Enzymatic Removal of Carboxyl Protecting Groups. Part II: Cleavage of the Benzyl and Methyl Moieties

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Supporting Information

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EXPERIMENTAL SECTION

Melting points were determined on a Buchi apparatus and are uncorrected. Specific rotations were measured at 25°C on a Perkin-Elmer 343 polarimeter using a 10 cm cell. NMR spectra were recorded on 200 MHz spectrometer. All amino acid derivatives were purchased from Fluka and Bachem. TLC plates (silica gel 60 F254) and silica gel 60 (70-230 or 230-400 mesh) for column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in EtOH stain. Esterase BsubpNBE was produced recombinantly (see main text), but is also available from Jülich Fine Chemicals (Esterase BS2, www.juelich-chemicals.com, Jülich, Germany). 50 mg of commercial available CAL-A correspond to 1830 Units (manufactures instructions (Roche Diagnostics): >30 U/mg_{Lyo} towards tributyrin as substrate). 50 mg lyophilisate of recombinant BsubpNBE / BS2 correspond to 20 mg protein or 7.5 mg BsubpNBE or 600 Units with *p*-nitrophenyl acetate (pNPA) as substrate ($80U/mg_{protein}$ towards pNPA).

Synthesis of substrates

General method for the preparation of benzyl esters

To a stirred solution of the acid (1 mmol) and benzyl alcohol (0.3 mL, 3 mmol) in CH_2Cl_2 (2 mL), 4-(dimethylamino)pyridine (0.01 g, 0.1 mmol) and subsequently *N,N′*-dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) were added at 0°C. The reaction mixture was stirred for 1 h at 0°C and overnight at room temperature. After filtration, the solvent was evaporated under reduced pressure and EtOAc (20 mL) was added. The organic layer was washed consecutively with brine, 1N HCl, brine, 5% $NaHCO_3$, and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography using $CHCl_3$ as eluent.

Benzyl 4-(benzyloxycarbonyl)butanoate (**11,** Table 1)

Yield 51%; Oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.44-7.26 (m, 10H), 5.12 (s, 2H), 5.10 (2H, s), 4.91 (m, 1H), 3.25 (m, 2H), 2.42 (t, 2H, J = 7.4 Hz), 1.86 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.0, 156.4, 136.4, 135.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.5, 126.9, 66.6, 66.3, 40.3, 31.4, 25.0; Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.55; H, 6.53; N, 4.34.

(*S*)-1-benzyl 5-*tert*-butyl 2-(benzyloxycarbonyl)pentanedioate (**17**, Table 1)

Yield 71%; Oil; $[\alpha]_D$ = -0.6 (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36-7.31 (m, 10H), 5.46 (d, 1H, J = 7.2 Hz), 5.18 (s, 2H), 5.12 (s, 2H), 4.45 (m, 1H), 2.29 (m, 2H), 2.20 (m, 1H), 1.98 (m, 1H), 1.43 (br s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.0, 171.8, 155.9, 136.1, 135.2, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 80.8, 67.2, 67.0, 53.5, 31.3, 28.0, 27.5; Anal. Calcd for $C_{24}H_{29}NO_6$: C, 67.43; H, 6.84; N, 3.28. Found: C, 67.49; H, 7.05; N, 3.15.

Substrates **2**, **4**, **7**, **10** and **15** (Table 1) were prepared according to the procedure for benzyl esters described above. The analytical data were in accordance with those reported in the literature (substrate **2**[1], substrate **4**[2], substrate **7**[3], substrate **10**[4], substrate **15**[5,6]).

Methyl 4-(*tert*-butoxycarbonyl)butanoate (**8,** Table 1)

To a stirred solution of HCl.H-GABA-OMe (0.31 g, 2 mmol) in MeOH (18 mL), Et_3N (2 mL, 14.4 mmol) and subsequently Boc_2O (0.66 g, 3 mmol) were added. The reaction mixture was stirred for 1 h at room temperature. MeOH was evaporated under reduced pressure and the residue was purified by column chromatography using $CHCl_3$ as eluent.

Yield 0.27 g (61%); white solid; mp 47-49°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.69 (m, 1H), 3.66 (s, 3H), 3.14 (m, 2H), 2.35 (t, 2H, J = 7.4 Hz), 1.80 (m, 2H), 1.42 [br s, 9H]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.7, 155.9, 79.2, 51.6, 39.8, 31.2, 28.3, 25.2; Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.45. Found: C, 55.45; H, 8.67; N, 6.48.

Methyl 4-(benzyloxycarbonyl)butanoate (**9,** Table 1)

To a stirred solution of HCl.H-GABA-OMe (0.31 g, 2 mmol) in 1,4-dioxane (3.6 mL), a 2M aqueous solution of Na_2CO_3 (2.2 mL, 4.4 mmol) was added, followed by benzyl chloroformate (0.3 mL, 2.1 mmol). The mixture was stirred at room temperature for 4 h and then partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography using $CHCl_3$ as eluent.

