JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2006; **49**: 109–123.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1031

Research Article

Synthesis and evaluation of a novel samarium-153 bifunctional chelating agent for radioimmunotargeting applications

Laurence Morandeau[1,*], Patricia Remaud-Le Saec[2], Ali Ouadi[3], Karine Bultel-Rivière[2], Marie Mougin-Degraef[2], Agnès de France-Robert[2], Alain Faivre-Chauvet[2] and Jean-François Gestin[2]
[1] *Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Nedlands 6009, Western Australia, Australia*
[2] *Chimie des Bioconjugués, Institut de Biologie, INSERM U. 601, 9, Quai Moncousu, 44093 Nantes Cedex, France*
[3] *Institut de Recherches Subatomiques, CNRS/IN2P3 and Université L. Pasteur, B. P. 28, 67037 Strasbourg Cedex 2, France*

Summary

A new bifunctional chelating agent (BCA), 3-(4-isothiocyanatobenzyl)triethylenetetraaminehexaacetic acid (**9**), has been synthesized in fast and easy conditions. An improved synthesis of its position isomer 1-(4-isothiocyanatobenzyl)triethylenetetraaminehexaacetic acid (**19**) is also described. Stability in serum media of the two corresponding aminobenzyl derivatives-samarium-153 complexes, respectively, 3-(4-aminobenzyl)triethylenetetraaminehexaacetic acid—samarium-153 and 1-(4-aminobenzyl)triethylenetetraaminehexaacetic acid—samarium-153, have been evaluated. The 3-(4-aminobenzyl)triethylenetetraaminehexaacetic acid complex revealed excellent stability in serum media, and therefore 3-(4-isothiocyanatobenzyl)triethylenetetraaminehexaacetic acid (**9**) appears useful for future *in vivo* radioimmunotherapy investigations. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: radioimmunotargeting; radioimmunotherapy; bifunctional chelating agent; samarium-153

*Correspondence to: Laurence Morandeau, Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Nedlands 6009, Western Australia, Australia. E-mail: Laurence.morandeau@health.wa.gov.au

Contract/grant sponsor: La Ligue Départementale Contre le Cancer de Vendée, France

Copyright © 2005 John Wiley & Sons, Ltd.

Received 18 March 2005
Revised 18 September 2005
Accepted 27 October 2005

Introduction

Radiolabelled antibodies have been commonly used in order to target tumour-cell antigens for application in immunoscintigraphy[1] (IS) and radioimmunotherapy[2,3] (RIT). However, this system often involves high irradiation of normal tissues, due in part to the low rate of monoclonal antibody uptake at the tumour site (0.01% of injected dose per gram of tumour) and the relatively slow clearance of excess activity (several hours). In order to improve efficacy of RIT, several strategies have been investigated. Among them are the development of pretargeting methods[4,5] and/or the replacement of iodine-131 by radionuclides possessing better radiophysical and chemical characteristics.

Iodine-131 is the main radionuclide currently used in RIT because it possesses satisfactory specific activity and is easy to link covalently to an aromatic ring. However, its radiophysical properties: low-energy β^- radiation, strong γ emission and long half-life ($E\beta\,\mathrm{avg} = 182\,\mathrm{keV}$, $E\gamma = 364\,\mathrm{keV}$ (81.2%), $T_{1/2} = 8.0$ days) do not make it the best β emitter for RIT applications. A way to improve RIT applications is to replace iodine-131 by radionuclides providing different LET (linear energy transfer) in order to adapt the delivered energy to the target nature and size.[6] Radionuclides such as yttrium-90 ($E\beta\,\mathrm{avg} = 935\,\mathrm{keV}$, $T_{1/2} = 2.67$ days), rhenium-188 ($E\beta\,\mathrm{avg} = 764\,\mathrm{keV}$, $E\gamma = 155\,\mathrm{keV}$ (15%), $T_{1/2} = 0.71$ days), holmium-166 ($E\beta\,\mathrm{avg} = 666\,\mathrm{keV}$, $E\gamma = 80.5\,\mathrm{keV}$ (6.3%), $T_{1/2} = 1.1$ days), rhenium-186 ($E\beta\,\mathrm{avg} = 323\,\mathrm{keV}$, $E\gamma = 137\,\mathrm{keV}$ (8.5%), $T_{1/2} = 3.8$ days), lutetium-177 ($E\beta\,\mathrm{avg} = 133\,\mathrm{keV}$, $E\gamma = 208\,\mathrm{keV}$ (11%), $T_{1/2} = 6.7$ days) or samarium-153 ($E\beta\,\mathrm{avg} = 225\,\mathrm{keV}$, $E\gamma = 103\,\mathrm{keV}$ (28%), $T_{1/2} = 1.9$ days) are of great interest. However, the replacement of iodine-131 by such radionuclides requires the synthesis of different types of bifunctional chelating agents (BCA), to ensure the formation of a stable complex *in vivo* with the selected radionuclide. These BCAs also present an activated function allowing the coupling with an antibody or other vector biomolecule.

