Total Solid-Phase Synthesis of 1,4,7,10-Tetraazacyclododecane- N , N ‘ , N ‘ ‘ , N ‘ ‘ ‘ -tetraacetic Acid-Functionalized Peptides for Radioimmunotherapy

ARTICLE *in* BIOCONJUGATE CHEMISTRY · MARCH 1999

Impact Factor: 4.51 · DOI: 10.1021/bc980118t · Source: PubMed

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Total Solid-Phase Synthesis of 1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic Acid-Functionalized Peptides for Radioimmunotherapy

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Received September 25, 1998; Revised Manuscript Received December 12, 1998

A convenient approach to the functionalization of peptides with the macrocyclic 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) moiety has been developed. Protected components (using *tert*-butyl or *tert*-butyloxycarbonyl groups) of both the peptide and the chelate were assembled on the same solid resin support. Deprotection and cleavage of the resin-bound DOTA−peptides were performed in one step using a trifluoroacetic acid cleavage mixture to yield free DOTA−peptide amides.

INTRODUCTION

Bifunctional chelating agents derived from the macrocyclic 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA)[1] have been used for studies in vivo due to the extraordinary stability of its complexes with many useful metals under physiologic conditions (*1*−*4*). Introduction of peptide/protein conjugation functionality to DOTA can originate either from the ring methylene carbons (*4*−*6*) or, more simply, through one of the four carboxylates of the macrocycle (*3*, *6*, *10*).[2] For the latter strategy, activation of a carboxylate group with isobutyl chloroformate (*7*−*9*) allows conjugation to a peptide (*10*). The DOTA should be monofunctionalized to prevent cross-linking of peptides. One disadvantage of this method is the low solubility of DOTA (<20 mM) (*8*, *10*) in anhydrous organic solvents.[3] We were able to circumvent this difficulty by synthesizing DOTA stepwise on the peptide N-terminus, using solid-phase synthesis methodology (Scheme 1). The selectivity in the *alkylation* of polyazamacrocycles (*12*) and the high solubility (>4 M) of the macrocycle cyclen in an appropriate organic solvent were also important.

EXPERIMENTAL PROCEDURES

Reagents. Fmoc-protected amino acids and BOP reagent for manual synthesis were purchased from BACHEM California, Inc. Fmoc-protected amino acids in 1 mmol cartridges, HMP resin, and reagents for automated synthesis (using "FastMoc" chemistry) were purchased from ABI. Cyclen sulfate, anhydrous NMP, anhydrous DMF, bromoacetyl bromide, chloroacetyl chloride, *tert*-butyl-2-bromoacetate, redistilled DIEA, piperidine, acetic anhydride, NBP, and HOBt were purchased from Aldrich Chemical Co. PEGA Rink amide resin was purchased from Nova-Biochem. Polypropylene chromatography columns (fritted) used for solid-phase peptide synthesis were purchased from Bio-Rad (parts 731-0003 and 732-1010). TFA and TIPS were purchased from Acros Organics or Fisher Scientific. Ammonia gas was purchased from Matheson. All buffers and cleavage cocktails were made using pure water (18 MΩ cm^{-1} resistivity). The ninhydrin test kit was purchased from ABI.

High-Performance Liquid Chromatography. For analytical HPLC, a Dynamax 60 Å (8 μm, 4.6 mm × 250 mm) C_{18} reversed-phase column was used at a flow rate of 1 mL/min. For semipreparative HPLC, a Phenomenex Magellan 100 Å (5 μm, 10 mm × 250 mm) C_{18} reversed-phase column was used at a flow rate of 3 mL/min. HPLC buffers are as follows: buffer system A, 0.068 M NH_4OAc (pH 4.8) versus CH_3CN; and buffer system B, 0.1% TFA/H_2O versus 0.1% TFA/CH_3CN. Eluting samples were detected at 280 nm unless otherwise indicated. For gradient 1, hold at 0% CH_3CN for 5 min and proceed to 50% by 35 min. For gradient 2, hold at 20% CH_3CN for 5 min and proceed to 50% by 23 min. For gradient 3, hold at 25% CH_3CN for 5 min and proceed to 50% by 25 min. For gradient 4, hold at 0% CH_3CN for 5 min, proceed to 50% in 10 min, and proceed to 70% in 15 min. Samples collected during HPLC were lyophilized at least twice on a Savant model SVC-100H Speed-Vac lyophilizer before analysis.

