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Supporting Information

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Supporting Information

for

Kinetic Resolution of Aliphatic β-Amino Acid Amides by β-Aminopeptidases

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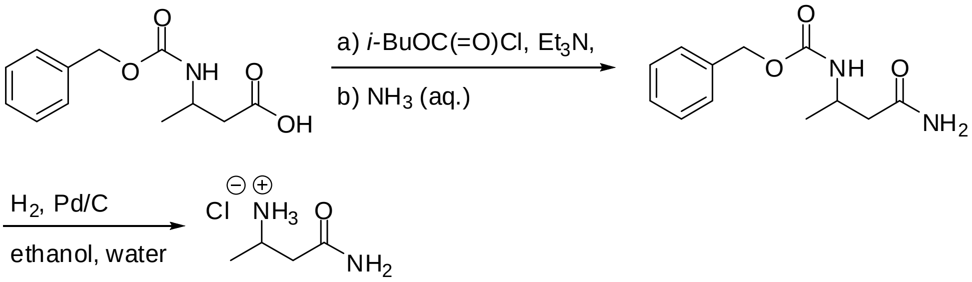
Preparation of Substrates

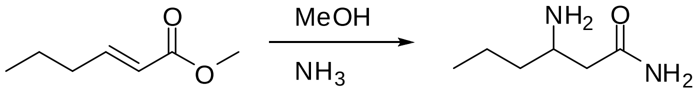
3-Aminobutanamide (rac-1a)



N-3-(Benzyloxycarbonylamino)butanamide: *N*-3-(Benzyloxycarbonylamino)butanoic acid[1] (50.0 g, 211 mmol) and triethylamine (21.5 g, 213 mmol) were dissolved in MTBE (1480 mL), and cooled to 3℃ by an ice-bath. *iso*-Butylchloroformiate (29.0 g, 213 mmol) was added slowly (exothermic reaction), resulting in the formation of a precipitate. After the addition was complete, the reaction mixture was stirred for an additional 30 min and diluted with MTBE (500 mL). Aqueous ammonia (25%, 80 mL) was added quickly (exothermic) and stirring was continued for an additional 45 min at room temperature. After cooling to 5℃, the precipitated solids were collected and washed with MTBE (250 mL). The moist product was then suspended in water (1.8 L) and the mixture was brought to reflux. Most of the solids dissolved, and a small amount of solvent was removed by distillation. After cooling to room temperature, the precipitated white solid was collected by filtration and washed with water. The desired amide was dried at 60℃ in vacuo (72.0 g, 174 mmol, 82.2%); ^{1}H NMR (500 MHz, [D$_6$]DMSO) δ 1.05 (d, *J*= 6.8 Hz, 3H), 2.11 (dd, *J*= 14.2, 8.1 Hz, 1H), 2.27 (dd, *J*= 14.2, 5.8 Hz, 1H), 3.85 (m, 1H), 5.00 (s, 2H), 6.77 (br s, 1H), 7.13-7.38 (m, 7H); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 20.3, 41.9, 44.1, 65.0, 127.7 (2x), 128.3, 137.2, 155.1, 172.1; IR (KBr), λ^{-1} (cm^{-1}): 3386, 3328, 3191, 1683, 1650, 1537,1275, 1258, 1066, 695; m.p. 166℃.

3-Aminobutanamide hydrochloride: *N*-3-(Benzyloxycarbonylamino)butanamide (31.7 g, 134 mmol) was dissolved in *n*-butanol (200 mL) and water (200 mL). Pd/C (0.9 g, 10%) was added and the mixture was pressurized with hydrogen gas and heated for 1 h at 67℃. The Pd/C was removed by filtration over celite, and the celite washed with water (100 mL). The phases were separated, and the aqueous phase was concentrated under reduced pressure on a rotary evaporator. The oily residue was taken up in *i*-propanol and conc. HCl (13.5 g) was added. The solution was con-centrated again under reduced pressure and the resulting material was recrystallized from *i*-propanol to give an off-white material that was dried at 50℃ in vacuo (14.4 g, 104 mmol, 77.5%); ^{1}H NMR (400 MHz, [D$_6$]DMSO) δ 1.20 (d, *J*= 6.6 Hz, 3H), 2.38 (dd, *J*= 15.8, 7.3 Hz, 1H), 2.49-2.53 (dd, overlapping with DMSO, 1H), 3.41-3.47 (m, 1H), 7.1 (br s, 1H), 7.7 (br s, 1H), 8.1 (br s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 18.0, 38.7, 44.1, 171.4; IR (KBr), λ^{-1} (cm^{-1}): 3363, 3178, 2985, 1665, 1640, 1424, 1189, 623; m.p. 141℃.

