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Solid-Phase Synthesis of Amine-Bridged Cyclic Enkephalin Analogues via On-Resin Cyclization Utilizing the Fukuyama−Mitsunobu Reaction

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Received July 3, 2002

An efficient solid-phase synthetic route is described for the preparation of 13-membered amine-bridged cyclic enkephalin analogues (ABEs) **1a** and **1c**−**1j** (Figure 1) resulting from a sulfonamide-containing peptide whose backbone is bound to a resin. The Fukuyama−Mitsunobu reaction of the 2-nitrobenzenesulfonyl-protected amine bound to the solid support with protected aminoethanol in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) is utilized to prepare a resin-bound sulfonamide-protected secondary amine. After peptide cyclization, this protected amine functionality becomes the "amine bridge" of the target molecule. In addition, the reagent DIAD was found to be a superior reagent compared to diethyl azodicarboxylate (DEAD) in the solid-phase Fukuyama−Mitsunobu reaction.

Introduction

The focus of our opioid project has been on the synthesis of cyclic enkephalin analogues bridged by heteroatoms such as sulfur or nitrogen.[1−5] Amine-bridged enkephalin analogues represent a new approach in the design of cyclic peptide opioids.[5] It is an advantage that the trivalent amine offers a handle by which to functionalize the bridge while maintaining the required array of the pharmacophores. In a preliminary effort, a cyclic analogue having a methylamine bridge, Tyr-c[($N_{\beta}CH_3$)-D-A_2pr-Gly-Phe-$NHCH_2CH_2$-] [MABE(I), **1a**],[5] and Tyr-c[($N\gamma CH_3$)-D-A_2bu-Gly-Phe-$NHCH_2CH_2$-] [MABE(II), **1b**][1,2] were synthesized. They were found to be potent but nonselective opioid agonists (Figure 1).

The potencies of **1a** and **1b** make them promising new lead compounds in our research to prepare enkephalin analogues with selectivity for a specific receptor. However, the synthetic routes for the synthesis of **1a** and **1b** described in the previous papers[1,2,5] were not appropriate to construct libraries of amine-bridged enkephalin analogues, which are needed for the study of structure−activity relationships. In this paper we present a novel combinatorial synthetic route for the derivatization of amine-bridged cyclic enkephalins (ABEs) **1c**−**1j** (Figure 1) on a solid support utilizing the Fukuyama−Mitsunobu reaction.

Result and Discussion

Synthetic Strategy. We have chosen a backbone amide linker initially developed by Jensen and coworkers[6] which is versatile and does not require the presence of side chain functionality. It is the key strategy in the synthetic route of ABEs on the solid support to utilize the Fukuyama−Mitsunobu reaction[7−9] to prepare a resin-bound sulfonamide-protected secondary amine which becomes the tertiary amine bridge in the target analogues. The Fukuyama−Mitsunobu reaction provides an efficient route for the synthesis of various secondary amines protected by the 2-nitrobenzenesulfonyl (nosyl) group which can be deprotected under mild conditions. Therefore, the derivatization is designed to be carried out by removal of the nosyl protecting group and alkylation on the cyclic peptide attached on the resin. The target peptidomimetic structures are obtained after cleavage of the resulting cyclic molecules from the solid support (Scheme 1).

Synthesis. The development of the synthetic route was initiated by the preparation of the orthogonally protected

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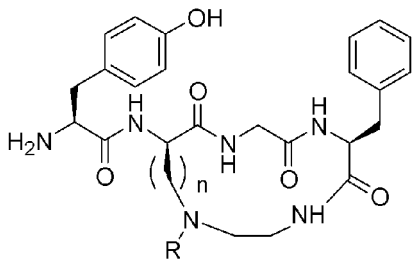
(1) Goodman, M.; Zapf, C.; Rew, Y. *Biopolymers* **2001**, *60*, 229−245.
(2) Baker, T. J.; Rew, Y.; Goodman, M. *Pure Appl. Chem.* **2000**, *72*, 347−354.
(3) Goodman, M.; Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T. L.; Chung, N. N.; Schiller, P. W.; Daubert, J. D.; Cassel, J. A.; DeHaven, R. *Abstract of Papers*, 221st ACS National Meeting, San Diego, April 1−5, 2001; American Chemical Society: Washington, DC, 2001; ORGN-109.
(4) Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T. L.; Chung, N. N.; Schiller, P. W.; Cassel, J. A.; DeHaven, R. N.; Goodman, M. *J. Med. Chem.* **2002**, *45*, 3746−3754.
(5) Shreder, K.; Zhang, L.; Dang, T.; Yaksh, T. L.; Umeno, H.; DeHaven, R.; Daubert, J.; Goodman, M. *J. Med. Chem.* **1998**, *41*, 2631−2635.
(6) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452.
(7) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373−6374.
(8) Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831−5834.
(9) Han, Y.; Albericio, F.; Barany, G. *J. Org. Chem.* **1997**, *62*, 4307−4312.

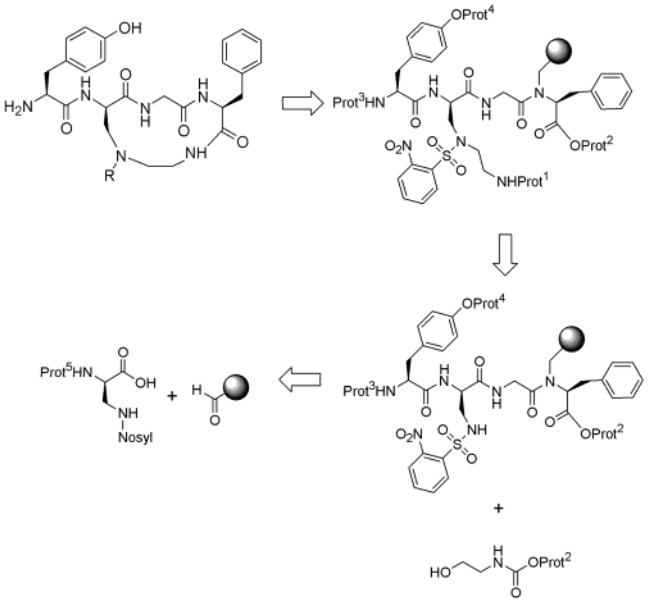
10.1021/jo0204471 CCC: $22.00
Published on Web 11/20/2002