NMR Spectroscopy. ^1H NMR spectra were recorded on a General Electric QE 300 spectrometer at 300 MHz. Chemical shifts are reported relative to HDO (4.80 ppm) or $CHCl_3$ (7.26 ppm).

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[1]Abbreviations: ABI, Applied Biosystems, Inc.; BOP, (benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate; cyclen, 1,4,7,10-tetraazacyclododecane; DCM, dichloromethane; DIEA, diisopropylethylamine; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; ESI-MS, electrospray ionization mass spectrometry; Et_2O, diethyl ether; F*, (*S*)-*p*-nitrophenylalanine; Fmoc, fluoren-9-ylmethyloxycarbonyl; HMP, hydroxymethylphenoxy resin; HOBt, 1-hydroxybenzotriazole; NMP, 1-methyl-2-pyrrolidone; NBP, (nitrobenzyl)pyridine; NO_2Phe, (*S*)-*p*-nitrophenylalanine; PEG, polyethylene glycol; PEGA, bis(2-acrylamidoprop-1-yl) polyethylene glycol cross-linked dimethylacrylamide and mono(2-acrylamidoprop-1-yl)-(2-aminoprop-1-yl) polyethylene glycol (800); SPPS, solid-phase peptide synthesis; TIPS, triisopropylsilane.

[2]This is a very practical alternative, since the resulting loss of metal−chelate stability (*8*) has been found to be insignificant in serum (with ^{111}In or ^{90}Y), or when subjected to DTPA challenge (*3*).

[3]The purity and counterion form of DOTA must be carefully defined and reliable. For example, we purchased DOTA that was only 25% pure as determined with a ^{57}Co metal binding assay (*11*).

