Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 2597–2600

TETRAHEDRON LETTERS

S-Trityl protection of bis-amino bis-thiol (BAT) chelator enables flexible derivatisation and facile labelling with technetium-99m

Bernard J. Cleynhens, Guy M. Bormans, Hubert P. Vanbilloen, Dominique V. Vanderghinste, Davy M. Kieffer, Tjibbe J. de Groot and Alfons M. Verbruggen*

Laboratory for Radiopharmaceutical Chemistry and Nuclear Medicine, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium

Received 15 January 2003; accepted 21 January 2003

Abstract—We have coupled *S*,*S*′-bis-trityl *N*-BOC protected 1,2-ethylenedicysteamine, a bis-amino bis-thiol (BAT) tetraligand, via a propylene or ethylene spacer to several biologically active molecules including 2-nitroimidazole, desethylflumazenil, a beta-CIT analogue, glucose and 2-(2′-hydroxy-4′-aminophenyl)-1,3-benzothiazole. The conjugates were efficiently labelled with technetium-99m by consecutive heating of the *S*,*S*′-bis-trityl protected ligand in hydrochloric acid followed by neutralisation and heating in the presence of ^{99m}Tc-tartrate. The *S*,*S*′-bis-trityl BAT chelator is an interesting synthon that allows both flexible derivatisation with various biologically active molecules and facile labelling with technetium-99m.

Bis-amino bis-thiol (BAT, N_2S_2) tetraligands are frequently used bifunctional chelating agents which form stable neutral complexes with technetium-99m and can be conjugated to biologically active molecules.[1–5] The latter may exhibit affinity for specific targets such as receptors, transporters or enzymes and can vectorise the technetium-99m radiolabel to tissues expressing these targets after intravenous administration of the radiolabelled conjugate. The conjugation chemistry of this kind of technetium binding bifunctional ligands is often hampered by the instability of the thiol groups. The thiols must be protected during conjugation and storage, since in unprotected form they are easily oxidised to the corresponding disulfide. A frequently used method of thiol protection in BAT-type compounds is their conversion to a disulfide, which can be reduced to the corresponding bis-thiol using $LiAlH_4$ or $NaBH_4$ prior to labelling with technetium-99m.[6–8] Alternatively, the thiol groups have been protected with a *p*-methoxybenzyl group, which is removed using a mixture of CF_3COOH, methanesulfonic acid and anisole,[9] or a trityl group in which case the thiols are deprotected mostly with CF_3COOH under cation trapping conditions (triethylsilane and anisole)[10] (Fig. 1). Such deprotection methods are laborious and it is difficult to isolate and store the deprotected ligands. The time between deprotection and labelling with technetium-99m should be kept as short as possible to avoid disulfide formation.

Therefore, we have developed a method in which deprotection of the ligand and labelling with ^{99m}Tc

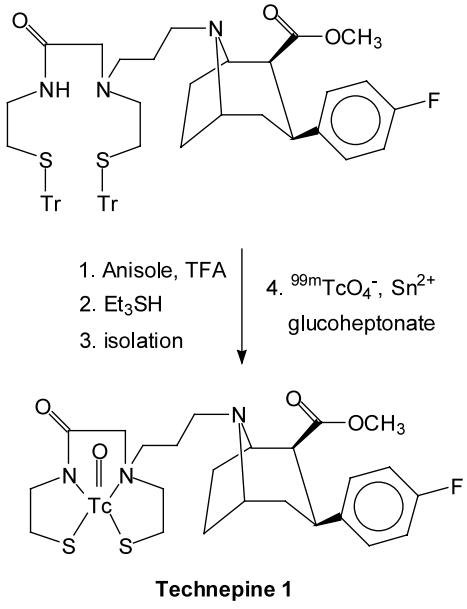


Figure 1. Synthesis of Technepine 1 by deprotection of the *S*,*S*′-bis-trityl protected precursor followed by labelling with ^{99m}Tc.[10]

* Corresponding author. Tel.: +32-16-34 37 30; fax: +32-16-34 38 91; e-mail: alfons.verbruggen@uz.kuleuven.ac.be

0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)00224-7

occur in a one-pot two-step procedure that is amenable to 'kit' formulation. The most simple BAT-ligand was chosen as the model, i.e. 1,2-ethylenedicysteamine.

