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Direct Solid-Phase Synthesis of Octreotide Conjugates: Precursors for Use as Tumor-Targeted Radiopharmaceuticals

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Abstract—Somatostatin analogues, such as octreotide, are useful for the visualization and treatment of tumors. Unfortunately, these compounds were produced synthetically using complex and inefficient procedures. Here, we describe a novel approach for the synthesis of octreotide and its analogues using *p*-carboxybenzaldehyde to anchor Fmoc-threoninol to solid phase resins. The reaction of the two hydroxyl groups of Fmoc-threoninol with *p*-carboxybenzaldehyde was catalyzed with *p*-toluenesulphonic acid in chloroform using a Dean–Stark apparatus to form Fmoc-threoninol *p*-carboxybenzacetal in 91% yield. The Fmoc-threoninol *p*-carboxybenzacetal acted as an Fmoc-amino acid derivative and the carboxyl group of Fmoc-threoninol *p*-carboxybenzacetal was coupled to an amine-resin via a DCC coupling reaction. The synthesis of protected octreotide and its conjugates were carried out in their entirety using a conventional Fmoc protocol and an autosynthesizer. The acetal was stable during the stepwise elongation of each Fmoc-amino acid as shown by the averaged coupling yield (>95%). Octreotide (74 to 78% yield) and five conjugated derivatives were synthesized with high yields using this procedure, including three radiotherapy octreotides (62 to 75% yield) and two cellular markers (72 to 76% yield). This novel approach provides a strategy for the rapid and efficient large-scale synthesis of octreotide and its analogues for radiopharmaceutical and tagged conjugates.

Introduction

Receptors for somatostatin, a cyclic tetradecapeptide originally isolated from bovine hypothalamus,[1–3] are found to be expressed in approximately 90% of carcinoid tumors.[4–7] Consequently, somatostatin receptor scintigraphy and radiotherapy have been applied to the clinical visualization and treatment of tumors, particularly for nonoperable acromegaly[8] or gastroentero pancreatic tumors.[9] The use of somatostatin itself for medical treatment is limited, however, due to its short half-life in vivo (2 to 3 min).[10,11] This has led to the development of several conformation-restricted cyclic peptide analogues. For example, octreotide **1**, consisting of D-amino acids (D-Phe[1], D-Trp[4]), amino alcohol (threoninol), and a disulfide bond (Cys[2]-S-S-Cys[7]), exhibits a prolonged half-life in vivo (60 to 90 min in human)[12] and is functionally 2000 times more effective than somatostatin.[13] Furthermore, [Tyr[3]]-octreotide labeled with iodine-123 ([^{123}I]-Tyr[3]-octreotide) and ^{111}In-labeled derivatives of octreotide ([^{111}In]-diethylenetriaminepenta acetic acid [DTPA]-D-Phe[1]-octreotide) have been developed as effective radiolabels for the visualization of neuroendocrine tumors in humans using single photon emission tomography (SPET).[14–20] In addition, FDA approval of the use of [^{111}In]-DTPA-octreotide has accelerated the development of somatostatin-derived radiopharmaceuticals.[21–32]

Large-scale synthesis of these analogues has been difficult because the existing methods of synthesis are inefficient and complex. The first reported synthesis of DTPA-D-Phe[1]-octreotide **2** by solid-phase peptide synthesis[33–35] (SPPS) had an overall yield of only 5% based on the starting Cys(Acm)-resin.[36] Field et al.[36] concluded that the low yield was resulted from (1) the inevitable undesired peptide bond formation between octreotide and the two anhydride groups of DTPA and (2) the poor solubility of DTPA in coupling solvents such as DMF or *N*-methylpyrolindone (NMP). They also isolated side chain protected [D-Trp(Boc),[4] Lys(-Boc),[5] Thr(Bu^t)[6]]-octreotide with a 14% yield. This indicated that the harsh cleavage conditions and poor rate of peptide cleavage by aminolysis with threoninol

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were responsible for the low total yield. Recently, Arano et al.[37,38] and Schmidt et al.[39] utilized modified resins, such as 2-chlorotrityl chloride resin and Rink acid resin, to load Fmoc-Thr(Bu^t)-ol as the first amino acid residue to synthesize **2**. The total yield of **2** reported by Arano et al.[37,38] was still relatively low (31.8% based on the starting Fmoc-Thr(Bu^t)-ol-resin), while Schmidt et al.[39] did not mention the yield. Deferoxamine-conjugated octreotide (DFO-octreotide **4**) has also been synthesized with a relatively low yield, using a relatively complex procedure.[27,28]