10.1021/bc980118t CCC: $18.00
Published on Web 02/11/1999

Scheme 1. Solid-Phase Synthesis of DOTA−Peptides

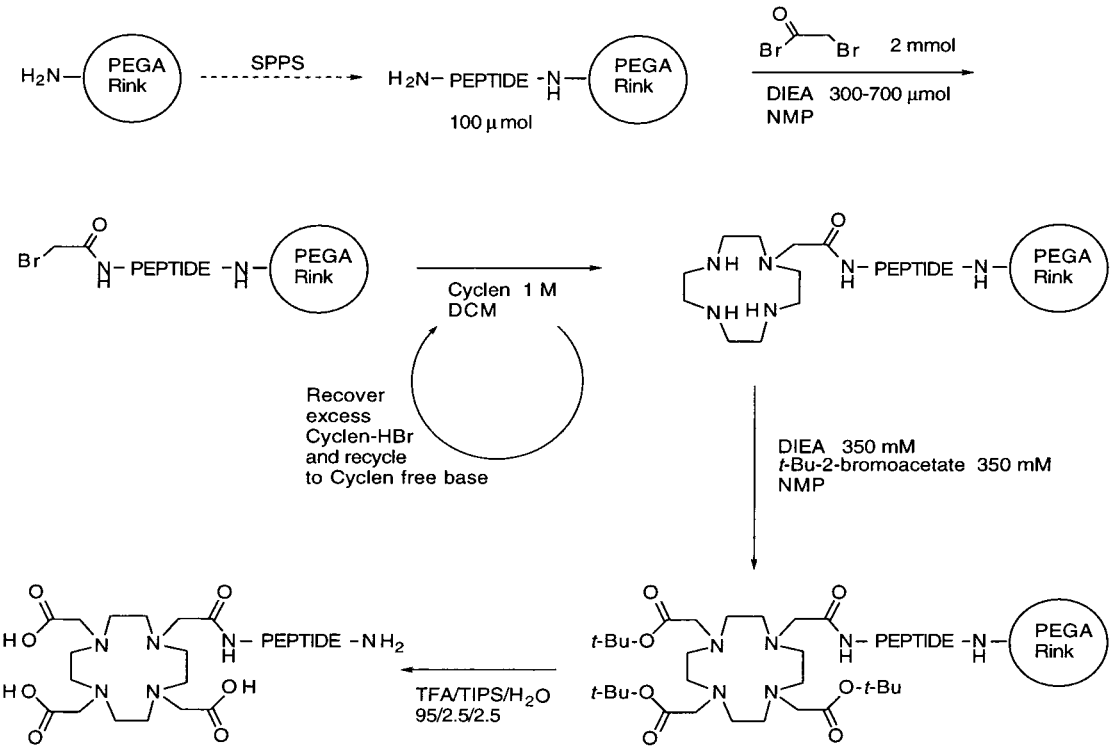

Table 1. Sequence, HPLC, and ESI-MS Data of DOTA−Peptide Amides

compd/sequence	purity of crude product by HPLC (%)[a]	HPLC retention time (min)[b]	purified yield (%)[c]	ESI-MS $[M + H]^+$ *m/z* (calcd/found)
1/DOTA−GGGF*-NH_2	65−84	14.8	32	767.3/768.0
2/DOTA−GFQGVQFAGF*-NH_2	40−60	27.4	20	1486.7/1486.0
3/DOTA−GFGSVQFAGF*-NH_2	40−75	26.8	12	1446.7/1445.0
4/DOTA−GFGSTFFAGF*-NH_2	58−87	28.3	20	1467.7/1468.0
5/DOTA−GLVGGAGAGF*-NH_2	62−63	23.0	31	1235.6/1236.0
6[d]/DOTA−GGVLRAGFK-NH_2	48	23.7	37	667.8/667.6[e]
7/DOTA−GGFLGLGAGF*-NH_2	52−84	29.0	23	1325.7/1325.7
8/DOTA−KLLKLLLKLYKKLLKLLK-NH_2	28	20.1	12	2594.6/2594.4[f]
9[d]/DOTA−GKLLKLLLKL*p*YKKLLKLLK-NH_2	47	19.3	18	2729.8/2730.0[f]

[a] Range of purities found by integration of the peak area of an HPLC trace (detection at 280 nm) for different batches (at least two) of each DOTA−peptide. [b] For peptides **1**−**7**, we used gradient 1 and buffer system A, while for peptides **8** and **9**, we used gradient 4 and buffer system B. [c] The purified yield is based on the substitution level of completed peptide resins. [d] Milder conditions used for synthesis (chloroacetyl chloride). [e] Mass calculations are for the $[M + 2H]^{2+}$ species. [f] Due to a distribution of charged states for this species, the spectrum was deconvoluted using MaxEnt software to determine the mass on the true mass scale.

Mass Spectrometry. ESI-MS was performed on a Fisons Instruments VG Quattro triple-quadrupole spectrometer using an eluent of 0.1% HCOOH/49.95% H_2O/49.95% CH_3CN at a flow rate of 5 μL/min. The cone voltage was set to 50 V. The source temperature was set to 80 °C. Calibration of the mass scale was performed using a solution of PEG 300, PEG 600, and PEG 1000 as a calibration standard. Molecular weights of multiply charged peptides were determined by mathematical transformation (deconvolution) of the electrospray spectra to a true mass scale spectrum using the maximum entropy transformation algorithm MaxEnt.

Peptide Synthesis. Peptides were synthesized using either a multiple parallel manual synthesis apparatus (peptides **1**−**7**, see Table 1) or an ABI model 430A automated peptide synthesizer (peptides **8** and **9**). All parent peptides were fully characterized by amino acid analysis and mass spectrometry before the parent peptide−resin was subjected to DOTA functionalization.

Parallel Manual Synthesis of Peptide−Resins 1−7. Using the multiple synthesis apparatus described previously (*13*), 17 peptides were manually synthesized in parallel within 10 days. Standard Fmoc SPPS was carried out according to the preactivation method of Hudson et al. (*14*). A simple method of assaying the activity of the HOBt esters used for SPPS was also employed (*13*).

PEGA Rink resin (10 g, 2 mmol, stored in CH_3OH) was delivered to a 250 mL polypropylene-fritted reaction vessel fashioned from a Bio-Rad Polyprep Column. Dry argon was introduced to the reaction column from below the frit to bubble-stir the slurry using the frit as a bubbler. The resin was washed with 4 × 60 mL of DCM (5 min/wash) and then with 5 × 60 mL of DMF (5 min/wash). Solvent was drained through the frit into a vacuum filter flask using positive argon pressure from above the swollen resin. Fmoc-L-NO_2Phe-OH (4.51 g, 10.4 mmol) and HOBt (1.41 g, 10.4 mmol) were placed in a dry pear-shaped flask and dissolved in 25 mL of dry DMF. To this solution was added 2.61 mL of redistilled DIEA (15 mmol). BOP reagent (5 g, 11.3 mmol) was dissolved in a separate dry pear-shaped flask with 5 mL

