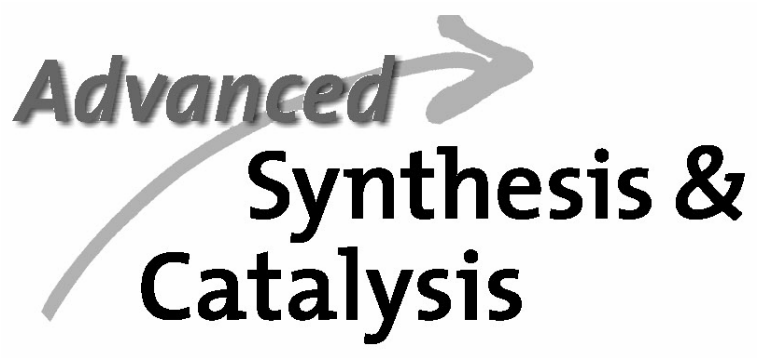

Supporting Information

One-Pot, Regioselective Synthesis of Substituted Arylglycines for Kinetic Resolution by Penicillin G Acylase

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Supporting Information

Materials

Penicillin G amidase and glutaryl acylase (purified soluble proteins from recombinant *E. coli*; both as suspensions in aqueous ammonium sulphate) were generous gifts from Roche Diagnostics (Mannheim, Germany) and used as received. All other reagents and solvents were from Merck (Darmstadt, Germany), Acros Organics (Gent, Belgium) or Carl Roth (Karlsruhe, Germany). Hydrolytic reactions on *N*-phenylacetyl-arylglycines were performed at room temperature using a Titroline Alpha automatic titrator (Schott) in pH-stat mode. HPLC analyses were performed using a Shimadzu LC-6 chromatograph equipped with a SPD-6A UV/vis detector. NMR-spectra were recorded on Bruker ARX-300, AC-300 and AVANCE 500 spectrometers. Mass spectra were determined on a Bruker Esquire LC spectrometer.

Phenylacetamide (4)

A mixture of benzylcyanide (82.00 g, 0.70 mol) and concentrated hydrochloric acid were heated to 50°C with continuous stirring for 40 min. The mixture was cooled to 4°C, ice (400 g) was added and stirring continued for 30 min. The precipated solid was collected, washed with cold water (2 x 500 mL) and dried under reduced pressure; yield 68.03 g (72%); mp 153°C; ^{1}H-NMR (300 MHz, D_2O): δ = 3.39 (2H, d, -CH_2), 6.94 (1H, s, -NH_2), 7.22 (5H, m, -CH_{ar}), 7.53 (1H, s, -NH_2); ^{13}C-NMR (75 MHz, D_2O): δ = 42.18 (-CH_2), 126.16 (-CH_p), 128.07 (-CH_m), 128.98 (-CH_o), 136.41 (-C_i), 172.21 (-$CONH_2$).

N-Phenylacetyl-DL-(4-hydroxyphenyl)-glycine (7a)

From phenol (**2a**, 47.00g 0.50 mol); yield 44.75 g (63%). ^{1}H-NMR (500 MHz, d_6-DMSO): δ = 3.56 (2H, s, 2'-C*H*$_2$), 5.67 (1H, d, -C*H*N), 6.95-7.35 (9H, m, -C*H*$_{ar}$), 8.41 (1H, d, -CON*H*), 9.81 (1H, bs, -O*H*); $J_{2,\text{-CONH}}$ = 7.5 Hz; ^{13}C-NMR (125.7 MHz, d_6-DMSO): d = 41.61 (2''-*C*H$_2$), 56.03 (-*C*HN), 111.65 (3'-*C*H), 115.25 (2'-*C*H), 126.25 (-*C*H$_m$''), 127.56 (-*C*H$_p$''), 128.19 (1'-*C*), 129.00 (-*C*H$_o$''), 136.30 (-*C*$_i$CH$_2$), 146.23 (4'-*C*), 169.81 (-*C*ONH), 172.26 (-*C*O$_2$H).

_N_-Phenylacetyl-DL-(2-thienyl)-glycine (7b)

From thiophene (**2b**, 40 mL, 0.50 mol); yield 26.57 g (39%). ^{1}H-NMR (300 MHz, d_6-DMSO): d = 3.56 (2H, s, 2''-C*H*$_2$), 5.58 (1H, d, 2-C*H*N), 7.02 (1H, m, 4'-C*H*), 7.10 (1H, dt, 3'-C*H*), 7.28 (5H, m, -C*H*$_{ar}$''), 7.50 (1H, dt, 5'-C*H*), 8.99 (1H, d, -CON*H*); $J_{2,\text{-CONH}}$ = 7.5, $J_{4',5'}$ = 5.1 Hz; ^{13}C-NMR (75.4 MHz, d_6-DMSO): d =41.45 (2''-*C*H$_2$), 51.75 (2-*C*HN), 126.03 (5'-*C*H), 126.31 (3'-*C*H), 126.60 (4'-*C*H), 126.77 (-*C*H$_p$), 128.10 (-*C*H$_o$), 129.00 (-*C*H$_m$), 136.07 (-*C*$_i$CH$_2$), 139.02 (1'-*C*), 169.95 (-*C*ONH), 170.99 (-*C*OOH).

