G 2319-M1

Supplementary Material

Methyl 4-(4-(1-hydroxyiminoethyl)-2-methoxyphenoxy)butanoate (3). A slurry of acetovanillone (41.00 g, 246.7 mmol), methyl 4-bromobutyrate (49.63 g, 274.1 mmol), and K_2CO_3 (51.1 g, 370 mmol) in 200 mL of DMF was stirred at room temperature for 16 hours. Water was added to the reaction mixture until all the K_2CO_3 was dissolved and the solution was then partitioned between EtOAc and sat. NaCl. The organic phase was dried ($MgSO_4$), filtered and evaporated to dryness to afford 67.90 g (100% crude yield) of intermediate keto-ester as a colorless oil which slowly solidified: mp 48-49 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (pentet, J = 7.3 Hz, 2 H), 2.56 (t, J = 7.3 Hz, 2 H), 2.56 (s, 3 H), 3.70 (s, 3 H), 3.91 (s, 3 H), 4.15 (t, J = 7.3 Hz, 2 H), 6.89 (d, J = 8.4 Hz, 1 H), 7.51-7.78 (m, 2 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.2, 26.1, 30.2, 51.5, 55.9, 67.6, 110.4, 111.2, 123.1, 130.4, 149.2, 152.5, 173.3, 196.7; MS (EI) *m/z* 266 (M^+). Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 62.81; H, 6.83.

To a solution of the keto-ester (68.4 g) in 225 mL of 2:1 pyridine:H_2O was added hydroxylamine hydrochloride (21.46 g, 309 mmol). After stirring at room temperature for 14 h the reaction mixture was partitioned between EtOAc and sat. NaCl. The organic phase was dried ($MgSO_4$), filtered and evaporated to dryness to afford oxime 3 (69.94 g, 100% crude yield) as a white solid: mp 82-83 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (pentet, J = 8.0 Hz, 2 H), 2.26 (s, 3 H), 2.55 (t, J = 8.0 Hz, 2 H), 3.68 (s, 3 H), 3.88 (s, 3 H), 4.09 (t, J = 8.0 Hz, 2 H), 6.87 (d, J = 8.8 Hz, 1 H), 7.12 (dd, J = 2.1, 8.8 Hz, 1 H), 7.25 (d, J = 2.1 Hz, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.9, 24.4, 30.4, 51.6, 55.9, 67.8, 109.0, 112.4, 119.1, 129.5, 149.3, 149.4, 155.6, 173.6; MS (APCI) *m/z* 282 (MH^+). Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98. Found: C, 59.62; H, 6.75; N, 4.81.

Methyl 4-(2-methoxy-4-(1-trifluoroacetamidoethyl)phenoxy)butanoate (4). A slurry of the oxime 3 (146.7 mmol) and 10% palladium on charcoal (2.5 g) in 400 mL of glacial acetic acid was degassed twice by placing the flask under reduced pressure (aspirator) and subsequently filling the flask with H_2. The reaction mixture was placed under 1.1 atmosphere of H_2 via a balloon and vigorously stirred at room temperature. An additional 2 g of catalyst was added after 18 h and the balloon was refilled with hydrogen as the gas was consumed. An additional 2 g of catalyst was added after 2 days. The reaction mixture was filtered after 5 days and the solvent was removed under vacuum. The oily residue was taken up in 600 mL of water and acidified to pH 1 with 6 N HCl. The aqueous phase was extracted with Et_2O (washings discarded) and the aqueous phase was basified with solid NaOH to pH 11 and was extracted with EtOAc. The EtOAc phase was dried ($MgSO_4$), filtered and evaporated to dryness to afford the intermediate amine as a colorless oil.

The crude amine was taken up in 300 mL of pyridine, cooled to 0 °C with an ice bath and was treated with trifluoroacetic anhydride (31 mL, 219 mmol) for 1 h. The reaction mixture was worked up by partitioning between EtOAc and sat. NaCl. The organic phase was dried ($MgSO_4$), filtered and evaporated to give the crude trifluoroacetate as a light yellow solid. The solid was recrystalized from CH_2Cl_2/hexanes to afford trifluoroacetate 4 (71.62 g, 80% overall yield from acetovanillone) as a white solid: mp 96-97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.58 (d, J = 7.5 Hz, 3 H), 2.15 (pentet, J = 7.1 Hz, 2 H), 2.54 (t, J = 7.1 Hz, 2 H), 3.68 (s, 3 H), 3.86 (s, 3 H), 4.05 (t, J = 7.1 Hz, 2 H), 5.09, (dq, J = 7.5, 7.5 Hz, 1 H), 6.38 (br d, J = 7.5 Hz, 1 H), 6.80-6.90 (m, 3 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.8, 24.4, 30.4, 49.5, 51.6, 56.0, 67.9, 110.4, 113.4, 118.2, 133.7, 148.2, 149.7, 173.6, (the resonances for N$\underline{C}$OCF$_3$ and NCO$\underline{C}$F$_3$ were not observed); MS (APCI) *m/z* 364 (MH^+), 251

$(MH - H_2NCOCF_3)^+$. Anal. Calcd for $C_{16}H_{20}F_3NO_5$: C, 52.89; H, 5.55; N, 3.86. Found: C, 52.76; H, 5.45; N, 3.59.