of dry DMF and syringed into the Fmoc-L-NO_2Phe-OH solution. After preactivation for 10 min, a 20 μL aliquot containing the active ester was checked for acylating activity and the Fmoc-L-NO_2Phe active ester solution was added to the swollen resin in the polypropylene reaction column. After 2.5 h, the peptide−resin tested nearly negative for free amines (<5 μmol/g) as determined by using the ABI ninhydrin test kit. After 3 h, the resin was washed with 5 × 60 mL of dry DMF and residual amines were capped using 2 mL of acetic anhydride (21 mmol) and 1 mL of DIEA (5.7 mmol) in a total slurry volume of 125 mL. After 1.5 h, the resin was washed with 5 × 60 mL of DMF (5 min/wash) and then treated with 20% piperidine in DMF in a total slurry volume of 125 mL for 20 min. The resin was then washed with 5 × 100 mL of DMF (5 min/wash).

Two more cycles of coupling and deprotection as described above were utilized until the common resin L-Ala-Gly-L-NO_2Phe-PEGA Rink was constructed. The resin was then portioned equally into 17 smaller 50 mL reaction vessels fashioned from 50 mL polypropylene conical flasks and Bio-Rad disposable polypropylene columns (part 732-1010). These reaction vessels were fitted to an 18-reaction vessel capacity multiple SPPS apparatus, and the balance of each peptide was synthesized in parallel at a rate of one amino acid per day. Each night, resins were stored in DCM in their amino-terminal form. At the conclusion of the syntheses, resins were stored in their amino-terminal form in ethanol at −20 °C. Resins were not dried, but stored swollen in solvent according to the manufacturer's suggestions. Completed resins were found to swell to 0.16 g/mL in DMF (average of four measurements). To determine the final substitution level for each resin, ninhydrin analyses (average of five measurements) were performed at the conclusion of the syntheses.

Synthesis of Peptide−Resins 8 and 9 by Automated Peptide Synthesis. Two peptide−resins were synthesized on an ABI model 430A automated peptide synthesizer on a 0.1 mmol scale using the manufacturer's "FastMoc" methodology, which involves HBTU activation of amino acids. Solid support was PEGA Rink amide resin (0.05 mmol/g). An automated resin sampler was programmed to aliquot samples of resin after coupling steps were completed. These resin samples were typically in the range of 3−5 mg and were tested by ninhydrin analysis (ABI test kit) to determine the completeness of coupling which was determined to be >98% for all steps. Peptide−resin **9**, Fmoc-GKLLKLLLKL*p*YKKLLKLLK−PEGA Rink, was initially synthesized with a free hydroxyl on the Tyr residue, which was subsequently protected as the *tert*-butyl phosphotriester upon global phosphorylation using the method of Perich (*15*). All other Fmoc-amino acids were incorporated with side chain protecting groups. After synthesis was complete, Fmoc-peptide−resins were stored at −20 °C in anhydrous NMP until further use. Immediately prior to subsequent alkylation steps, the Fmoc-peptide−resins were amino-terminus deprotected by treatment with 5 mL of 20% piperidine/DMF for 5 min followed by another treatment for 10 min. The peptide−resin was then washed with 5 × 5 mL of DMF (5 min/wash) and 5 × 5 mL of NMP (5 min/wash).

Preparation of Cyclen Free Base. Cyclen sulfate (5.12 g, 13.9 mmol) was stirred vigorously in DCM (125 mL) in a 250 mL round-bottom flask fitted to a reflux condenser and septum for the gas inlet tube and vent needle. Ammonia gas was filtered and dried through a 300 mm × 50 mm column of KOH pellets and bubbled into the suspension (being stirred) for 9 h. The precipitated $(NH_4)_2SO_4$ was filtered off, and the supernatant was again treated with ammonia gas for an additional 2 h. The flask was sealed and the mixture allowed to stir overnight. A second batch of $(NH_4)_2SO_4$ precipitate was filtered off, and the supernatant was rotary evaporated to give white cyclen free base in quantitative yield. ^{1}H NMR ($CDCl_3$): δ 2.61 (s). ESI-MS: *m*/*z* calcd $(M + H)^+$ 173.2, found 173.5.