1a, n = 1, R = methyl, MABE(I)
1b, n = 2, R = methyl, MABE(II)
1c, n = 1, R= 2-nitrobenzenesulfonyl
1d, n= 1, R = H
1e, n= 1, R = allyl
1f, n= 1, R = benzyl
1g, n= 1, R = cyclopropylmethyl
1h, n= 1, R = acetyl
1i, n= 1, R = benzoyl
1j, n= 1, R = 1-naphthylmethyl

FIGURE 1. Structures of **1a**, **1b**, and ABEs **1c**−**1j**.

SCHEME 1. Retrosynthetic Analysis of On-Resin Synthesis of Amine-Bridged Cyclic Enkephalin Analogues



Fmoc-D-A_2pr(nosyl)-OH (**2**), where A_2pr represents 2,3-diaminopropionic acid (Scheme 2).

Attempts to protect the amine directly in Fmoc-D-A_2pr-OH with 2-nitrobenzenesulfonyl chloride (nosyl chloride) under various conditions were unsuccessful because of the presence of the free carboxylic acid. Thus, we tried to mask the carboxylic acid with a temporary protecting group. The intermediate Fmoc-D-A_2pr-OH was first converted into the silyl ester, which is soluble in CH_2Cl_2 using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and triethylamine (TEA) in refluxing CH_2Cl_2 under argon.[10] The sulfonylation was then achieved at room temperature without isolation of the silylated amino acid by adding a slight excess of nosyl chloride and 1 equiv of TEA. After the hydrolysis of the silyl ester of the orthogonally protected amino acid with methanol, the target amino acid building block **2** was readily obtained.

The synthesis of **1a** on a solid support is summarized in Scheme 3. We employed 2-(4-formyl-3-methoxy)phenoxyethyl polystyrene resin (**3**), which is a commercially available aldehyde resin, to prepare a linear peptide backbone with an allyl ester and a Boc group as the acid and amine protecting groups. The first amino acid was anchored to the aldehyde resin by on-resin reductive amination of phenylalanine allyl ester. The next residue was incorporated with the Trt protecting group protocol, which circumvents diketopiperazine formation upon addition of the third residue, **4**.[6] Coupling of Trt-Gly-OH to the secondary amine was mediated by 2-chloro-1-methylpyridinium iodide (CMPI), 1-hydroxy-7-azabenzotriazole (HOAt), and diisopropylethylamine (DIEA) in CH_2Cl_2/DMF (v/v = 9/1). Selective removal of the Trt group was accomplished by treatment with CH_2Cl_2/TFA/triisopropylsilane (TIS) (v/v/v = 96/2/2).

The third residue, **2**, was found to be very susceptible to racemization. The building block **2** was introduced successfully by an in situ neutralization/coupling protocol mediated by HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate), HOBt (1-hydroxybenzotriazole monohydrate), and a mild base, 2,6-lutidine in CH_2Cl_2/DMF (v/v = 1/1). Under the same coupling conditions with DIEA, the epimerization of **1a** occurred at an unacceptable level (>10%).[11] Finally, after removal of the N^{α}-Fmoc protecting group, addition of Boc-Tyr(tBu)-OH was achieved using the same conditions mentioned above to yield a linear tetrapeptide backbone, **5**.

The subsequent alkylation to the sulfonamide **5** with *N*-(allyloxycarbonyl)ethanolamine (**6**) was achieved using the Fukuyama−Mitsunobu reaction (diisopropyl azodicarboxylate (DIAD), Ph_3P, THF, 0 °C, 10 min, then room temperature, 12 h). The reaction was monitored by periodic resin cleavage, with product analysis by HPLC, and was complete after 12 h. Interestingly, the solid-phase Fukuyama−Mitsunobu reaction under the same conditions using diethyl azodicarboxylate (DEAD) always gave the ethylated product **10** in more than 40% yield in addition to the desired product **9** (Scheme 4).

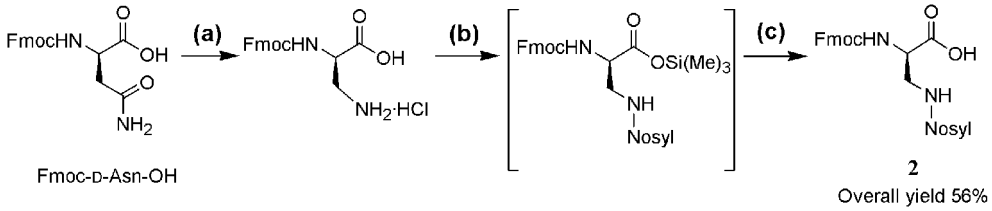
The ethylated product was formed presumably because of the ease in the hydrolysis of DEAD. We did not observe this side reaction when DIAD was used. Thus, the use of DIAD is preferred to DEAD in the solid-phase Mitsunobu reaction, while it is reported that both DIAD and DEAD work equally well as oxidants and can be used interchangeably for reactions in solution.[12] This result indicates that even a simple reaction such as the Mit-

(10) Raillard, S. P.; Mann, A. D.; Baer, T. A. *Org. Prep. Proced. Int.* **1998**, *30*, 183−186.

(11) Epimerization was confirmed by analytical HPLC after cleavage of the target cyclic peptide **1a** from the solid support.

