Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 1193-1204 (2000)

SYNTHESIS AND ^{99m}Tc-LABELLING OF bz-MAG3-TRIPROLINYL-PEPTIDES, THEIR RADIOCHEMICAL EVALUATION AND IN VITRO RECEPTOR-BINDING

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SUMMARY

Here we describe a convenient and general solid-phase synthetic method to prepare bifunctional peptides for efficient complexation with ^{99m}Tc. This approach permits the reproducible, efficient, and convenient preparation of very pure (typically >90%) peptide-chelator-radiometal complexes by incorporating a triproline (P_3) spacer between a benzoyl-protected mercaptoacetyltriglycine (bz-MAG3) bifunctional chelating agent (BFCA) and the targeting peptide. Synthesis and radiochemical evaluation of a number of different bz-MAG3-P_3-peptide conjugates show that they can be efficiently labeled with ^{99m}Tc under mild conditions, can bind to their cellular targets, are resistant to cysteine challenge, and show good stability in plasma.

Key Words: Technetium-99m, peptide synthesis, radiolabeling, nuclear medicine, MAG3, radiopharmaceutical

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Received 6 April 2000
Reised 21 June 2000
Accepted 10 July 2000

INTRODUCTION

Over the last twenty years, many researchers in nuclear medicine have endeavored to label receptor-binding peptides with ^{99m}Tc, in the interest of developing targetable, pharmacokinetically favorable radioimaging agents.[1,2] Unfortunately, many peptide-chelator conjugates can give rise to multiple isomers upon complexation with ^{99m}Tc. These isomers typically can only be separated by sophisticated and time-consuming methods such as HPLC. The short half-life of ^{99m}Tc, however, makes post-labeling HPLC purification impractical in a clinical setting.[3] One strategy for reducing the number of isomeric species generated when radiolabeling peptides is to incorporate a linker between the targeting peptide and the BFCA,[4,5] thus reducing the potential for misdirected chelation. A second strategy is to use an achiral, polydentate BFCA to chelate the technetium core, thus restricting the formation of isomeric species to *syn* and *anti* forms.[3] Here we describe a convenient solid phase peptide synthesis (SPPS) method that incorporates both of these strategies to produce stable, efficiently labeled (<60 min, >90% labeling yield), and radiochemically pure (>90%) technetium-labeled radiopharmaceutical complexes of several bioactive peptides. We also show that these peptides retain biological activity and desirable technetium chelating properties.

BFCA-Peptide	Sequence
bz-MAG3-P_0-$(KLA)_3$	bz-mAc G G G ——— K L A K L A K L A NH_2
bz-MAG3-P_1-$(KLA)_3$	bz-mAc G G G P —— K L A K L A K L A NH_2
bz-MAG3-P_2-$(KLA)_3$	bz-mAc G G G P P — K L A K L A K L A NH_2
bz-MAG3-P_3-$(KLA)_3$	bz-mAc G G G P P P K L A K L A K L A NH_2
Bombesin	pE Q R L G N Q W A V G H L M NH_2
bz-MAG3-Bombesin	bz-mAc G G G ——— Q R L G N Q W A V G H L M NH_2
bz-MAG3-P_3-Bombesin	bz-mAc G G G P P P Q R L G N Q W A V G H L M NH_2
αM2	Y C A R E P P T R T F A Y G NH_2
bz-MAG3-P_3-αM2	bz-mAc G G G P P P Y C A R E P P T R T F A Y G NH_2
bz-MAG3-P_3-αM2-a	bz-mAc G G G P P P Y A A R E P P T R T F A Y G NH_2

Table 1. Peptide sequences used in this study. The sequences have been aligned to better display the common and modified regions.

