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A Solid-Phase Labeling Strategy for the Preparation of Technetium and Rhenium Bifunctional Chelate Complexes and Associated Peptide Conjugates

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A new solid-phase labeling strategy for the preparation of ^{99m}Tc and Re chelate complexes and associated peptide derivatives, was developed. Resin-bound monoamide monoamine (MAMA) chelates were prepared in such a manner that upon the addition of a suitable Re(V) and Tc(V) precursor the target metal complexes were selectively released from the resin. The desired products were isolated from unreacted ligand by a simple filtration/solid-phase extraction procedure. In addition to the preparation of a series of functionalized ligands, a peptide conjugate was constructed from one of the resin-bound chelates using a conventional automated peptide synthesizer. The yields of the Re chelate complexes were typically greater than 70%, while the maximum yield for reactions run at the tracer level using ^{99m}Tc was 50%. The reported approach has a number of attractive features, including the opportunity to prepare libraries of novel agents, the ability to isolate macroscopic amounts of Re complexes for use in in vitro screening studies and as well-characterized standards for tracer level work, and the ability to produce ^{99m}Tc complexes that are free of any unreacted starting material without having to employ preparative HPLC.

INTRODUCTION

Solid-phase labeling strategies (SPLS) are an attractive means of preparing radiotracers because they facilitate the process of separating the desired product from the excess starting materials used during standard labeling procedures (*1*). Removal of unlabeled ligands to give high effective specific activity formulations is particularly important in situations in which a precursor (i.e., unlabeled ligand) can compete with the tracer for the target receptor. It is also relevant to studies involving small animal positron emission tomography (PET) and single photon emission computed tomography (SPECT) scanners where minute amounts of unlabeled material can be toxic to the animal and/or have a significant impact on the distribution of a tracer (*2*). SPLS are easy to automate, which is important when high levels of activity are needed, and, although it has not been widely exploited, SPLS also engender the opportunity to incorporate modern drug discovery techniques, including combinatorial chemistry, into the radiopharmaceutical development process.

SPLS typically involve covalent attachment of a substrate to an insoluble support through a linker that is cleaved when the resin-bound material reacts with a particular radioisotope. For instance, Hunter and co-workers developed a convenient strategy for radio-iodinating aromatic rings by attaching benzoic acid and benzylamine derivatives to a solid support through a tin linker (*3*). In this way, iodinated compounds, including [^{131}I]-meta-iodobenzylguanidine (MIBG), were prepared in excellent yield, purity, and specific activity.

Similar strategies for labeling with ^{99m}Tc, the most widely used radionuclide in diagnostic medicine, have also been developed (*4*). Dunn-Dufault et al. showed that it was possible to label an N_3S chelate that was connected via the thiol residue (through a succinimidyl linker) to a variety of different supports (*5*). Although these systems afforded high (effective) specific activity formulations, radiochemical yields varied significantly (13−80%). Alternatively, the same chelate, derivatized with a peptide, was linked through a thiol group to a support consisting of gold particles (*6*). Upon labeling with ^{99m}Tc, the desired product was isolated in excellent radiochemical yield. More recently, Alberto et al. published a solid-phase labeling system for the technetium(I) tricarbonyl ($[Tc(CO)_3]^+$) core (*7*). In this approach, a derivative of diethylene triamine was attached to the solid support via the secondary amine. Upon the addition of $[^{99m}Tc(OH_2)_3(CO)_3]^+$, the technetium bound to the triamine ligand, and it was postulated that one of the displaced water molecules reacts with the carbon−nitrogen bond, thus freeing the coordination complex from the solid support.

Existing SPLS for technetium are often designed for incorporating ^{99m}Tc into compounds, but they have not been particularly effective at producing macroscopic quantities of Re or ^{99}Tc complexes which are needed for screening potential radiopharmaceuticals in vitro and as reference compounds for work done at the tracer level. Furthermore, there has been only limited investigation into the use of the supported synthons as platforms for preparing receptor targeted radiopharmaceuticals using automated solid-phase synthesis (*8*−*10*).

The objective of the work described herein was to develop a SPLS that could be used to prepare both Re and ^{99m}Tc complexes of functionalized chelates. The methodology was designed to enable the preparation of discrete compounds in useful quantities while also offering the opportunity to prepare libraries of peptide−chelate conjugates. Merrifield-type resins were selected as the support because an extensive collection of prefunctionalized derivatives are commercially available and the polystyrene-based resins are already widely used in automated peptide and organic synthesizers. The monoamide monoamine chelate (MAMA) (*11*, *12*) was chosen as one of many possible ligand systems because it forms neutral, stable, and well-defined complexes with both Tc(V) and Re(V), and it can be easily derivatized, regioselectively, with a wide range of different functional groups (*13*−*15*), which imparts a great deal of flexibility when designing libraries.

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EXPERIMENTAL PROCEDURES

Instrumentation. All peptides were prepared on an Advanced ChemTech 348 Ω Peptide synthesizer using a 40-well reaction block. Electrospray ionization (ESI) mass spectrometry experiments were performed on a Micromass Quattro Ultima instrument where samples were dissolved in 1:1 CH_3OH/ H_2O or 1:1 CH_3CN/H_2O mixtures. High-resolution MS was obtained using a Micromass Global Q-TOF Ultima instrument. FTIR spectra were acquired on a Bio-Rad FTS-40 FTIR spectrometer. NMR experiments were performed using Bruker Avance DRX-500, Avance AV-600 or AC-200 spectrometers. For a pair of geminal protons, A refers to the proton associated with the higher frequency signal, while B refers to the proton giving rise to the lower frequency resonance. For the Re complexes, α refers to ring protons that are endo relative to the Re−oxygen bond, while β refers to protons that are oriented exo.

Analytical HPLC studies for nonradioactive samples were performed using a Varian Pro Star model 230 solvent delivery system, 330 PDA detector fitted with a C-18 Microsorb column (4.6 × 250 mm, 300 Å-5 μm). The elution protocol for nonpeptide based compounds was a gradient of 25 to 40% CH_3CN (containing 0.1% TFA) over 15 min at a flow rate of 1.0 mL/min. All analytical HPLC experiments were monitored at λ = 254 and 214 nm. For peptides, a gradient range of 5 to 20% CH_3CN over 15 min was used. For labeling reactions with ^{99m}Tc, approximately 100 μL of sample was removed from a reaction mixture and diluted to 1 mL with distilled water. A total of 10 μL of this solution was injected onto a Nucleosil C-18 column (250 × 4.6 mm, 5 μm). The runs employed the same gradients used for the Re analogues.

Materials, Methods, and Characterization Data. Unless otherwise stated, all reagents and solvents were ACS grade or higher and used without further purification from commercial suppliers. Polystyrene-based *N*-α-9-fluorenylmethoxycarbonyl (Fmoc)-glycine loaded SASRIN resin (0.69 mmol g^{-1}, 1% divinylbenzene, 200−400 mesh) was obtained from Bachem Inc. Fmoc-protected amino acids were purchased from Novabiochem, Bachem Inc, and Advanced ChemTech Inc. Cysteamine 4-methoxytrityl resin was purchased from Novabiochem and TentaGel S Trt-OH resin from Advanced ChemTech. ^{99m}Tc was obtained by elution of a generator obtained from Bristol-Myers Squibb.

***S*-(((4-Methoxyphenyl)diphenyl)methyl)-2-aminoethanethiol.** To a solution of cysteamine hydrochloride (7.7 g, 68.1 mmol) in TFA (100 mL) was added *p*-anisylchlorodiphenylmethane (20 g, 64.8 mmol). After 3 h of stirring of the sample under an atmosphere of N_2, the TFA was removed under reduced pressure, and the resulting colorless oil was suspended in CH_2Cl_2 (200 mL) and extracted with 3 N NaOH (3 × 100 mL) (Note: the oil does not go into CH_2Cl_2 until the addition of NaOH). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to yield a white solid (16.6 g, 70%). TLC R_f = 0.11 (3% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 3058, 3001, 2955, 2927, 2855; ^{1}H NMR (200 MHz, $CDCl_3$): δ7.27 (bm, 12 H, H-aryl), 6.77 (d, J = 8.8, 2H, H-aryl), 3.69 (s, 3H, OCH_3), 2.54 (t, J = 6.3, 2H, C**H$_2$**NH_2), 2.29 (t, J = 6.3, 2H, TrS**CH$_2$**), 1.12 (b s, 2H, NH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ158, 145, 137, 130, 129, 128, 126, 113, 66, 55, 41, 36; MS (ESI): *m*/*z* 273 [$(MeOTr)^+$], 350 [$(M + H)^+$].