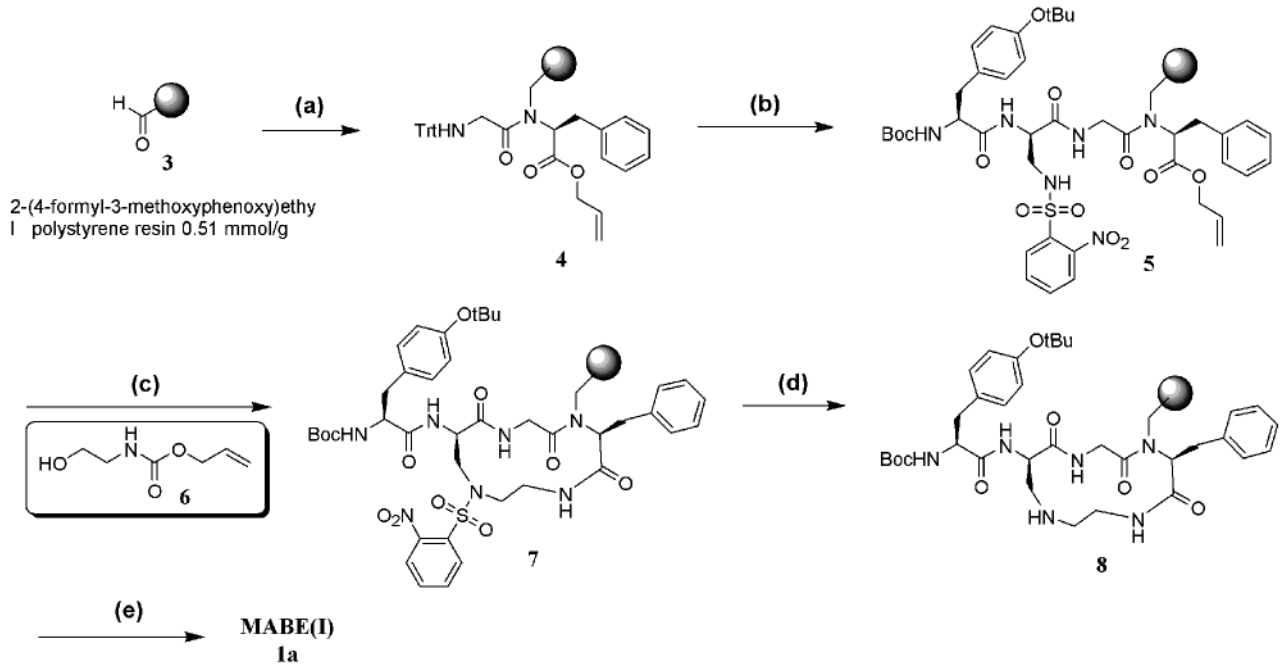
(12) Wisniewski, K.; Koldziejczyk, A. S.; Falkiewicz, B. *J. Pept. Sci.* **1998**, *4*, 1−14.

SCHEME 2. Preparation of 2[a]



[a] Reagents and conditions: (a) (i) IBTFA (1.1 equiv), pyridine (3.0 equiv), CH_2Cl_2, rt, 24 h; (ii) 1 N aqueous HCl; (b) (i) TEA (1.0 equiv), MSTFA (2.2 equiv), CH_2Cl_2, reflux for 1 h; (ii) nosyl chloride (1.1 equiv), TEA (1.0 equiv), rt, 4 h; (c) MeOH, rt, 1 h.

SCHEME 3. On-Resin Synthesis of 1a[a]



[a] Reagents and conditions: (a) (i) H-Phe-OAll·*p*-tosylate (8.0 equiv), $NaBH(OAc)_3$ (8.0 equiv), 1,2-dichloroethane, 18 h; (ii) Trt-Gly-OH (5.0 equiv), CMPI (5.0 equiv), HOAt (5.0 equiv), DIEA (8.0 equiv), CH_2Cl_2/DMF (8/1), 12 h; (b) (i) CH_2Cl_2/TFA/TIS (96/2/2), 1 min, three times; (ii) **2** (3.0 equiv), HBTU (3.0 equiv), HOBt (3.0 equiv), 2,6-lutidine (4.0 equiv), CH_2Cl_2/DMF (1/1), 8 h; (iii) 20% piperidine in NMP, 10 min, two times; (vi) Boc-Tyr(tBu)-OH (3.0 equiv), HBTU (3.0 equiv), HOBt (3.0 equiv), 2,6-lutidine (4.0 equiv), CH_2Cl_2/DMF (1/1), 8 h; (c) (i) **6** (5.0 equiv), THF 12 h; (ii) $PhSiH_3$(24 equiv), $Pd(Ph_3)_4$ (0.1 equiv), CH_2Cl_2, 10 min, two times; (iii) HBTU (3.0 equiv), HOBt (3.0 equiv), 2,6-lutidine (4.0 equiv), CH_2Cl_2/DMF (1/1), 8 h; (d) DBU (5.0 equiv), mercaptoethanol (10 equiv), DMF, 30 min; (e) (i) 38% formaldehyde (40 equiv), THF/TMOF (1/1), 12 h; (ii) $NaBH(OAc)_3$ (20 equiv), 1,2-dichloroethane, 12 h; (iii) TFA/TIS/H_2O (92/5/3), 2 h.

sunobu reaction may result in different products and should be carefully considered when applied to solid-phase synthesis. This variability in solid-phase reactions arises from the requirement of great excess for the amounts of reagents necessary to "push" the reactions forward to satisfactory yields.

Deprotection of the allyl ester and Alloc groups was easily carried out under mild conditions, using excess $PhSiH_3$ as an allyl acceptor and a catalytic amount of $Pd(PPh_3)_4$ in CH_2Cl_2.[13,14] The nosyl-protected cyclic peptide attached to the solid support, **7**, was prepared by cyclization with HBTU/HOBt in the presence of 2,6-lutidine in CH_2Cl_2/DMF (v/v = 1/1). No detectable epimerization product was found in the analytical HPLC (Scheme 3).