Some BCAs are already known for their chelation properties towards a specific radionuclide such as 4-aminobenzyl-DOTA[7] (2-(4-aminobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) for yttrium-90 and lutetium-177, 4-aminobenzyl-HEHA[8] (2-(4-aminobenzyl)-1,4,7,10,13,16-hexaazacyclohexadecane-1,4,7,10,13,16-hexaacetic acid) for actinium-225 or CHX-A″-DTPA[9] (*N*-[(*R*)-2-amino-3-(4-aminophenyl)propyl]*trans*-(*S*,*S*)cyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid) for bismuth-213. The structural differences between these BCAs (linear, pre-organized, macrocyclic) lead to their specificity towards a radionuclide. The common point between these BCAs is their functionalization by a terminal aromatic amine easily convertible in isothiocyanate, in order to react with a vector presenting an amine function.

Copyright © 2005 John Wiley & Sons, Ltd.

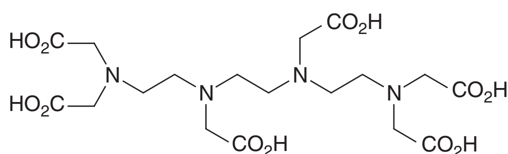


Figure 1. TTHA (triethylenetetraaminehexaacetic acid)

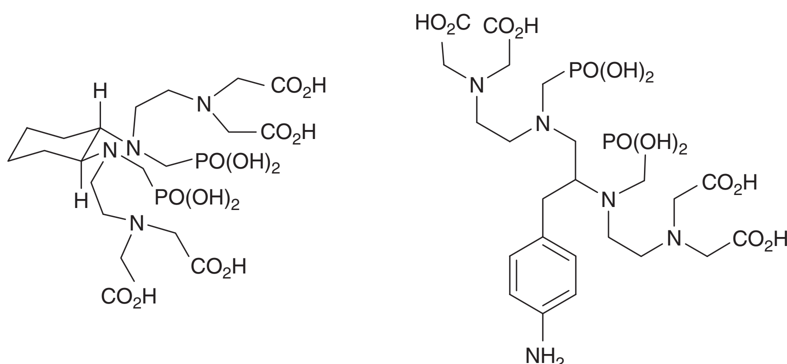
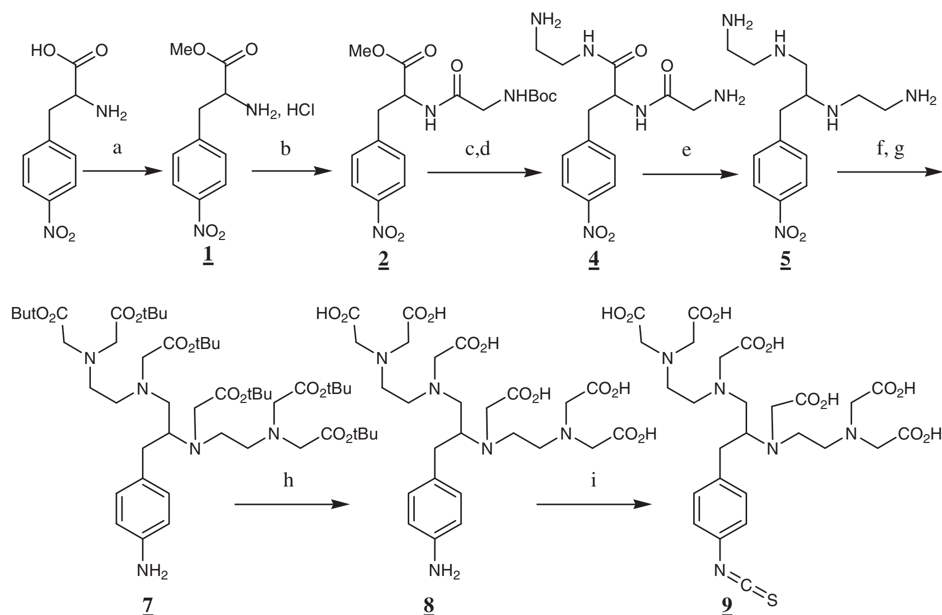


Figure 2. cyclohexane-*trans*-1,2-*bis*(aminomethylphosphonic)-*N,N′*-*bis*(ethyl-2-iminodiacetic) acid and 1-(4-aminobenzyl)-ethylenediamine-*N,N′*-*bis*(ethyl-2-iminodiacetic)-*N,N′*-*bis*(methylphosphonic) acid

Prior studies on TTHA (triethylenetetraaminehexaacetic acid) (Figure 1) and analogues[10,11] indicate that this family of compounds is suitable for the complexation of metallic ions such as Sm^{3+} and Ga^{3+}. We have previously described the synthesis of a series of different chelating agents for samarium-153 based on a triethylenetetraamine structure, varying the pre-organization of the chelating sites and the nature of the chelating functions (carboxylic and/or phosphonic acids). Among them, cyclohexane-*trans*-1,2-*bis*(aminomethylphosphonic)-*N,N′*-*bis*(ethyl-2-iminodiacetic) acid[12,13] (Figure 2), a pre-organized monofunctionalized chelating agent based on a cyclohexyl diamine backbone possessing two phosphonic and four carboxylic acid functions showed promising results for future application to metastatic bone-cancer therapy. Synthesis of its bifunctionalized analogue allowing coupling to vectors is in progress in our laboratory. A similar mixed TTHA-like BCA was also synthesized, where a 4-aminobenzyl group was linked to the ethylene group in third position[14] (Figure 2). Unfortunately, despite interesting complexation studies with samarium-153, the resulting ^{153}Sm-complex showed to be unstable in serum media.