***S*-Linked Resin-Bound MAMA Chelate (4).** Cysteamine-4-methoxytrityl resin (1.0 g, 0.31 mmol/g) was suspended in 10 mL of dry DMF in a fritted vessel and argon was bubbled through the suspension for 15 min. The solution was filtered, and fresh DMF (10 mL) containing NMM (375 μL, 3.41 mmol) was added. In a separate flask, bromoacetic acid (431 mg, 3.1 mmol), HOBT (1.0 g, 3.1 mmol), and HBTU (1.17 g, 3.1 mmol) were dissolved in DMF (4 mL), and the sample was stirred for 5 min at room temperature. This solution was added to the suspension containing the polymer and the mixture agitated for 3 h. After filtration of the sample and thoroughly washing the resin with CH_2Cl_2, DMF, MeOH, DMF, and CH_2Cl_2, a solution of NMM (1.25 g, 12.4 mmol) in DMF (10 mL) was added, and the suspension was agitated with a flow of argon for 15 min. Methoxytrityl-protected cysteamine (4.7 g, 13.5 mmol) was subsequently added, and the reaction was agitated for an additional 48 h. Filtration followed by thorough washing with CH_2Cl_2, DMF, MeOH, DMF, and CH_2Cl_2 yielded a yellow solid (1.12 g, 89%). IR (KBr, cm^{-1}): 2926, 2865, 2363, 2337, 1685.

ReO-MAMA (1). Compound **4** (100 mg) was allowed to swell in a solution (v/v/v) of 5% MeOH, 1% pyridine in DMF (5 mL) for 5 min prior to the addition of $[NBu_4][ReOCl_4]$ (19 mg, 0.031 mmol). The resulting green suspension was stirred at 85 °C for 16 h. The solution was then filtered, and the DMF was removed under reduced pressure (55 °C at 1 Torr). The product (10 mg, 85%), a reddish-brown solid, was isolated by silica gel column chromatography (5% MeOH/CH_2Cl_2). TLC R_f = 0.15 (10% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 1654, 966; ^{1}H NMR (600 MHz, CD_3OD): δ5.00 (m, $J_{4\beta,4\alpha}$ = −18.6, H-4β), 4.07 (m, H-4α), 4.03 (m, $J_{2\beta,2\alpha}$ = −12.0, $J_{1\beta,2\beta}$ = 6.0, $J_{1\alpha,2\beta}$ = 6.0, H-2β), 3.83 (m, $J_{6\beta,6\alpha}$ = −10.2, $J_{5\alpha,6\beta}$ = 6.6, H-6β), 3.44 (m, $J_{1\beta,2\alpha}$ = 7.2, $J_{1\beta,2\alpha}$ = 7.2, H-2α), 3.21 (m, H-5 β), 2.96 (m, H-1β), 2.89 (m, $J_{1\beta,1\alpha}$ = −11.4, H-1α), 2.84 (m, $J_{5\beta,6\alpha}$ = 6.6, $J_{5\alpha,6\alpha}$ = 12.6, H-6α), 2.70 (m, $J_{5\beta,5\alpha}$ = −10.8, H-5α); ^{13}C NMR (151 MHz, $CDCl_3$): δ 191.1, 70.0, 66.1, 39.9, 39.8, 38.6; HRMS (TOF-EI) calcd. for $C_6H_{11}N_2S_2O_2Re$: 393.9820. Found 393.9807.

Methyl [2-(*S*-4′-methoxytrityl)-thioethyl)amino]acetate (6). Compound **5** (2.0 g, 5.73 mmol), *N*-methylmorpholine (700 μL) and methylbromoacetate (493 μL, 5.16 mmol) were dissolved in CH_2Cl_2 (50 mL). Following stirring for 24 h under N_2, the solution was extracted with water three times (50 mL each) and dried over Na_2SO_4. Removal of the solvent by rotary evaporation followed by silica gel column chromatography (50% ethyl acetate/hexanes) yielded the product as a colorless oil (1.96 g, 90%). TLC R_f = 0.50 (5% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 3336, 3059, 1742; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.30 (m, 12H, H-aryl), 6.80 (d, J = 8.9 Hz, 2H, H-aryl), 3.79, (s, 3H, phenyl-OCH_3), 3.70 (S, 3H, (CO)-OCH_3), 3.30 (s, 2H, (CO)CH_2NH), 3.55 (t, J = 7.7 Hz, 2H, NHCH_2CH_2S), 3.36 (t, 2H, $NHCH_2CH_2$S), 1.97 (s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.5, 158.0, 145.0, 136.8, 130.7, 129.4, 127.8, 126.5, 113.1, 55.2, 51.8, 50.2, 47.9, 32.0; $ESMS^+$: *m*/*z* 273 [$(4\text{-MeOTr})^+$], 422 [$(M + H)^+$].

Methyl [2-(*S*-4′-methoxytrityl)-thioethyl)-*N*-benzyl-amino]-acetate (7). Compound **6** (410 mg, 0.973 mmol), NMM (1 mL), and benzyl bromide (1 mL, 8.36 mmol) were stirred in CH_2Cl_2 (75 mL) for 16 h. The solvent was removed under reduced pressure, and the product was purified by silica gel column chromatography (30% ethyl acetate/hexanes) yielding the product as a colorless oil (497 mg, 99%); TLC R_f = 0.90 (5% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 3059, 3030, 1742; ^{1}H NMR: (200 MHz, $CDCl_3$): δ7.30 (m, 17H, H-aryl), 6.80 (d, J = 8.9 Hz, 2H, H-aryl), 3.78, (s, 3H, phenyl-OCH_3), 3.65 (s, 2H, benzyl-CH_2), 3.63 (s, 3H, OCH_3), 3.18 (s, 2H, (CO)CH_2-NH), 2.66 (t, J = 7.7 Hz, 2H, NHCH_2CH_2S), 2.32 (t, 2H, $NHCH_2CH_2$S); ^{13}C NMR (50 MHz, $CDCl_3$): δ172.1, 158.2, 145.3, 130.8, 129.5, 128.9, 128.2, 127.8, 127.1, 126.5, 113.1, 111.7, 57.7, 55.2, 53.7, 53.0, 51.1, 30.4; MS (ESI): *m*/*z* 273 [$(4\text{-MeOTr})^+$], 512 [$(M + H)^+$].

Potassium [2-(*S*-4′-methoxytrityl)-thioethyl)-*N*-benzyl-amino]acetate (8). Compound **7** (497 mg, 0.973 mmol) was dissolved in diethyl ether (5 mL) and potassium trimethylsilanoate (156 mg, 1.22 mmol) was added and the suspension was stirred for 6 h. The resulting colorless precipitate was dissolved

in EtOAc and purified by silica gel column chromatography (3% MeOH/EtOAc then 10% MeOH/EtOAc) to give the product as a white foam (416 mg, 80%). TLC $R_f = 0.15$ (10% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 3351, 3059, 3031, 1654; ^1H NMR (200 MHz, $CDCl_3$): δ7.24 (m, 17H, H-aryl), 6.73 (d, $J = 8.9$ Hz, 2H, H-aryl), 3.77 (s, 3H, phenyl-OCH_3), 3.77 (s, 2H, benzyl-CH_2), 3.02 (s, 2H, $(CO)CH_2NH$), 2.45 (bs, 4H, SCH_2CH_2NH); ^{13}C NMR (50 MHz, $CDCl_3$): δ173.1, 158.0, 144.8, 136.6, 134.1, 130.6, 130.1, 129.4, 128.5, 127.8, 126.6, 113.1, 66.4, 57.4, 55.5, 55.1, 52.7; MS (ESI): *m*/*z* 497 $[(M)^-]$.