Cyclen Recovery Procedure. Excess cyclen in DCM, which had been recovered after reaction with alkylating peptide resin (see below), was concentrated by rotary evaporation and recycled to the free base. As an example, 1.6 g of recovered cyclen oil was taken up in 80 mL of CH_3OH, and 15 mL of concentrated HCl was added dropwise. The mixture was then stirred for 6 h, and the CH_3OH was rotary evaporated. Cyclen hydrochloride was then converted to the free base in the same manner as cyclen sulfate (see above). Peptide **2** DOTA−GFQGVQ-FAGF*-NH_2 was synthesized using recycled cyclen.

General Synthesis of DOTA−Peptides. A general method for the synthesis of DOTA−peptides is shown by example using the synthesis of DOTA−GGFLGLGAGF*-NH_2. All other DOTA−peptides followed a similar synthetic procedure.

Step 1. Acylation with Bromoacetyl Bromide. NH_2-GGFLGLGAGF*−PEGA Rink resin (0.59 g, 92 μmol) was washed with 5 × 5 mL of dry NMP. The resin slurry volume was adjusted to 5.8 mL with NMP and vortex mixed. To the vortexing slurry were added 80 μL (460 μmol) of DIEA, and then 180 μL (2 mmol) of bromoacetyl bromide. The resin was vortex mixed for 1 h, and washed with 5 × 5 mL of dry NMP and 10 × 5 mL of dry DCM. A 5 mg sample of resin was assayed by ninhydrin for free amines and found to be negative for free amines (<5 μmol/g). A 1 mg sample was removed and treated with NBP (3 g in 60 mL of acetone). The yellow resin turned royal blue upon treatment with NH_3(g), indicating the presence of alkylating species (the reactive bromoacetyl moiety).

Alternate Step 1. Acylation with Chloroacetyl Chloride. NH_2-GGVLRAGFK−PEGA Rink resin (0.1 g, 16 μmol) was washed with 5 × 2 mL of dry NMP. The resin slurry volume was adjusted to 1.1 mL and the slurry vortex mixed. To the vortexing slurry were added 80 μL (115 μmol) of DIEA, and then 29 μL (364 μmol) of chloroacetyl chloride. The resin was vortex mixed for 72 min, and then washed with 5 × 2 mL of dry NMP and 10 × 2 mL of dry DMSO. A 5 mg sample of resin was assayed by ninhydrin for free amines and found to be negative for free amines (<10 μmol/g). A 1 mg sample was removed and soaked with NBP (3 g in 60 mL of acetone) for 30 min at 35 °C. The yellow resin turned violet upon treatment with NH_3(g), indicating the presence of alkylating species (the reactive chloroacetyl moiety).

Step 2. Immobilization of Cyclen by the Haloacetyl-Peptide−Resin. The bromoacetyl-GGFLGLGAGF*−PEGA Rink resin was gravity drained and vortex mixed. Over a period of less than 10 s, 1.4 mL of 4.3 M cyclen free base in dry DCM was added to the resin that was being vortexed. This gave a total volume of 5.4 mL for a final cyclen concentration of 1.1 M. The slurry was vortex mixed for 2.5 h, checked for alkylating species via NBP test (negative), and washed with 6 × 5 mL of dry DCM (the washes pooled for the recycling procedure), 5 × 5 mL of dry DMSO (to remove any insoluble cyclen mixed salts), and 5 × 5 mL of dry NMP. A 43 mg sample of the cyclen−peptide−resin was removed, washed with 8 × 5 mL of DCM, and dried with flowing argon gas. Standard

peptide cleavage (see below) resulted in a product that was purified on HPLC (gradient 1, buffer system A). The major peak was collected and determined to be cyclen−GGFLGLGAGF*-NH_2. HPLC purity: 69%. ESI-MS: *m/z* calcd for $C_{53}H_{82}N_{16}O_{13}$ $(M + H)^+$ 1151.7, found 1151.0; calcd for $(M + 2H)^{2+}$ 576.4, found 576.2.

Step 3. Alkylation of Immobilized Cyclen−Peptide with tert-Butyl 2-Bromoacetate. The slurry volume of cyclen−GGFLGLGAGF*−PEGA Rink resin was adjusted to 5.3 mL with dry NMP. DIEA (366 μL, 2.1 mmol) and then *tert*-butyl 2-bromoacetate (310 μL, 2.1 mmol) were added to give final concentrations of 0.35 M. The resin was vortex mixed for 13.5 h, and then washed with 5 × 5 mL of DMF and 6 × 5 mL of DCM. An analytical sample of resin was removed and the product cleaved from the resin (see below). After collection of product fractions from C_{18} HPLC (gradient 1, buffer system A), ESI-MS of the cleaved DOTA−peptide amide indicated completion of the reaction and the absence of significant impurity corresponding to incomplete alkylation. The remaining resin was then dried by flowing argon over the drained bed for several hours.