In addition, we investigated the synthesis of the non-mixed structural analogue carrying six carboxylic acid functions.

Copyright © 2005 John Wiley & Sons, Ltd.



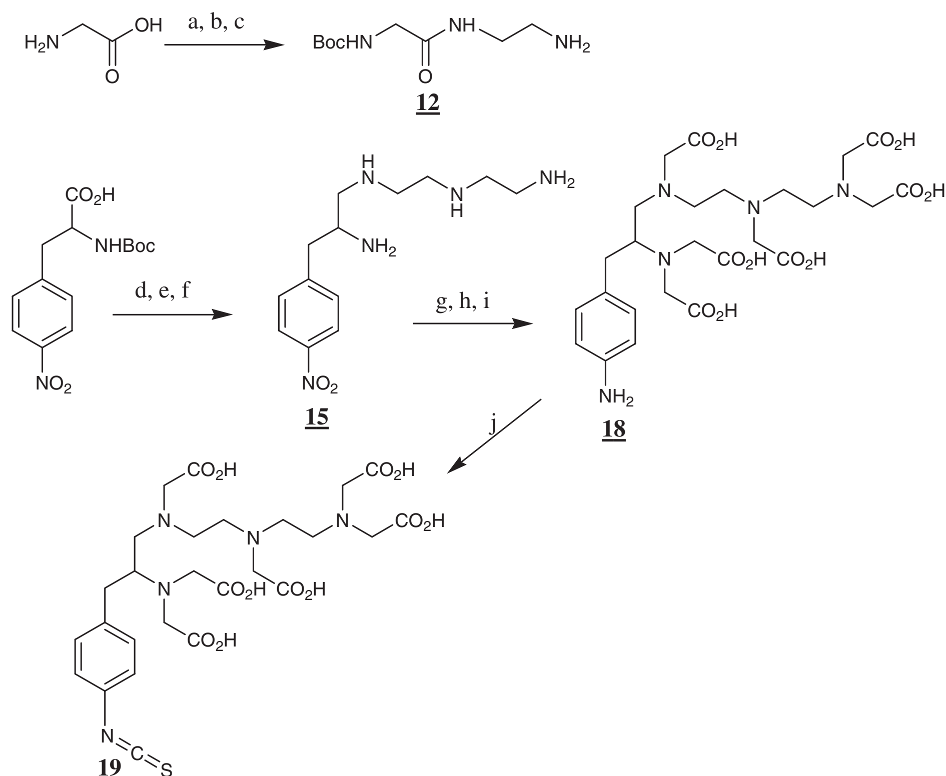
Scheme 1. Synthetic scheme of 3-(4-isothiocyanatobenzyl) triethylenetetraaminehexaacetic acid 9. (a) MeOH, HCl(g), 91%; (b) (1) Et_3N, MeOH; (2) *N*(Boc)glycine, CDI, THF, 95%; (c) ethylenediamine, MeOH, 99%; (d) (1) HCl 3 M, (2) NaOH 3 M, 71%; (e) (1) BH_3/THF, (2) NaOH(s), 78%; (f) KI, 1,8-*bis*-(dimethylamino)naphthalene, *t*Bu-bromoacetate, DMF, 62%; (g) H_2, Pd/C, MeOH, 100%; (h) HCl 6 M, acetone, 100%; (i) $CSCl_2$, HCl 0.5 M, $CHCl_3$, 97%

The present paper reports the synthesis of a new BCA, the 3-(4-isothiocyanatobenzyl) triethylenetetraaminehexaacetic acid or (3-(4-isothiocyanatobenzyl)TTHA) (Scheme 1) and an improved synthesis of its position isomer 1-(4-isothiocyanatobenzyl)TTHA[15] (Scheme 2), potential candidates for applications in radioimmunotargeting. Further studies on the aminobenzyl precursor of these two BCAs were then carried on: 3-(4-aminobenzyl)TTHA and 1-(4-aminobenzyl)TTHA have been radiolabelled with samarium-153 to provide additional information on the influence of the position of the aromatic ring on the TTHA backbone. Serum stability studies have been performed in serum media at 37°C at different time intervals in order to compare the *in vitro* stability of these different ^{153}Sm-radiolabelled chelating agents.

Results and discussion

The methyl ester of 4-nitrophenylalanine 1 was obtained after treatment of 4-nitrophenylalanine with HCl gas in methanol (Scheme 1). The amine function of compound 1 was regenerated after treatment with triethylamine in

Copyright © 2005 John Wiley & Sons, Ltd.



