 (1H, dd, *J* 8.4 Hz, *J* 17.3 Hz, H-3), 3.69 (1H, dd, $J_{5,6}$ 5.3 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 3.73-3.88 (4H, H-6, C<u>H</u>H'O, C<u>H</u>$_2$NH), 4.29 (1H, d, $J_{1,2}$ 7.7 Hz, H-1), 4.38 (1H, dd, $J_{CH,\alpha H}$ 4.2 Hz, $J_{CH,CH'}$ 10.2 Hz, CH<u>H</u>'O), 4.50 (1H, ad, *J* 3.4 Hz, αH), 5.13 (2H, s, C<u>H</u>$_2$Ph), 7.35-7.38 (5H, m, Ar-H); dC (100 MHz, CD_3OD) 21.3 (t, CH_2), 29.0 (t, CH_2), 51.8 (t, CH_2NH), 54.8 (d, αC), 61.1 (t, C-6), 66.9 (t, <u>C</u>H_2Ph), 69.5 (t, CH_2O), 70.3, 76.6 (2 x d, C-4, C-5), 73.3 (d, C-2), 76.4 (d, C-3), 103.1 (d, C-1), 127.9 (d, 5 x Ar-C), 138.1 (s, Ar-C), 156.3, 163.3, 171.2 (3 x s, 3 x C=O); *m/z* (ESI+) 518 ($M+MeOH+H^+$, 100 %).

Glycolipid mimic 11



11

$[\alpha]_D^{21}$ -1.8 (c, 0.1 in MeOH); δ_H (500 MHz, CD_3OD) 0.92 (3H, t, *J* 6.8 Hz, CH_3), 1.25-1.45 (24H, m, 12 x CH_2), 1.53 (2H, m, C<u>H</u>$_2$$CH_2$NH), 3.16-3.29 (2H, m, C<u>H</u>$_2$NH), 3.41 (1H, bs, H-2), 3.53 (1H, bs, H-4), 3.65-3.72 (2H, m, H-3, H-6), 3.81-3.89 (2H, m, αH, C<u>H</u>H'O), 4.10-4.11 (2H, m, H-6', CH<u>H</u>'O), 4.48 (1H, ad, *J* 5.5 Hz, H-5), 5.11 (2H, s, C<u>H</u>$_2$Ph), 5.31 (1H, bs, H-1), 7.30-7.40 (5H, m, Ar-H); δ_C (100 MHz, CD_3OD) 11.6 (q, CH_3), 27.8-30.0 (t, 12 x CH_2), 30.5 (t, <u>C</u>$H_2$$CH_2$NH), 38.4 (t, <u>C</u>$H_2$NH), 57.8 (t, CH_2O), 62.8 (d. αC), 65.3 (t, C-6), 65.9 (t, <u>C</u>H_2Ph), 70.9 (d, C-2),

71.3 (d, C-4), 73.1 (d, C-3), 76.9 (d, C-5), 103.4 (d, C-1), 127.5 (d, 5 x Ar-C), 137.1 (s, Ar-C), 157.2, 170.5 (2 x s, 2 x C=O); *m/z* (ESI+) 638 (M+MeCN+H^+, 100 %).

Enzyme modification

SBL-S166C (approximately 10 mg of enzyme) was added to 0.5 mL of modifying buffer (70 mM CHES, 5 mM $CaCl_2$, pH 9.5). To this solution was added the appropriate MTS reagent (100 μL of a 20 mM aqueous solution). The solution was sealed, vortexed and placed on an end-over-end rotator at room temperature. After a 30 min period a further portion of the MTS reagent (100 μL of a 10 mg in 200 μL of buffer) was added and rotated for a further 30 min. Completion of the modification was determined by titration with Ellman's reagent showing no free thiol present. The reaction was poured onto a pre-equilibrated desalting column (Amersham PD-10, Sephadex G25) and eluted with water (3.5 mL) and the eluant was dialysed at 4 ^oC against distilled water (2 x 1 L, 2 x 45 min) to afford the CMMs. MS (ES-MS) *m/z*: S166C-S-**g**: calcd 26832, found 26839; S166C-S-**e**: calcd 26861, found 26869; S166C-S-**a**: calcd 26864, found 26867; S166C-S-**c**: calcd 26929, found 26924.

***In situ* enzyme screening**

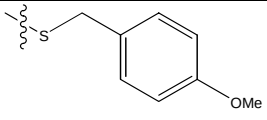
In a 96 well plate 20 μl of enzyme solution (in 5 mM MES, 2 mM $CaCl_2$, pH 6.5, 8 x 10^{-5} mM), 40 μL of CHES buffer (70 mM CHES, 5 mM MES, 2 mM $CaCl_2$, pH 9.5) and 10 μL of the MTS reagent in acetonitrile (10 mM). The reactions were left at room temperature for 2 h. Residual thiol groups were tested by using Ellman's reagent. 10 μL of the reaction mixture was added to 60 μL of Ellmans reagent in Tris buffer (pH 8.6, 0.375 mM). The plate was monitored at 414 nm 15 min later. The reaction was quenched with 10 μL of MES pH 6.5 buffer.