We have developed a method to facile synthesis of octreotide.[40,41] In this report we describe a novel procedure for the rapid and the efficient synthesis of octreotide conjugated derivatives: DTPA-octreotide **2**, 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA)-octreotide **3**, DFO-octreotide **4**, and two cell-visualization octreotide derivatives, biotin-octreotide **5** and fluorescein-octreotide **6**.[42–50] The process utilizes the Fmoc-chemistry of SPPS to rapidly synthesize octreotide and its derivatives with high yields and purity.

Results

In designing a simple method of synthesis of octreotide and its conjugates, we considered the following: (i) direct linkage between the Fmoc-threoninol and the solid phase resin, (ii) the use of a dilute solution during oxidation of the disulfide bond to prevent intermolecular cross linkage, and (iii) preparation of the conjugated compounds to have a single free carboxyl group that can directly couple to the octreotide in advance.

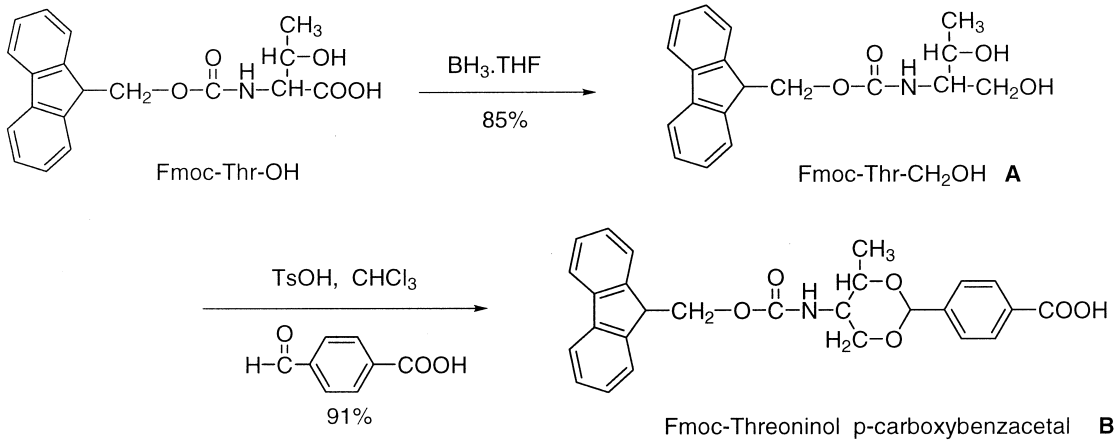
Fmoc-threoninol *p*-carboxybenzacetal

It has been known that acetal[51] resin can be prepared by reacting benzaldehyde resin[52] with glycerol and can be used to generate a C-terminal diol derivative. In the synthesis of octreotide, our approach was to utilize *p*-carboxybenzaldehyde to anchor Fmoc-threoninol to solid-phase resins. The 1,3-dihydroxyl groups of Fmoc-threoninol reacted with *p*-carboxybenzaldehyde to form Fmoc-threoninol *p*-carboxybenzacetal. The acetal derivative was used as an Fmoc-amino acid derivative in SPPS. The feasibility of this reaction was examined in the synthesis of **1** and five conjugated octreotides (**2, 3, 4, 5,** and **6**). Scheme 1 shows the synthesis of Fmoc-threoninol *p*-carboxybenzacetal **B**. Fmoc-threonine was reduced with BH_3/THF to produce Fmoc-threoninol **A** with an 85% yield. **B** was prepared with 91% yield from **A** and *p*-carboxybenzaldehyde (2.5 equiv amounts) in chloroform catalyzed by *p*-toluenesulfonic acid.