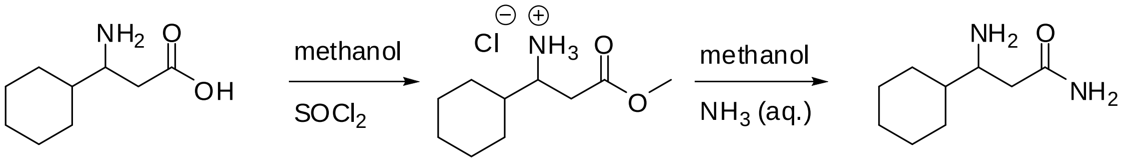
3-Aminohexanamide (*rac*-1b):



Methyl hex-2-enoate[2] (38.0 g, 297 mmol) was dissolved in methanol (250 mL). The mixture was filled into an autoclave and pressurized with 20 bar of NH$_3$ overnight at 60℃. After cooling to room temperature, all volatiles were removed from the now yellow solution under reduced pressure. The oil solidified and was taken up in a mixture of MTBE (200 mL) and ethanol (18 mL), to which *n*-hexane (100 mL) was added dropwise. While being stirred, the mixture was cooled to 0℃ in an ice-bath and crystals appeared. The off-white crystals were collected by filtration, washed with a small amount of cold *n*-hexane and finally dried *in vacuo* at room temperature to give the desired product (26.4 g, 203 mmol, c.y. 68.4%); ^{1}H NMR (400 MHz, [D$_6$]DMSO) δ 0.85 (t, *J*= 7.3 Hz, 3H), 1.16-1.40 (m, 4H), 1.95 (dd, *J*= 14.5, 8.6 Hz, 1H), 2.10 (dd, *J*=

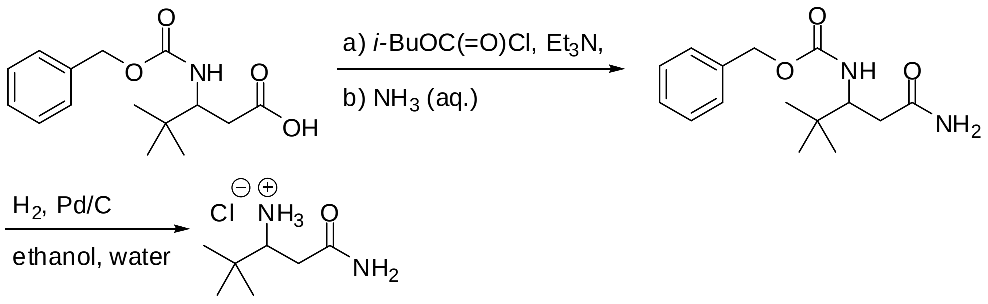
14.5, 4.7 Hz, 1H), 2.90-2.96 (m, 1H), 6.70 (br s, 1H), 7.35 (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 14.0, 18.6, 39.6, 43.6, 48.0, 173.5; IR (KBr), λ^{-1} (cm^{-1}): 3351, 2956, 1668, 1603, 1409, 728; m.p. 67℃.

3-Amino-3-cyclohexylpropanamide (rac-1c)



3-Amino-3-cyclohexylpropanoic acid (29.7 g, 173 mmol) was suspended in methanol (300 mL) and cooled to 5℃. Thionyl chloride (24.8 g, 208 mmol) was added dropwise to the suspension while keeping the temperature at 5℃ by cooling with ice. Stirring was continued for 1 h at 5℃ and 1 h at 40℃, after which all volatiles were removed under reduced pressure, yielding the intermediate amino acid ester as a white solid (37.9 g, 171 mmol). The solid was taken up in methanol (180 mL), added to aqueous ammonia (25% in water, 360 mL) and stirred for 24 h at room temperature. All methanol was removed under reduced pressure and the pH of the solution was set to 13. The resulting liquid was concentrated under reduced pressure, leaving a sticky solid after the removal of most of the water, to which toluene (250 mL) was added. The contents of the flask were stripped free of water, and then most of the remaining toluene was removed as well under reduced pressure leaving a white, sticky solid. The solid was taken up in hot MTBE (800 mL), filtered over celite, and the celite was washed with an additional amount of MTBE (300 mL). The combined MTBE solutions were collected and slowly cooled to 0℃. The precipitate was collected, washed with cold MTBE (200 mL) and dried at 60℃ under reduced pressure to give the product as a white solid (14.5 g, 85.2 mmol, c.y. 49.2%); ^{1}H NMR (500 MHz, [D$_6$]DMSO) δ 0.91-1.20 (m, 6H), 1.33 (br s, 2H), 1.59-1.71 (m, 5H), 1.90-1.94 (dd, J= 14.3, 9.4 Hz, 1H), 2.11-2.15 (dd, J= 14.3, 3.9 Hz, 1H), 2.74-2.78 (m, 1H), 6.69 (br s, 1H), 7.36 (br s, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 25.6, 25.7, 27.8, 28.0, 35.0, 39.9, 52.5, 172.3; IR (KBr), λ^{-1} (cm^{-1}): 3351, 2956, 1668, 1603, 1409, 729; m.p. 80℃.

