Experimental Procedures

Materials

2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and Boc–amino acids were obtained from Peptides International, Kentucky. 2-(7-Aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) was purchased from GL Biochem (Shanghai). Bis(trichloromethyl) carbonate (BTC) was obtained from Fluka. Boc-Leu-OCH2Pam-copoly(styrene-1%m-divinylbenzene) resin and N,N-diisopropylethylamine (DIEA) were obtained from Applied Biosystems, Foster City. N,N-Dimethylformamide (DMF), dichloromethane, methanol, tetrahydrofuran (THF), diethyl ether, ethyl acetate (EtOAc), chloroform, and HPLC-grade acetonitrile were purchased from Fisher. Trifluoroacetic acid (TFA) was obtained from Halocarbon Products, New Jersey. HF was purchased from Matheson. All other reagents were obtained from Sigma-Aldrich except phthalimide potassium salt, which was from Fluka, and bromoacetic acid, which was purchased from Acros.

N(2-Nb)alanine

N(2-Nb)alanine was synthesized according to the procedure described by Tatsu et al. Briefly, a solution of 2-nitrobenzaldehyde (3.32 g, 0.022 mol) in 10 ml MeOH was added to a solution of alanine (1.782 g, 0.02 mol) in 5 ml MeOH and 5 ml 2M NaOH, and stirred at room temperature for 5 hrs. NaBH₄ (600 mg, 0.063 mol) was added at 0°C and allowed to react for 2 hours. The volume was reduced and the solution washed with cold diethyl ether, the aqueous phase adjusted to pH 4 with HCl, and the solution washed again with diethyl ether. The amino acid was then recrystallized from water.

N(2-Nb)leucine and N(2-Nb)valine

The same procedure was used as for N(2-Nb)alanine.

N(2-Nb)alanine, N(2-Nb)leucine, and N(2-Nb)valine were not Boc-protected because reaction of the N(2-Nb)amino acid was extremely slow with itself (i.e., the HOBT-activated N(2-Nb)amino acid) during peptide synthesis, and did not give rise to significant side reactions.

N(2-Nb)glycine

N(2-Nb)glycine was synthesized on-resin using the submonomer method of Zuckermann et al. Briefly, bromoacetic acid (445 mg, 3.2 mmol) was activated with diisopropylcarbonyldimide (252 µL, 1.6 mmol) in DCM for 10 min, diluted 1:1 with DMF, added to the neutralized resin (0.1 mmol), and allowed to react for 10 minutes. After washing with DMF, 2-nitrobenzylamine (755 mg, 4 mmol) in 3 mL DMF and 775 µL (4.4 mmol) diisopropylethylamine were added to the resin and allowed to react overnight.
N(4-methoxy-2-Nb)glycine

N(4-methoxy-2-Nb)glycine was prepared by the submonomer method as for N(2-Nb)glycine, using 4-methoxy-2-nitrobenzylamine. 4-methoxy-2-nitrobenzylamine was prepared from 4-methoxy-2-nitrobenzonitrile using a procedure from Meanwell et al. 4-methoxy-2-nitrobenzonitrile (1.78 g, 10 mmol) was dissolved in 12 ml of dry THF and 20 ml of BH$_3$-THF complex was added slowly under argon. After 19 hrs, 24 ml of 10% HCl was slowly added and the solution was refluxed for 30 minutes. The solution was then filtered, and 6 ml of NH$_4$OH (30% NH$_3$ in water) was added. The solution was extracted with ethyl ether (2 x 50 ml), the extracts washed with water (2 x 50 ml), dried over potassium carbonate, and concentrated to an oil.