Step 4. Cleavage of the DOTA−Peptide Amide from Solid Support and HPLC Purification. The dried DOTA−peptide−resin was weighed (0.576 g, 82 μmol), and a cleavage cocktail of TFA, TIPS, and H_2O (95:2.5:2.5, v/v) was added (10 mL). After vortex mixing for 2.5 h, the eluent was collected and the resin was washed with 5 × 4 mL of cocktail. The combined eluents were rotary evaporated to approximately 500 μL. Cold Et_2O (10 mL) was added and the solution cooled for 30 min at −20 °C. The precipitate was centrifuged at 800*g* at 4 °C for 5 min. The Et_2O was decanted and the pellet resuspended in another 10 mL of cold Et_2O. This centrifuge/wash procedure was repeated for a total of five washes. The precipitate was dried under vacuum and weighed (70 mg). The precipitate was taken up in 5 mL of 20% DMSO/water, filtered through a 0.45 μm membrane, and HPLC analyzed using gradient 1 with buffer system A on a Dynamax analytical C_{18} column. The major peak (retention time of 29 min) comprised 54% of the integrated chromatogram. The crude material was HPLC purified using gradient 2 with buffer system A on a Magellan C_{18} semipreparative column (retention time of 13 min). The product was lyophilized twice. The yield was 25 mg, or 19 μmol (23% based on ninhydrin-quantitated substitution of 156 μmol/g × 0.91 for *t*-Bu-DOTA−GGFLGL-GAGF*−resin).[4] ESI-MS: *m/z* calcd for $C_{59}H_{88}N_{16}O_{19}$ $(M + H)^+$ 1325.6, found 1325.7; calcd for $(M + 2H)^{2+}$ 663.3, found 663.7. Amino acid analysis confirmed the correct composition of amino acids. A stock solution of DOTA−GGFLGLGAGF*-NH_2 was prepared in 1:1 DMSO/0.1 M triethylammonium acetate, and the concentration was determined with a ^{57}Co metal binding assay (11.7 mM). This value compared well with the spectrophotometrically determined value (13.4 mM) obtained from absorbance at 280 nm when 2.5 μL of this stock was diluted in 500 μL of 0.1 M sodium phosphate (pH 7.0) using an ϵ of 9050 M^{-1} cm^{-1} (280 nm) for *p*-nitrophenylalanine at pH 7.

RESULTS

A variety of peptide−resins were synthesized, by manual and automated methods, for use as scaffolds for the construction of the DOTA moiety on their amino termini. The crude unmodified peptides, when cleaved from the resin, were typically >90% pure as determined by HPLC.

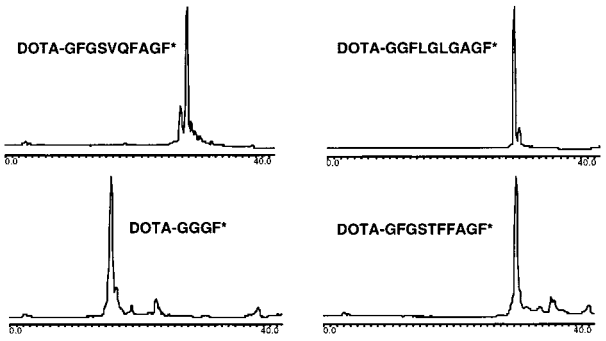

Figure 1. HPLC chromatograms of the crude cleavage products for several DOTA−peptide amides on an analytical C_{18} column. All DOTA−peptide amides were deprotected and cleaved from PEGA Rink amide resin using a cleavage cocktail of 95% TFA/2.5% TIPS/2.5% H_2O. After precipitation from cold Et_2O, DOTA−peptides were dissolved in 20% DMSO/H_2O and filtered before HPLC analysis. The major peak in each chromatogram was determined to be the desired product.