S-Linked Resin-Bound (*N*-Bn)-MAMA Chelate (9). Compound **8** (215 mg, 0.402 mmol), HOBt (540 mg, 4.02 mmol), and HBTU (1.52 g, 4.02 mmol) were dissolved in DMF (5 mL) and mixed using an orbital shaker for 5 min. At the same time, cysteamine-4-methoxytrityl resin (100 mg, 0.31 mmol/g) was swelled in a mixture of DMF (5 mL) and NMM (34 μL). The solution containing the carboxylic acid was then added to the resin, and the resulting mixture was shaken for 4 h. The resin was washed with DMF (2 × 20 mL, 10 min), MeOH (2 × 20 mL, 10 min), DMF (2 × 20 mL, 10 min), and CH_2Cl_2 (2 × 20 mL, 10 min).

ReO-MAMA-(*N*-Bn)(10). Compound **9** (200 mg) was allowed to swell in 5 mL of a solution of 5% MeOH and 1% pyridine in DMF (v/v/v). $[NBu_4][ReOCl_4]$ (37 mg, 0.063 mmol) was added, and the vial was heated at 85 °C for 16 h. The solvent was then removed, and the brownish red residue was dissolved in dichloromethane and purified by passing it through a short plug of silica (50:50 hexanes/EtOAc, then EtOAc) to give the product as a red solid (21 mg, 70%). TLC $R_f = 0.25$ (5% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 1654, 966; ^1H NMR (syn isomer, 600 MHz, $CDCl_3$): δ 7.72 (m, $J_{9,10} = 2.4$, $J_{10,11} = 2.4$, H-10), 7.49 (m, H-9, H-11), 5.11 (d, $J_{7A,7B} = -13.5$, H-7A), 5.00 (d, $J_{4\beta,4\alpha} = -16.2$, H-4$\beta$), 4.74 (m, $J_{5\alpha,7B} = 1.1$, H-7B), 4.44 (m, $J_{2\beta,2\alpha} = -12.4$, $J_{1\beta,2\beta} = 1.0$, $J_{1\alpha,2\beta} = 5.6$, H-2β), 4.12 (m, $J_{1\beta,1\alpha} = -11.3$, $J_{1\beta,2\beta} = 1.1$, $J_{1\beta,2\alpha} = 5.2$, H-1β), 3.82 (d, H-4α), 3.73 (m, $J_{6\beta,6\alpha} = -13.0$, $J_{5\beta,6\beta} = 3.8$, $J_{5\alpha,6\beta} = 13.0$, H-6$\beta$), 3.25 (m, H-1$\alpha$, H-2$\alpha$), 3.09 (m, $J_{5\beta,5\alpha} = -12.9$, $J_{5\beta,6\alpha} = 2.3$, H-5β), 2.91 (m, $J_{5\alpha,6\alpha} = 4.5$, H-6$\alpha$), 1.48 (m, H-5$\alpha$); ^{13}C NMR (syn isomer, 151 MHz, $CDCl_3$): δ 187.5, 132.3, 131.2, 129.4, 128.7, 65.4, 64.8, 62.7, 59.6, 46.9, 38.0. HRMS calcd. for $C_{13}H_{17}N_2S_2O_2Re$: 484.0289. Found 484.0274.

***S*-(Triphenylmethyl)-2-aminoethanethiol (11).** The method of O'Neil et al. was followed with minor modifications. To a solution of cysteamine hydrochloride (19.2 g, 170 mmol) in TFA (120 mL) was added triphenylmethanol (44.0 g, 169 mmol). After 3 h of stirring under an atmosphere of N_2, the TFA was removed under reduced pressure. The resulting white solid was suspended in CH_2Cl_2 (300 mL) and extracted with 3 N NaOH (4 × 150 mL) (Note: the solid does not go into CH_2Cl_2 until the addition of NaOH), H_2O (3 × 150 mL), $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to yield the product (45 g, 85%) as a white solid. TLC $R_f = 0.12$ (3% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 3056, 3020; ^1H NMR (200 MHz, $CDCl_3$): δ7.27 (m, 15 H, H-aryl), 2.54 (t, $J = 6.3$, 2H, CH_2NH_2), 2.29 (t, $J = 6.3$, 2H, $TrSCH_2$), 1.12 (bs, 2H, NH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ158.1, 129.7, 127.8, 126.6, 125.6, 39.4, 34.4; MS (ESI): *m*/*z* 243 $[(Tr)^+]$, 320 $[(M+H)^+]$.

***S*-(Triphenylmethyl)-2-aminoethanethiol-*N*,*N*-diacetate-(13).** To a stirred solution of compound **11** (1.84 g, 5.77 mmol) in CH_2Cl_2 (20 mL) containing triethylamine (4.7 g, 46.16 mmol), bromo methyl acetate (7.1 g, 46.16 mmol) was added dropwise. The solution was stirred for 12 h under an atmosphere of nitrogen whereupon the solvent was concentrated in vacuo, and the resulting solution was loaded onto a column of silica (64 cm × 10 cm). The column was washed with hexanes (250 mL), 10% ethyl acetate/hexanes (250 mL), and 50% ethyl acetate/hexanes (250 mL). The solvent in the final fraction was evaporated, and the dimethyl ester (2.55 g) was dissolved in methanol (35 mL) and a solution of KOH (2.48 g, 44.2 mmol) in water (5 mL) was added. The resulting colorless solution was stirred for 12 h under argon. Upon removal of the solvent, the resulting semisolid was dissolved in water (75 mL), and the pH was adjusted to 2.0 by the dropwise addition of HCl which was accompanied by rapid stirring. The resulting precipitate (2.3 g, 92%) was isolated by filtration and washed with 10 mM HCl (3 × 10 mL). TLC $R_f = 0.05$ (10% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 3300, 3062, 3022, 1676; ^1H NMR (200 MHz, $CDCl_3$): δ7.21 (m, 15H, H-aryl), 3.70, (s, 4H, $CH_2(CO)$), 2.00 (m, 4H, S-CH_2CH_2NH); ^{13}C NMR (50 MHz, D_2O): δ171.5, 145.8, 130.74, 129.10, 128.06, 56.28, 55.58, 28.77, 28.72; MS (ESI): *m*/*z* 434 $[(M - H)^-]$.

***S*-Linked Resin-Bound (*N*-CH_2CO_2H)-MAMA Chelate (14).** Cysteamine-4-methoxytrityl-PS resin (100 mg, 0.31 mmol/g) was stirred with CH_2Cl_2 (2 × 10 mL, 10 min per wash) and DMF (2 × 10 mL, 10 min per wash), filtered, and then suspended for 5 min in DMF (5 mL) containing NMM (40 μL). Meanwhile, compound **13** (126 mg, 0.31 mmol), HBTU (117 mg, 0.31 mmol), and HOBT (42 mg, 0.31 mmol) were dissolved in DMF (1 mL) and mixed using an orbital shaker for 5 min. The solution containing the di-carboxylic acid was subsequently added to the amine and stirred by bubbling Ar through the mixture for 4 h at which time the resin was negative to the Kaiser test. The resin was washed with DMF (2 × 20 mL, 10 min per wash), CH_2Cl_2 (2 × 20 mL, 10 min per wash), and MeOH (2 × 20 mL, 10 min per wash) and then treated with a mixture of acetic anhydride in DMF (1:9 v/v) for 30 min. The solution was filtered, and the resin was washed with DMF (2 × 20 mL, 10 min per wash), CH_2Cl_2 (2 × 20 mL,10 min per wash), and MeOH (2 × 20 mL, 10 min per wash) and finally dried in vacuo. MS (ESI) (cleaved sample): *m*/*z* =251 $[(M - H)^-]$.