N(4,5-dimethoxy-2-Nb)glycine

N(4,5-dimethoxy-2-Nb)glycine was prepared by the submonomer method as for N(2-Nb)glycine, using 4,5-dimethoxy-2-nitrobenzylamine. 4,5-dimethoxy-2-nitrobenzylamine was prepared from 4,5-dimethoxy-2-nitrobenzyl bromide using the method described in Rajagopalan et al. To a solution of potassium phthalimide (338.23 mg, 1.83 mmol) in 7 mL DMF was added 4,5-dimethoxy-2-nitrobenzyl bromide (495.2 mg, 1.79 mmol), and the solution stirred for 18 hours at room temp. Chloroform (10 ml) was added, and the solution poured into water. The aqueous layer was separated and extracted with chloroform, and the combined chloroform layers were washed with 0.2 M NaOH solution, water, and brine, then dried over MgSO$_4$. The product was concentrated, and the precipitate triturated with diethyl ether and allowed to dry. To the 4,5-dimethoxy-2-nitrobenzyl phthalimide (568 mg, 1.66 mmol) in 10 ml MeOH was added 120 µL hydrazine hydrate (2.49 mmol), and the solution refluxed for 15 hrs. After cooling, the solution was acidified with 5 M HCl and extracted with EtOAc, and the combined EtOAc layers were washed with 10 ml 5 M HCl. The combined aqueous layers were filtered, adjusted to pH 9 with 6 M NaOH, and extracted with chloroform. The combined chloroform layers were washed with brine, dried over NaSO$_4$, and concentrated to yield a dark yellow oil.

Peptide Synthesis

Peptides were synthesized on a Leu-OCH$_2$-Pam-resin using the manual Boc chemistry in situ neutralization/HBTU protocol described previously, except for coupling to the N-substituted amino acids. Formation of HOAt active esters using DIC and HATU was performed as described by Angell et al., except using 4 equivalents of amino acid and activating reagent. Boc-protected amino acid (0.4 mmol) was dissolved in DMF containing HOAt (0.4 mmol), DIC (0.4 mmol) was added and allowed to react for 10 minutes, then DIEA (0.4 mmol) added and the solution added to the resin. Alternatively, HATU (0.4 mmol) was dissolved in DMF containing Boc-protected amino acid (0.4 mmol), DIEA (0.5 mmol) was added for 1 minute to activate, and the solution added to the resin. Amino acid symmetric anhydrides were formed by reacting Boc-protected amino acid (0.8 mmol) with DIC (0.4 mmol) in DCM for 10 minutes, removing the DCM under a stream of nitrogen, and redissolving the anhydride in a minimal volume of DMF. Amino acid chlorides were prepared in situ using the methods described by Falb et al. BTC (0.132 mmol) and Boc-protected amino acid (0.4 mmol) were dissolved in 1.2 ml THF, collidine
(14 mmol) was added and allowed to react for 1 min, and the solution was then added to the resin prewashed with dry THF. Activated amino acids were allowed to react with the N-substituted amino acids for 3 hours, the peptide-resin was washed with DMF, and SPPS was continued. After chain assembly was complete, the Nα-Boc group was removed with neat TFA, and the peptide-resin was washed with dichloromethane and dried by aspiration. The peptide was cleaved from the resin by treatment with anhydrous HF at 0°C for 1 hour, with 10% p-cresol added as a scavenger. After removal of HF by evaporation, the peptide was precipitated and washed with cold diethyl ether and dissolved in aqueous acetonitrile + 0.1% TFA, then lyophilized.

**LC-MS analysis**

Analytical reverse-phase HPLC was performed on an Agilent 1100 system with either Vydac C-18 or C-4 (5 µm 2.1 x 50 mm) silica columns. Peptide masses were obtained using online electrospray MS detection. Semi-preparative HPLC was performed on a Vydac C-18 (10 x 250 mm) column. Peptides were eluted from the column using an appropriate shallow gradient of acetonitrile/0.08% TFA versus water/0.1% TFA. Fractions containing the desired purified peptide product were identified by analytical LC-MS, then combined and lyophilized.

**Removal of N-protection**

N-protected peptides MG(X-G)FL were dissolved at a concentration of 1 mg/ml in 1:1 CH₃CN/H₂O + 0.1% TFA, pH 2. Photolysis was performed by exposing the peptide solutions to low-intensity 366 nm UV light in a 1 cm quartz cell 0.5 cm from the UV source (Entela UVGL-58, 18.4 W), at room temperature. The reaction was monitored by removing aliquots at the desired time points and analyzing by RP-HPLC. Scavenging was performed by addition of 200 eq L-cysteine HCl•H₂O.