RESULTS AND DISCUSSION

Chelator Selection

In this study, we chose to use the S-benzoyl protected derivative of mercaptoacetyltriglycine (MAG3) as the thiol-protected BFCA. MAG3 is a

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well-known, FDA-approved N_3S amidothiol-based ^{99m}Tc chelator and has been used as a renal imaging agent for a number of years.[6] Besides its achiral structure and polydentate binding character, MAG3 possesses many additional beneficial qualities as a preformed chelator including: high labeling yield, excellent solution stability, high resistance to transchelation by cysteine, and long shelf-life.[7] We found the S-benzoyl protected mercaptoacetic acid to be completely stable to SPPS coupling conditions and TFA cleavage, as judged by analytical reversed-phase HPLC and ES-MS. As a consequence, bz-MAG3 can be directly incorporated into peptide sequences from its subunits using Fmoc-glycine-OH and benzoylmercaptoacetic acid. However, the benzoyl group's thioester linkage is not stable in conditions required for Fmoc deprotection (20%piperidine in DMF), therefore the benzoylmercaptoacetylation step must be restricted to the last step of peptide synthesis. It should also be noted that exposure of the thioester to the alkaline conditions (pH 8-12) used during technetium labeling could theoretically result in an S→N transfer of the benzoyl group from MAG3 to any side chain amino groups present in the targeting peptide, leading to peptide inactivation. However, as seen below, this phenomenon was not observed.

Linker Selection

Linker composition is an important consideration in the design of peptide based radiopharmaceuticals, as it can modify the pharmacokinetic profile and receptor-binding capability of the final construct.[1] Examples of linkers used successfully to improve radiochemical purity include 6-aminocaproic acid[4] and 4-aminobutyric acid.[5] For this study we chose to use multiple proline residues as a linker, primarily for its low cost, amenability to SPPS, non-chelating imide backbone structure, and biocompatibility.

The minimum number of proline residues required to isolate the MAG3 chelator from the targeting peptide was determined by building proline linkers of increasing length onto the N-terminus of a model peptide with the sequence K-L-A-K-L-A-K-L-A-NH_2, appending the spacers with bz-MAG3, and then characterizing the ^{99m}Tc-labeled BFCA-peptide by radio-HPLC. The results of these studies are presented in Figure 1. The chromatograms display a clear trend of increasing radiochemical purity with increasing proline number, and suggest that a minimum spacer length of three proline residues is required in order to achieve >90% radiochemical purity.

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The multiple lysine residues present in this sequence allow one to estimate the degree of benzoyl S→N transfer during labeling. If substantial lysine amino benzoylation were to occur, a population of peaks should appear in the HPLC traces. The presence of a single radiochemical peak (Figure 4d), and corresponding UV trace (data not shown) suggests that side chain amino benzoylation is not significant under the alkaline labeling conditions used in this study.

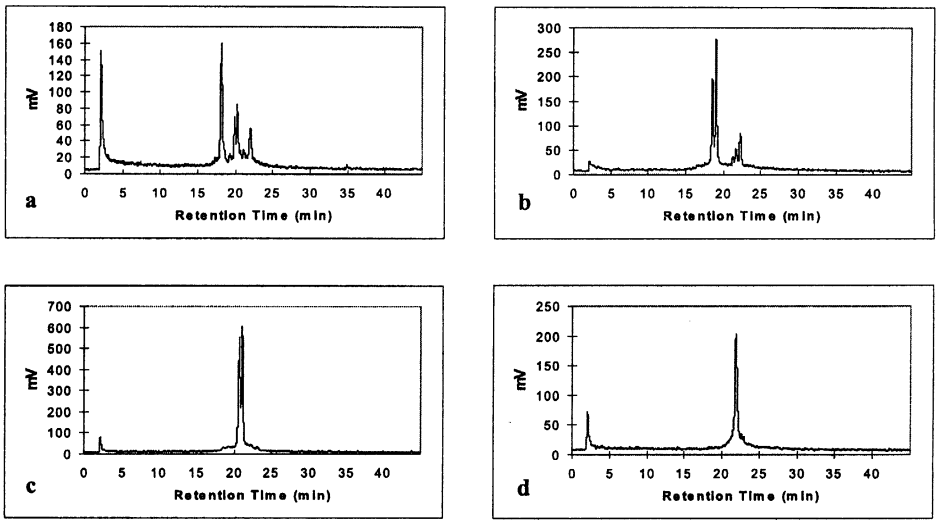

Figure 1. Effect of spacer length on radiochemical purity. BFCA-peptides were constructed with zero (a), one (b), two (c), and three (d) proline spacers between the bz-MAG3 BFCA and a model peptide with the sequence KLAKLAKLA-NH_2. The radiochromatograms for the ^{99m}Tc labeled constructs display a clear trend of increasing radiochemical purity with increasing number of prolines in the spacer.