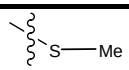
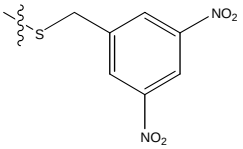
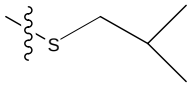
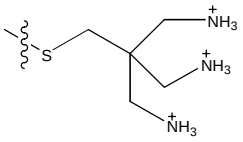
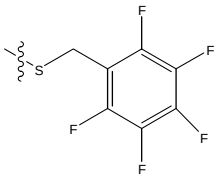
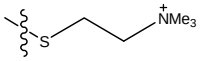
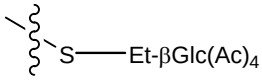
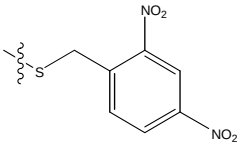
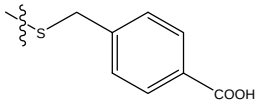
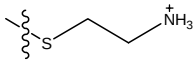
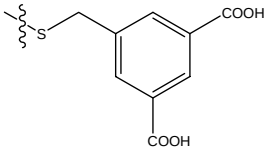
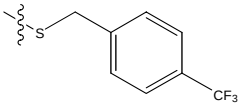
In a 96 well plate 5 μL of modified enzyme was added to 95 μL of Tris buffer (pH 8.6) containing 0.1 mM chromophoric substrate **4** and 5% DMSO. The absorbance was recorded every 30 s for 30 min at 410 nm.

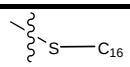
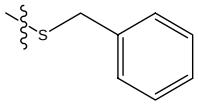
Kinetic measurement

For amidase activity Michaelis-Menten constants were determined at 25 ^oC by curve fitting (GraFit 3.03) of the initial rate data determined at seven concentrations (0.01 mM-8.0 mM) of chromophoric substrate **4** in 0.1 M Tris-HCl buffer (0.005% Tween 80, 1% DMSO, pH 8.6).

Data for *in situ* modification

No	Introduced modification	intial rate data(Ms^{-1})	k_{cat}/K_M ($M^{-1}s^{-1}$) estimated
a		3.79e-9 ± 2.09e-9	33.2±18.8

b		2.13e-9 ± 2.06e-9	18.7±18.4
c		1.67e-9 ± 3.73e-10	14.6±3.16
d		1.24e-9 ± 2.33e-10	10.9±2.1
e		8.43e-10 ± 9.38e-11	7.39±0.90
f		8.32e-10 ± 6.27e-10	7.30±5.61
g		7.74e-10 ± 1.06e-10	6.79±1.00
h		7.68e-10 ± 1.10e-10	6.74±1.03
i		7.23e-10 ± 2.47e-10	6.34±2.24
j		6.34e-10 ± 2.19e-10	5.56±1.99
k		6.32e-10 ± 2.03e-11	5.54±0.23
l		4.25e-10 ± 1.48e-10	3.73±1.34
m	WT	4.13e-10 ± 3.72e-10	3.62±3.33
n		2.93e-10 ± 1.25e-10	2.57±1.13

o		1.54e-10 ± 1.73e-10	1.35±1.54
p		1.24e-10 ± 7.27e-11	1.09±0.65
q	Control	-1.41e-10 ± 5.45e-11	-1.23±0.26

Data for full Michaelis-Menten kinetics

Table 1: Full kinetic parameters for Wild Type SBL and CMMs

Enzyme	**V_{max} (Ms^{-1})**	**K_M (M)**	**k_{cat}(s^{-1})**	**k_{cat}/K_M ($M^{-1}s^{-1}$)**
SBL-WT	3.92e-8 ± 3.08e-9	0.0023±0.0005	7.00e-3	3.04
S166c-**g**	4.35e-8 ± 4.28e-9	0.0036±0.0007	7.77e-3	2.16
S166c-**e**	5.01e-8 ± 4.72e-9	0.0020±0.0005	8.95e-3	4.47
S166c-**a**	3.02e-9±4.21e-10	0.0014±0.0005	5.39e-4	0.39
S166c-**c**	4.59e-8±3.59e-9	0.0054±0.0008	8.17e-3	1.51

References:

1. E. Wuensch, W. Graf, O. Keller, W. Keller, G. Wersin, *Synthesis*, 1986, **11**, 958.
2. C. H. Hassall, J. O. Thomas, *J. Chem. Soc.*, 1968, **4**, 1495-1501.