_N_-Phenylacetyl-DL-(3,4-dimethoxyphenyl)-glycine (7c)

From 1,2 dimethoxybenzene (**2c**, 69.08 g, 0.50 mol); yield 71.05 g (86%). ^{1}H-NMR (300 MHz, d_6-DMSO): δ = 3.58 (2H, d, 2''-C*H*$_2$), 3.75 (6H, s, -OC*H*$_3$), 5.26 (1H, d, 2-C*H*N), 6.93 (5H, m, -C*H*$_{ar}$''), 7.27 (1H, s, 2'-C*H*), 7.29 (2H, s, 5'-C*H*, 6'-C*H*), 8.78 (1H, d, -CON*H*); $J_{2,\text{-CONH}}$ = 7.4, $J_{gem.\ 2''}$ = 1.9 Hz; ^{13}C-NMR (75.4 MHz, d_6-DMSO): δ = 41.64 (2''-*C*H$_2$), 55.45 (-OCH$_3$), 55.53 (-OCH$_3$), 55.95 (2-C*H*N), 111.20 (2'-*C*H), 111.68 (5'-*C*H), 119.90 (6'-*C*H), 126.28 (-*C*H$_p$), 128.11 (-*C*H$_o$), 129.01 (-*C*H$_m$), 129.27 (1'-*C*), 136.27 (-*C*$_i$CH$_2$), 148.55 (4'-*C*), 148.77 (3'-*C*), 169.88 (-*C*ONH), 172.12 (-*C*OOH); ESI-MS: m/z (%) = 352 (100) [M+Na$^+$], 328 (50) [M-H$^+$], 284 (100) [$C_{17}H_{18}NO_3^+$], 195 (4) [$C_{10}H_{11}O_4^+$], 91 (10) [$C_7H_7^+$].

_N_-Phenylacetyl-DL-(2,4-dimethoxyphenyl)-glycine (7d)

From 1,3-dimethoxybenzene (**2d**, 69.08 g 0.50 mol); yield 50.00 g (61%). R_f = 0.47 [EtOAc-MeOH-H$_2$O. 17:3:1]; ^{1}H-NMR (300 MHz, d_6-DMSO): d = 3.50 (2H, s, 2''-C*H*$_2$), 3.76 (6H, s, -OC*H*$_3$), 5.55 (1H, d, 2-C*H*N), 6.53 (2H, dd, 5'-CH), 6.58 (1H, d, 3'-CH), 7.18 (1H, d, 6'-C*H*), 7.26 (5H, m, -CH$_{ar}$), 8.50 (1H, d, -CON*H*); $J_{2,\text{-CONH}}$ =

7.4, $J_{gem.\ 2''}$ = 1.9 Hz; ^{13}C-NMR (75.4 MHz, d_6-DMSO): d = 41.63 (2''-*C*H_2), 50.10 (2-C*H*N), 55.26 (-O*C*H_3), 55.64 (-O*C*H_3), 104.80 (5'-*C*H), 117.67 (5'-*C*H), 126.25 (-*C*H_p''), 128.10 (-*C*H_o), 129.02 (-*C*H_m), 129.20 (-C_{ar}OCH$_3$), 136.35 (-C_{ar}OCH$_3$), 157.74 (4'-*C*), 160.42 (3'-*C*), 169.82 (-*C*ONH), 172.42 (-*C*OOH); ESI-MS: m/z (%) = 332 (6) [M^++3].