1,2-Ethylenedicysteamine (**a**, Scheme 1) was obtained in high yields via a reductive dimerisation of thiazolidine by the action of sodium in liquid ammonia, in a similar way as described for the synthesis of 1,2-ethylenedicysteine (EC).[11,12] Subsequent thiol protection of this BAT compound was achieved by reaction with triphenylmethanol in trifluoroacetic acid. Trityl groups were preferred over other *S*-protecting groups (such as benzoyl, benzyl, benzamidomethyl, *p*-methoxybenzyl)[13] as they combine a good stability during conjugation with the ease of removal prior to labelling with technetium-99m.

The developed synthesis method of **a** is a good alternative to the conventional synthesis of BAT compounds. An earlier method for the synthesis of **a** consisted of reaction of 1,2-ethylenediamine with *S*-protected 2-mercaptoacetyl chloride followed by reduction of the amide groups with BH_3.[14] This reduction step is not feasible when *S*-trityl protecting groups are used, as they are not stable under these conditions.

One amino group of *S*,*S*′-bis-trityl-1,2-ethylenedicysteamine (**b**, Scheme 1) was protected with a *tert*-butyloxycarbonyl (BOC) group by treatment with 1 equiv. of $(BOC)_2O$ to afford a *S*,*S*′-bis-trityl, *N*-BOC protected BAT synthon (**c**, Scheme 1). This synthon could efficiently be coupled through a propylene or ethylene spacer to several vectors including nitroimidazole (NI), desethylflumazenil (FZ, acid analogue of flumazenil, RO-15-3890), 3β-(4-chlorophenyl)tropane-2β-carboxylic acid (TROP), glucose (GLU) and 2-(2′-hydroxy-4′-aminophenyl)-1,3-benzothiazole (BT) (Fig. 2).

Coupling to NI was done by reaction of **c** with 1-bromo-3-chloropropane and subsequent *N*-alkylation of 2-nitroimidazole yielding conjugate **1** (Scheme 1, Fig. 2). Coupling of **c** to TROP and FZ involved alkylation of **c** with 3-bromo-1-propanol and subsequent ester formation with the carboxylic acid of, respectively, TROP and RO-15-3890 after activation with oxalyl chloride to obtain **2** and **3**.

Conjugate **4** was synthesised by reaction of **c** with 2-[2′-(3″-tosyloxypropoxy)-4′-aminophenyl]-1,3-benzothiazole. For preparation of a thiol protected BAT-glucose conjugate, 2-iodoethyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucoside was reacted with **c** and the conjugate was subsequently deacetylated using sodium methanolate to obtain **5**.

The resulting conjugates were deprotected and isolated for characterisation (LC–MS) by reaction with trifluoroacetic acid and anisole. For labelling with ^{99m}Tc, however, we found that it was possible to deprotect a sufficient fraction of the ligand by addition of 0.5 ml HCl 0.5 M to a solution of 1 mg of the ligand in 0.3 ml