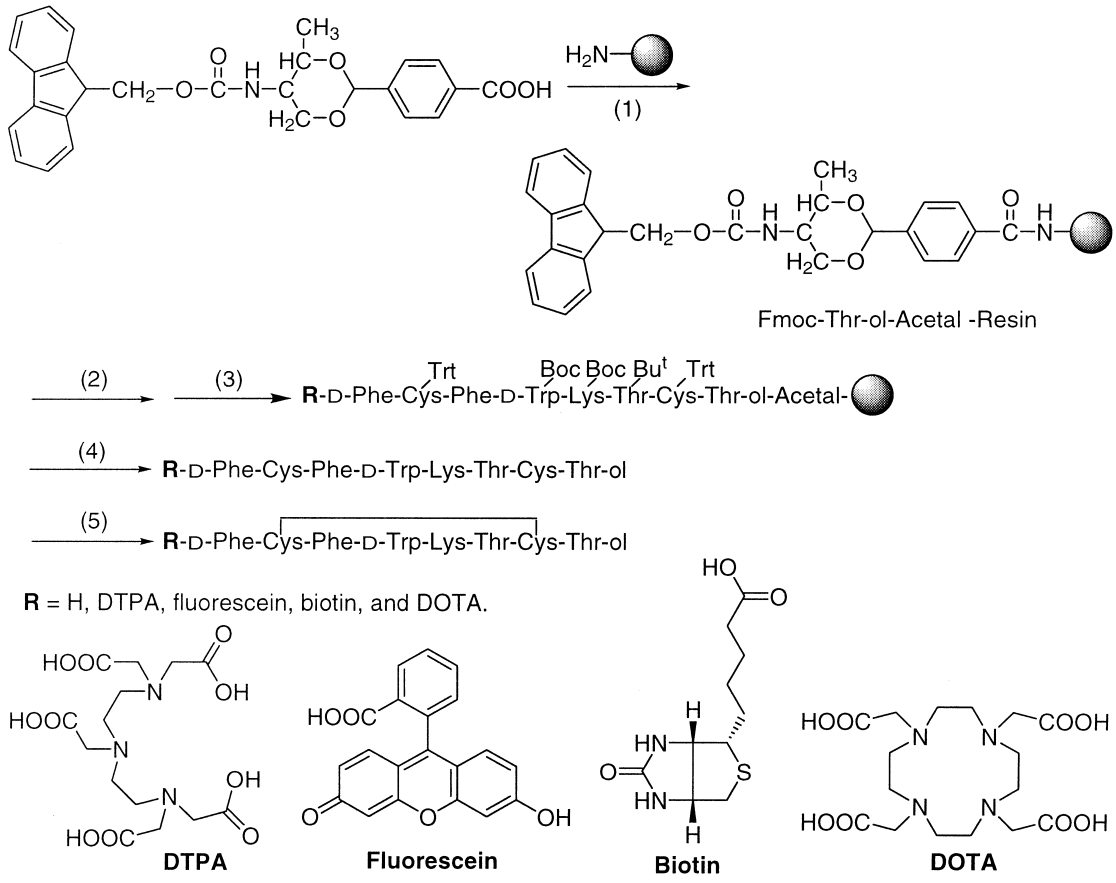
Octreotide 1

The carboxyl group of **B** as the first amino acid derivatives was anchored onto the solid-phase resin using DCC/HoBt in NMP. The peptide bond elongation was carried out using an autosynthesizer with a standard FastMoc protocol, as depicted in Scheme 2. After the peptide bond formation was completed, the weight of the elongated peptide on the resin was used to calculate the average yields at each coupling step (Table 1). The average coupling was higher than 95%. This was consistent with the recorded coupling yield at each step on the autosynthesizer. The results of the coupling yields also indicated that the acetal linker was stable in the stepwise elongation of each corresponding Fmoc-amino acid. The cleavage of the side chain protecting groups of Cys(Trt), Lys(Boc), D-Trp(Boc), Thr(Bu^t) and of the elongated peptide from the resin was performed simultaneously using the TFA (trifluoroacetic acid) method. After evaporation of the TFA solution under vacuum, the resulting residue was washed with ice-cooled dry ether and the reduced **1** was extracted with acetic acid solution (20%). Figure 1 shows the chromatogram of the reduced **1** directly after extraction with acetic acid solution. The major peak with retention time of 30.1 min was collected and identified (refer to Experimental).

The formation of the disulfide bond was performed using a dilute solution of octreotide (1 mM) at 4°C, pH 7, in 5% ammonium acetate. The folding solution was prepared by dilution of the extraction of acetic acid solution (20%) to **1** mM octreotide followed by adjusting the pH to 7 with ammonium hydroxide (25%). Progress



Scheme 1. The synthesis of Fmoc-threoninol *p*-carboxybenzacetal **B**.