Targeting Peptide Selection

To establish the generality of this technology, we prepared and labeled several bz-MAG3-P_3-peptides, using analogs of bombesin[8] and the αM2 sequence[9] as our representative targeting peptides. Bombesin is a 14-residue peptide that binds to the gastrin-releasing peptide (GRP) receptor with a K_d of approximately 4 nM.[10] The GRP receptor is overexpressed in small-cell lung carcinoma (SCLC), glioblastoma, gastric cancer, as well as cancers of the pancreas, prostate, and breast.[11] αM2 is a 15-residue peptide derived from the third heavy chain complementarity determining region (CDR 3H) of the ASM2 murine IgG1 monoclonal antibody.[9] The αM2 peptide has a K_d of approximately 25 μM to tumor-associated antigen mucin core protein (MUC1), which

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is expressed on over 90% of all epithelial carcinomas.[9] Analogs of bombesin[12] and native αM2 have both been shown to label ^{99m}Tc at their N-terminus without significantly affecting their receptor-binding ability.

Radiochemical Purity Characterization

Radiochemical purity was judged primarily by radio-HPLC. Concurrent monitoring of the UV chromatogram at 210 nm was used to confirm that the observed radiochemical profiles were due to interaction with ^{99m}Tc at the tracer level. All the BFCA-peptides used in the present study were labeled by an exchange labeling approach.[7] Radiochemical purity and yield were optimized by adjusting the pH of the reaction mixture and then observing the radiochromatographic profile. Nearly all the BFCA-peptides showed optimal radiochemical purity when using the $SnCl_2$/tartrate labeling mixture at pH 12, with the exception of bz-MAG3-P_3-bombesin, which showed optimal radiochemical purity at pH 8 using dithionite/gluconate. As can be seen in Figure 2, the radio-HPLC chromatograms for bz-MAG3-bombesin and bz-MAG3-P_3-bombesin show the marked improvement in radiochemical purity achievable by using a triproline spacer.

The radiolabeling characteristics for the αM2 series of experiments were found to be consistent with those of bombesin. αM2 contains a cysteine residue near its N-terminus, which imparts a natural chelating ability to this sequence, although the technetium-labeled product is quite impure when radiolabeling by this method is employed (data not shown). Appending the bz-MAG3-P_3 BFCA-spacer to this sequence improved radiochemical purity, but not radiochemical yield (50%). By

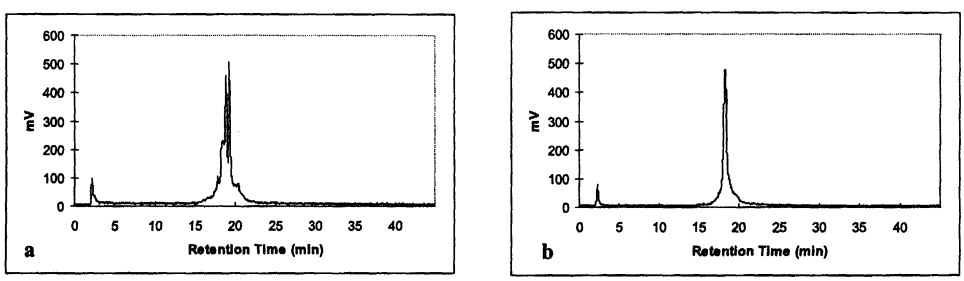

Figure 2. Optimized radio-HPLC profiles for MAG3-bombesin (a) and MAG3-P_3-bombesin (b). Addition of the triproline spacer considerably improves radiochemical purity.

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replacing the native cysteine from this sequence with alanine, then adding the bz-MAG3-P_3 BFCA-spacer, radiochemical yield improved, with over 90% radioactivity present as a single peak. These results suggest that the presence of cysteine in bz-MAG3-P_3-peptide sequences may interfere with technetium chelation of the MAG3 moiety. Chelation interference from other residues appears to be well suppressed by inclusion of the P_3 spacer.

