Supporting Information

Single Step Formation of Structurally Defined Bicyclic Peptides Via S_NAr Cyclization

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Peptide Synthesis

Protected Fmoc-amino acid derivatives were purchased from GlycoPep Chemicals Inc. (Chicago, IL), Novabiochem (San Diego, CA), Bachem Bioscience (Philadelphia, PA) or PerSeptive Biosystems (Foster City, CA). Side chain protecting groups were: tBu (Tyr); trityl (Gln); Pbf (Arg); and tBoc (Lys, Trp). Fmoc-AM RAM amide resin (0.65-0.76 mmol/g) was obtained from Rapp Polymere (Tübingen, Germany). DMF and other solvents were obtained from EM Science (Gibbstown, NJ). Peptide synthesis was carried out at a 0.2 mmol scale on a Rainin Symphony multiple peptide synthesizer (Protein Technologies Inc., Tucson, AZ) using standard 9-fluorenylmethoxycarbonyl (Fmoc) protocols. Specifically, Fmoc deprotection was carried out with two 10 min treatments of 20 % piperidine/DMF, and couplings were allowed to go for 1½ hr. Amino acids (1.1 mmol, 5 fold excess) were activated *in situ* with equal amounts of N,N-diisopropylcarbodiimide (DIC) (Aldrich) and 1-hydroxybenzotriazole (HOBt) (Novabiochem). 3,5-dihydroxybenzoic acid (20-fold excess) was preactivated with DIC in a 2:1 ratio in DMF for 10 min to form a 10-fold excess of symmetric anhydride and then added manually to the N-terminal deprotected peptide-resin. Removal of Allyloxycarbonyl (Aloc) protecting groups from Lys side chains was carried out in a mixture of anhydrous chloroform/piperidine/acetic acid 92:5:3. The solvent mixture was first sparged with argon and 3 equivalents of $(Ph_3P)_4Pd(0)$ was then added. The solution was then added to the peptide resin (0.05 mmol, based on initial substitution level) in a 12 mL polypropylene plastic syringe fitted with porous frit and capped under argon. The deprotection was generally complete in 1.5 hr. The resin was then washed with DMF followed by a mixture of 5% diisopropylethylamine (DIEA), 5% (w/v) sodium diethyldithiocarbamate /DMF. The diethyldithiocarbamate ion acts as a chelating agent to remove Pd containing compounds which adsorb onto the resin. The presence of free amino groups was confirmed by the use of the Kaiser ninhydrin test. Acylation with 2-fluoro-5-nitro benzoic acid (Aldrich) was carried out as for the 3,5-dihydroxybenzoic acid (10 min preactivation with DIC in a 2:1 ratio in DMF). A 10-fold excess of symmetric anhydride per free amino group (two free amino groups per peptide) was prepared and allowed to couple for 1 hour. Cyclization was carried out by addition of the peptide resin to a solution of 95:5 DMF:1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (Aldrich) for 4-5 hr at room temperature. Alternatively, the cyclization was carried out in 0.02 M K_2CO_3/DMF. Reduction of nitro groups was carried out with 5 mL of a solution of 2 M $SnCl_2 \cdot 2H_2O$/DMF at room temperature for 4-5 hr, during which time, the orange color of the resin was greatly diminished. Acetylation was done with a 25;5:70 mixture of Ac_2O/DIEA/ DCM for 1 hr. Peptides were cleaved from the solid support (~20 mg peptide-resin was

cleaved) with concomitant removal of acid-labile side chain protecting groups by treatment at room temperature for 2 hr with 2.5 mL TFA/ anisole/H_2O/iPr_3SiH 91:3:3:3. The TFA solution was then filtered away from the resin and concentrated in a Genevac HT-4 evaporator (GeneVac Ltd. West Nyack, NY). The peptides were then precipitated with cold diethyl ether and collected by centrifugation and decanting of the ether.

Synthesis of Fmoc-Lys(3,5-dihydroxybenzoyl)-OH

10 g Fmoc-Lys(tBoc)-OH was treated with 50 mL of 48.5:48.5:1 TFA:DCM:anisole for 1 hr at room temperature. The mixture was concentrated on a rotary evaporator and the product Fmoc-Lys-OH was precipitated with hexane and dried under vacuum. 2 g Fmoc-Lys-OH (5.44 mmol) was dissolved in 60 mL of 2:1 dioxane:water. Triethylamine (about 0.5 mL) was added to bring the pH to ~ 8.5. Subsequently, 2.2 equivalents of 3,5-dihydroxybenzoic acid was combined with 1.1 equivalents of DIC in 10 mL acetonitrile (3,5-dihydroxybenzoic acid is insoluble in dioxane) and allowed to preactivate for 10 min then added to the Fmoc-Lys-OH solution and allowed to react at room temperature for 30 min. The solution was concentrated to about 25 mL on a rotory evaporator and the pH was adjusted to 2 with 1 M HCl. The product precipitated and was extracted with ethyl acetate (2 x 50 mL), dried over $MgSO_4$ and concentrated to dryness. The product was purified by flash chromatography on a Biotage system with a hexane: ethyl acetate: HOAc 85:14.5:0.5 mobile phase, and 1.2 g purified product (44 % yield) was obtained in > 97 % purity as judged by reversed-phase HPLC.