3-Amino-4,4-dimethylpentanamide (*rac*-1d)



β^{3}-*Cbz-Neopentylglycinamide*: To a solution of *β^{3}-Cbz*-Neopentylglycine[3] (5.0, 17.9 mmol), toluene (50 mL) and triethylamine (1.82 g, 18.0 mmol) *iso*-butylchloroformiate (2.58 g, 18.0 mmol) was added at -3℃. The gel-like suspension was diluted with a small amount of toluene (30 mL), stirred for an additional 5 min and concentrated aqueous ammonia (6.8 mL, 25%) was added. After stirring for 30 min at 5-10℃, all volatiles were removed under reduced pressure giving a white solid that was taken up in MTBE (100 mL) and a saturated solution of NaHCO$_3$ (25 mL). The biphasic mixture was shortly heated up to reflux and then cooled to room temperature. The precipitated solid was collected by filtration and washed with MTBE (50 mL) and water (50 mL) to give the desired compound as a white solid (3.2 g, 11.5 mmol, c.y. 64.2%) after drying at 60℃ in vacuo. ^{1}H NMR (500 MHz, [D$_6$]DMSO) δ 0.83 (s, 9H), 2.05 (dd, *J*= 14.3, 10.3 Hz, 1H),), 2.26 (dd, *J*= 14.3, 3.5 Hz, 1H), 3.71-3.76 (m, 1H), 4.97 (d, *J*= 12.0 Hz, 1H), 5.03 (d, *J*= 12.0 Hz, 1H), 6.72 (br s, 1H), 6.96 (d, *J*= 9.5 Hz, 1H), 7.08 (br s, 1H), 7.29-7.36 (m, 5H); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 26.2, 35.0, 36.5, 56.4, 64.9, 127.4, 127.5, 128.2, 137.4, 156.0, 172.8; IR (KBr), λ^{-1} (cm^{-1}): 3464, 3320, 2964, 1693, 1656, 1542, 1282, 1052; m.p. 152°C.

3-Amino-4,4-dimethylpentanamide hydrochloride: *β^{3}-Cbz*-Neopentylglycinamide (18.1 g, 65.0 mmol) was suspended in water (100 mL) and Pd/C (0.46 g, 10%) was added. The whole was filled into an autoclave and hydrogenated for 4 h at 50℃. The catalyst was removed by filtration of celite and the resulting solution was concentrated to dryness to give a white solid (10.2 g). This material (9.4 g) was dissolved in a mixture of *i*-propanol (200 mL) and water (10.5 mL) to which were added slowly MTBE (265 mL) and concentrated HCl (7.0 g) at 5℃. Seeding crystals were added followed by additional MTBE (85 mL). The resulting suspension was stirred for 15 min at 0-5℃ and then the product was collected by filtration. After washing with MTBE : *i*-propanol (1:1, 100 mL), the material was dried *in vacuo* at 45℃ to give the desired amide as a white solid (10.0 g, 55.3, c.y. 85.1%); ^{1}H NMR (500 MHz, [D$_6$]-

DMSO) δ 0.94 (s, 9H), 2.26 (dd, J= 16.3, 8.3 Hz, 1H), 2.55 (dd, J= 16.3, 4.1, 1H), 3.23 (dd, J= 8.3, 4.1 Hz, 1H), 7.18 (br s, 1H), 7.69 (br s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO) δ 25.6, 33.1, 33.3, 56.5, 172.5; IR (KBr), λ^{-1} (cm^{-1}): 3388, 3079, 2073, 1681, 1617, 1527, 1426, 1381, 590; m.p. 183℃.

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