ReO-MAMA-(*N*-CH_2CO_2H) (15a). Compound **14** (100 mg) was washed with CH_2Cl_2 (2 × 10 mL, 10 min per wash) and DMF (2 × 10 mL, 10 min per wash) and then suspended in a 5 mL solution of 5% MeOH and 1% pyridine in DMF (v/v/v) containing $[NBu_4][ReOCl_4]$ (18.6 mg, 31.2 μmol). After the green suspension was heated to 85 °C in an oil bath for 16 h, the solution was filtered and concentrated in vacuo. The resulting red/brown solid was dissolved in 50/50 MeOH/CH_2Cl_2 and purified by passing it through a plug of silica (CH_2Cl_2, 50/50 MeOH/CH_2Cl_2). Concentration of the solvent by rotary evaporation gave the product as a red solid (10 mg, 70%). TLC $R_f = 0.25$ (5% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 1654, 966; ^1H NMR (600 MHz, CD_3OD): δ5.12 (d, $J_{4\beta,4\alpha} = -17.6$, H-4$\beta$), 4.65 (d, H-4$\alpha$), 4.47 (d, $J_{2\beta,2\alpha} = -12.8$, H-2$\beta$), 4.47 (d, $J_{7A,7B} = -16.6$, H-7β), 4.22 (m, $J_{5\alpha,7B} = 1.3$, H-7α), 4.12 (m, $J_{5\beta,5\alpha} = -12.3$, H-5$\beta$), 3.98 (m, $J_{1\beta,1\alpha} = -10.8$, $J_{1\beta,2\alpha} = 4.6$, H-1β), 3.42 (m, $J_{6\beta,6\alpha} = -13.3$, $J_{6\beta,5\beta} = 3.8$, $J_{6\beta,5\alpha} = 13.3$, H-6$\beta$), 3.11 (m, $J_{1\alpha,2\alpha} = 12.3$, H-2$\alpha$), 3.04 (m, $J_{1\alpha,2\beta} = 6.0$, H-1α), 2.88 (m, $J_{5\alpha,6\alpha} = 4.4$, H-6$\alpha$), 1.57 (m, H-5$\alpha$); ^{13}C NMR (151 MHz, CD_3OD): δ189.8, 170.1, 67.8, 64.4, 61.1, 58.6, 46.2, 38.2; HRMS (TOF-ESI) calcd. for $C_8H_{12}N_2S_2O_4Re$: 450.9793. Found 450.9797.

***S*-Linked Resin-Bound (*N*-$CH_2CONHCH_2CO_2Bn$)-MAMA Chelate (16).** Compound **14** (100 mg) was washed with CH_2Cl_2 (2 × 10 mL, 10 min per wash) and DMF (2 × 10 mL, 10 min per wash) and then suspended for 5 min in DMF (5 mL) containing HBTU (117 mg, 0.31 mmol) and HOBT (42 mg, 0.31 mmol). Meanwhile, O-Bn-glycine (62 mg, 0.31 mmol) and NMM (40 μL) were dissolved in DMF (1 mL), and the mixture was agitated for 5 min. The solution containing the amine was

added to the resin, and the mixture was stirred by bubbling Ar through the solution for 3 h at which time the resin was negative to the Kaiser test. The solution was filtered, and the resin was washed with DMF (2 × 20 mL, 10 min per wash), CH_2Cl_2 (2 × 20 mL, 10 min per wash), and MeOH (2 × 20 mL, 10 min per wash). MS(ESI) (cleaved sample): *m*/*z* 400 [(M + H)$^+$].

ReO-MAMA-(*N*-CH$_2$CONHCH$_2$CO$_2$Bn) (17). Compound **16** (100 mg) was washed with CH_2Cl_2 (2 × 10 mL, 10 min per wash) and DMF (2 × 10 mL, 10 min per wash) and then suspended in 5 mL of a solution of 5% MeOH and 1% pyridine in DMF (v/v/v) containing [NBu$_4$][ReOCl$_4$] (19 mg, 31.2 μmol). After the green suspension was heated to 85 °C with an oil bath for 16 h, the resin was collected by filtration and the filtrate concentrated by rotary evaporation. The resulting red/brown solid was dissolved in CH_2Cl_2, washed with water, and then purified by passing it through a plug of silica (50/50 hexanes/EtOAc; 95/5 CH_2Cl_2/MeOH). Removal of the solvent by rotary evaporation gave the product as a red solid (12 mg, 68%). TLC R_f = 0.25 (5% MeOH/CH_2Cl_2); IR (NaCl disk, cm^{-1}): 1654, 966; ^{1}H NMR (600 MHz, $CDCl_3$): δ7.39 (m, H-aryl), 5.22 (d, J = −10, H-11A), 5.19 (d, H-11B), 4.92 (d, $J_{4\beta,4\alpha}$ = −17.4, H-4β), 4.73 (d, H-4α), 4.71 (d, $J_{7\beta,7\alpha}$ = −15.9, H-7A), 4.65 (m, $J_{2\beta,2\alpha}$ = −10.4, $J_{1\alpha,2\beta}$ = 4.1, H-2β), 4.29 (d, H-7B), 4.16 (d, J = −15, H-9A), 4.12 (m, $J_{1\beta,1\alpha}$ = −10.6, $J_{1\beta,2\alpha}$ = 4.6, H-1β), 4.07 (d, H-9B), 3.86 (m, $J_{5\beta,5\alpha}$ = −12.3, $J_{5\beta,6\beta}$ = 3.5, H-5β), 3.39 (m, $J_{6\beta,6\alpha}$ = −13.7, $J_{6\beta,5\alpha}$ = 13.6, H-6β), 3.25 (m, $J_{1\alpha,2\alpha}$ = 12.3, H-2α), 3.18 (m, H-1α), 2.86 (m, $J_{5\alpha,6\alpha}$ = 5.5, H-6α), 1.56 (m, H-5α); MS(ESI): *m*/*z* 598 [(M + H)$^+$], 600 [(M + H)$^+$].

***S*-Linked Resin-Bound (*N*-CH$_2$CONHCH$_2$CH$_2$NH$_2$)-MAMA Chelate (18).** Compound **14** (100 mg) was washed CH_2Cl_2 (2 × 10 mL, 10 min per wash) and DMF (2 × 10 mL, 10 min per wash) and then suspended for 5 min in DMF (5 mL) containing HBTU (200 mg, 0.53 mmol) and HOBT (100 mg, 0.73 mmol). Ethylenediamine (2 mL) was then added to the resin, and the mixture was stirred by bubbling Ar through the solution for 3 h. The resin was collected by filtration and washed with DMF (2 × 20 mL, 10 min per wash), CH_2Cl_2 (2 × 20 mL, 10 min per wash), and MeOH (2 × 20 mL, 10 min per wash).

Resin-Bound MAMA−Peptide Conjugate (20). Compound **18** (150 mg) was added to two reaction wells, suspended in DMF (2 mL/well), and shaken at 900 rpm for 1 min. The wells were subsequently filtered, suspended in THF (2 mL/well), shaken at 900 rpm for 1 min, and drained for 90 s. The THF wash was repeated two more times. The DMF wash was then repeated a final two times to complete the general wash cycle. This procedure was used between every deprotection and coupling step. Fmoc deprotection was brought about through the addition of 20% v/v piperidine−DMF solution to the active vessels (2 mL/well) and shaking for 5 min at 900 rpm. Following filtration, the process was repeated, shaking for 10 min. The deprotected resin-bound amino acid was washed using the general wash procedure and subsequently coupled to the next Fmoc-protected amino acid using a standard HBTU coupling technique. Coupling reactions initially involved adding DMF (200 μL) to the active vessels followed by the addition of a 4-fold excess of the protected amino acid as a 0.5 M solution in DMF. Four equivalents of HBTU as a 0.5 M solution in DMF was then added followed by a 8-fold excess of DIPEA as 2.0 M solution in DMF. The reaction block was subsequently shaken for 80 min at 900 rpm. Following filtration, the resin was washed using the general washing procedure prior to the start of the next cycle.

Once the final amino acid had been added and the Fmoc group removed, a pause was introduced into the peptide program so that 10 equiv of the *n*-butyl isocyanate in DMF (0.5 M solution) could be added. The reaction block was then shaken at 900 rpm under nitrogen for 12 h. The reaction vessels were emptied, and the resins were washed using the general wash cycle. The cleavage cocktail, which consisted of 20% TFA and 2% TIS in DCM (v/v/v), was cooled to 0 °C and added to the resin. The mixture was allowed to warm to room temperature over 60 min and subsequently filtered into cold diethyl ether. The resulting heterogeneous solution was centrifuged at 3000 rpm and −5 °C for 10 min. MS (ESI): *m*/*z* 767 [(M + H)$^+$].

ReO-MAMA Peptide Conjugate (21a). Compound **20** (100 mg) was washed with CH_2Cl_2 (2 × 10 mL, 10 min per wash) and DMF (2 × 10 mL, 10 min per wash) and then suspended in 2 mL of a solution of 5% MeOH and 1% pyridine in DMF (v/v/v) containing [NBu$_4$][ReOCl$_4$] (18.6 mg, 31.2 μmol). After the green suspension was heated to 85 °C in an oil bath for 16 h, the resin was collected by filtration and the filtrate was concentrated by rotary evaporation. The resulting red/brown solid was dissolved in a mixture of 10% MeOH in CH_2Cl_2 (v/v) and purified by passing the mixture through a plug of silica (CH_2Cl_2 then 50/50 MeOH/CH_2Cl_2). Concentration of the later fractions gave a red solid (14 mg, 47%). MS(ESI): *m*/*z* 966, 968 [(M + H)$^+$].