HPLC and Mass Spec Analysis

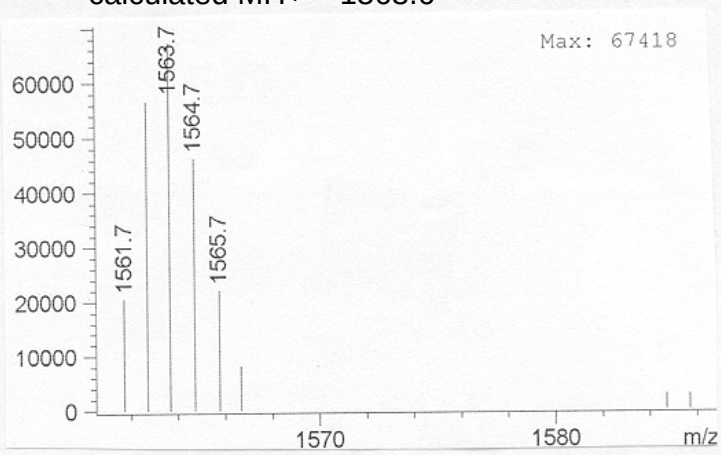
Analytical HPLC was performed on an HP 1100 system (Agilent Technologies Palo Alto, CA) with a flow rate of 1 mL/min in all cases. For reversed phase HPLC runs, a Zorbax SB-C18 4.6 mm i.d. x 15 cm column with 5 μm particle size and 80 Å pore size was used. The mobile phase was an AB linear gradient of 10 to 100% B over 15 min followed by 100% B for an additional 3 min, where A = 0.05% TFA/H_2O and B = 0.05% TFA in 60:40 CH_3CN:H_2O. Size-exclusion HPLC was carried out on a TSKgel G2000 SWXL 7.8 mm i.d. x 30 cm column with a mobile phase of 60:40 CH_3CN:H_2O containing 0.05 % TFA. LC-MS was run on an HP 1100 HPLC system equipped with an HP Series 1100 MSD single quadrupole mass spectrometer operating in positive mode with electrospray ionization. The column and gradient program were the same as listed above. Reversed-phase HPLC of Fmoc-Lys(3,5-dihydroxybenzoyl)-OH was carried out on the same Zorbax SB-C18 column listed above. The program employed was an AB gradient from 25% to 100% B over 25 min where A = 0.05% TFA/H_2O and B = 0.05% TFA/CH_3CN.

Mass Spectral Data

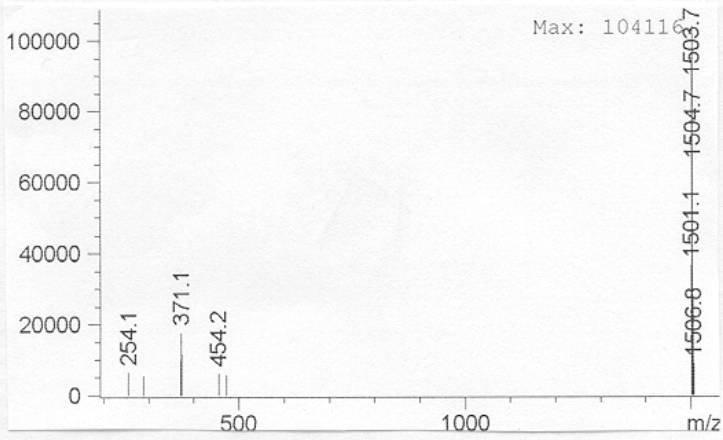
Compound	Calculated MH^+	Observed MH^+
1a	1503.75	1503.6
1b	1566.80	1566.3
1c	1503.75	1503.4
1d	1453.69	1453.3
1e	1484.71	1484.3
1f	1484.71	1484.8
1g	1453.69	1453.7
1h	1503.75	1503.9
1i	1503.75	1503.8
2a	1453.69	1453.6
2b	1566.80	1566.8

Sample Mass Spectra for peptides shown in Figure 2

(1) analog 1a (bis-nitro compound)
calculated MH+ = 1563.6



(2) analog 1a (bis-aniline compound)
calculated MH+ = 1503.6



(3) analog 1a (bis-acetyl compound)
calculated MH+ = 1587.7