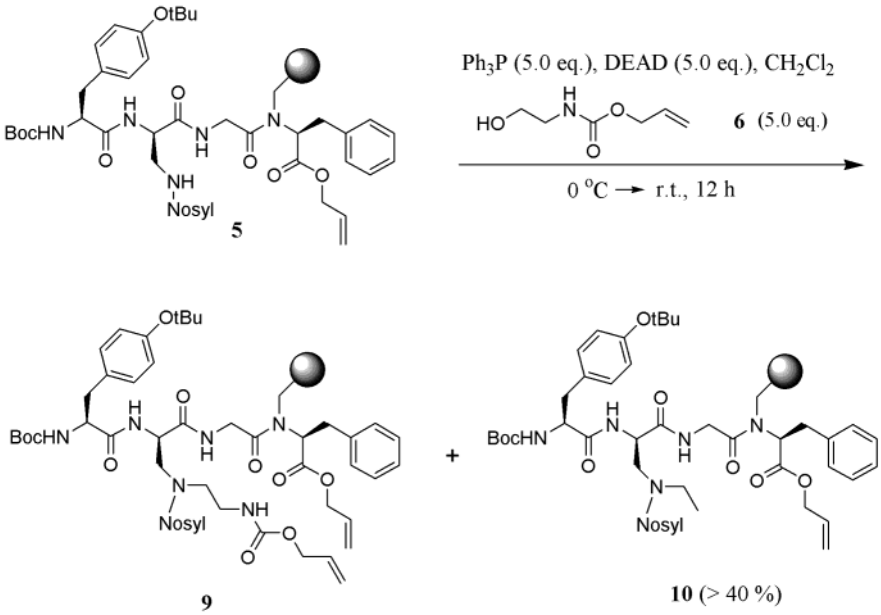
On-resin derivatization commenced with selective removal of the nosyl group with 2-mercaptoethanol and DBU, a nonionic strong base, to produce **8**.[15] Attempts to cleave the nosyl group with other well-known conditions such as Fukuyama's thiophenol/K_2CO_3 and 2-mercaptoethanol/LiOH conditions failed.[7] Methylation of the on-resin cyclic peptide with a secondary amine bridge was successfully achieved by a two-step reductive amination: (1) immonium ion formation using 38% aqueous formaldehyde (40 equiv) in THF/trimethylorthoformate (TMOF) (v/v = 1/1) and (2) $NaBH(OAc)_3$ (20 equiv) in 1,2-dichloroethane. The target compound **1a** was obtained by the acidolytic cleavage of the resulting cyclic molecule from the resin using TFA/TIS/H_2O (v/v/v = 92/5/3) with 94% purity as evidenced by analytical HPLC, and in 78% yield based on the aldehyde resin (Scheme 3). The NMR spectrum of the compound is identical with that reported

(13) Thieriet, N.; Alsina, J.; Giralt, E.; Guibe, F.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 7275−7278.

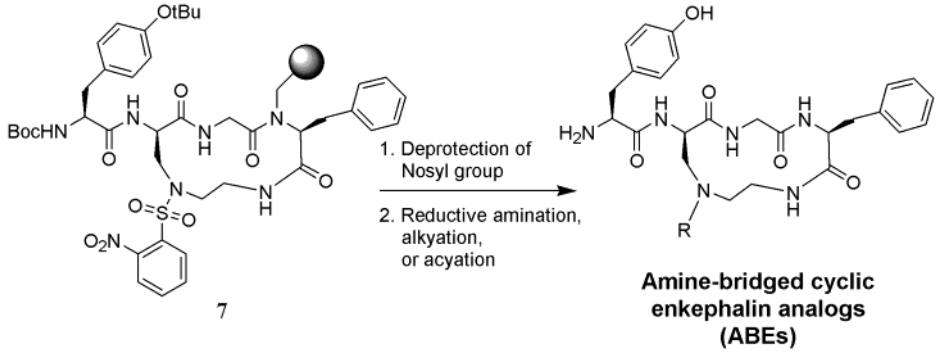
(14) Dessolin, M.; Guillerez, M.-G.; Thieriet, N.; Guibe, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*, 5741−4.

(15) Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1998**, *120*, 2690−2691.

SCHEME 4. Fukuyama−Mitsunobu Reaction of the Peptide Attached on the Resin, 5, with 6 in the Presence of DEAD



SCHEME 5. On-Resin Derivatization of Amine-Bridged Cyclic Enkephalins



earlier.[5] On the basis of this chemistry, we have created a general route for the solid-phase synthesis of diverse amine-bridged opioid structures and accomplished the derivatization of ABEs by reductive amination, alkyation, or acylation (Scheme 5).

The quality of the synthesis was verified at several stages, by cleaving portions of resins **4**, **5**, **7**, and **8** with TFA and analyzing the products by HPLC and MS techniques. HPLC profiles of crude peptides **1a**, **1c**, and **1d** are given in Figure 2.

Conclusions

We have devised efficient routes for the synthesis of ABEs using an aldehyde resin and the Fukuyama−Mitsunobu reaction and demonstrated that this synthetic route is appropriate for the parallel synthesis of an array of target structures for structure−activity relationship studies. A variety of amine-bridged molecules were prepared in high purity by these techniques. In addition, we found that the use of DIAD is preferred to DEAD for the solid-phase Fukuyama−Mitsunobu reactions.

The in vitro and in vivo biological test results and the conformational analysis using NMR and computer simulations of the analogues are in progress and will be reported elsewhere.

Experimental Section

General Procedures and Notes. The Kaiser test[16] was used for the qualitative test for the presence or absence of the free amino group. The final products were purified and analyzed by RP-HPLC using protein peptide C_{18} columns. Column dimensions were 4.5 × 250 mm (90 Å silica, 5 μm) for analytical and 22 × 250 mm (90 Å silica, 10 μm) for preparative HPLC, and UV absorbance was monitored at 220 nm. A binary system of water and acetonitrile, both containing 0.1% TFA, was used throughout. Purity analysis of the crude final products was carried out on a PDA system using a linear gradient of 10−90% acetonitrile over 30 min (condition A, t_R[a]) at a 1 mL/min flow rate. Two analytical HPLC profiles of purified products were obtained using a linear gradient of 10−50% acetonitrile over 30 min (condition B, t_R[b]) and one of the following isocratic conditions: an isocratic elution of 16% acetonitrile (condition C, t_R[c]), an isocratic elution of 20% acetonitrile (condition D, t_R[d]), an isocratic elution of 23% acetonitrile (condition E, t_R[e]), an isocratic elution of 27% acetonitrile (condition F, t_R[f]) at a 1 mL/min flow rate. Prepara-

(16) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−8.