Methyl 4-(2-methoxy-5-nitro-4-(1-trifluoroacetamidoethyl)phenoxy)-butanoate (5). Trifluoroacetate **4** (9.40 g, 25.9 mmol) was slowly added to 200 mL of 70% HNO_3 cooled to 0 °C. The solution turned orange in color and was quenched after 2 hours by pouring into water and adjusting the total volume to 2 L. The resultant slurry was chilled to 4 °C overnight and filtered to give a light yellow solid. The solid was washed with water and recrystalized from $MeOH/H_2O$ to afford the nitrated acetate **5** (9.07 g, 86% yield) as a light yellow solid: mp 156-157 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.62 (d, J = 7.9 Hz, 3 H), 2.18 (pentet, J = 7.5 Hz, 2 H), 3.70 (s, 3 H), 3.94 (s, 3 H), 4.12 (t, J = 7.5 Hz, 2 H), 5.50 (dq, J = 7.9, 7.9 Hz, 1 H), 6.87 (s, 1 H), 7.37 (br d, J = 7.9 Hz, 1 H), 7.61 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.1, 24.2, 30.3, 48.4, 51.7, 56.4, 68.2, 110.2, 111.0, 130.9, 140.5, 147.6, 154.0, 156.6, 173.3, the resonance for $NCOCF_3$ was not observed; MS (APCI) *m/z* 409 (MH^+). Anal. Calcd for $C_{16}H_{19}F_3N_2O_7 \bullet 0.05\ H_2O$: C, 46.96; H, 4.70; N, 6.85. Found: C, 46.59; H, 4.78; N, 6.89.

4-(4-(1-(9-Fluorenylmethoxycarbonylamino)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (7). To a solution of **5** (12.36 g, 30.27 mmol) in 250 mL of warm MeOH was added 1 N NaOH (100 mL, 100 mmol) and the resultant solution was heated to reflux for 5 hours. The solution was cooled to room temperature and concentrated to approximately 100 mL with a rotary evaporator. p-Dioxane (150 mL) and H_2O (100 mL) were added and the pH of the solution adjusted to pH 9 with 6 N HCl. A solution of Fmoc-Cl (9.83 g, 38.0 mmol) in 100 mL of dioxane was added and an additional 25 mL of dioxane was added to create a homogeneous solution. The pH of the solution was adjusted with 1N NaOH to pH 8 over the next 30 minutes and a light yellow precipitate formed during this time. The reaction was quenched after 18 h by adding 100

mL of 1 N HCl and adjusting the total volume to 1 L with H_2O. The precipitate was collected, taken up in 1 L of hot EtOAc, dried over $MgSO_4$ and was filtered while hot. The solvent was removed under reduced pressure affording a light yellow solid which was triturated with 1 L of hot Et_2O. The solid was collected and was recrystalized from MeOH to afford the Fmoc linker 7 (12.78 g, 81% yield for two steps) as a light yellow solid: mp 200-201 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.43 (d, J = 7.4 Hz, 3 H), 1.95 (pentet, J = 7.3 Hz, 2 H), 2.39 (t, J = 7.3 Hz, 2 H), 3.87 (s, 3 H), 4.05 (t, J = 7.3 Hz, 2 H), 4.16 (t, J = 6.9 Hz, 1 H), 4.27 (m, 2 H), 5.19 (dq, J = 7.4 Hz, 1 H), 7.25 (s, 1 H), 7.29 (t, J = 7.3 Hz, 2 H), 7.39 (t, J = 7.3 Hz, 2 H), 7.48 (s, 1 H), 7.64 (d, J = 8.3 Hz, 2 H), 7.87 (d, J = 8.3 Hz, 2 H), 8.06 (d, J = 8.3 Hz, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 21.9, 24.0, 29.9, 46.0, 46.7, 56.2, 65.2, 67.9, 108.2, 109.4, 120.1, 125.0, 126.9, 127.6, 135.5, 139.9, 140.7, 143.6, 143.9, 146.3, 153.4, 155.3, 174.0; MS (APCI) *m/z* 551 (MH^+), 282, 179. Anal. Calcd for $C_{28}H_{28}N_2O_8 \cdot 0.3\ H_2O$: C, 63.94; H, 5.48; N, 5.33. Found: C, 63.57; H, 5.44; N, 5.54.