Stability in Plasma & Cysteine Challenge

Since the efficacy of a diagnostic test largely depends on the *in vivo* stability of the radiopharmaceuticals, an important objective of this study was to determine the stability of these radiolabeled peptides in plasma. The results of these experiments demonstrate that the ^{99m}Tc-MAG3-P_3-peptide complexes remained stable when incubated with human plasma at 37 °C for 18h. A maximum of 20% dissociated ^{99m}Tc activity was found for both the ^{99m}Tc-MAG3-P_3-peptides when compared with the radiochemical yield of original compound.

The chelating strength of each ^{99m}Tc complex was evaluated by transchelation with excess free cysteine. Radiolabeled peptides were incubated with 100-fold and 500-fold molar excess of cysteine for 1h and 6h intevals, and analyzed for transchelation from labeled peptide to free cysteine by radio-TLC. A maximum displacement of 11% (1h) and 20% (6h) was found after incubation with 500-fold molar excess free cysteine. These studies show that the incubation of ^{99m}Tc-BFCA-peptides with excess amount of cysteine can result in a gradual decomposition of radiolabeled peptide with the simultaneous formation of ^{99m}Tc-cysteine. In contrast, ^{99m}Tc-MAG3 alone tends to remain stable under these conditions. Nevertheless, these transchelation studies show that the ^{99m}Tc-MAG3 peptides are nearly as stable as ^{99m}Tc-MAG3.[14]

In Vitro Receptor Binding

^{99m}Tc-MAG3-P3 peptides of bombesin and αM2-a were tested for their ability to bind the two human tumor cell lines: PC-3, and MCF-7-III. A dose-dependent binding curve was found for both cell lines (Figure 3). The binding of the two bioactive peptides was inhibited by the presence of an excess of its corresponding unlabeled competing peptide showing that the binding was specific. The nonspecific binding progressively increases at higher concentrations for both peptides. However, specific binding was demonstrable at lower concentrations. The double-reciprocal plot of the

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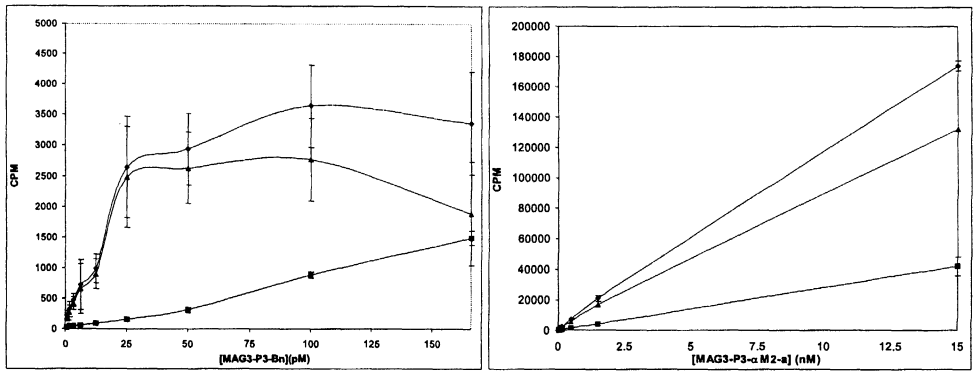

Figure 3. *In vitro* binding data for a) ^{99m}Tc-labeled MAG3-P_3-bombesin with the MCF-7-III breast carcinoma cell-line, and b) ^{99m}Tc-labeled MAG3-P_3-αM2-a with PC-3. Specific binding (triangles) is calculated as the difference between the total binding (diamonds) and non-specific binding (squares).

^{99m}Tc-MAG3-P_3-bombesin specifically bound versus the amount of free peptide provides a K_d of ~6.3 nM. This value is similar to the K_d of ~4 nm reported for ^{125}I-Tyr^4-bombesin[12], demonstrating that receptor binding is not substantially affected by the addition of MAG3-P_3 to the targeting peptide. For ^{99m}Tc-MAG3-P_3-αM2-a, saturation was not evident at the relatively low concentrations employed, however, specific binding was clearly demonstrable at nanomolar peptide levels. This observation suggests the binding constant of MAG3-P_3-αM2-a is close to that of native αM2 (25 μM).