Scheme 2. Improved synthetic pathway for 1-(4-isothiocyanatobenzyl) triethylenetetraaminehexaacetic acid 19. (a) MeOH, HCl(g), 94%; (b) Di-*tert*-butyl dicarbonate, Et_3N, 75%; (c) ethylenediamine, MeOH, 89%; (d) C.D.I., compound 12, 78%; (e) (1) HCl 3 M, (2) NaOH 3 M 92%; (f) (1) BH_3/THF, (2) NaOH(s), 79%; (g) KI, 1,8-*bis*-(dimethylamino)naphthalene, *t*Bu-bromoacetate, DMF, 78%; (h) H_2, Pd/C, MeOH, 100%; (i) HCl 6 M, acetone, 100%; (j) $CSCl_2$, HCl 0.5 M, $CHCl_3$, 98%

methanol. Compound 2 was obtained after activation of 1.2 equivalents of the *N*-(Boc)-glycine with C.D.I. (carbonyldiimidazole) and amidic coupling with the amine function of compound 1 in a 95% yield. Given that the purity of commercial C.D.I. is variable due to its water sensitivity, a small excess of activated *N*-(Boc)-glycine was used, in order to ensure complete reaction of compound 1. Solution of *N*[*N*(Boc)glycyl]4-nitrophenylalanine methyl ester 2 in methanol was added dropwise to a large excess of ethylenediamine (40 eq.) in order to avoid amidic coupling between two molecules of compound 2 on ethylenediamine. The tertiobutoxycarbonyl group of compound 3 was removed by acidic hydrolysis (HCl 3 M). Compound 4 was finally obtained, after regeneration of the free amine functions, in a 71% yield. Reduction of the carbonyl groups of compound 4 was achieved in BH_3/THF. Dissolution of 4

Copyright © 2005 John Wiley & Sons, Ltd.

was observed progressively while adding the BH_3/THF solution. It was followed by treatment with HCl 6 M under reflux affording 3-(4-nitrobenzyl)-triethylenetetraamine **5** in a 77% yield. A silica gel column was necessary to purify the tetraamine **5**. Hexa-*t*-butylester **6** was then obtained in a 62% yield by reacting 7 equivalents of 1,8-*bis*-(dimethylamino)naphthalene, 1.1 equivalents of potassium iodide, 8 equivalents of *t*-butylbromoacetate[16,17] with 1 equivalent of the tetraamine **5**. The nitroaromatic function of compound **6** was reduced by catalytic hydrogenation in presence of palladium on active carbon to give **7** in quantitative yield. Hydrolysis in acidic conditions (HCl 3 M) was then conducted to give 3-(4-aminobenzyl)-triethylenetetraaminehexaacetic acid **8** in quantitative yield. 3-(4-isothiocyanatobenzyl)-triethylenetetraaminehexaacetic acid **9** was finally obtained after treatment with thiophosgene.

In Scheme 2, glycine methyl ester hydrochloride **10** was obtained after treatment of glycine with HCl gas in MeOH. The amine function was protected with a Boc function in a 75% yield. Compound **11** was then reacted with ethylene diamine as described for compound **3** for a 89% yield. Resulting compound **12** was then coupled to the carboxylic acid function of *N*(Boc)4-nitrophenylalanine after activation with C.D.I., with a 78% yield. The Boc protective groups were cleaved after treatment with HCl 3 M and the amine functions regenerated (92%). Carbonyl groups were reduced with BH_3/THF as described for compound **4**, to yield 79% of compound **15**. Tetraamine **15** was reacted with *t*-butylbromoacetate, as described for compound **6**, leading to compound **16** in 78% yield. The nitroaromatic function was then reduced by catalytic hydrogenation (98%), followed with the acid hydrolysis of the *t*-butylacetate ester functions to yield compound **18** (100%). Treatment of **18** with thiophosgene finally gave 1-(4-isothiocyanatobenzyl)-triethylenetetraaminehexaacetic acid **19** (98%).

We synthesized a new BCA, 3-(4-isothiocyanatobenzyl)TTHA **9**, with a 28% global yield. We also improved the synthesis of its position isomer, 1-(4-isothiocyanatobenzyl)TTHA **19** with a global yield of 27%, in comparison with the synthesis Bhargava *et al.*[15] who obtained a global yield of 17%. Serum media stability of 3-(4-aminobenzyl)TTHA-^{153}Sm complex (**8**-^{153}Sm) has been compared to one of its position isomer 1-(4-aminobenzyl)TTHA- ^{153}Sm complex (**18**-^{153}Sm) (Figure 3).

Three solutions have been prepared, one as a control solution containing $^{153}SmCl_3$ in a 0.02 M citrate buffer (pH5), the second one containing ^{153}Sm-1-(4-aminobenzyl)TTHA complex and the third one containing ^{153}Sm-3-(4-aminobenzyl)TTHA complex. These solutions were then transferred to human serum and incubated at 37°C. Aliquots of each were taken at various time intervals (0, 1, 4, 24, and 144 h) and purified through a PD-10 column in order to separate free samarium-153 from chelated samarium-153. As shown in

Copyright © 2005 John Wiley & Sons, Ltd.