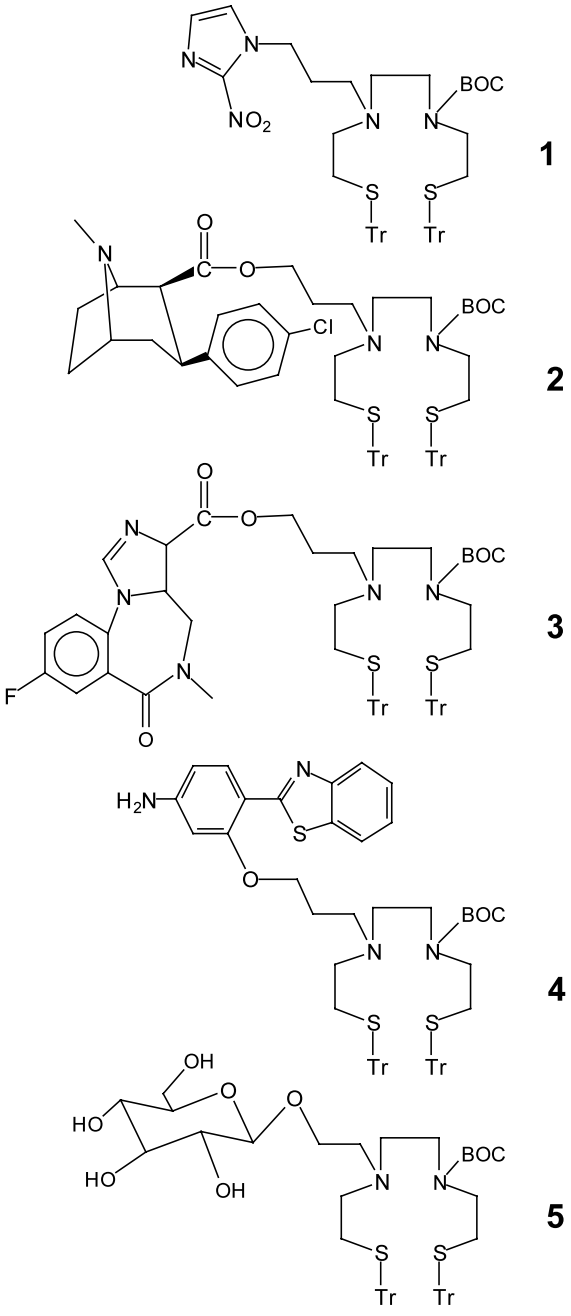
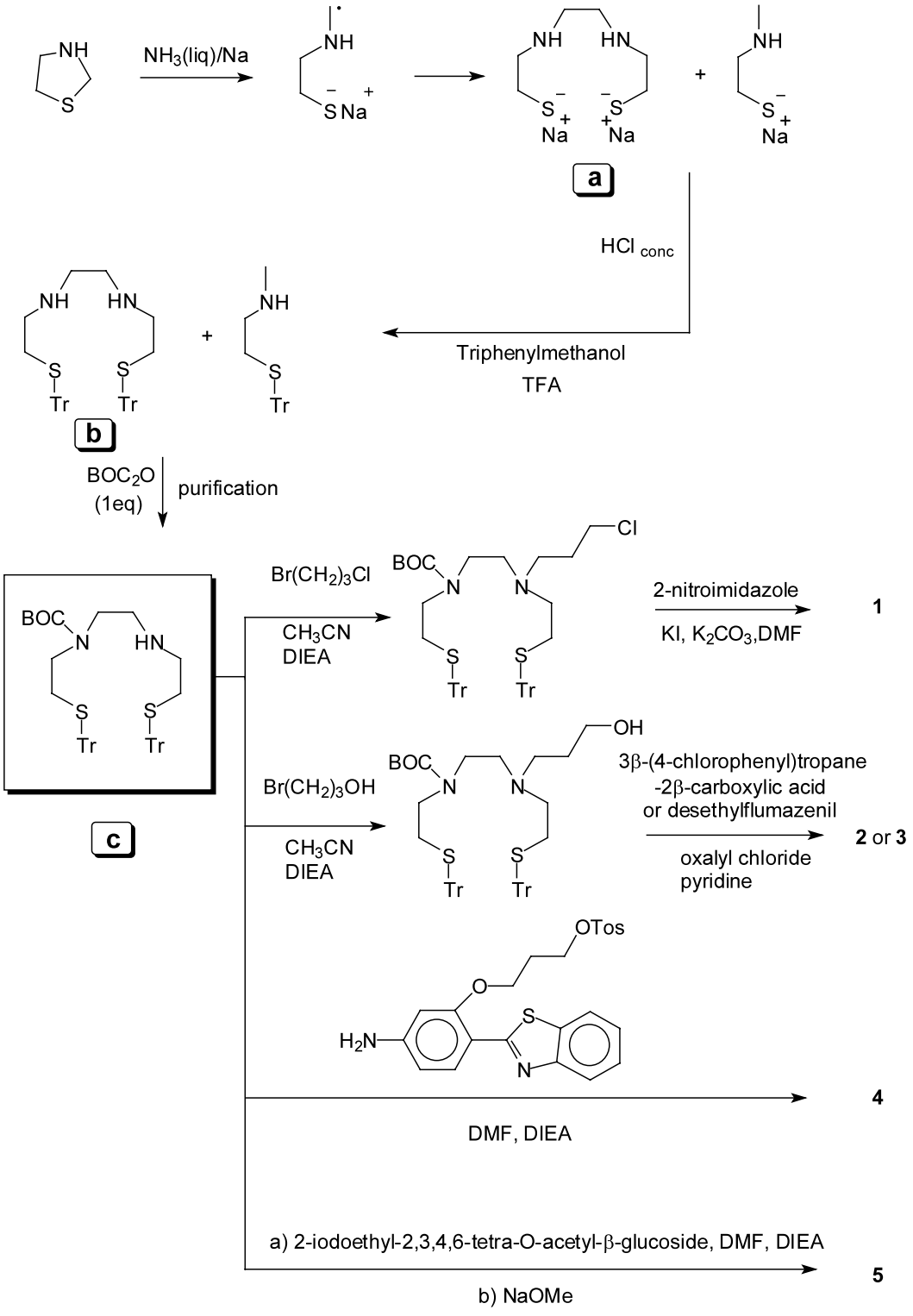