Yield 0.33 g (66%); Oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.32 (m, 5H), 5.20 (m, 1H), 5.07 (s, 2H), 3.63 (s, 3H), 3.20 (m, 2H), 2.34 (t, 2H, J = 7.4 Hz), 1.81 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.5, 156.3, 136.4, 128.3, 127.9, 66.4, 51.5, 40.1, 31.0, 24.9; Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.38; H, 6.75; N, 5.76.

Benzyl 4-(((9H-fluoren-9-yl)methoxy)carbonyl)butanoate (**13,** Table 1)

To a stirred suspension of Fmoc-GABA-OH (0.5 g, 1.54 mmol) in benzene (5 mL), benzyl alcohol (1.0 g, 9.24 mmol) and *p*-toluenesulfonic acid (0.08 g, 0.46 mmol) were added. The reaction mixture was stirred under reflux for 48 h. The homogeneous solution was cooled down, washed with water, 5% $NaHCO_3$, dried over Na_2SO_4 and the organic solvent was evaporated under reduced pressure. The residue was purified by column chromatography using Petroleum ether (60-80°C)/EtOAc (6:4) as eluent.

Yield 0.46 g (72%); oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.76 (d, 2H, J = 6.8 Hz), 7.58 (d, 2H, J = 7 Hz), 7.35 (m, 9H), 5.12 (s, 2H), 4.92 (m, 1H), 4.40 (d, 2H, J = 6.6 Hz), 4.20 (t, 2H, J = 6.8 Hz,), 3.23 (m, 2H), 2.40 (t, 2H, J = 7.4 Hz), 1.85 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.0, 156.4, 143.9, 141.3, 135.8, 128.6, 128.5, 128.3, 128.2, 127.62, 127.56, 127.0, 126.9, 124.9, 119.9, 66.5, 66.4, 47.2, 40.3, 31.4, 25.1; Anal. Calcd for $C_{26}H_{25}NO_4$: C, 75.16; H, 6.06; N, 3.37. Found: C, 75.32; H, 6.17; N, 3.18.

Methyl 4-(((9H-fluoren-9-yl)methoxy)carbonyl)butanoate (**12,** Table 1)

To a stirred solution of HCl.H-GABA-OMe (0.50 g, 4.3 mmol) in 1,4-dioxane (4.5 mL), a 10% aqueous solution of Na_2CO_3 (11 mL, 10.4 mmol) was added, followed by 9-fluorenylmethyl chloroformate (1.16 g, 4.5 mmol). The mixture was stirred overnight at room temperature and then acidified until pH 2. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography using $CHCl_3$ as eluent.

Yield 0.67 g (46%); White solid; mp 98-100°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.69 (d, 2H, J = 7.6 Hz), 7.52 (d, 2H, J = 7.6 Hz), 7.29 (m, 4H), 4.92 (m, 1H), 4.33 (d, 2H, J = 6.6 Hz), 4.14 (t, 1H, J = 6.6 Hz), 3.60 (s, 3H), 3.15 (m, 2H), 2.29 (t, 2H, J = 7.4 Hz), 1.77 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.6, 156.4, 143.9, 141.2, 127.6, 126.9, 124.9, 119.9, 66.5, 51.6, 47.2, 40.3, 31.1, 25.0; Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.59; H, 6.28; N, 4.26.

Substrates **1**[7,8], **3**[7,2], **14**[9] and **16**[10] (Table 1) were synthesized according to the literature.

Dipeptides **1**, **2** and **3** (Table 2) were synthesized by the WSCI/HOBt method.[11] Their analytical data were in accordance with those reported in the literature (substrate **1**[12], substrate **2**[13], substrate **3**[14]).

Substrate **4** (Table 2) was prepared according to an already known procedure.[15]

Products of enzymatic hydrolysis

(*S*)-2-((*S*)-2-(benzyloxycarbonyl)-3-phenylpropanamido)-4-methylpentanoic acid

White solid; mp 126-128°C; $[\alpha]_D$ = -13.0 (*c* 1, CH_3OH); ^1H NMR (200 MHz, $CDCl_3$): δ 8.39 (br s, 1H), 7.33-7.15 (m, 10H), 6.60 (d, 1H, *J* = 7 Hz), 5.71 (d, 1H, *J* = 7.2 Hz), 5.02 (s, 2H), 4.51 (m, 2H), 3.03 (d, 2H, *J* = 6.6 Hz), 1.54 (m, 3H), 0.85 (d, 6H, *J* = 5.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.0, 171.5, 156.3, 136.2, 136.0, 129.3, 128.6, 128.5, 128.2, 127.9, 127.0, 67.2, 56.1, 50.9, 40.9, 38.2, 24.7, 22.7, 21.8; Anal. Calcd for $C_{23}H_{28}N_2O_5$: C, 66.97; H, 6.84; N, 6.79. Found: C, 66.84; H, 6.89; N, 6.83.

The other products of the enzymatic hydrolysis were identified by their analytical data in comparison with authentic samples.

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