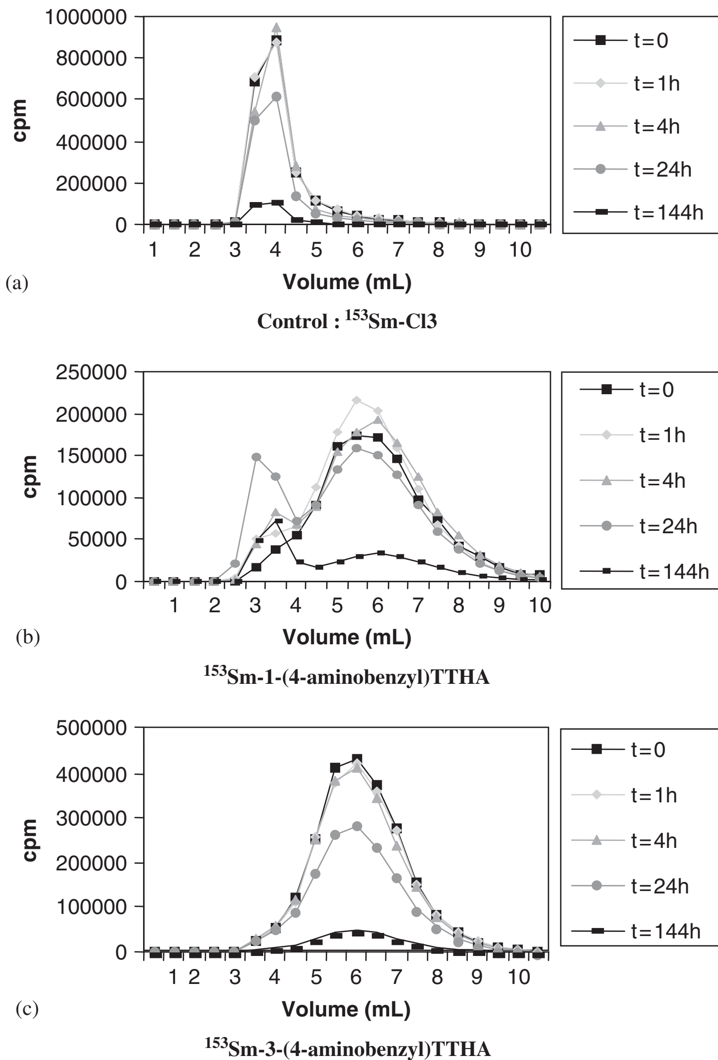


Figure 3. PD-10 elutions of serum media solutions incubated at 37°C: (a) control $^{153}SmCl_3$; (b) ^{153}Sm-1-(4-aminobenzyl)triethylenetetraaminehexaacetic acid and (c) ^{153}Sm-3-(4-aminobenzyl)triethylenetetraaminehexaacetic acid

Figure 3, free samarium-153 (control) is eluted in the first fractions, between 3 and 4.5 ml, while ^{153}Sm-BCA complexes are eluted between 4.5 and 8 ml. Serum stability of these two complexes at 37°C showed significant difference. While an important release (30%) of samarium-153 was observed for ^{153}Sm-1-(4-aminobenzyl)TTHA complex after 24 h in serum media, increasing to 42% at 144 h, ^{153}Sm-3-(4-aminobenzyl)TTHA complex was totally stable in serum at 37°C after 6 days. ^{153}Sm-3-(4-aminobenzyl)TTHA complex was very stable in serum media, unlike its isomer ^{153}Sm-1-(4-aminobenzyl)TTHA complex.

Copyright © 2005 John Wiley & Sons, Ltd.

The difference of stability of these two complexes could be explained by the position of the 4-aminobenzyl group on the TTHA backbone. 3-(4-aminobenzyl)TTHA appears to be more symmetrical than its structural isomer 1-(4-aminobenzyl)TTHA. Thus, the position of the 4-aminobenzyl group may induce a stronger pre-organization of the TTHA backbone than the one observed for 1-(4-aminobenzyl)TTHA.

This study shows once again that serum stability, and furthermore, *in vivo* stability of a complex is not definitely predictable. Serum stability studies are thus essential prior to any evaluation of *in vivo* stability and behaviour of new radiopharmaceuticals. It is assumed that the amino precursor **8** or isothiocyanato derivative **9**, once coupled to an antibody or other vector biomolecule would exhibit the same binding and serum stability properties after labelling with samarium-153, as described in the present study for compound **8**. 3-(4-isothiocyanatobenzyl)TTHA **9** was successfully coupled to a bivalent peptide and labelled with Indium-111 in our laboratory.[18] However, this needs to be confirmed in further experiments with samarium-153.

In summary, we synthesized a novel BCA, the 3-(4-isothiocyanatobenzyl) TTHA with satisfactory yields, in fast and easy conditions. We also improved the synthesis of its position isomer 1-(4-isothiocyanatobenzyl)TTHA by 10%. Serum stability of the two amino precursors of these BCAs labelled with samarium-153 were evaluated and compared. The ^{153}Sm-1-(4-aminobenzyl)TTHA complex (**18**-^{153}Sm) was found to be very labile in serum media at 37°C, while the ^{153}Sm-3-(4-aminobenzyl)TTHA complex (**8**-^{153}Sm) showed remarkable serum media stability.