Tentagel-*S*-Tr-OH Resin MAMA−Peptide Conjugate. Tentagel-S-Trt-OH resin (1.0 g, 0.4 mmol/g) was suspended in a solution of 2-aminoethane thiol hydrochloride (1.0 g, 0.9 mmol) in TFA (10 mL). The solution was gently agitated with a stream of Ar, and after 2 h, the solution was filtered and the resin washed with CH_2Cl_2 (2 × 10 mL, 10 min per wash) and DMF (2 × 10 mL, 10 min per wash) and then suspended for 5 min in DMF (5 mL) containing DIP (40 μL). Meanwhile, compound **13** (1.26 g, 3.1 mmol), HBTU (1.17 g, 3.1 mmol), and HOBT (420 mg, 0.31 mmol) were dissolved in DMF (10 mL) and shaken for 5 min. The solution containing the di-carboxylic acid was then added to the resin-bound amine, and the mixture was stirred by bubbling Ar through the solution for 4 h at which time the resin was negative to the Kaiser test. The resin was collected by filtration, washed with DMF (2 × 20 mL, 10 min per wash), CH_2Cl_2 (2 × 20 mL, 10 min per wash), and MeOH (2 × 20 mL, 10 min per wash), and then treated with a solution of acetic anhydride−DMF (1:9 v/v). After 30 min, the resin was collected by filtration, washed with DMF (2 × 20 mL, 10 min per wash), CH_2Cl_2 (2 × 20 mL, 10 min per wash), and MeOH (2 × 20 mL, 10 min per wash), and finally dried in vacuo. MS(ESI) (cleaved sample): *m*/*z* 251 [(M − H)$^-$].

General Labeling Procedure. Na[^{99m}TcO$_4$] was eluted from a ^{99}Mo/^{99m}Tc generator using a solution of 0.9% saline. Transfer chelate-tin solutions were prepared by dissolving 12.5 g of the transfer chelate in 75 mL of nitrogen sparged water. Stannous chloride dihydrate (200 mg) was dissolved in 1 mL of 1 M HCl and added to the solution containing the transfer chelate. The pH of the resulting solution was adjusted to 7.2 using 1 N NaOH, and the volume was increased to 100 mL with nitrogen sparged water. Note that the solution must be used within 3 h of preparation to preserve the stannous chloride dihydrate.

The transfer chelate solution (0.1, 1 or 2 mL) was added to a serum vial containing the resin (25, 100, or 200 mg) and a stir-bar. The vial was crimp-sealed, and the headspace was replaced with nitrogen. Sodium pertechnetate was then added to each vial (2−15 mCi, 0.05−0.41 MBq), and the volume was adjusted to 2.5 mL with nitrogen sparged water. EtOH (250 μL) was then added, and the reaction mixture was allowed to proceed for 2.5−3 h. The reaction mixture was then heated to 80 °C in a Reacti-Therm heater-stirrer under a stream of N_2 to remove the residual ethanol, and the reaction mixture was subsequently cooled in an ice−water bath. The mixture was then loaded onto a C-18 Sep-Pak (Waters), which was washed

Scheme 1

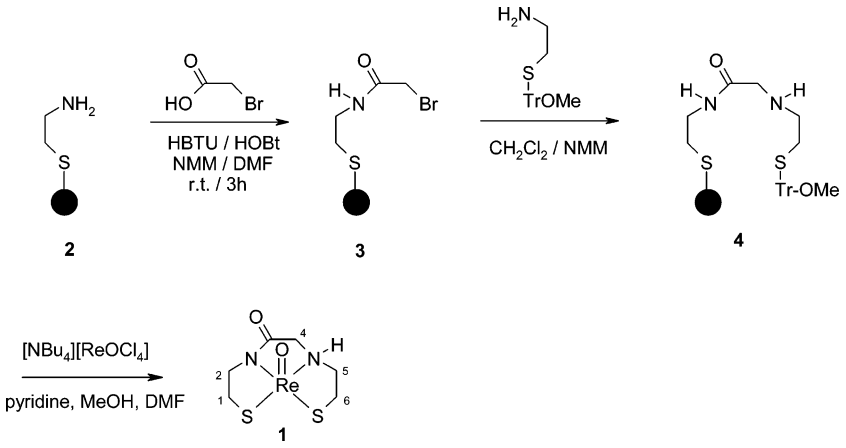


Table 1

solvent	reagents[a]	temp (°C)	yield (%)
CH_2Cl_2	[TBA][$ReOCl_4$]	25	7
DMF	[TBA][$ReOCl_4$]	25	9
DMF	[TBA][$ReOCl_4$]	85	40
DMF	[TBA][$ReOCl_4$], Et_3SiH	85	0
DMF	[TBA][$ReOCl_4$], 5% MeOH	85	47
DMF	[TBA][$ReOCl_4$], 1% pyridine	85	55
DMF	[TBA][$ReOCl_4$], 5% MeOH, 1% pyridine	85	85

[a] TBA = tetra-*n*-butylammonium.

two times with 10 mL of water, and then the product was eluted using 100% ethanol.

RESULTS AND DISCUSSION

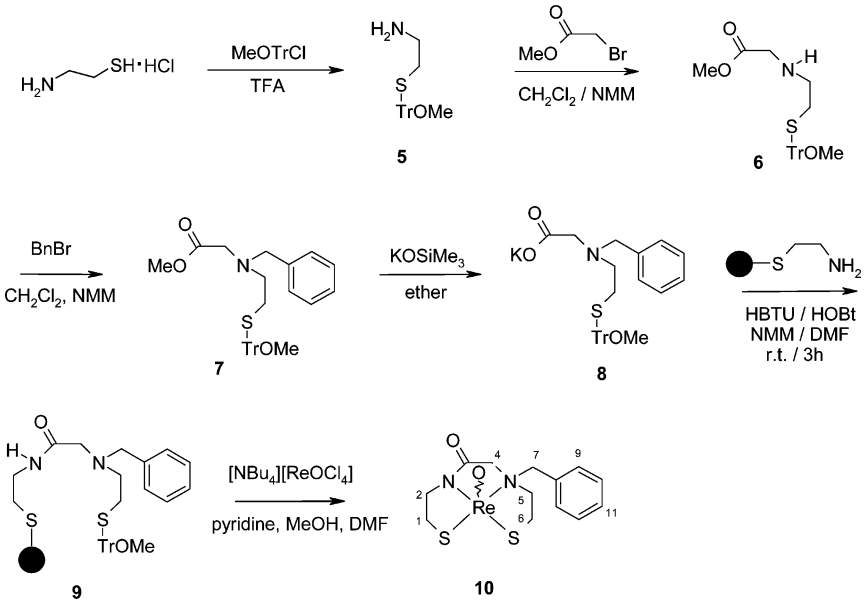
From some preliminary solution-phase studies, we found that *S*-*p*-methoxytrityl (PMT) groups on the MAMA chelate were readily cleaved in the presence of $[TcOCl_4]^-$ and $[ReOCl_4]^-$. Consequently, the MAMA chelate was prepared on a solid support, in which one of the pendant thiol groups was linked to a polystyrene resin through a PMT group. Commercially available 2-aminoethanethiol loaded-4-MeOTr-polystyrene resin was placed into a reaction chamber of a solid-phase synthesis vessel and swelled in DMF. Following the solution phase approach, bromoacetyl bromide was added at −30 °C in the presence of triethylamine under a constant bubbling of Ar. Despite a number of attempts using a variety of conditions, the yields of **3** were unacceptably low. In turn, success was achieved when bromoacetic acid was coupled to the resin-bound amine using HBTU−HOBt (*16*) (Scheme 1). After 2 h under these coupling conditions, the Kaiser test (*17*) indicated the reaction had gone to completion. The desired chelant **4** was prepared by adding a large excess of the thiol-protected cysteamine to drive the reaction to completion. The progress of the reaction was followed by cleaving small amounts of the resin using 5% TFA in CH_2Cl_2 and analyzing the products by HPLC-MS. After 48 h, the major peak in the chromatogram corresponded to that of the MAMA chelate.