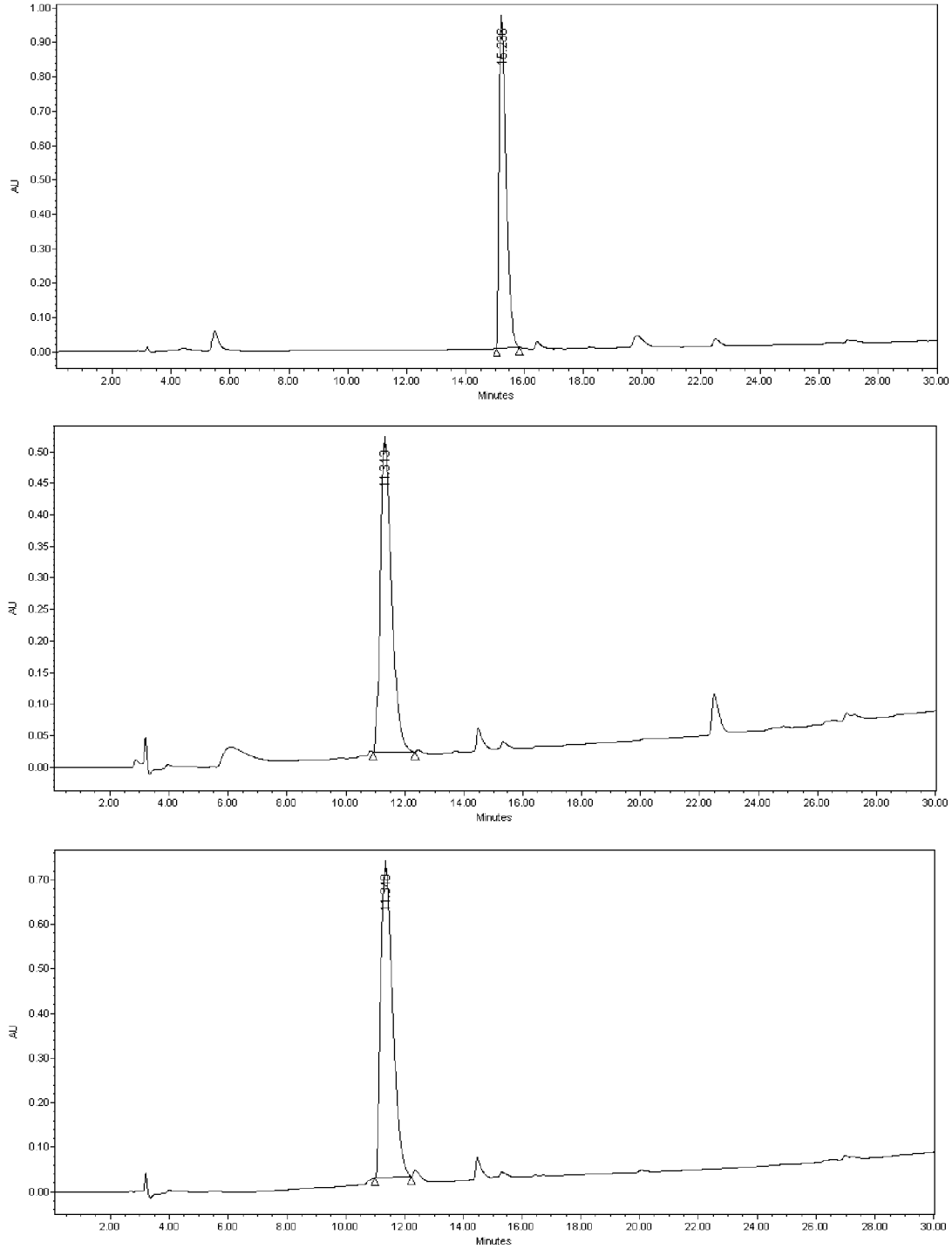


FIGURE 2. HPLC profiles of crude peptides cleaved from resin-bound peptides: nosyl-ABE(I) (**1c**) cleaved from the peptide-bound resin **7** (top), H-ABE(I) (**1d**) cleaved from the peptide-bound resin **8** (middle), and **1a** cleaved from the peptide-bound resin **8** after reductive amination (bottom) using formaldehyde with a gradient of 10−90% (0.1% TFA/CH_3CN in 0.1% TFA/H_2O) over 30 min at 1 mL/min.

tive HPLC was carried out at a 10 mL/min flow rate using condition B, and the materials so obtained were further purified by one of the isocratic conditions mentioned above at a 10 mL/min flow rate.

Fmoc-D-A_2pr(nosyl)-OH (2). To a solution of Fmoc-D-Asn-OH (11.3 g, 31.9 mmol) in DMF/water (2/1, v/v, 250 mL) was added bis(trifluoroacetoxy)iodobenzene (IBTFA) (15.1 g, 35.1 mmol) at 0 °C. The reaction was then stirred for 10 min at 0 °C, and pyridine (7.75 mL, 95.8 mmol) was added. After being stirred for 24 h at room temperature, the reaction mixture was concentrated under reduced pressure. The concentrated reaction mixture was dissolved in 1 N aqueous hydrochloric acid solution (100 mL) and then extracted with ether to remove the organic impurity (100 mL). The aqueous layer was lyophilized, and the resulting crude product was recrystallized from ether/EtOH (3/1, v/v). The crystals were filtered and washed with cold ether to give Fmoc-D-A_2pr-OH·HCl (10.3 g, 28.4 mmol, 89%) as a yellow solid: mp 145−149 °C (lit.[17] mp 146 °C); $[\alpha]^{25}_D = 19.5°$ ($c = 0.96$, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.13 (br s, 3H, NH_3^+), 7.89 (d, $J = 7.6$ Hz, 2H), 7.81 (d, $J = 8.4$

Hz, 1H, NH), 7.73 (d, J = 6.8 Hz, 2H), 7.42 (t, J = 7.2 Hz, 2H), 7.33 (t, J = 7.6 Hz, 2H), 4.40−4.20 (m, 4H), 3.20 (br, 1H), 3.00 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 170.7, 156.1, 143.6, 140.6, 127.6, 127.1, 125.2, 120.1, 66.1, 51.9, 46.7, 34.3; MS (ESI) m/z 327 $[M + H]^+$, 325 $[M - H]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{18}H_{19}N_2O_4$ 327.13448, found 327.13480; IR (KBr pellet, cm^{-1}) 3326, 3038, 2964, 1701, 1540, 1450, 1306, 1261, 1107, 761, 739.