(1) DCC/HoBt; (2)Stepwise elogation and final deprotection;
(3) Conjugation;
(4) CF_3COOH, thioanisole, ethanedithiol, phenol, H_2O;
(5) 5% CH_3COONH_4 aq., pH 7.0.

Scheme 2. Synthetic procedure for octreotide and its derivatives.

Table 1. Data and yields of octreotide and conjugated derivatives

No.	Peptide	Calcd MW	FAB $(M+H)^+$ m/z	Peptide-resin[a] (mg)	Average coupling [b] (%)	Isolated yields[c] (%)
1	Octreotide	1018	1019.5	899	97	76
2	DTPA-octreotide	1393	1394.6	394	96	75
3	DOTA-octreotide	1404	1405.6	393	94	71
4	DFO-octreotide	1756	1756.5	1002	85	62
5	Biotin-octreotide	1244	1245.4	238	96	76
6	Fluore-octreotide	1322	1323.4	485	96	72

[a] Synthesis scale is (1) 0.24 mmol, (2) 0.1 mmol, (3) 0.1 mmol, (4) 025 mmol, (5) 0.062 mmol, and (6) 0.125 mmol, respectively.
[b] Calculated from the increase in weight of the elongated petide-resin divided by the weight of protected peptide.
[c] Obtained after RP-HPLC purification using a Vydac C18 preparative column. Experimental procedures and conditions in text. Amino acid analysis of octreotide after hydrolysis with 6 N HCl:TFA (4:1, v/v) for 3 h at 130°C was listed as: amino acid/found/calcd = Lys/1.0/1; 1/2Cys/0.97/1; Phe/1.94/2; Thr/0.65/1.

of the disulfide bond formation was monitored by high-performance liquid chromotography (HPLC). Figure 2 shows the time course for folding of **1**. The reduced form of **1** had a retention time of 8.6 min and the oxidized form of **1** had a retention time of 8.0 min. The relative concentration of reduced **1** decreased gradually with a corresponding increase in oxidized **1**. Conversion was complete after 48 h. Pure octreotide was isolated using a preparative column. The fractions corresponding to the desired peaks were collected and lyophilized. The weight of **1** at this stage was 193 mg (approximately 76% yield, based on a 0.25 mmol scale). The peptide was further characterized using amino acid composition analysis and Fab-Mass (Table 1).

Conjugated derivatives

Direct coupling of the conjugated compounds on the autosynthesizer was an effective procedure. Both fluorescein and biotin have a carboxyl group that can be

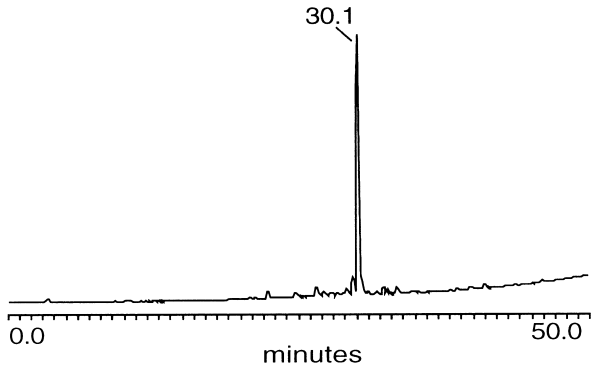


Figure 1. RP-HPLC analysis of reduced octreotide. Sample was taken from the extraction (20% acetic acid solution). The retention time was 30.1 as monitored at UV 214 nm. The elution: A = 5% acetonitrile aqueous containing 0.1% TFA; B = 90% acetonitrile aqueous containing 0.1% TFA. See Experimental for HPLC conditions.