The optimal conditions for preparing the Re-MAMA complexes were identified by varying the choice of solvent, base, and reaction temperature (Table 1). In a typical experiment, the resin was swelled in a solvent or solvent mixture and $[NBu_4][ReOCl_4]$ was added, and the contents of the vial were agitated using a platform mixer (300 rpm). For reactions run at room temperature in the absence of base, the color of the resin changed to deep green within 3 h. This, in our experience, indicated the presence of a partially chelated intermediate. The greenish resin was analyzed by IR, and a strong absorbance at 909 cm^{-1} was observed, which is indicative of a Re═O stretch (*18*). When the reaction mixture was left shaking overnight in CH_2Cl_2 in the absence of base, the solution took on a light orange color, and small amount of a red solid was seen in the bottom of the reaction vial. The precipitate was in fact the target compound, which was established by comparing the characterization data of the isolated product with that obtained from an authentic standard prepared by following literature procedures (*12*). The yield of the metal complex improved substantially when the temperature of the reaction was increased to 85 °C and when the solvent was changed to a mixture of DMF and MeOH. Methanol was added to trap the cation that is formed upon liberation of the protecting group and release of the chelate from the resin. In contrast, the use of triethylsilane as a scavenger in place of methanol did not improve the yield. An additional increase in yield to 85% was achieved when a small amount of pyridine was added to the reaction. Pyridine prevented disproportionation of the M(V) chlorides at the higher reaction temperatures and likely facilitated deprotonation of the amide group on the chelate. From this reaction, the product was isolated through a plug of silica gel or a silica gel Sep-Pak, which was necessary to separate the cleaved protecting group from the target compound.

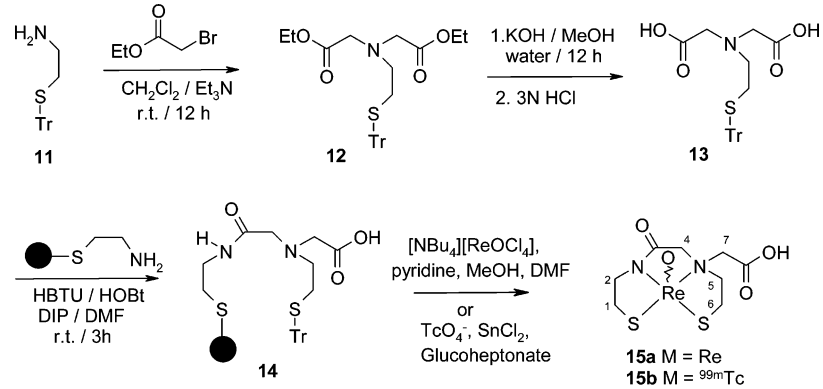
Direct functionalization of the amino group of **4** proved to be very difficult. The efficiency of the derivatization with a variety of reactive alkylating agents, including benzyl bromide and allyl bromide, was highly variable, and despite numerous attempts involving an array of reaction conditions, the yields of the desired products were not acceptable. To circumvent this issue, an alternate route was developed whereby a large portion of the chelate was synthesized in solution and then coupled to the resin in the final step using a robust amide bond forming reaction.

The initial target for the convergent approach was the *N*-benzyl MAMA derivative, which was previously prepared in solution by O'Neil et al. (*12*). The requisite precursor, compound **6**, was prepared in 90% yield by reacting methyl bromoacetate with *S*-(4-methoxytrityl)-2-aminoethane thiol (Scheme 2). Alkylation of **6** with benzyl bromide to give **7** occurred quantitatively in the presence of triethylamine. Conversion of the methyl ester to the acid was carried out by treating **7** with potassium trimethylsilanoate in ether (*19*). Over the course of 4 h, a thick white paste formed in the bottom of the reaction vessel, which was the potassium salt **8**. The product was collected by filtration and passed down a short column of silica to separate it from any excess potassium trimethylsilanoate. Compound **8** was subsequently coupled to the resin-bound amine using HBTU and HOBt. After 4 h, the resin gave a negative Kaiser test and was therefore filtered, washed rigorously, and dried under vacuum.

Scheme 2



Scheme 3



The complexation−cleavage reaction was run under the optimum conditions identified for the unsubstituted MAMA complex. The resin **9** (200 mg) was suspended in 5 mL of a solution containing MeOH (5%), pyridine (1%), and DMF. [TBA][$ReOCl_4$] was added and the vial was heated to 85 °C for 16 h. The Re complex **10** was isolated in 70% yield through a simple plug of silica. The spectroscopic data for the product were consistent with reported values. Interestingly, the ^{1}H NMR of the product showed that compound **10** was isolated as a mixture of syn and anti diastereomers in approximately a 12:1 ratio. In the case of compound **1** and all subsequent N-substituted ligands, only the syn isomers were observed in the ^{1}H NMR.

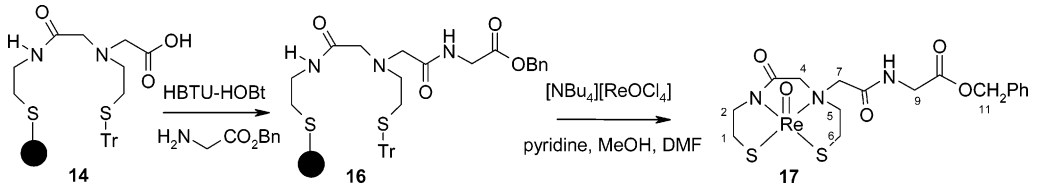
To introduce a functional group that can be used to link to amine groups, the resin-bound chelate **14** was prepared (Scheme 3). The synthesis involved first preparing the di-ester **12** by condensing 2 equiv of protected 2-aminoethanethiol with an excess of methylbromoacetate. Unfortunately, the reaction conditions required to prepare and isolate compound **12** necessitated the use of the trityl (Tr) protecting group in place of the PMT group. Compound **12** was converted to the diacid **13**, which was subsequently attached to the resin-bound amine via a HBTU−HOBt coupling procedure taking advantage of the inherently dilute nature of the resin to ensure monosubstitution. The resin-bound chelate was subsequently suspended in DMF containing 1% pyridine and 5% MeOH and treated with [NBu_4]-[$ReOCl_4$]. After 16 h, the dark orange solution was concentrated in vacuo yielding the desired product, as a red solid, in 70% yield.

The IR spectrum of **15a** showed a carbonyl stretch at 1654 cm^{-1} and the rhenium oxo stretch at 966 cm^{-1}. The splitting patterns present in the chelate portion of the ^{1}H NMR spectrum of **15a** are similar to those for compound **10**. The main difference was that the chemical shifts of the methylene protons in the chelate were for the most part shifted downfield relative to those in the benzyl derivative. The methylene protons for the pendent acid are diastereotopic and appear as a doublet (H-7A, 4.47 ppm) and a doublet of doublets (H-7B, 4.22 ppm), which in the latter case includes weak coupling to H-5α. The good yield of **15a** was achieved despite the fact that the trityl protecting group is much less reactive than the methoxy-trityl protecting group present in compound **9**.