To a suspension of Fmoc-D-A_2pr-OH·HCl (8.00 g, 22.0 mmol) in CH_2Cl_2 (100 mL) were added TEA (3.07 mL, 22.0 mmol) and MSTFA (8.56 mL, 46.2 mmol) successively at 0 °C, and the reaction was refluxed until a clear solution was obtained. The clear reaction mixture was then cooled to room temperature, and nosyl chloride (5.36 g, 24.2 mmol) was added followed by TEA (3.07 mL, 22.0 mmol) and stirred for 4 h. After the addition of MeOH (70 mL), the reaction mixture was stirred for 1 h and then concentrated under reduced pressure. The reaction was diluted with EtOAc (100 mL) and washed with 10% aqueous citric acid solution (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2/MeOH/AcOH = 70/1/0.1 to 30/1/0.1, v/v/v) afforded 7.01 g (13.8 mmol, 63%) of the target compound **2** as a light yellow solid. A further analytical sample was recrytallized from ether/EtOH: mp 137−141 °C; R_f = 0.15 (CH_2Cl_2/MeOH/AcOH = 20/1/0.1); $[\alpha]^{25}_D$ = 17.1°(c = 0.95, MeOH); ^{1}H NMR (DMSO-d_6) δ 8.16 (br, 1H, NH), 8.00 (m, 2H), 7.88 (d, J = 7.6 Hz, 2H), 7.84 (m, 2H), 7.70 (d, J = 7.6 Hz, 2H), 7.57 (d, J = 8.8 Hz, 1H, NH), 7.41 (t, J = 7.6 Hz, 2H), 7.32 (t, J = 7.6 Hz, 2H), 4.25 (m, 3H), 4.08 (dd, J = 12.8 and 7.6 Hz, 1H), 3.26 (m, 2H); ^{13}C NMR(DMSO-d_6) δ 171.1, 155.7, 147.5, 140.6, 134.0, 132.7, 132.5, 129.4, 127.6, 127.0, 125.2, 124.5, 120.1, 65.9, 54.0, 46.7, 43.8; MS (ESI) m/z 512 $[M + H]^+$, 534 $[M + Na]^+$, 510 $[M - H]^-$; HRMS (MALDI) m/z $[M + Na]^+$ calcd for $C_{24}H_{21}N_3O_8NaS$ 534.0949, found 534.0942; IR (KBr pellet, cm^{-1}) 3325, 3068, 2955, 2893, 1704, 1541, 1450, 1344, 1166, 1085, 760, 740, 587.

Preparation of Trt-Gly-(resin)-Phe-OAll (4). To a stirred suspension of 2-(4-formyl-3-methoxy)phenoxyethyl polystyrene resin **3** (10.0 g, 0.51 mmol/g, 5.10 mmol) in 1,2-dichloroethane (200 mL) with a mechanical stirrer were added H-Phe-OAll·TsOH (8.0 equiv) and $NaBH(OAc)_3$ (8.0 equiv) successively at room temperature. After being stirred for 9 h at room temperature, the reaction was quenched with MeOH (40 mL) carefully. The resin was then filtered, washed with MeOH, CH_2Cl_2, 20% piperidine in DMF, MeOH, and CH_2Cl_2 (three times), and dried in vacuo. The dried intermediate resin was suspended in CH_2Cl_2/DMF (v/v, 8/1, 200 mL) and allowed to react with Trt-Gly-OH (5.0 equiv), CMPI (5.0 equiv), HOAt (5.0 equiv), and DIEA (8.0 equiv) for 12 h. After being washed with DMF, MeOH, and CH_2Cl_2 (3 times), the resin product was dried in vacuo to provide the dipeptide attached on the solid support, **4** (12.4 g, 5.06 mmol, theoretical yield 12.5 g, 99%). The loading level of resin **5** was 0.41 mmol/g.

Preparation of Boc-Tyr(tBu)-D-A_2pr(nosyl)-Gly-(resin)-Phe-OAll (5). The peptide-bound resin **4** (5.02 g, 2.06 mmol) was treated with CH_2Cl_2/TFA/TIS (v/v/v = 96/2/2, 80 mL, 3 × 1 min) and then washed again with DMF, MeOH, and CH_2Cl_2 (three times). The resin was suspended in DMF/CH_2Cl_2 (1/1, v/v, 80 mL), treated with Fmoc-D-A_2pr(nosyl)-OH (2.5 equiv), HBTU (2.5 equiv), HOBt (2.5 equiv), and 2,6-lutidine (3.5 equiv) for 8 h. After being washed with DMF, MeOH, and CH_2Cl_2 (three times), the resin was treated with 20% piperidine in NMP (10 mL, 2 × 10 min) and washed with CH_2Cl_2, MeOH, CH_2Cl_2, and DMF (three times). The washed resin was suspended again in DMF/CH_2Cl_2 (1/1, v/v, 80 mL) and treated with Boc-Tyr(tBu)-OH (2.5 equiv), HBTU (2.5 equiv), HOBt (1.0 equiv), and 2,6-lutidine (3.5 equiv) for 8 h. After being washed successively with DMF, MeOH, and CH_2Cl_2 (three times), the resin was dried in vacuo to provide **5**.