Figure 2. Synthesised conjugates with the *S*,*S*′-bis-trityl protected BAT synthon.

EtOH and heating for 10 min in a boiling water-bath. Neutralisation followed by addition of $SnCl_2$, sodium tartrate and ^{99m}Tc-generator eluate (containing $^{99m}TcO_4^-$) and heating for 10 min in a boiling water-bath yielded the corresponding Tc(V)O-BAT-vector complex. Radio-LC-MS was used to confirm the identity of all mentioned ^{99m}Tc-labelled conjugates.

Mass spectrometric analysis of the reaction mixture after deprotection of **1** (with HCl 0.5 M at 100°C) showed the presence of (i) unmodified conjugate **1** (57%), (ii) the ligand without the *N*-BOC group (38%), (iii) the ligand without the *N*-BOC and one of the trityl



Scheme 1. Synthesis of *S*,*S*′-bis-trityl protected BAT conjugates.

groups (2.9%) and (iv) the ligand without one of the trityl groups (1.4%) (Fig. 3). The conjugate with a fully deprotected BAT was not detected.

Labelling with ^{99m}Tc requires preceding removal of at least one trityl group as labelling starting from the *S*,*S*′-bis-trityl protected ligand without the deprotection step with HCl 0.5 M did not yield the desired complex. This suggests that deprotection of the derivative still containing one thiol protective group may proceed further during the complexation step. In earlier studies we have shown that *S*-benzyl or *S*-benzamidomethyl protected mercaptoacetyltriglycine can be labelled efficiently with ^{99m}Tc using a similar labelling proce-

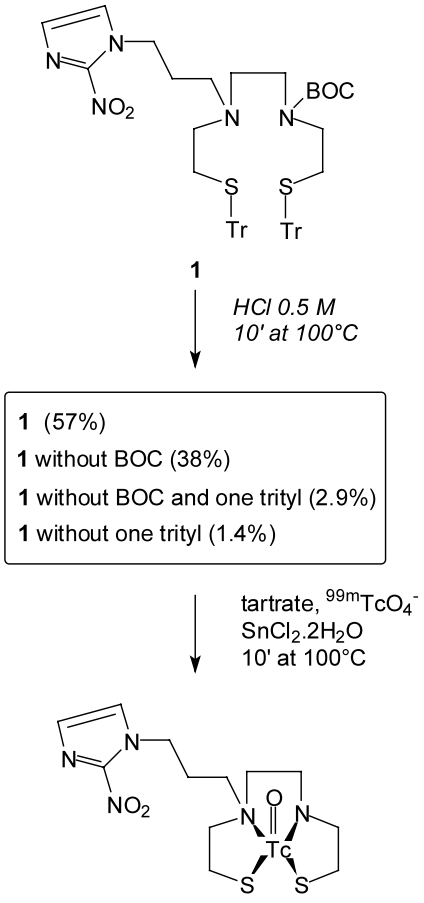


Figure 3. Reaction products formed after deprotection of **1** (heating 10 min in a boiling water-bath in the presence of 0.5 ml HCl 0.5 M and 0.3 ml ethanol), followed by labelling with ^{99m}Tc.

dure (10 min heating in the presence of tartrate and $^{99m}TcO_4$-).[15] On the other hand, it is also possible that only a minute amount of ligand, lower than the detection threshold, is fully deprotected. However, this may still be sufficient to chelate technetium, which is typically present in nanogram amounts.