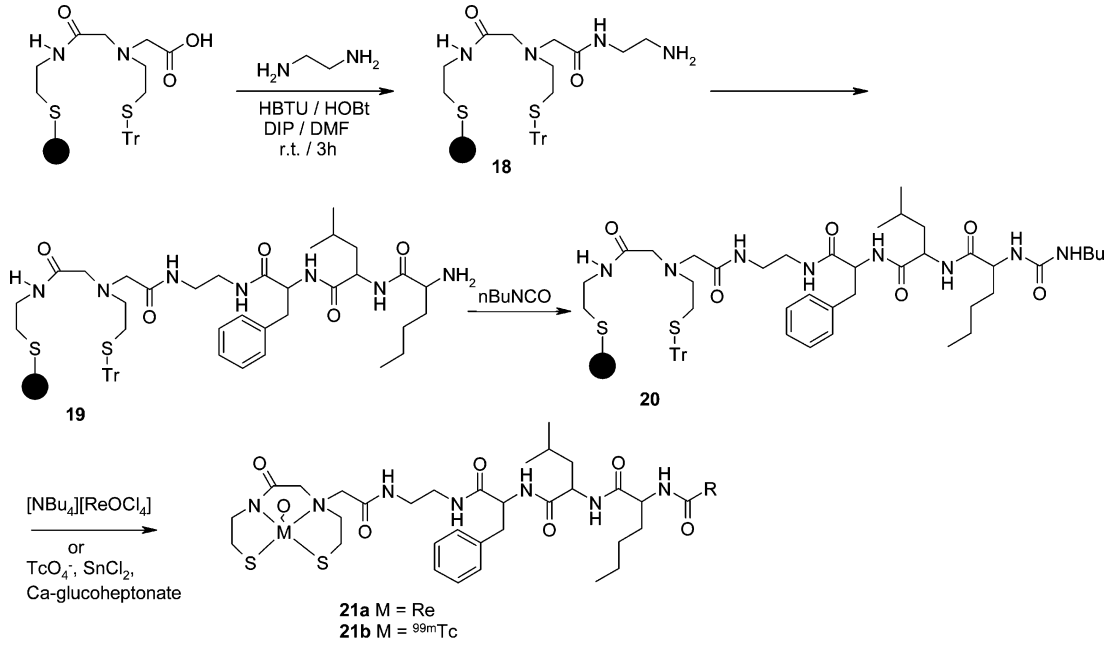
Prior to automated solid-phase synthesis work, the reactivity of the pendent group was investigated by attempting to couple O-benzyl glycine, as a model amine, to the resin-bound chelate **14** (Scheme 4). The amine and *N*-methyl morpholine (NMM) in DMF was added to the carboxylic acid, which had been premixed with HOBt/HBTU, and the mixture was agitated using a flow of Ar for 3 h. After the sample was filtered and washed, the Re-O-benzyl glycine-MAMA complex **17** was prepared by reacting the O-benzyl glycine functionalized resin **16** with [TBA][$ReOCl_4$]. The product, a red solid, was again isolated through a plug of silica in 68% yield. The ease with which the acid can be derivatized affords the opportunity to attach a wide array of targeting agents, beyond simply peptides, to the resin-bound chelate.

Peptide Conjugation. The next goal was to develop a system that would be suitable for the preparation of peptide−MAMA

Scheme 4



Scheme 5



conjugates. Although it is conceivable to build peptides off the carboxylic acid funtionalized MAMA derivative, automated peptide synthesis is more often performed starting from a primary amine using Fmoc-protected amino acids. A primary amine functionalized MAMA chelate was prepared by coupling a diamine to the carboxylic acid of **14** (Scheme 5). An excess of ethylenediamine was added to the carboxylic acid functionalized resin **14** in the presence of HBTU and HOBt and the reaction was allowed to proceed for 3 h at room temperature. A small sample of resin was cleaved and analyzed by ESMS, which showed a peak corresponding to the product and no signs of unreacted acid.

Compound **18** was subsequently added to the reaction well of an Advanced Chemtech automated synthesizer and a model peptide was built up using a standard Fmoc/HBTU protection/coupling method. The target peptide was a *n*-butyl urea derivative of Phe-Leu-Nle, which is an antagonist for the formyl peptide receptor (FPR) (*20*). This ligand was of interest because radiolabeled compounds that are capable of binding selectively to the FPR on white blood cells can be used to image sites of infection and inflammation (*21*). The synthesis of the model peptide began by reacting a 4-fold excess of Fmoc-protected phenyl alanine with **18** using HBTU as the coupling agent. After the resin was washed and the Fmoc group removed using 20% piperidine in DMF, the next two amino acids, Fmoc-leucine and Fmoc-norleucine were added in an analogous manner. After deprotection of the terminal amine, conversion to the urea was carried out by adding a 10 fold excess of *n*-butyl isocyanate and shaking the mixture for 12 h. To ensure the desired conjugate had formed, a sample was cleaved from the support using a cocktail consisting of 20% TFA and 2% triisopropylsilane in DCM and the solution analyzed by LC-MS, which showed only the desired product. The peptide-functionalized resin was subsequently treated with [TBA][$ReOCl_4$] and after the green suspension was heated in an oil bath for 16 h, the resin was removed by filtration and the filtrate concentrated by rotary evaporation. The resulting red/brown solid was dissolved in a mixture of methanol and dichloromethane and purified by passing it through a plug of silica to remove the residual protecting group. The product, which showed a single peak in the HPLC, was isolated in 47% yield.

Radiolabeling. Initial attempts at labeling with ^{99m}Tc were performed using the carboxylic acid functionalized chelate **14**. The resin (100 mg) was suspended in saline, and a solution of stannous chloride dihydrate and calcium glucoheptonate was added. The latter compound, which was selected by screening a number of transfer chelates, was added to prevent premature reduction of TcO_4^- to $TcO_2 \cdot 2H_2O$. To the suspension, freshly eluted pertechnetate was subsequently added, and the mixture was analyzed by HPLC as a function of time. The desired product appeared after 6 h at room temperature; however, it took 16 h to reach a maximum yield of 40% with the other major product being TcO_4^-. The addition of EtOH as a cosolvent and heating did not increase the overall yield of **15b** or change the kinetics of the reaction. The long reaction time and suboptimal yield is likely a result of the poor compatibility of the polystyrene-based resin with water. Despite the low concentration of technetium in the reaction, it also appeared as though there were insufficient numbers of accessible reaction sites on the support to afford high yields of the desired product. In the absence of the complexation reaction, oxidation becomes a competing reaction pathway, which explains the appearance of TcO_4^-.

In an attempt to improve the yield and/or reduce the reaction time, the polystyrene support was substituted with a Tentagel resin. Tentagel-type resins are copolymers consisting of a low cross-linked polystyrene matrix on which poly(ethylene glycol) (PEG) is grafted. The presence of PEG enables the support to

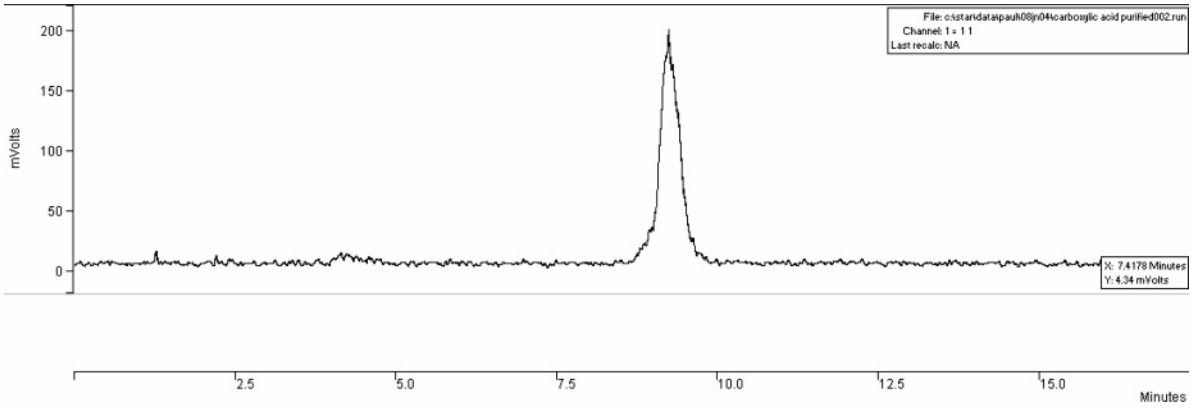


Figure 1. HPLC γ-radiochromatogram of **15b**.