Preparation of Nosyl-Protected Cyclic Peptide Attached to the Resin, 7. To an argon-agitated suspension of the peptide-bound resin **5** (2.06 mmol) and *N*-(allyloxycarbonyl)ethanolamine (**6**) (5 equiv) in THF (80 mL) were added carefully DIAD (5.0 equiv) and Ph_3P (5.0 equiv) at 0 °C, and the reaction was agitated at room temperature for 12 h. After successive washings with DMF, MeOH, and CH_2Cl_2 (three times), a solution of $PhSiH_3$ (24 equiv) and a solution of Pd(PPh_3)$_4$ (0.1 equiv) in CH_2Cl_2 (20 mL) were added to the resin under Ar. The resin was shaken for 10 min, the peptide−resin was washed with DMF, MeOH, and CH_2Cl_2 (three times), and then the deprotection process was repeated once. The deprotected peptide−resin was suspended again in DMF/CH_2Cl_2 (1/1, v/v, 15 mL) and treated with HBTU (3.0 equiv), HOBt (3.0 equiv), and 2,6-lutidine (4.0 equiv) for 12 h. After being washed successively with DMF, MeOH, and CH_2Cl_2 (three times), the resin was dried in vacuo to provide the peptide−resin **7** (5.67 g, 1.95 mmol, theoretical yield 5.71 g, 95% from peptide-bound resin **4**). The loading level of the resin **7** was 0.34 mmol/g.

Synthesis of Nosyl-ABE(I) (1c) from the Peptide−Resin 7. Target peptide was cleaved from the peptide-bound resin **7** (0.50 g, 0.17 mmol) by treatment with TFA/TIS/H_2O (v/v/v = 92/5/3, 12 mL) at room temperature for 2 h. The filtrate from the cleavage reaction was collected and combined with TFA washes (2 × 10 mL) of the cleaved peptide−resin. Concentration of the combined filtrates under reduced pressure, precipitation in IPE (10 mL), and centrifugation yielded a crude peptide·TFA salt as a yellow solid (94 mg, 0.12 mmol, 71% from the peptide-bound resin **7**) with 88% purity from the analytical HPLC (t_R[a] = 15.24 min) and in 67% overall yield based on the aldehyde resin **3**. Compound **1c** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 682 $[M + H]^+$, 704 $[M + Na]^+$, 680 $[M - H]^-$, 716 $[M + Cl]^-$; HRMS (MALDI) m/z $[M + Na]^+$ calcd for $C_{31}H_{35}N_7O_9NaS$ 704.21092, found 704.20867; RP-HPLC t_R[b] = 20.97 min, t_R[d] = 22.21 min.

Synthesis of H-ABE(I) (1d) from the Peptide−Resin 7. The peptide-bound resin **7** (0.50 g, 0.17 mmol) was suspended in DMF (6 mL) and treated with DBU (5 equiv) and mercaptoethanol (10 equiv) for 30 min at room temperature. A bright yellow solution was indicative of nosyl cleavage. After the deprotected peptide-bound resin **8** was extensively washed with DMF, MeOH, and CH_2Cl_2 (three times), the target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1c.** A crude peptide·2TFA salt was obtained as a yellow solid (82 mg, 0.11 mmol, 65% from the peptide-bound resin **7**) with 89% purity from the analytical HPLC (t_R[a] = 11.31 min). Compound **1d** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 497 $[M + H]^+$, 519 $[M + Na]^+$, 495 $[M - H]^-$, 531 $[M + Cl]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{25}H_{33}N_6O_5$ 497.25070, found 497.24916; RP-HPLC t_R[b] = 13.29 min, t_R[c] = 7.62 min.

Synthesis of MABE(I) (1a) from the Peptide−Resin 7. The nosyl protecting group of the peptide-bound resin **7** (0.50 g, 0.17 mmol) was removed using the described procedure for the synthesis of compound **1d**. The deprotected peptide-bound resin **8** was treated with 37% formaldehyde (40 equiv) in THF/TMOF (v/v, 1/1, 10 mL) at room temperature for 12 h and washed again with 1,2-dichloroethane. The resin was then suspended in 1,2-dichloroethane and treated with $NaBH(OAc)_3$ (20 equiv) at room temperature for 12 h. After the peptide-bound resin **8** was washed with DMF, MeOH, and CH_2Cl_2 (three times), the target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1c.** A crude peptide·2TFA salt was obtained as a yellow solid (72 mg, 0.098 mmol, 57% from the peptide-bound resin **7**) with 94% purity from the analytical HPLC (t_R[a] = 11.35 min). Compound **1a** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 511 $[M + H]^+$, 533 $[M + Na]^+$, 509 $[M - H]^-$, 545 $[M + Cl]^-$;

(17) Zhang, L.-h.; Kauffman, G. S.; Pesti, J. A.; Yin, J. *J. Org. Chem.* **1997**, *62*, 6918−6920.

HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{26}H_{35}N_6O_5$ 511.26635, found 511.26507; RP-HPLC t_R[b] = 13.47 min, t_R[c] = 7.85 min.

Synthesis of Allyl-ABE(I) (1e) from the Peptide−Resin 7. The nosyl protecting group of the peptide-bound resin **7** (0.50 g, 0.17 mmol) was removed using the described procedure for the synthesis of compound **1d**. The deprotected peptide-bound resin **8** was treated with allyl bromide (20 equiv) and $NaHCO_3$ (40 equiv) in DMF (10 mL) at room temperature for 24 h and washed again with DMF, H_2O, and benzene (three times). The target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1e.** A crude peptide·2TFA salt was obtained as a yellow solid (80 mg, 0.10 mmol, 59% from the peptide-bound resin **7**) in 96% purity as determined by analytical HPLC (t_R[a] = 12.42 min). Compound **1e** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 537 $[M + H]^+$, 559 $[M + Na]^+$, 535 $[M - H]^-$, 571 $[M + Cl]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{28}H_{37}N_6O_5$ 537.28200, found 537.28494; RP-HPLC t_R[b] = 15.72 min, t_R[c] = 12.18 min.