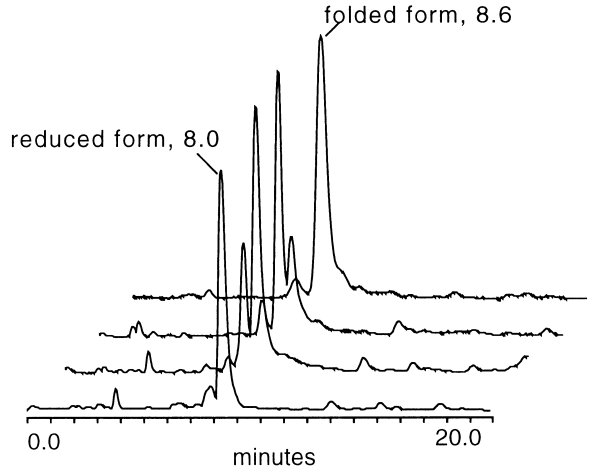
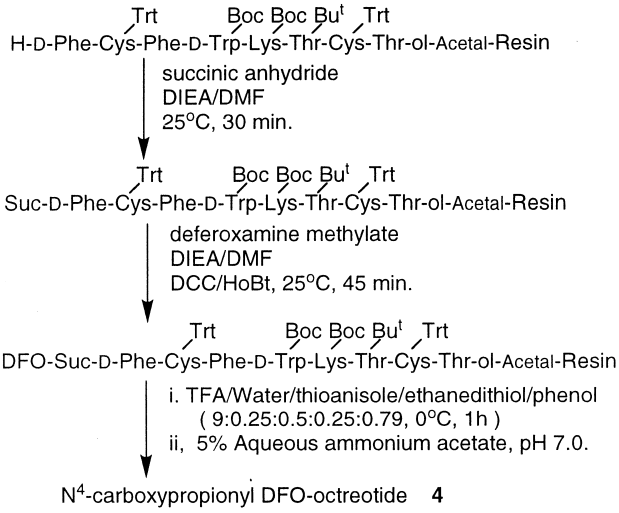


Figure 2. The time course for folding of reduced octreotide **1**. The progress was followed by RP-HPLC monitored at 280 nm. The reduced form had a retention time of 8.6 min and the oxidized form had a retention time of 8.0 min. The elution: A = 5% acetonitrile aqueous containing 0.1% TFA; B = 90% acetonitrile aqueous containing 0.1% TFA. See Experimental for HPLC conditions.

coupled to **1** without modification. The DTPA derivative (monoreactive DTPA derivative) was obtained with a 24% total yield starting from diethylenetriamine according to the procedures of Arano et al.[38] The DOTA derivative was prepared as previously described[53,54] by dissolving three equivalents of tetrabutylammonium hydroxide with DOTA in water and lyophilizing to yield the salt of the partially protected DOTA. The fluorescein, biotin, DTPA and DOTA derivatives were used as the ninth amino acid derivatives and were successfully coupled to octreotide using the autosynthesizer (Scheme 2). DFO has no carboxyl group to be linked with **1**. Scheme 3 shows the procedure for synthesis of N^4-carboxypropionyl DFO-octreotide **4**. Protected octreotide was first incubated with succinic anhydride to produce 4-carboxypropionyl octreotide on the autosynthesizer, followed by coupling DFO to 4-carboxypropionyl octreotide using the DCC method.

The same procedures for cleavage and disulfide formation described above were used to complete the synthesis of



Scheme 3. The synthesis of N^4-carboxypropionyl DFO-octreotide **4**.

2–**6** with yields of 65 to 72%. All analogues **2**–**6** were characterized by amino acid composition and Fab-Mass for confirmation (Table 1).

Discussion

There is a growing interest in the use of octreotide conjugates as precursors in tumor-targeted radiopharmaceuticals. The present study demonstrates the feasibility of the use of *p*-carboxybenzaldehyde to anchor Fmoc-threoninol to solid-phase amine resin. This linker is compatible with the Fmoc peptide synthesis protocol and can be easily applied to larger scale preparation of octreotide derivatives. The first residue **B** can be easily and cost-effectively prepared from Fmoc-threonine prepared via the direct reaction of threonine with Fmoc-OSu.

Disulfide bond formation of the octreotide derivatives in a dilute solution was effective and avoided intermolecular disulfide bond cross linkage. Most importantly, DTPA, DOTA, DFO, biotin, and fluorescein can be directly incorporated with fully protected octreotide on the resin without the necessity for protecting the ε-amino group of Lys[5]-octreotide as reported previously,[36] thus avoiding a more complex procedure. The method presented in the present study provides a simplified synthesis and results in dramatically improved yields of conjugated octreotide analogues. These results may aid in the development of these compounds for use in the diagnosis and therapy of cancer.