Experimental

Materials and methods

All chemicals were of the highest purity commercially available (Sigma-Aldrich company). The HPLC solvents were purchased from Carlo-Erba. TLC was performed using precoated Kieselgel 60 plates F_{254} (TLC plates, Carlo-Erba) and was visualized by UV or iodine. Silica gel (230–400 mesh, Carlo-Erba) was purchased from VWR. High-performance liquid chromatography was carried out at 211 nm on a Waters 600 HPLC System using analytical reverse-phase HPLC (SymmetryShield 5 μm RP-18 4.5 × 150 mm, Waters). NMR spectra were recorded on a BRUKER AC 250 apparatus (250.133 MHz for ^{1}H). Chemical shifts are indicated in δ values (ppm) downfield from internal TMS, and coupling constants (*J*) are given in Hertz (Hz). Multiplicities were recorded as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded using a BRUKER Esquire LC electrospray mass spectrometer with methanol or water as carrier solvent. IR spectra were recorded on an Avatar 320 Thermonicolet FTIR-ATR, using a

Copyright © 2005 John Wiley & Sons, Ltd.

germanium crystal. Samarium-153 was graciously provided by Schering-CisBio International in a solution of samarium-153 in HCl 0.04 M (volumetric activity: 5.2 GBq/ml, specific activity: 40 GBq/mg). Radioactivity was counted on a γ counter (WIZARD3 480, automatic γ counter, WALLAC).

4-Nitrophenylalanine hydrochloride methyl ester **1**

A suspension of 5 g of 4-nitrophenylalanine monohydrate (21.9 mmol) in 100 ml of MeOH was saturated with HCl gas and maintained at room temperature for 18 h. After cooling to 0°C, a white precipitate was filtrated off and washed with cold MeOH. Compound **1** (5.2 g, 91%) was obtained as a white powder.

^{1}H NMR (D_2O) (δ, ppm): 3.2–3.5 (m, 2H), 3.81 (s, 3H), 4.50 (t, 1H, $J = 6.5$ Hz), 7.51 (d, 2H, $J = 8.8$ Hz), 8.23 (d, 2H, $J = 8.8$ Hz). R_f (MeOH): 0.82. MS ($M + H^+$): 225.

N{N(Boc)glycyl}4-nitrophenylalanine methyl ester **2**

To 1.28 g (4.9 mmol) of **1** and 0.753 ml of triethylamine (5.4 mmol) in 3 ml of MeOH, 20 ml of diethyl ether was added. After 4 h at 0°C, salts of triethylamine hydrochloride were removed by filtration. The filtrate was evaporated to dryness, to give 1.1 g of yellow oil.

An amount of 0.955 g of C.D.I. (5.9 mmol) was added to a solution of 1.03 g of *N*(Boc) glycine (5.9 mmol) in 5 ml of anhydrous THF, under nitrogen atmosphere. After 10 min, the 4-nitrophenylalanine methyl ester in solution in 3 ml of anhydrous THF was added. The reaction mixture was stirred for 1 h at room temperature. The THF was evaporated and the residue dissolved in 50 ml of $CHCl_3$ and washed twice with 50 ml of HCl 0.5 M. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. Compound **2** (1.78 g, 95%) was obtained as a white powder.

^{1}H NMR ($CDCl_3$) (δ, ppm): 1.45 (s, 9H), 2.16 (s, 2H), 3.16 (d.d, 1H, $J = 13.7$ Hz, and 5.8 Hz), 3.31 (d.d, 1H, $J = 13.7$ Hz and $J = 5.8$ Hz), 3.79 (s, 3H), 4.90 (d.d, 1H, $J = 13.4$ Hz and $J = 5.8$ Hz), 5.11 (s, 1H), 6.75 (d, 1H, $J = 7.0$ Hz), 7.30 (d, 2H, $J = 8.6$ Hz), 8.14 (d, 2H, $J = 8.6$ Hz). R_f (AcOEt): 0.75. MS ($M + H^+$): 382.

N-(2-Aminoethyl)-{N(Boc)glycyl}4-nitrophenylalaninamide **3**

An amount of 1.8 g of **2** (4.7 mmol) dissolved in 20 ml of MeOH was added dropwise to 12.6 ml of ethylene diamine (188 mmol), under vigorous stirring. The reaction mixture was kept for 3 h at room temperature. MeOH and the excess of ethylene diamine were then evaporated under vacuum. The residue was taken in a minimum of H_2O and extracted five times with 25 ml of $CHCl_3$. The organic layers were dried over Na_2SO_4, filtrated and concentrated under

Copyright © 2005 John Wiley & Sons, Ltd.

vacuum. Purification on silica gel column ($MeOH/NH_4OH$ 10/0.2) gave compound **3** (1.94 g, 99%) as a white solid.