_N_-Phenylacetyl-DL-(2,3,4-trimethoxyphenyl)-glycine (7e)

From 1,2,3-trimethoxybenzene (**2e**, 16.80 g, 0.10 mol); yield 6.50 g (36 %). R_f = 0.81 [EtOH]; ^{1}H-NMR (300 MHz, d_6-DMSO): d = 3.51 (2H, s, 2''-C*H*$_2$), 3.75 (6H, s, -OC*H*$_3$), 3.79 (3H, s, -OC*H*$_3$), 5.55 (1H, d, -C*H*N), 6.82 (1H, d, -C*H*), 7.00 (1H, d, -C*H*), 7.26 (5H, m, -C*H*$_{ar}$''), 8.59 (1H, d, -CON*H*); $J_{2,\text{-CONH}}$ = 7.7, $J_{5',6'}$ = 8.7 Hz; ^{13}C-NMR (75.4 MHz, d_6-DMSO): d = 41.59 (2''-*C*H_2), 50.22 (2-*C*HN), 55.85 (3'-O*C*H_3), 60.24 (4'-O*C*H_3), 60.78 (2'-O*C*H_3), 107.83 (5'-*C*H), 122.69 (1'-*C*), 123.35 (6'-*C*H), 126.23 (-*C*H_p), 128.05 (-*C*H_o), 128.98 (-*C*H_m), 136.25 (-C_iCH$_2$), 141.57 (3'-*C*), 151.07 (4'-*C*), 153.32 (2'-*C*), 169.71 (-*C*ONH), 172.21 (-*C*OOH).

_N_-Phenylacetyl-DL-(4-hydroxy-5-isopropyl-2-methylphenyl)-glycine (7f)

From thymol (**2f**, 75.11 g, 0.50 mol); after washing, the remainder was additionally extracted with cyclohexane in a Dean-Stark apparatus for 2 d to remove unreacted **2f**; yield 62.43 g (73%). ^{1}H-NMR (500 MHz, d_6-DMSO): δ = 1.14 (6H, dd, -CHC*H*$_3$), 2.18 (3H, s, 2'-C*H*$_3$), 3.14 (1H, sept., -C*H*CH$_3$), 3.52 (2H, q, 2''-C*H*$_2$), 5.40 (1H, d, 2-C*H*N), 6.60 (1H, s, -C*H*$_{ar}$), 7.03 (1H, s, -C*H*$_{ar}$), 7.19 (1H, m, -C*H*$_p$), 7.27 (4H, m, -C*H*$_o$, -C*H*$_m$), 8.64 (1H, d, -CON*H*), 9.23 (1H, bs, -O*H*), 12.45 (-COO*H*); $J_{\text{-CH,-CH3}}$ = 6.9, $J_{gem.2''a,2''b}$ = 13.9, $J_{2,\text{-CONH}}$ = 7.6 Hz; ^{13}C-NMR (125.7 MHz, d_6-DMSO): δ = 18.90 (2'-*C*H$_3$), 22.82 (-CH*C*H$_3$), 22.88 (-CH*C*H$_3$), 26.67 (-*C*HCH$_3$), 42.00 (2''-*C*H$_2$), 52.98 (-*C*HN), 117.02 (3'-*C*H), 125.06 (-*C*H$_p$), 126.29 (1'-*C*), 126.60 (6'-*C*H), 128.45 (-*C*H$_o$), 129.34 (-*C*H$_m$), 132.04 (5'-*C*), 134.70 (-C_iCH$_2$), 136.81 (2'-*C*), 154.27 (4'-*C*OH), 170.10 (-*C*ONH), 173.08 (-*C*OOH).

_N_-Phenylacetyl-DL-(3-hydroxy-4-methoxyphenyl)-glycine (7g)

From 1-hydroxy-2-methoxybenzene (**2g**, 25.22 g, 0.20 mol); yield 10.27 g (32 %). ^{1}H-NMR (300 MHz, d_6-DMSO): d = 3.53 (2H, s, 2''-C*H*$_2$), 3.74 (3H, s, -OC*H*$_3$), 5.18

(1H, d, -C*H*N), 6.74 (2'C*H*), 6.80 (5'-C*H*), 6.94 (6'-C*H*), 7.23 (5H, m, -CH_{ar}"), 8.72 (1H, d, -CON*H*), 9.08 (1H, s, 3'-CO*H*); $J_{2,\text{-CONH}}$ = 7.3, $J_{5',6'}$ = 8.1 Hz; ^{13}C-NMR (75.4 MHz, d_6-DMSO): d = 41.59 (2"-*C*H_2), 55.55 (-O*C*H_3), 56.02 (-*C*HN), 111.65 (5'-*C*H), 115.25 (2'-*C*H), 120.13 (6'-*C*H), 126.25 (-*C*H_m"), 127.56 (-*C*H_p"), 128.09 (1'-*C*), 129.00 (-*C*H_o"), 136.30 (-*C*$_i$CH$_2$), 146.38 (4'-*C*), 147.42 (3'-*C*), 169.83 (-*C*ONH), 172.23 (-*C*O_2H); ESI-MS: m/z = 338 [M+Na^+], 314 [M-H^+].