Synthesis of Benzyl-ABE(I) (1f) from the Peptide−Resin 7. The nosyl protecting group of the peptide-bound resin **7** (0.50 g, 0.17 mmol) was removed using the described procedure for the synthesis of compound **1d**. The deprotected peptide-bound resin **8** was treated with benzyl bromide (20 equiv) and $NaHCO_3$ (40 equiv) in DMF (10 mL) at room temperature for 24 h and washed again with DMF, H_2O, and benzene (three times). The target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1c.** A crude peptide·2TFA salt was obtained as a yellow solid (95 mg, 0.12 mmol, 59% from the peptide-bound resin **7**) with 91% purity from the analytical HPLC (t_R[a] = 15.91 min). Compound **1f** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 587 $[M + H]^+$, 609 $[M + Na]^+$, 585 $[M - H]^-$, 621 $[M + Cl]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{32}H_{39}N_6O_5$ 587.29764, found 587.29928; RP-HPLC t_R[b] = 22.00 min, t_R[e] = 14.33 min.

Synthesis of Cyclopropylmethyl-ABE(I) (1g) from the Peptide−Resin 7. The nosyl protecting group of the peptide-bound resin **7** (0.50 g, 0.17 mmol) was removed using the described procedure for the synthesis of compound **1d**. The deprotected peptide-bound resin **8** was treated with cyclopropylmethyl bromide (20 equiv) and $NaHCO_3$ (40 equiv) in DMF (10 mL) at 80 °C for 24 h and washed again with DMF, H_2O, and benzene (three times). The target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1c.** A crude peptide·2TFA salt was obtained as a yellow solid (90 mg, 0.12 mmol, 59% from the peptide-bound resin **7**) with 85% purity from the analytical HPLC (t_R[a] = 14.00 min). Compound **1g** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 551 $[M + H]^+$, 573 $[M + Na]^+$, 549 $[M - H]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{29}H_{39}N_6O_5$ 551.29764, found 551.29825; RP-HPLC t_R[b] = 17.49 min, t_R[d] = 9.12 min.

Synthesis of Acetyl-ABE(I) (1h) from the Peptide−Resin 7. The nosyl protecting group of the peptide-bound resin **7** (0.50 g, 0.17 mmol) was removed using the described procedure for the synthesis of compound **1d**. The deprotected peptide-bound resin **8** was treated with acetyl chloride (5 equiv) and DIEA (5 equiv) in CH_2Cl_2 (10 mL) for 24 h and washed again with DMF, MeOH, and CH_2Cl_2 (three times). The target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1c.** A crude peptide·TFA salt was obtained as a yellow solid (65 mg, 0.10 mmol, 59% from the peptide-bound resin **7**) with 72% purity from the analytical HPLC (t_R[a] = 11.61 min). Compound **1h** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 539 $[M + H]^+$, 561 $[M + Na]^+$, 537 $[M - H]^-$, 573 $[M + Cl]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{27}H_{35}N_6O_6$ 539.26126, found 539.26228; RP-HPLC t_R[b] = 15.01 min, t_R[c] = 10.07 min.

Synthesis of Benzoyl-ABE(I) (1i) from the Peptide−Resin 7. The nosyl protecting group of the peptide-bound resin **7** (0.50 g, 0.17 mmol) was removed using the described procedure for the synthesis of compound **1d**. The deprotected peptide-bound resin **8** was treated with benzoyl chloride (5 equiv) and DIEA (5 equiv) in CH_2Cl_2 (10 mL) for 24 h and washed again with DMF, MeOH, and CH_2Cl_2 (three times). The target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1c.** A crude peptide·TFA salt was obtained as a yellow solid (75 mg, 0.11 mmol, 65% from the peptide-bound resin **7**) with 81% purity from the analytical HPLC (t_R[a] = 14.31 min). Compound **1i** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 601 $[M + H]^+$, 623 $[M + Na]^+$, 599 $[M - H]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{32}H_{37}N_6O_6$ 601.27691, found 601.27624; RP-HPLC t_R[b] = 20.01 min, t_R[d] = 17.60 min.

Synthesis of 1-Naphthylmethyl-ABE(I) (1j) from the Peptide−Resin 7. The nosyl protecting group of the peptide-bound resin **7** (0.50 g, 0.17 mmol) was removed using the described procedure for the synthesis of compound **1d**. The deprotected peptide-bound resin **8** was treated with 1-naphthylmethyl bromide (20 equiv) and $NaHCO_3$ (40 equiv) in DMF (10 mL) at 80 °C for 24 h and washed again with DMF, H_2O, and benzene (three times). The target peptide was cleaved from the resin and isolated using the described procedure for the synthesis of compound **1c.** A crude peptide·2TFA salt was obtained as a yellow solid (100 mg, 0.12 mmol, 71% from the peptide-bound resin **7**) with 92% purity from the analytical HPLC (t_R[a] = 18.10 min). Compound **1j** was further purified by RP-HPLC as described in the General Procedures and Notes: MS (ESI) m/z 637 $[M + H]^+$, 659 $[M + Na]^+$, 635 $[M - H]^-$, 671 $[M + Cl]^-$; HRMS (MALDI) m/z $[M + H]^+$ calcd for $C_{36}H_{41}N_6O_5$ 637.31330, found 637.31250; RP-HPLC t_R[b] = 25.81 min, t_R[f] = 20.28 min.

Acknowledgment. This work was supported by NIH Grant R01-DA 05539 (M.G.). We also thank Dr. Chiara Perazzolo and Dr. Antonia DeCapua for the interpretation of NMR spectra and Sandra Blaj Moore for her helpful assistance in preparation of this manuscript.

Supporting Information Available: ^1H NMR spectra and their peak assignments, HR mass spectra, and analytical HPLC profiles of both crude and purified final products. This material is available free of charge via Internet at http://pubs.acs.org.

JO020447L