^1H NMR ($CDCl_3$) (δ, ppm): 1.42 (s, 9H), 2.27 (s, 2H), 2.72 (m, 2H), 3.21 (m, 4H), 3.74 (m, 2H), 4.78 (m, 1H), 5.4 (s, 1H), 7.36 (d, 2H, $J = 8.5$ Hz), 7.43 (m, 1H), 7.56 (m, 1H), 8.09 (d, 2H, $J = 8.5$ Hz). R_f ($MeOH/NH_4OH$, 10/0.2): 0.35. MS ($M+H^+$): 410.

N-(2Aminoethyl)-glycyl 4-nitrophenylalaninamide **4**

A solution of 1.89 g (4.6 mmol) of **3** in 46 ml of HCl 3 M (138 mmol) was stirred for 8 h at room temperature. After evaporation of the mixture, the residue was taken in 5 ml of water and the pH adjusted to 11–12 with a solution of NaOH 3 M. This aqueous layer was extracted six times with 30 ml of $CHCl_3$. Organic layers were dried over Na_2SO_4, filtrated off and dried under reduce pressure to yield compound **4** (1.4 g, 71%) as a white solid.

^1H NMR (MeOD) (δ, ppm): 2.56 (m, 2H), 3.1–3.3 (m, 6H), 4.53 (m, 1H), 7.41 (d, 2H, $J = 8.8$ Hz), 8.09 (d, 2H, $J = 8.8$ Hz). R_f ($MeOH/NH_4OH$ 10/1): 0.35. MS ($M+H^+$): 310.

3-(4-Nitrobenzyl)triethylenetetraamine **5**

An amount of 40 ml of a solution of BH_3/THF 1 M (40 mmol) was added dropwise to a suspension of 1.1 g of **4** (3.56 mmol) in 10 ml of anhydrous THF, at 0°C and under nitrogen atmosphere. The reaction mixture was kept under reflux for 36 h. After cooling and slow addition of MeOH (5 ml), the solvent was evaporated and the residue taken in 15 ml of a solution of HCl 6 M. The mixture was maintained under reflux for 5 h and then concentrated under vacuum. The residue was taken in 3 ml of water and solid sodium hydroxide was added until the pH raised to 11–12. The aqueous layer obtained was then extracted three times with 20 ml of $CHCl_3$. The organic layers were dried over Na_2SO_4, filtrated and concentrated. Purification on silica gel column ($MeOH/CHCl_3/NH_4OH$, 7/1/3) led to compound **5** (0.776 g, 78%) as a yellow oil.

^1H NMR ($CDCl_3$) (δ, ppm): 2.4–3.0 (m, 13H), 1.25 (s, 6H), 7.36 (d, 2H, $J = 8.8$ Hz), 8.15 (d, 2H, $J = 8.8$ Hz). R_f ($MeOH/CHCl_3/NH_4OH$, 7/1/3): 0.15. SM ($M+H^+$): 282.

3-(4-Nitrobenzyl)-triethylenetetraaminepentaacetyl tertiobutyl ester **6**

An amount of 0.2 g of **5** (0.71 mmol), 0.13 g of potassium iodide (0.782 mmol), 1.1 g of 1,8-*bis*-(dimethylamino)naphthalene (5.1 mmol) and 30 ml of anhydrous DMF were stirred under nitrogen atmosphere. 0.95 ml of tertiobutylbromoacetate (6.4 mmol) was added and the reaction mixture was maintained at 65°C for 16 h. After cooling, the DMF was removed under vacuum and the residue was taken in 10 ml of $CHCl_3$ and washed twice with 10 ml of HCl

Copyright © 2005 John Wiley & Sons, Ltd.

0.6 M and once with 10 ml of water. The organic layer was dried over Na_2SO_4, filtrated off and concentrated under vacuum. Compound **6** (0.424 g, 62%) was finally obtained as a yellow oil after purification on silica gel column (Hexane/AcOEt 2/1).

^1H NMR ($CDCl_3$) (δ, ppm): 1.45 (s, 54H), 2.3–3.1 (m, 13H), 3.25 (s, 2H), 3.33 (s, 4H), 3.34 (s, 2H), 3.44 (s, 4H), 7.46 (d, 2H, $J = 8.8$ Hz), 8.01 (d, 2H, $J = 8.8$ Hz). R_f (Hexane/AcOEt 2/1): 0.35. SM ($M + H^+$): 966.

3-(4-Aminobenzyl)-triethylenetetraaminepentaacetyl tertiobutyl ester **7**

Catalytic hydrogenation of 0.424 g of **6** (0.44 mmol) in 25 ml of MeOH was carried out over 42 mg of 10% Pd/C (0.1eq.; w/w) at atmospheric pressure. After 3 h, the catalyst was filtered off (Celite) and the filtrate was removed under reduced pressure. Compound **7** (0.411 g, 100%) was obtained as a yellow oil.

^1H NMR ($CDCl_3$) (δ, ppm): 1.42 (s, 9H), 1.43 (s, 9H), 1.45 (s, 36H), 2.4–3.5 (m, 27H), 6.57 (d, 2H, $J = 8.2$ Hz), 6.98 (d, 2H, $J = 8.2$ Hz). R_f (AcOEt/hexane 2/1): 0.4. MS ($M + H^+$): 936.