Experimental

Fmoc protected amino acids were purchased from either Bachem or Novabiochem (Germany). Rink Amide AM resin (loading 0.56 mmol/g) was obtained from Novabiochem (Germany). Trifluoroacetic acid (TFA), tetrabutyl ammonium hydroxide (20% solution in water), and DOTA were from Merck Chemical Co. (Germany).

1 M BH_3/THF, toluenesulfonic acid, diethylenetriaminepentaacetatic acid and *p*-carboxybenzaldehyde were purchased from Aldrich Chemical Corporation (USA). Solvents were obtained from ALPS Chemical Co. (Taiwan).

Synthesis of *N*-Fmoc-threoninol (A). To a solution of *N*-Fmoc-L-threonine (*N*-Fmoc-L-Thr, 1.7 g, 5 mmol) dissolved in anhydrous THF (50 mL) was added dropwise of 1 M BH_3/THF (50 mL) through an additional funnel over a period of 10 min. The resulting mixture was then stirred at room temperature for 8 h and quenched by the addition of 1 N HCl (50 mL). After stirring at room temperature for 1 h, the mixture was diluted with water (40 mL) and followed by the addition of a mixture of THF:diethyl ether (1:1; 100 mL). The organic layer was separated and the aqueous layer was extracted with THF:ether (1:1; 2×60 mL). The combined organic layers were then washed with brine (2×125 mL), 1.5 N KOH (2×125 mL), and brine (2×125 mL), dried with Na_2SO_4, filtered, concentrated under vacuum, and purified by flash chromatography (ethyl acetate:*n*-hexane, 4:1) to afford white solid of *N*-Fmoc-L-threoninol (1.3 g, 80%; R_f 0.34 in ethyl acetate:*n*-hexane, 4:1). Mp 100–102°C; FABMS: *m*/*z* 328 (M + 1), 179, 178. ^{1}H NMR ($CDCl_3$) δ 7.73 (2H, d, 7.5 Hz), 7.57 (2H, d, 7.4 Hz), 7.37 (2H, t, 7.4 Hz), 7.28 (2H, t, 7.4 Hz), 5.58 (1H, d, 8.8 Hz), 4.43–4.37 (2H, m), 4.19–4.09 (2H, m), 3.73 (2H, d, 4.2 Hz), 3.58–3.50 (1H, br), 1.16 (3H, d, 6.3 Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$) δ 157.06, 143.75, 141.28, 141.24, 127.68, 127.03, 124.98, 119.95, 68.38, 66.71, 64.57, 56.05, 47.18, 20.22.

Synthesis of *N*-Fmoc-threoninol *p*-carboxybenzacetal (B). A mixture of *N*-Fmoc-threoninol (0.3 g, 0.9 mmol), *p*-carboxybenzaldehyde (0.34 g, 2.25 mmol), a few crystals of *p*-toluenesulfonic acid, and two drops of dimethylsulfoxide in $CHCl_3$ (30 mL) was heated overnight under nitrogen with a Dean–Stark trap to remove water. After the mixture cooled to room temperature, the solvent was evaporated under reduced pressure to provide yellowish oil. Flash chromatography of the residue over silica gel using methylene chloride:ethyl acetate (3:1) as eluent furnished the desired product (385 mg, 91%) ($R_f = 0.28$ in methylene chloride:ethyl acetate, 3:1). Mp 86–88°C; $[\alpha]_D^{25}$ −33° (*c* 0.02, dichloromethane). FABMS *m*/*z* 460 (M + 1), 179, 178, 154, 136. ^{1}H NMR ($CDCl_3$) δ 10.11 (1H, COOH), 8.13 (2H, d, 8.3 Hz), 7.75 (2H, d, 7.5 Hz), 7.60–7.55 (4H, m), 7.41–7.28 (4H, m), 5.63–5.56 (2H, m), 4.47–4.42 (2H, m), 4.25–4.12 (4H, m), 3.71 (1H, d, 4.2 Hz), 1.27 (3H, m, $-CH_3$); ^{13}C NMR ($CDCl_3$) δ 171.13, 156.54, 143.81, 143.05, 141.30, 130.21, 129.87, 127.70, 127.04, 126.13, 125.02, 119.98, 100.73, 75.44, 71.81, 66.96, 48.70, 47.24, 17.52.

