



SYNTHESIS AND TECHNETIUM-99M LABELING OF CYCLIC GP IIB/IIIA RECEPTOR ANTAGONISTS CONJUGATED TO 4,5-BIS(MERCAPTOACETAMIDO)-PENTANOIC ACID (MAPT)

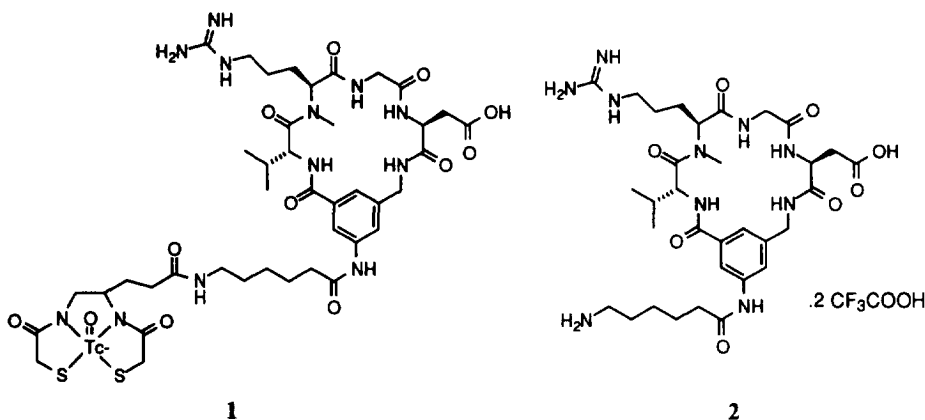
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Abstract: The synthesis of cyclic Arg-Gly-Asp GP IIB/IIIA receptor antagonists conjugated to 4,5-bis(S-1-ethoxyethyl-mercaptoacetamido)pentanoic acid is reported. The corresponding Tc-99m-labeled complexes were prepared by exchange labeling with Tc-99m-glucoheptonate. This indirect labeling approach is an alternative to the preformed chelate approach using mapt.

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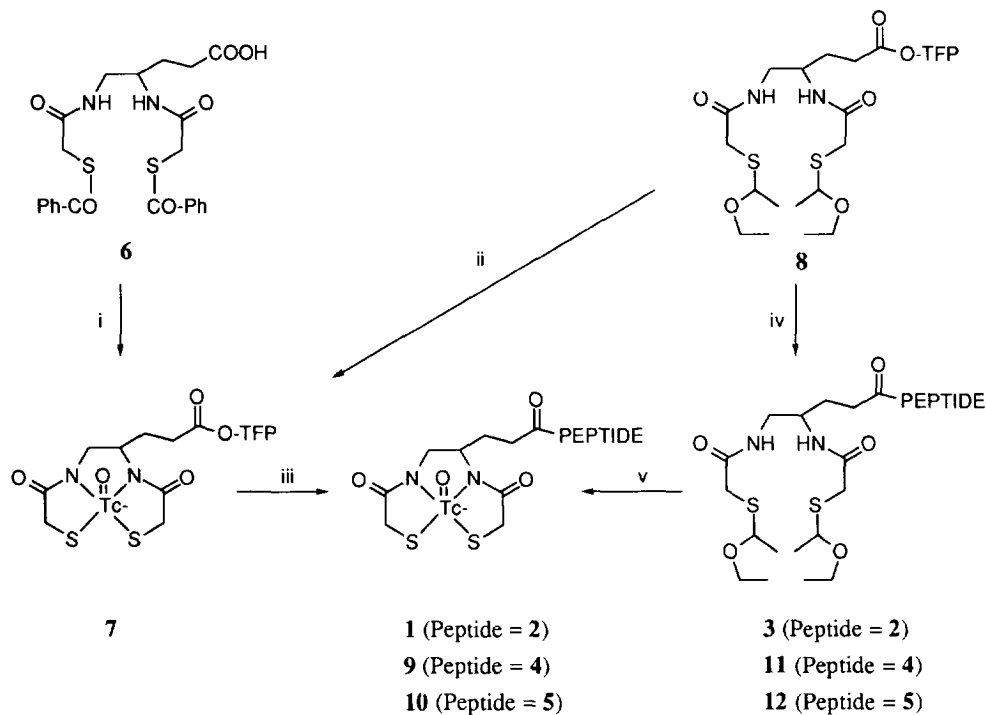
The unequivocal diagnosis, treatment and prevention of thromboembolic disease has gained considerable attention recently. During the past few years, advances have been made in non-invasive diagnosis of thromboembolism, notably deep vein thrombosis and pulmonary embolism.¹ A thrombus is an intravascular deposit predominantly comprising fibrin, and aggregates of platelets and red blood cells. Platelet aggregation is mediated by fibrinogen, which binds via the Arg-Gly-Asp (RGD) motif to the GP IIB/IIIA receptor expressed on activated platelets. Small molecules, containing the RGD motif and RGD mimetics, which are antagonists of the fibrinogen receptor GP IIB/IIIA, represent a rapidly growing class of antithrombotics.² Technetium-99m (Tc-99m) labeled fibrinogen receptor antagonists, which bind to the GP IIB/IIIA receptor on activated platelets with high specific activity are, therefore, potential radiopharmaceuticals for the detection of thrombi.