EXPERIMENTAL

Reagents

All Fmoc amino acids, Rink-methylbenzhydrylamine (MBHA) resin, O-benzotriazole-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU), and piperidine were purchased from Chem-Impex International Inc., (Woodale, IL) and used without further purification. Sequencing grade N,N′-dimethylformamide (DMF), was purified by passing over a biphasic alumina column prior to use. Thioglycolic acid was purchased from Aldrich Chemical Company (St. Louis, MO) and distilled prior to use. Sodium pertechnetate (Na^+ $^{99m}TcO_4^-$) was supplied by the Edmonton Radiopharmaceutical Centre (Edmonton, AB). All other chemicals were either of spectrophotometric or reagent grade and used without further purification.

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Instrumentation

Mass spectra were recorded using a Fisons VG Trio 2000 electrospray mass spectrometer (ES-MS). Analytical HPLC was carried out on a Beckman Gold Nouveau HPLC system fitted with a reversed-phase column (C8, 5μ, 2.1mm x 15 cm) and running a 2%B/min gradient (Solvent A: 0.1% aqueous TFA, Solvent B: 0.1% TFA in acetonitrile) at 0.5 mL/min. ^{1}H NMR spectra were acquired using a Varian Unity 500 MHz NMR spectrometer.

Peptide Synthesis

Sequences for all the peptides used in this study are provided in Table 1. The peptides were assembled using a combination of manual and automated synthesis, following standard Fmoc/HBTU methodology.[15] Peptides were synthesized using Rink-MBHA resin at a 0.2 mmol scale. Upon completion of synthesis the N-terminal Fmoc group was removed, and the peptide-resin washed to prepare for condensation with S-benzoylmercaptoacetic acid.

Preparation of S-benzoylmercaptoacetic acid

Preparation of S-benzoylmercaptoacetic acid has been previously reported.[16] Synthesis of this material was performed on a 0.1 mol scale. Briefly, 8.9 g (0.22 mol) sodium borohydride and 9.2 g (0.1 mol) thioglycolic acid were dissolved in a binary solvent mixture of 100 mL toluene and 100 mL ddH_2O. The solution was cooled to 5 °C in an ice bath. 14 g (0.1 mol) of benzoic acid chloride was slowly added to the solution (30 min). The reaction was allowed to proceed at 5 °C for an additional 30 min, then at room temperature for 1h. The toluene layer was removed and washed four times with ddH_2O, The combined aqueous phases were acidified (pH 1.0) by the addition of constant boiling (6N) HCl, causing the product to precipitate. The precipitate was filtered and dried *in vacuo* overnight. The precipitate was recrystallized from ethyl acetate to yield 11.5 g of product (0.059 mol, 59%). Purity (>99%) was confirmed by reversed-phase analytical HPLC. The product was characterized by ES-MS and ^{1}H NMR analysis.

Addition of S-benzoylmercaptoacetic acid

A four-molar excess (with respect to the peptide-resin) of S-benzoylmercaptoacetic acid was activated and manually coupled to the free N-terminus of the peptide-resin

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using standard HBTU/NMM protocol.[15] The reaction proceeded to completion within 15 min as determined by the Kaiser ninhydrin assay.[17] This step completed the synthesis of the bz-MAG3 moiety to yield the final BFCA-peptide-resin. The peptide resin was washed thoroughly with DCM (3x10 mL), DMF (5x10 mL) and methanol (5x10 mL), then dried *in vacuo* overnight to prepare for cleavage.

Cleavage and Side chain Deprotection

The peptide-resin (ca. 200 - 300 mg) was placed in a disposable polypropylene cartridge fitted with a polyethylene frit. A 10 mL solution of cleavage cocktail: TFA:H_2O:DTT (95:2.5:2.5) was added and shaken gently for 2h. The resin was then removed by filtration and washed with 2 mL TFA. The filtrate was evaporated in vacuo, then precipitated with cold ether (30 mL). After decanting the ether layer, the precipitated peptide was washed again with cold ether (2x20 mL), dissolved in ddH_2O, frozen, and lyophilized to yield the crude bz-MAG3-peptide.