_N_-Phenylacetyl-DL-1-(2-hydroxynaphthyl)-glycine (7h)

From 2-naphthol (**2h**, 28.50 g, 0.25 mol); yield 37.01 g (88%). R_f = 0.94 [EtOAc-MeOH-H_2O. 17:3:1]; ^{1}H-NMR (500 MHz, d_6-DMSO): δ = 3.52 (2''-CH_2), 5.84 (2-C*H*N), 7.18-7.53 (9H, m, -CH_{ar}), 7.77 (1H, d, 4'-C*H*), 7.98 (2H, d, 5'-C*H*, 8'-C*H*), 9.52 (1H, d, -CON*H*); $J_{2,\text{-CONH}}$ = 7.3 Hz; ^{13}C-NMR (125.7 MHz, d_6-DMSO): δ = 42.24 (2''-CH_2), 51.52 (2-*C*HN), 111.40 (3'-*C*H), 118.06 (1'-*C*), 123.05 (5'-*C*H), 125.38 (7'-CH), 127.25 (-*C*H_p), 128.13 (7'-*C*H), 128.95 (-*C*H_o), 129.40 (4b'-*C*), 129.66 (-*C*H_m), 129.97 (4'-*C*H), 131.04 (8'-*C*H), 131.08 (4a'-*C*), 135.28 (-*C*$_i$CH$_2$), 151.25 (2'-*C*OH), 170.19 (-*C*ONH), 173.71 (-*C*OOH); ESI-MS: m/z (%) = 223 (100) [M^+-112], 334 (6) [M-H^+].

Glutaric acid monoamide (5)

Under continuous stirring Et_2O was cooled to -60°C and a gentle stream of gaseous NH_3 was fed into the liquid for 30 min. Glutaric anhydride (57.10 g, 0.50 mol) was added in small portions while NH_3 feeding was continued for 30 min and the temperature kept at -60°C. The mixture was allowed to warm to room temperature and the precipitated **5** was collected by filtration. The colourless solid was dried under reduced pressure; yield 65.29 g (99%). ^{1}H-NMR (300 MHz, d_6-DMSO): δ = 1.64 (2H, quin., 3'-CH_2), 2.05 (4H, m, 2-CH_2, 4-CH_2), 7.09 (1H, s, -CON*H*), 7.27 (1H, s, -CON*H*); $J_{2,3}$ = 7.4, $J_{3,4}$ = 7.4 Hz; ^{13}C-NMR (75.4 MHz, d_6-DMSO): δ = 21.46 (3'-CH_2), 34.74 (2-CH_2), 35.35 (4-CH_2), 175.57 (-*C*ONH_2), 175.78 (-*C*OOH); ESI-MS: m/z = 131 [M^+].

_N_-Glutaryl-(3',4'-dimethoxyphenyl)-glycine (8c)

A 50% aqueous solution of glyoxylic acid (**3**, 37.00 g, 0.25 mol) was concentrated to 80% acid content under reduced pressure. The syrup was dissolved in acetic acid (60 mL) and glutaric monoamide (**5**, 32.75 g, 0.25 mol) was added to the solution. The mixture was kept at 50°C for 5 h, then cooled to 5°C and 1,2-dimethoxybenzene (**2c**, 69.08 g, 0.50 mol) was added. A gentle stream of hydrogen chloride was fed into the solution for 1 h while keeping the temperature below 10°C. The mixture was kept at 5°C for 16 h, then the volatile compounds were removed under reduced pressure. The remainder was thoroughly washed with water and Et_2O and dried under vacuum; yield of **8c** 7.40 g (9%). ^{1}H-NMR (300 MHz, d_6-DMSO): δ = 1.72 (2H, quin., 3'-C*H*$_2$), 2.22 (4H, m, 2-C*H*$_2$, 4-C*H*$_2$), 3.75 (3H, s, -OC*H*$_3$), 3.76 (3H, s, -OC*H*$_3$), 5.24 (1H, d, -C*H*N), 6.94 (2H, s, -C*H*$_{ar}$), 6.99 (1H, s, -C*H*$_{ar}$) 8.51 (1H, s, -CON*H*); $J_{\text{-CHN,-CONH}}$ = 7.4 Hz; ^{13}C-NMR (75.4 MHz, d_6-DMSO): δ = 20.58 (3'-CH_2), 32.84 (2'-CH_2), 33.84 (4'-CH_2), 55.42 (-OCH_3), 55.47 (-OCH_3), 55.86 (-*C*HN), 111.29 (-*C*H$_{ar}$), 111.59 (-*C*H$_{ar}$), 119.96 (-*C*H$_{ar}$), 129.21 (-*C*$_{ars}$), 148.49 (-*C*OCH_3), 148.51 (-*C*OCH_3), 171.56 (-*C*ONH), 172.20 (-*C*OOH), 174.15 (-*C*OOH).