We have reported³ the synthesis of Tc-99m labeled analogues (such as **1**, **9**, and **10**) of the GP IIB/IIIA receptor antagonists DMP728 and DMP757⁴ as potential thrombus imaging agents. The uptake in thrombi of several Tc-99m-labeled compounds was evaluated and **1** was the most promising compound.^{3, 5}



Earlier, we described in detail the synthesis of **1** and **9** via the preformed chelate approach⁶ utilizing the bifunctional chelator 4,5-bis-(S-benzoylmercapto-acetamido)-pentanoic acid (mapt, **6**). The coordination chemistry of Tc(O)-bis-(mercaptoacetamido)-pentanoate (Tc-99m-mapt, **7**) is well documented and this approach has been extensively applied previously towards the labeling of antibodies.⁷ In the preformed chelate approach, shown below, the Tc-99m-mapt chelate **7** was formed first (in two steps), purified by preparative HPLC, and then conjugated to peptide **2** at the tracer level to give **1** (path i/iii).⁶

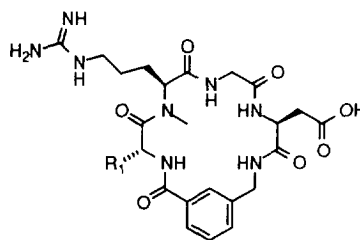
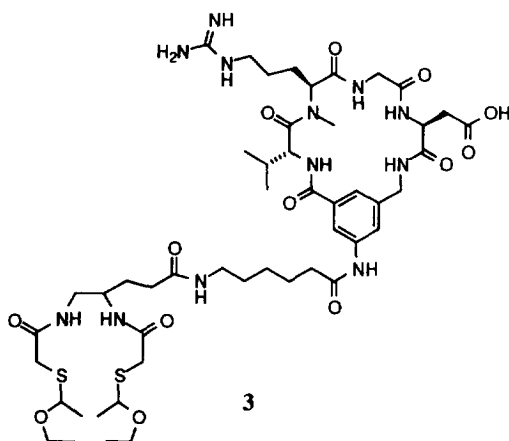
We report here, the utility of mapt and synthesis of **1** and analogues **9** and **10** via the *indirect labeling approach*. In this approach, the bifunctional chelator (**8**) is first attached to the peptide to form the mapt-peptide conjugate, followed by Tc-99m labeling by ligand exchange with Tc-99m glucoheptonate (path iv/v).



i. (a) $^{99m}\text{TcO}_4^-/\text{Na}_2\text{S}_2\text{O}_4$, pH 10-12; (b) TFP/WS-CDI, pH 6 (ref 6). ii. see ref 7 iii. Peptide **2** or **4** or **5**, pH 9.5-10 (as in ref 6). iv. Peptide **2** or **4** or **5**, DMF, TEA, rt. v. Tc-99m glucoheptonate

Thus, reaction of peptide **2** with the active ester 2,3,5,6-tetrafluorophenyl 4,5-bis(S-1-ethoxyethyl-mercapto-acetamido)pentanoate (**8**) in DMF in the presence of triethylamine at room temperature gave the mapt-peptide conjugate **3** in 56% yield after trituration with ethyl acetate. Similarly, mapt-peptide conjugates **11** and **12** were readily synthesized from **4** and **5**, respectively.^{8,9} The S-1-ethoxyethyl protecting group appeared to be stable during preparative HPLC of **11**. We believe these compounds (**3**, **11**, and **12**) are the first reported examples of the mapt chelator conjugated to such receptor targeted peptides, *prior* to formation of the Tc-99m complex. Therefore, for small molecules, this alternative approach also eliminates the need for multiple

preparative HPLC procedures performed at the tracer level using the preformed chelator approach.



DMP 728 $R_1 = \text{Et}$

DMP 757 $R_1 = \text{iPr}$

4 $R_1 = -(\text{CH}_2)_4\text{-NH}_2 \cdot 2\text{TFA}$

5 $R_1 = -(\text{CH}_2)_4\text{-NH-CO-(CH}_2)_5\text{-NH}_2 \cdot 2\text{TFA}$

Complexes **1**, **9**, and **10** were synthesized from compounds **3**, **11**, and **12**, respectively, by combining 0.25 mg of each compound dissolved in 150 μL of isopropanol-water (2:1), 150 μL of glacial acetic acid-0.2 M HCl (1:7), 0.5 mL of a Glucoscan® kit reconstituted with 2.5 mL of water and 0.3 mL of $^{99\text{m}}\text{TcO}_4^-$ in saline (40 mCi). Each reaction mixture was heated at 75 °C for 15 min and then analyzed by radio-HPLC¹⁰ (path v). The desired products were obtained in good yields. For comparison of retention times, complexes **1** and **9** were also synthesized by path ii/iii. Compound **8** (0.3 mg) was dissolved in 200 μL of acetonitrile, 150 μL of glacial acetic acid-0.2 M HCl (1:7), 0.5 mL of a Glucoscan® kit reconstituted with 2.5 mL of water and 0.2 mL of $^{99\text{m}}\text{TcO}_4^-$ in saline (70 mCi). The reaction mixture was heated at 75 °C for 15 min to form complex **7**. To the solution of complex **7**, was added 2-3 mg of peptide **2** or **4** dissolved in 0.5 mL of 1.0 M sodium bicarbonate buffer, pH 10, followed by 0.3 mL of 1.0 N NaOH. After 1 h at room temperature, formation of **1** or **9** was observed by radio-HPLC.¹⁰ Yield and analytical data of the radiochemistry are shown in Table 1.¹¹