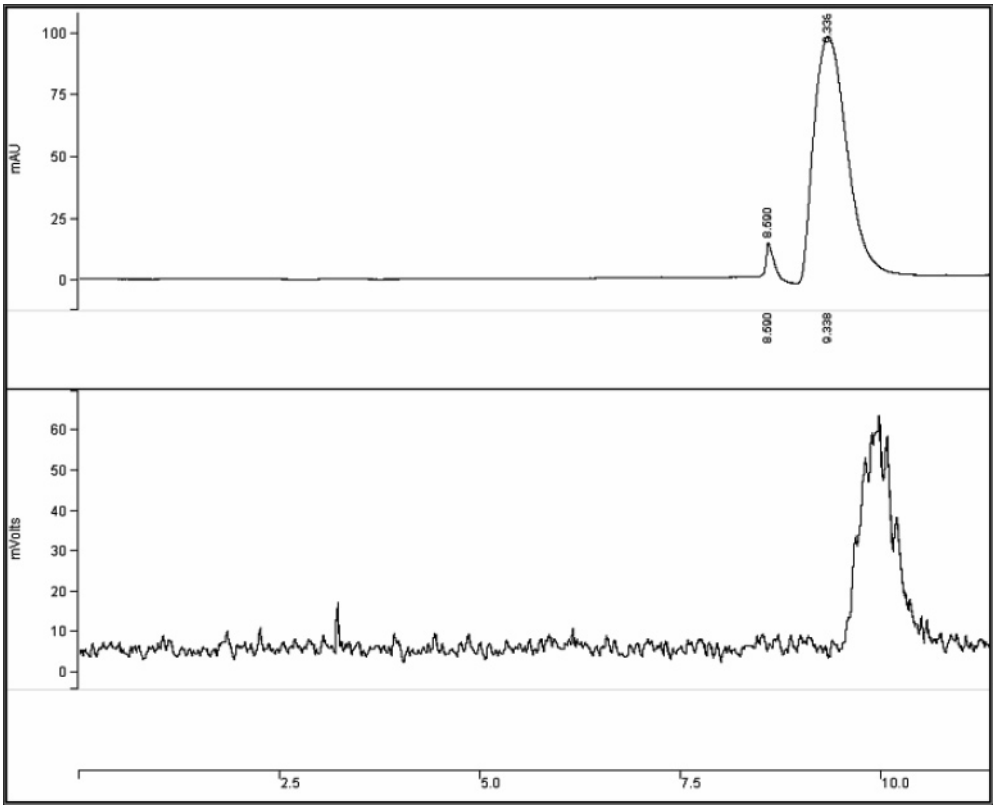


Figure 2. HPLC chromatograms of Re-peptide complex **21a** (UV, top) and ^{99m}Tc complex **21b** (γ). The difference in retention time corresponds to the separation of the detectors which are connected in series.

swell in polar solvents including water, a feature that was used successfully by Alberto et al. for the solid-phase synthesis of Tc(I) amine complexes (*7*). The one disadvantage of switching resins was that the commercially available form of the Tentagel resin has the trityl linker bound to the polymer via an electron with drawing carbonyl group. This could decrease the reactivity of the chelate derivative toward the complexation and cleavage reactions compared to the analogue bound to the methoxy-trityl substituted polystyrene resin.

After loading 2-aminoethane thiol was carried out, the carboxylic acid derivative was prepared on the Tentagel resin by coupling the resin-bound primary amine with the dicarboxylic acid **13** using HBTU, HOBt, and DIP following a similar methodology used to prepare the polystyrene analogue. After 3 h, a small amount of the resin was removed, filtered, washed, and tested for the presence of primary amines using the Kaiser test. No color (blue) developed even after extensively heating the resin, suggesting that the majority of the primary amine sites had reacted.

Radiolabeling of the TentaGel version of **14** was carried out using different amounts of resin in solutions containing varying amounts of calcium glucoheptonate and stannous chloride dihydrate. To prevent unwanted reoxidation, all reactions were performed in crimp-sealed vials in which the headspace gas was exchanged with nitrogen and all solvents were sparged with nitrogen. Reactions containing 2.5, 12.5, and 25 mg of the transfer chelator (and 100 mg of resin) were analyzed after 2 h by HPLC. There was three times the yield of the target when 12.5 mg of calcium glucoheptonate compared to the reaction with 2.5 mg of ligand, but no further increase in the amount of **15b** was observed when 25 mg was added to the reaction mixture. As a result, 12.5 mg of transfer chelate was used in all subsequent reactions.

Similar experiments were conducted to determine the optimal amount of resin. Reactions were carried out with 25, 100, or 200 mg of resin (10, 40, or 80 μmol of resin-bound substrate). Upon combining the resin with the calcium glucoheptonate−stannous chloride mixture and $^{99m}TcO_4^-$, the vials were heated

with stirring for 2 h. The radiochemical yield of the reaction with 200 mg of resin was 38%, while that for the 100 mg and 25 mg samples were 17 and 5%, respectively. The impact of changing the amount of resin on the radiochemical yield is somewhat surprising, given the large excess of reactive sites compared the amount of ^{99m}Tc even at the lowest amount of resin used. HPLC analysis of the reactions as a function of time revealed that after 2 h there was little change in radiochemical yield. At the time the yield reached a maximum there was still unreacted Tc-glucoheptonate present in solution, which suggests that even with the PEG modified resin, reactions were taking place only at a very small portion of the available reactive sites and that a kinetic barrier was limiting the overall yield.

To increase swelling as a means of improving accessibility to the chelate, a small amount of ethanol was added to the reaction immediately prior to heating. This resulted in a change in the appearance of the resin, which became an evenly distributed collection of beads as opposed to a congealed gellike mass. After 2 h of heating, HPLC indicated nearly 50% conversion versus 38% for the same reaction performed without EtOH present. Prior to purification, ethanol was removed by heating the reaction vial to 80 °C under a stream of nitrogen for 5 min. The reaction mixture was then loaded onto a C-18 Sep-Pak and washed two times with 10 mL of water. The activity that was eluted in these fractions corresponded to pertechnetate and Tc-glucoheptonate. Subsequent elution with 100% ethanol removed all of the remaining activity on the Sep-Pak where HPLC analysis (Figure 1) of the fraction showed a single peak whose retention time matched that for **15a**. The radiochemical purity of **15b** was 97%, which is greater than the radiochemical purity requirements for technetium kits and certainly acceptable for performing preclinical imaging studies.

The acid **14** attached to the Tentagel resin was subsequently derivatized with the model peptide in the same manner used to prepare **20**. Radiolabeling was conducted under the conditions developed for **14** using 555 MBq (15 mCi) of activity in saline containing 12.5 mg of glucoheptonate, 2 mg of $SnCl_2 \cdot 2H_2O$ and 250 μL of EtOH at pH 7.2. Purification was performed again using a simple solid-phase extraction on a C-18 cartridge where the product was isolated in 35% radiochemical yield with a radiochemical purity exceeding 99%. The peak in the γ-trace corresponded with the peak in the UV trace of the cold rhenium analogue (Figure 2). In the UV chromatogram of the HPLC trace, there was no evidence of unlabeled peptide.

The SPS labeling system described here is capable of producing Re-MAMA complexes in good yield and the corresponding ^{99m}Tc complexes in reasonable yield. The discrepancy between the two is likely associated with the fact that the Tc reactions are carried out at much lower concentrations and in a highly polar reaction medium using a hydrophilic starting material (Tc-gluconate complex). As part of the future work, the corresponding ^{186}Re and ^{188}Re complexes of the polymer bound ligands will be prepared, which will help validate our hypothesis in terms of the influences of the reaction medium and reagent concentrations. Despite the low yields of the ^{99m}Tc complexes, all products were isolated in high purity free from any unreacted starting material. The only impurities that had to be separated from the product were the cleaved protecting group and residual metal salts. This was accomplished by employing simple solid-phase extraction procedures, which can be easily automated. During the development of the solid-phase labeling system, a series of functionalized MAMA derivatives were prepared. Derivatives included both acid an amine functionalized resins. These compounds, which react efficiently with amines and carboxylic acids, respectively, can be used to prepare a wide range of other biomolecule conjugates and their corresponding Re and ^{99m}Tc complexes.

CONCLUSIONS

A convenient solid-phase synthesis methodology for the preparation of bifunctional Re and Tc chelate complexes was developed. The products, which can be prepared in parallel, can be isolated by a simple solid-phase extraction procedure that is easily automated. It was demonstrated that peptide conjugates of the functionalized resins can be prepared in high yield using traditional automated peptide synthesis protocols thereby creating the opportunity to prepare libraries of novel tracers. The yields of the products at tracer level were not quantitative, which is a barrier that will need to be overcome if this approach is to be used for the clinical production of radiotracers. This can be accomplished by screening different polymer supports and more reactive linkers. Notwithstanding, the ability to produce Re and high specific activity ^{99m}Tc complexes in parallel without the need to use HPLC along with the opportunity to prepare libraries of peptide−ligand conjugates using conventional automated peptide synthesis will render the reported method particularly useful for preclinical discovery work.

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Supporting Information Available: HPLC and ^{1}H NMR spectra for the Re complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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