Preparation of tri(tetrabutylammonium hydroxide) salt of 1,4,7,10-tetraazacyclo-dodecane *N,N′,N″,N‴*-tetraacetic acid (C). To a solution of 1,4,7,10-tetraazacyclododecane *N,N′,N″,N‴*-tetraacetic acid (2 mmol, 0.808 g) dissolved in water (10 mL) was added a solution of tetrabutylammonium hydroxide (0.75 mL, 6 mmol) with stirring until homogeneous. The solution was then lyophilized to afford a pale-yellowish oily residue of tri(tetrabutyl ammonium hydroxide) salt of DOTA. The salt was used directly without further purification.

Preparation of monoreactive DTPA derivative (D). The monoreactive DTPA derivative was prepared as the procedure reported by Arano et al.[38] Using diethylenetriamine as a starting material and following six reaction steps to provide expected monoreactive DTPA derivative as a yellowish oil in 24% total yield. FABMS *m*/*z* 618 (M + 1); ^{1}H NMR ($CDCl_3$) δ 5.17 (1H, br, COOH), 3.51 (2H, s, NCH_2COOH), 3.50 (2H, s, NCH_2COO-Bu^t), 3.48 (2H, s, NH_2COO-Bu^t), 3.45 (4H, s, NH_2COO-Bu^t), 2.83–3.01 (8H, m, $NCH_2CH_2NCH_2CH_2N$), 1.46–1.48 (36H, s, $-C(CH_3)_3$).

Solid-phase peptide synthesis of the peptides. Octreotide analogues were synthesized using FastMoc chemistry on Applied Biosystems (ABI) 433A peptide synthesizer. Fmoc protected amino acids were activated in situ in AA cartridges with HoBt/HBTU/DIEA in NMP. Four equivalent amounts of each amino acid derivative were used. Coupling yield of each amino acid coupling cycle was monitored by equipped conductivity sensor. The average coupling yield was also calculated from the increase in weight of the elongated peptide-resin divided by the weight of protected peptide.

Synthesis of octreotide 1. The sequence was synthesized on Rink Amide resin (loading 0.56 mmol/g resin). Resin (446 mg, 0.25 mmol) was washed with DCM:DMF (1:1) in reaction vessel for 20 min before starting the synthesis programme of the peptide synthesizer. *N*-Fmoc-threoninol *p*-carboxybenzacetal **B** (1 mmol, 459 mg) was put in the first AA cartridge followed by 1 mmol of each of all the other protected amino acids. The resin-bound sequence of this peptide was Fmoc-D-Phe-Cys(Trt)-Phe-D-Trp(Boc)-Lys(Boc)-Thr(Bu^t)-Cys(Trt)-Thr-ol-acetal-resin. After the final Fmoc deprotection with 20% piperidine and programmed wash procedure, the resin complex was dried in vacuo and weighted to be 899 mg. Its average coupling yield was computed to be 97% which was consistent with the recorded coupling yield at each step (higher than 95%) on the ABI 433A autosynthesizer. Peptide side chain deprotection and cleavage were carried out in TFA solution (containing TFA 9 mL, water 0.25 mL, thioanisole 0.5 mL, EDA 0.25 mL, and phenol 0.79 g). The dried side chain protected peptide-resin (899 mg) was added to 10 mL TFA solution at 0°C, and the mixture was stirred gently at room temperature for 1.5 h. After evaporation of TFA under vacuum, the peptide was precipitated by addition of ice-cold dry ether. The precipitate was filtered, washed with cold ether on a sintered glass funnel, extracted with 20% acetic acid solution, and dried by lyophilization to a white powder. RP-HPLC analysis (detail HPLC condition in the text) of the crude product at this stage showed a major peak at 30.1 min as monitored at UV 214 nm (Fig. 1). Dilute the peptide product to about 1 mM with 5% ammonium acetate solution and adjust pH to 7.0 with ammonium hydroxide (25%) to accomplish disulfide bond formation. During the folding process, an aliquot of 0.1 mL sample was taken at different times and injected to a Rainin (C18, 5 mm, 100A,

4.6×250 mm) analytical column for monitoring the disulfide oxidation of reduced octreotide. HPLC analysis showed the disulfide oxidation was above 90% complete in 46 h. After 48 h the solution was loaded onto a Vydac C18 preparative column and purified to give a white powder of octreotide **1** (193 mg). Amino acid analysis of octreotide after hydrolysis with 6 N HCl:TFA (4:1; v/v) for 3 h at 130°C was listed as: amino acid/found/ calcd, Lys/1.0/1; 1/2Cys/0.97/1; Phe/1.94/2; Thr/0.65/1. FABMS m/z 1019.5 $(M+H)^+$.