Purification and Analysis

Crude BFCA-peptides were purified by HPLC (Waters - System 501) on a reversed-phase C-8 2.1x25 cm Zorbax 300 preparative column using a binary gradient of aqueous 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B) at a flow rate of 8.0 mL/min. A shallow gradient of 0.2% B/min was then applied, UV (210 nm) absorbing fractions were collected and then characterized by analytical HPLC and ES-MS. Fractions of sufficient purity were combined and lyophilized to yield the desired BFCA-peptides in excess of 95% purity.

Radiolabeling

The BFCA-peptides were labeled with ^{99m}Tc by an exchange approach. For all peptides labelling with ^{99m}Tc was achieved by mixing 25 μL [1 mg/mL in 50:50 CH_3CN:H_2O (v/v)] of each BFCA-peptide with 100 μL of 0.1M citrate-phosphate buffer, 300 μL tartrate (40 mg/mL aqueous solution), 100 μL $SnCl_2$ (4 mg/mL in 0.05 N HCl) and 100 μL $^{99m}TcO_4^-$ (1 to 5 mCi) in saline[7]. The labeling mixture was heated at 100 °C in a water-bath for 10 min and cooled to room temperature prior to radiochromatographic analysis. Labeling conditions were optimized for all BFCA-peptides by adjusting the citrate-phosphate buffer from pH 2 to 12. Labeling conditions were further optimized for the biologically active BFCA-peptides (αM2 and

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bombesin) by examining their radiolabeling characteristics using sodium dithionite (100 μL of 50 mg/mL aqueous $Na_2S_2O_4$) as an alternative reducing agent, and sodium gluconate (100 μL of a 100mg/mL aqueous solution) as an alternate transfer ligand.[18]

Radiochemical Purity

Radiochemical purity was determined primarily by analytical radio-HPLC. A reversed-phase HPLC column (C-18, 5μ, 3.9 x 150 mm, DeltaPak) was equilibrated in 0.1% aqueous TFA, then a sample of the reaction mixture after labeling was applied and eluted with a linear 2.5%/min gradient (A, 0.1% aqueous TFA; B, 0.1% TFA in acetonitrile). The eluent was monitored for UV absorbance at 210 nm (Waters model 486) and for radioactivity by scintillation (Bioscan). For the serum stability and cysteine challenge experiments, radiochemical purity was determined by radio-TLC using Whatman chromatography paper No. 1 with acetone and saline (0.9% NaCl) as mobile phases.

In Vitro Plasma Stability and Transchelation

To assess the *in vitro* stability of the complexes, a 100 μL aliquot of both ^{99m}Tc-complexed MAG_3-P_3-peptides (αM2-a and bombesin) were incubated with 0.5 mL human plasma at 37 °C for 18h. The quantity of free versus bound ^{99m}Tc was determined by radio-TLC. As a control, the radiochemical purity of the ^{99m}Tc-MAG3-peptide complex was determined in saline (0.9% NaCl) at 30 min and 18h after radiolabeling.

A cysteine challenge assay ws used to measure the ability of cysteine to transchelate, or remove ^{99m}Tc from a given ^{99m}Tc-ligand complex. The relative strength of the ^{99m}Tc labeled BFCA-peptide complex was determined by incubating the labeled BFCA-peptides with varying molar concentrations of cysteine. Each ^{99m}Tc labeled peptide was mixed with excess cysteine at molar ratios of 1:100 and 1:500. The reaction mixtures (ca. 1 mL) were incubated at 37 °C. Samples were removed at 1h and 6h intervals and analyzed by radio-TLC to determine the percentage of transchelated ^{99m}Tc.

In Vitro Receptor Binding

The receptor-binding ability of the ^{99m}Tc-complexed MAG3-P_3-peptides bombesin and αM2-a were evaluated on human PC-3 prostate carcinoma [obtained from the

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American Type culture Collection (ATCC)] and MCF-7-III breast carcinoma cell lines (a bombesin-enriched receptor subline obtained from Dr. R. Clarke, Georgetown University Medical Center, Washington, D.C., USA) using direct-detection. Both cell lines were grown to confluence in 24 well plates in RPMI-1640 culture media with 10% FBS. Twenty-four hours prior to conducting the binding assays, the media was replaced with RPMI-1640 with 5% FBS. Serial dilutions were prepared from an accurately weighed sample of bz-MAG3-P_3-peptide and labeled with ^{99m}Tc. The radiolabeled peptides were incubated at 0 °C with each cell-line and washed with PBS to determine the total binding of the ^{99m}Tc-MAG3-P_3-peptide-complex. In parallel, equal amounts of each ^{99m}Tc-labeled peptide were incubated with the cells together with 100 times excess of corresponding unlabeled MAG3-P_3-peptide to determine non-specific binding. The difference between the total and non-specific binding (averaged over six replicates) was used to determine specific receptor binding.