In conclusion, we developed a new synthetic pathway using *S*,*S*′-bis-trityl, *N*-BOC 1,2-ethylenedicysteamine which can be coupled to a variety of biologically interesting molecules. The conjugates can be stored in thiol protected form and labelled with ^{99m}Tc in a simple one-pot two-step procedure. This labelling method is also amenable to kit formulation.

Acknowledgements

Financial support was provided by the Fund for Scientific Research-Flanders, Belgium and by a grant of the Onderzoeksraad K.U. Leuven (OT.00.39).

References

1. Kung, H. F.; Meegalla, S.; Kung, M.-P.; Plössl, K. *Transition Met. Chem.* **1998**, *23*, 531–536.
2. Chumpradit, S.; Kung, M.-P.; Mu, M.; Kung, H. F. In *Technetium and Rhenium in Chemistry and Nuclear Medicine*; Nicolini, M.; Bandoli, G.; Mazzi, U., Eds. Design and synthesis of ^{99m}Tc-labelled benzamides as potential dopamine D2/D3 receptor imaging agents. Cortina International: Verona, 1999; pp. 497–502.
3. Mahmood, A.; Kronauge, J. F.; Barbarics, E.; Freiberg, E.; Madras, B. K.; Li, J.; Davison, A.; Jones, A. G. In *Technetium and Rhenium in Chemistry and Nuclear Medicine*; Nicolini, M.; Bandoli, G.; Mazzi, U., Eds. Technetium(V) and rhenium(V) analogues of Way 100635 $5HT_{1A}$ receptor-binding complexes. Cortina International: Verona, 1999; pp. 393–399.
4. Chumpradit, S.; Choi, R. S.; Hou, C.; Acton, P. D.; Kung, H. F. *J. Labelled Comp. Radiopharm.* **2001**, *44*, S524–S526.
5. Chumpradit, S.; Kung, M.-P.; Mu, M.; Hou, C.; Kung, H. F. *J. Labelled Comp. Radiopharm.* **1997**, *39*, S437–S438.
6. Lever, S. Z.; Baidoo, K. E.; Kramer, A. V.; Burns, H. D. *Tetrahedron Lett.* **1988**, *29*, 3219–3222.
7. Samnick, S.; Brandau, W.; Sciuk, J.; Steinstrasser, J.; Schober, O. *Nucl. Med. Biol.* **1995**, *22*, 573–583.
8. Misra, H. K.; Virzi, F.; Hnatowich, D. J.; Wright, G. *Tetrahedron Lett.* **1989**, *30*, 1885–1888.
9. Meegalla, S. K.; Plössl, K.; Kung, M. P.; Chumpradit, S.; Stevenson, D. A.; Kushner, S. A.; McElgin, W. T.; Mozley, D.; Kung, H. F. *J. Med. Chem.* **1997**, *40*, 9–17.
10. Meltzer, P. C.; Blundell, P.; Jones, A. G.; Mahmood, A.; Garada, B.; Zimmerman, R. E.; Davison, A.; Holman, B. L.; Madras, B. K. *J. Med. Chem.* **1997**, *40*, 1835–1844.
11. Blondeau, P.; Berse, C.; Gravel, D. *Can. J. Chem.* **1967**, *49*, 49–52.
12. Vanbilloen, H. P.; Cleynhens, B. J.; Verbruggen, A. M. *Nucl. Med. Biol.* **1998**, *25*, 569–575.
13. Okarvi, S. M.; Adriaens, P.; Verbruggen, A. *J. Labelled Comp. Radiopharm.* **1997**, *39*, 853–874.
14. Oya, S.; Plössl, K.; Kung, M. P.; Stevenson, D. A.; Kung, H. F. *Nucl. Med. Biol.* **1998**, *25*, 135–140.
15. Bormans, G.; Cleynhens, B.; Adriaens, P.; Vanbilloen, H.; De Roo, M.; Verbruggen, A. *Nucl. Med. Biol.* **1995**, *22*, 339–349.