Synthesis of octreotide conjugated derivatives. Two 0.1 mmol scale and two 0.25-mmol scale of fully protected octreotide-resin was synthesized separately by the above-described procedure and used for conjugation experiments.

DTPA-octreotide 2. Coupling of DTPA to octreotide was operated on ABI 433A as previous described protocol. Fully side chain protected octreotide-resin (0.1 mmol scale) was first synthesized by the previous method to give 364 mg of resin complex. Four equivalent amount of DTPA derivative **D** (0.4 mmol, 247 mg) was added into AA cartridges and was used as the ninth AA residue. Using the same treatment, analysis and purification procedure for octreotide, 104 mg of pure DTPA-octreotide was obtained. FABMS m/z 1394.6 $(M+H)^+$.

Synthesis of DOTA-octreotide 3. Application of the same procedure above, except using tri(tetrabutyl ammonium hydroxide) salt of 1,4,7,10-tetraazacyclo-dodecane N,N',N'',N'''-tetraacetic acid **C** (200 mg) prepared previously as the ninth amino acid residue. Using the same treatment, analysis and purification procedure for octreotide, DOTA-octreotide **3** was obtained in 99 mg. FABMS m/z 1405.6 $(M+H)^+$.

Synthesis of DFO-octreotide 4. 0.25 mmol of side chain protected octreotide-resin, synthesized previously (weighted 885 mg), was first incubated with 1 mmol of succinic anhydride to produce 4-carboxypropionyl octreotide on the autosynthesizer, followed by coupling DFO (1 mmol, 660 mg was used) to 4-carboxypropionyl octreotide with 433A preset loading module (DCC method). Following the same treatment, analysis and purification procedure for octreotide, pure DFO-octreotide was obtained in 272 mg. FABMS m/z 1756.5 $(M+H)^+$.

Synthesis of biotin-octreotide 5. The procedure was exactly as that for DTPA-octreotide **2** but using biotin (0.25 mmol, 61 mg) as the ninth residue. 220 mg of side chain protected octreotide-resin, synthesized previously, was weighted and used for the synthesis of biotin-octreotide **5**. The yield after HPLC purification was 59 mg. FABMS m/z 1245.4 $(M+H)^+$.

Synthesis of fluorescein-octreotide 6. 440 mg of side chain protected octreotide-resin, synthesized previously, was weighted and used for the synthesis of fluorescein-octreotide **5**. The yield after HPLC purification was 119 mg. FABMS m/z 1323 $(M+H)^+$.

Purification and characterization of peptides. Analytical and preparative RP-HPLC were performed on a Hitachi L-6200A system equipped with L-6000 pump and L-4250 UV–vis detector. The RP columns of Rainin (C18, 5 mm, 100A, 4.6×250 mm) analytical column and Vydac (C18, 22×250 mm) preparative column was used. For analysis of the synthesized octreotide derivatives by RP-HPLC, the elution gradient of 0–100–100–0% **B** in 0–30–35–40 min at a flow rate of 1.0 mL/min was utilized, where **A** was 5% acetonitrile aqueous with 0.1% TFA and **B** was 90% acetonitrile aqueous with 0.1% TFA. For monitoring the folding of reduced octreotide, the elution gradient of 50–60–60–50% **B** in 0–20–25–30 min at a flow rate of 1.0 mL/min was used, where **A** was 5% acetonitrile aqueous with 0.1% TFA and **B** was 90% acetonitrile aqueous with 0.1% TFA. Peptide purification was conducted on a Vydac C18 preparative column. The peptide solution was first loaded to the column with a flow rate of 4 mL/min then collected by flowing 75% of acetonitrile aqueous through the column at a flow rate of 8 mL/min, monitored at 280 nm. Amino acid analysis and FABMS were performed at the Precision Instrument Development center of the National Science Council, Taiwan.

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