CONCLUSION

The combined use of benzoyl-protected MAG3 with a triproline spacer allows for the preparation of ^{99m}Tc-based peptide radiopharmaceuticals with many desirable qualities. Synthesis by SPPS affords simultaneous peptide synthesis, linker addition, and chelator addition while reducing the number of synthetic steps and purification steps required to prepare the final product. Incorporation of MAG3 as the chelating agent allows one to obtain all its associated advantages: FDA approval, low isomerism, good stability in plasma, resistance to transchelation, and 15 years of clinical experience. Synthesis of benzoyl-protected MAG3-peptides imparts long shelf life and high labeling efficiency under mild labeling conditions. Our studies indicate that the synthesis, stability, labeling protocols, and biological activity of peptides prepared and labeled by this method are all good, suggesting that these techniques are generally applicable and largely sequence independent.

ACKNOWLEDGMENTS

Dr. David Wishart thanks the Alberta Heritage Foundation for Medical Research (AHFMR), the Protein Engineering Network of Centres of Excellence (PENCE) and Bristol-Myers Squibb for their support. Dr. M.R. Suresh would like to thank the Alberta Cancer Board, the Medical Research Council (MRC) and Biomira Inc. for

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financial support. G.H. Van Domselaar is supported by a PMAC-HRF/MRC Graduate Research Scholarship in Pharmacy.

REFERENCES

1. Liu S. and Edwards D.S. *Chem. Rev.* **99:** 2235-2268 (1999)
2. Thakur M.L. *Nucl. Med. Commun.* **16:** 724-732 (1995)
3. Liu S., Edwards D.S. and Barrett J.A. *Bioconj. Chem.* **8:** 621-636 (1997)
4. Harris T.D. *et al. Bioorg. Med. Chem. Lett.* **6:** 1741-1746 (1996)
5. Pallela V.R., Thakur M.L., Chakder S. and Rattan S. *J. Nucl. Med.* **40:** 352-360 (1999)
6. Schaap G.H., Alferink T.H., de Jong R.B., Oe P.L., Roos J.C. and Donker A.J. *Eur. J. Nucl. Med.* **14:** 28-31 (1988)
7. Okarvi S., Adriaen P. and Verbruggen A.M. *J. Label. Compds. Radiopharm.* **39:** 853-874 (1997)
8. Anastasi A., Erspamer V. and Bucci M. *Arch. Biochem. Biophys.* **148:** 443-446 (1972)
9. Sivolapenko G.B. *et al. The Lancet* **346:** 1662-1666 (1995)
10. Reile H., Cai R., Armatis P. and Schally A. *Int. J. Onc.* **7:** 749-754 (1995)
11. Preston S.R., Miller G.V. and Primrose J.N. *Crit. Rev. Oncol. Hematol.* **23:** 225-238 (1996)
12. Baidoo K.E., Lin K.S., Zhan Y., Finley P., Scheffel U. and Wagner H.N. Jr. *Bioconj. Chem.* **9:** 218-225 (1998)
13. Zamora P.O. and Rhodes B.A. *Bioconj. Chem.* **3:** 493-498 (1992)
14. Stalteri M.A., Bansal S., Hider R. and Mather S.J. *Bioconj. Chem.* **10:** 130-136 (1999)
15. Welings D.A. and Atherton E. *Methods Enzymol.* **289:** 44-67 (1997)
16. Brandau W., Bubek B., Eisenhut M. and Taylor D.M. *Appl. Radiat. Isot.* **39:** 121-129 (1988)
17. Kaiser E., Colescott R.L., Bossinger C.D. and Cook P.I. *Analytical Biochem.* **34:** 595-598 (1970)
18. Bormans E., *et al. Nucl. Med. Biol.* **17:** 499-506 (1990)

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