Table 1. Yield and Analytical Data for the Tc-99m-mapt Conjugates.

Complex	Yield (%) (path v)	Ret. Time (min) (path v)	Ret. Time (min) (path ii/iii)	Ret. Time (min) (ref 6, path i/iii)
1	68	16.8	16.5	16.2
9	77	15.6	15.5	15.0
10	60	15.0	not determined	15.5

Thus, we have demonstrated a new application of the mapt chelator, namely in the synthesis of receptor-directed (GP IIb/IIIa) Tc-99m labeled peptides *via the indirect labeling method*. The retention times observed for the complexes synthesized by two the different paths (indirect labeling and the preformed chelate) are in good agreement and are in accord with the preformed chelate data previously reported.⁶ These data also provide strong confirmation of the identity of the Tc-99m labeled mapt-peptide complexes.

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5. Barrett, J. A.; Damphousse, D. J.; Heminway, S. J.; Liu, S.; Edwards, D. S.; Looby, R. J.; Carroll, T. R. *Bioconj. Chem.* **1996**, *7*, 203. Compound **1** was actively incorporated into a growing thrombus (canine thrombus model) with images (gamma scintigraphy) clearly detectable. Thrombus/blood and thrombus/muscle ratios at 2 h were approximately 7:1 and 10:1, respectively.
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8. Compound **3**, yield 56%; purity by analytical HPLC: 100%; HRMS-FAB: for C₄₉H₈₀N₁₂O₁₃S₂ + H, *m/z* calc. 1109.5487, found 1109.5487. **11**, post HPLC purification yield 52%; purity: 94%; HRMS-FAB: for C₄₄H₇₁N₁₁O₁₂S₂ + H, *m/z* calc. 1010.4803, found 1010.4813. **12**, yield 81%, purity: 100%; HRMS-FAB: for C₅₀H₈₂N₁₂O₁₃S₂ + H, *m/z* calc. 1123.5644, found 1123.5655. The analytical HPLC method used a Hewlett Packard Model 1090M instrument and a Vydac C₁₈ column (4.6 mm x 25 cm) at a flow rate of 1.0 mL/min.; a gradient mobile phase from 98% A (0.1% TFA in water) to 100% B (0.1% TFA in 90% acetonitrile, HPLC grade) at 45 min was used. UV detection was set at 220 nm.
9. The authors wish to thank Danuta Glowacka and Thomas Harris for providing peptides (described in ref 3) **4** and **5**, and Steven Johnson for peptide **2**.
10. The radio-HPLC method used a Hewlett Packard Model 1090 instrument and a Vydac C₁₈ column (4.6 mm x 25 cm) at a flow rate of 1.0 mL/min with a gradient mobile phase from 100% A (10 mM phosphate buffer, pH 6.0) to 30% B (acetonitrile) over 15 min then to 70% B over 10 min. The detector system was a Ludlum Model 44-2 NaI probe, connected to a Ludlum Model 177 ratemeter and a Multichrom® data system. ^{99m}TcO₄⁻ in saline was obtained from a DuPont Merck Technelite® generator (eluate age < 2 h, prior elution < 24 h). A Glucoscan® kit contains 200 mg sodium glucoheptonate and 60 µg stannous chloride. Deionized water was obtained from a Millipore Milli-Q Water Purification System and was of > 18 MΩ quality.
11. (a) Together with radio-HPLC, simultaneous detection by UV was also performed. The differences in the retention times, using the method described above, of the Tc-^{99m} labeled mapt-peptide complexes and the unlabeled peptides (with or without mapt) is adequate for a facile separation. For example, in the indirect labeling method the mapt-peptide conjugates eluted about 2 min after the Tc-^{99m} labeled mapt-peptide complexes (**1** and **9**). In the preformed chelate experiment described here, the unlabeled peptides (**2** and **4**) eluted 5-7 min prior to the Tc-^{99m} mapt-peptide complexes. (b) As discussed in ref 6 the diastereomers of the Tc-^{99m} mapt-peptide complexes, arising from the use of racemic mapt and formation of a stereocenter at Tc(O), were not resolved under the HPLC conditions utilized. In all cases the diastereomeric mixture was evaluated in the canine thrombus model (described in ref 5). (c) It should be noted that differences in the retention times of the mapt-peptide conjugates and the Tc-^{99m} mapt-peptide complexes (and diastereomers) will vary depending on the peptide used.