4-Thiazolidinone-Photolinker-TentaGel. Commercially available H_2N-S-TentaGel (Rapp Polymere, Tübingen, Germany) (1 g, 0.30 mmol/g loading) was washed with DMF and treated with 3 mL of a 0.15 M solution of OBt-activated Fmoc-photolinker 7 (prepared from 310 mg of 7, 92 mg of HOBt, 95 μL of DIC in 3 mL of DMF) for 1.5 hour. Ninhydrin test indicated a complete reaction had taken place. The resin was washed with DMF and CH_2Cl_2, and was then capped with 20% Ac_2O, 30% pyridine, 50% CH_2Cl_2 for 30 minutes. The resin was washed (3 x 5 mL DMF, 3 x 5 mL CH_2Cl_2), and dried under vacuum for 1 hour. A portion of the resin (200 mg) was deprotected with 30% piperidine/DMF for 30 minutes and then washed with DMF. A 0.5 M solution of Fmoc-Glycine symmetrical anhydride (prepared from 182 mg of Fmoc-Gly-OH and 50 μL of DIC in 0.6 mL of DMF) was coupled to the resin for 1 hour, by

which time ninhydrin had revealed that a complete reaction had taken place. The resin was washed and capped as above for 30 minutes. Deprotection with piperidine, washing and drying as above gave roughly 150 mg of dry resin. A portion of the resin (40 mg) was transferred into a 4-mL vial and acetonitrile (2 mL), 3 Å molecular sieves (20-30 pellets), benzaldehyde (152 μL), and mercaptoacetic acid (300 μL) were added and the vial was heated to 70 °C for 2 hours. The vial was cooled to room temperature and the resin was transferred to a disposable filter tube and washed extensively (3 x 5 mL CH_2Cl_2, 3 x 5 mL DMF, 3 x 5 mL CH_2Cl_2, 3 x 5 mL MeOH, 3 x 5 mL CH_2Cl_2, 3 x 5 mL Et_2O).

General photolysis conditions. The resin (10-20 mg) was suspended in 100 μL of pH 7.4 PBS buffer containing 5% of DMSO. Photolysis were conducted by irradiated the samples with a 500 W Hg ARC lamp fitted with a 350-450 nm dichroic mirror at a 10 mW/cm^2 power level measured at 365 nm. The samples were irradiated from above for 3 h with gentle mixing from an orbital shaker table. After photolysis the supernatant was analyzed by reverse-phase HPLC and the amount of product calculated by area based on standards of authentic material.

Stability towards TFA treatment. A support containing an internal standard was prepared from TentaGel and Fmoc-Gly-OH labeled at the α-carbon with ^{13}C (2-^{13}C, 99% from Cambridge Isotope Laboratories, Inc., Andover, MA), followed by Fmoc-linker **7** under standard conditions. A 4-thiazolidinone was assembled on the resin after deprotection from glycine, benzaldehyde labeled at the carbonyl (carbonyl-^{13}C, 99%), and mercaptoacetic acid in analogy to the above procedures. A portion of the resin was treated with 95% TFA/5% H_2O for 1 h, followed by washing with CH_2Cl_2, MeOH, and Et_2O. In a separate experiment, an additional 20 mg of resin was treated for 2 h at room temperature with a solution of phenol (75 mg), thioanisole (50 μL), water (50 μL),

ethanedithiol (25 μL), and TFA (1 mL) followed by washing as above. Gel ^{13}C NMR analysis of the resin indicated no loss of thiazolidinone or photolabile linker, as evidenced by the relative integration of the two labeled carbons.

H-Met-Gly-Trp-Met-Asp-Phe-resin. The peptide was prepared on TentaGel resin (25 mg) bearing the photolabile linker **7** according to the synthesis cycle described below. All amino acids were N-Fmoc protected. The side chain functionalities of Asp and Trp were protected as the *tert*-butyl ester and Boc carbamate, respectively.

Coupling. To a 0.11 M solution of amino acid in DMF (0.50 mL) were added 0.20 M HATU in DMF (0.25 mL) and 0.60 M N,N-diisopropylethylamine in DMF (0.25 mL). The resulting solution was added to TentaGel bearing an unprotected amine, and the suspension stirred at room temperature. After 20 minutes the supernatant was decanted and the resin washed successively with DMF and THF.

Capping. To the resin was added a commercially prepared solution of N-methylimidazole (16%) in THF (0.50 mL) followed by a commercially prepared solution of acetic anhydride (10%) and 2,6-lutidine (10%) in THF (0.50 mL). The resulting suspension was stirred 5 minutes at room temperature. The supernatant was then decanted and the resin washed successively with THF and DMF.

Amine deprotection. 20% piperidine in DMF (1.00 mL) was added to the resin and the resulting suspension was stirred 10 minutes at room temperature. The supernatant was then decanted and the resin washed with DMF.

Following Fmoc removal from the final methionine residue, the resin was washed thoroughly with THF and the solvent evaporated. Side chain protection was removed by treating the resin with 1000:75:50:50:25 TFA/phenol/water/thioanisole/ethanedithiol (0.40 mL) and allowing the

resulting suspension to stand 1 hour at room temperature with occasional agitation.

Photolytic release of H-Met-Gly-Trp-Met-Asp-Phe-NH_2. 50 beads bearing the fully-deprotected peptide were placed in one well of a 96-well polystyrene microtiter plate and covered with 75 µl of a 1:1 solution of DMSO and PBS containing 0.1% hydrazine. A glass slide was fixed on the top of the plate, which was then irradiated for one hour. After irradiation the supernatant was decanted from the beads and analyzed directly by reverse phase HPLC. The product peptide, which co-eluted with authentic material, was obtained in 70% yield. MS (ESI) *m/z* 786 (MH^+).