Functionalization of the amino terminus of the peptide−resin was provided through the use of the bifunctional bromoacetyl bromide or chloroacetyl chloride. Since the acyl halide functionality is much more reactive than the alkyl halide with respect to amine nucleophilic attack, acylation was the preferred reaction pathway. Initial acylation experiments using peptide−HMP resins (polystyrene-based) followed by cyclen alkylation resulted in a complex mixture of byproducts. Analysis of the bromoacetyl-peptide−HMP resins using 1.1 M NH_3 as the nucleophile showed that simple polystyrene-based peptide−resins treated with bromoacetyl bromide were poor alkylating agents in DCM when compared to peptide−poly(ethylene glycol) acrylamide resins. We therefore used the PEGA−Rink resins as the solid phase for our peptide syntheses.

Milder reaction conditions in which chloroacetyl chloride was used were also successful, despite the less reactive chloroacetyl electrophile. Preliminary tests of the alkylation step in which NH_3 was used as the nucleophile showed that the chloroacetyl group is significantly less reactive toward NH_3 than the bromoacetyl group in DCM. Nevertheless, alkylation of cyclen by the chloroacetyl group was accomplished in both DMSO and DCM. Recovery of unreacted cyclen was further simplified by the fact that the resulting cyclen mixed salt was in the hydrochloride form and required one less step for recycling to the free base form.

During the cyclen alkylation step, it was feared that a considerable amount of cross-linked peptide−cyclen−peptide products might have resulted since cyclen has four nucleophilic sites. However, products such as peptide−DOTA−peptide were not detected by mass spectrometry (sub-picomole limits of detection). Rather, the most significant impurity for the multistep DOTA functionalization of peptide−resin was typically one with a mass corresponding to underalkylation of immobilized cyclen−peptide by *tert*-butyl 2-bromoacetate (i.e., two groups instead of three). The purity of the cleaved DOTA−peptides as measured by integration of their HPLC chromatograms was high enough to yield useful amounts of pure material. Table 1 displays the sequences, HPLC purities, yields, and ESI-MS data of the DOTA−peptide amides. Figure 1 displays the HPLC

[4]The factor of 0.91 is derived from the fact that *t*-Bu-DOTA adds to the weight of the peptide resin, and the substitution level of 156.1 μmol/g must be adjusted accordingly to 142 μmol/g.

chromatograms of several of these DOTA−peptide amides.

DISCUSSION

Peptide linkers can potentially improve the efficacy of targeting radionuclides to tumors (*10*, *13*); practical synthesis of peptides bearing strong metal-binding groups makes it possible to test various strategies efficiently. A combined procedure where both the DOTA and the DOTA-linked peptide amide are synthesized on a single support offers many advantages. The peptidyl solid support can be thought of as a purification column, eliminating the costly and laborious HPLC steps utilized to purify the chelate, the peptide, and the chelate-modified peptide. The time to synthesize one DOTA−peptide amide is significantly reduced, since byproducts and unreacted starting materials are simply eluted during the wash steps. The procedure can take as little as 4 days from bare PEGA Rink resin to HPLC-purified DOTA−peptide.

The gravimetric yields of DOTA−peptide realized with this method are not yet characteristic of what can be expected of SPPS. For example, solid-phase synthesis yields of the peptides alone are typically >90%. However, the DOTA−peptide yields are comparable to those realized with other methods where only one of multiple sites on a macrocycle is modified. Using a solid-phase chemistry solvent such as DCM provides practical advantages for the use of cyclen in our synthetic route. Not only does the resin swell effectively in DCM, but also cyclen is highly soluble in this solvent, allowing us to use the principle of excess and avoid cross-linking. The recovery of unreacted cyclen is also easily and rapidly accomplished in DCM. The use of *tert*-butyl 2-bromoacetate (*16*) affords a carboxyl-protected DOTA moiety which, when utilized in an orthogonal protection scheme, allows further manipulation of the resin-bound peptide. This may prove useful in the synthesis of peptides with multiple functionalities.

The variety of peptides that have been DOTA-functionalized on the solid phase testifies to the general utility of this synthetic route. Milligram quantities of DOTA peptides have been rapidly and efficiently made and purified. These DOTA−peptides can be further investigated for use in radioimmunoconjugates or targeted delivery vehicles for radioisotopes.

ACKNOWLEDGMENT

This work was supported by NIH Research Grants CA16861 (C.F.M.) and CA47829 (G. L. DeNardo, principal investigator). R.H.P. was supported by NIH Postdoctoral Fellowship CA71177. We thank Dr. Vincent Jacques for helpful discussions.

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BC980118T