3-(4-Aminobenzyl)-triethylenetetraaminepentaacetic acid **8**

To 0.214 g of **7** (0.23 mmol) dissolved in 1 ml of acetone was added 1 ml of HCl 6 M. After 16 h under stirring at room temperature, the reaction mixture was dried under vacuum, to give a white solid (0.178 g, 100%).

^1H NMR (D_2O) (δ, ppm): 2.7–3.6 (m, 13H), 3.81 (s, 6H), 4.04 (s, 6H), 7.40 (m, 4H). MS ($M + H^+$): 600.

3-(4-Isothiocyanatobenzyl)-triethylenetetraaminepentaacetic acid **9**

To a solution of 12 μl of thiophosgene (0.16 mmol) in 1 ml of $CHCl_3$ under vigorous stirring was added dropwise a solution of 2.5 mg of **8** (3.2 μmol) in 1 ml of HCl 0.5 M. The reaction mixture was maintained at room temperature for 12 h. The aqueous layer was then washed twice with 2 ml of $CHCl_3$, concentrated on a rotary vacuum and dried *in vacuo*. Compound **9** (2.5 mg, 97%) was obtained as a pale yellow solid.

MS ($M + H^+$): 642. HPLC: solvent A: TFA 0.1%, Solvent B: CH_3CN, flow rate: 1 ml/min, gradient: 0–5 mn 100%A isocratic, 5–35 mn 100%A to 100%B. Retention time: 19.1 min. IR: 2100 cm^{-1} (SCN).

Glycine methyl ester hydrochloride **10**

Synthesis was identical to **1** starting with 5 g of glycine (66.6 mmol). Compound **10** was obtained (7.9 g, 94%) as a white powder.

^1H NMR (D_2O) (δ, ppm): 3.81 (s, 3H), 3.91 (s, 2H). R_f ($CHCl_3$/MeOH 4/1): 0.36. MS ($M + H^+$): 90.

Copyright © 2005 John Wiley & Sons, Ltd.

N(Boc) glycine methyl ester **11**

An amount of 1.22 ml of triethylamine (8.75 mmol) was added to a suspension of 1 g of **10** (7.96 mmol) in 2 ml of MeOH. 5 ml of ether was then added and the mixture was placed in an ice bath. The salts were removed by filtration and the filtrate evaporated. The resulting oil was dissolved in 10 ml of AcOEt. A solution of 1.91 g of di-*tert*-butyl dicarbonate (8.75 mmol) in 10 ml of AcOEt was added dropwise and the reaction maintained at room temperature for 18 h. The reaction mixture was washed twice with 10 ml of water. The organic layer was dried over Na_2SO_4, filtrated off and concentrated to yield compound **11** (1.12 g, 75%) as a yellow oil.

^{1}H NMR ($CDCl_3$) (δ, ppm): 1.45 (s, 9H), 3.75 (s, 3H), 3.92 (d, 2H, $J = 5.5$ Hz), 6.65 (s broad signal, 1H). R_f (MeOH): 0.65. MS ($M+H^+$): 190.

N-(2-Aminoethyl) N(Boc)glycinamide **12**

Synthesis was identical to compound **3** starting with 4.38 g of **11** (23.2 mmol) in 3 ml of MeOH and 62 ml (927 mmol) of ethylenediamine. After treatment, a yellow oil (4.46 g, 89%) was obtained.

^{1}H NMR ($CDCl_3$) (δ, ppm): 1.45 (s, 9H), 1.52 (s, 2H), 3.15 (m, 2H), 3.31 (m, 2H), 3.78 (d, 2H, $J = 5.8$ Hz), 5.33 (s broad signal, 1H), 6.64 (s broad signal), 1H). R_f (MeOH): 0.5. MS ($M+H^+$): 218.

{N(Boc)glycyl-N-(2-aminoethyl)}4-N(Boc)nitrophenylalaninamide **13**

An amount of 0.3 g of *N*(Boc)-4-nitro-*L*-phenylalanine (0.97 mmol) was dissolved in 5 ml of anhydrous DMF under nitrogen atmosphere. 0.17 g of C.D.I. (1.07 mmol) was added. After 10 min, 0.21 g of **12** (0.97 mmol) dissolved in 5 ml of anhydrous DMF was added dropwise. The reaction was allowed to proceed for 12 h at room temperature. DMF was evaporated and the residue was purified on a silica gel column (AcOEt), to give compound **13** as a yellow oil (0.38 g, 78%).

^{1}H NMR ($CDCl_3$) (δ, ppm): 1.40 (s, 9H), 1.42 (s, 9H), 3.23 (m, 8H), 4.40 (s, 1H), 4.94 (s, 1H), 5.17 (m, 1H), 6.86 (s, 2H), 7.38 (m, 2H), 8.15 (m, 2H). R_f (AcOEt/MeOH 1/1): 0.5. MS ($M+H^+$): 510.

Glycyl-N-(2-aminoethyl)-4- nitrophenylalaninamide hydrochloride **14**

Synthesis was identical to compound **4** starting with 0.38 g of **13** (0.746 mmol). A white powder (0.21 g, 92%) was obtained.

^{1}H NMR (D_2O) (δ, ppm): 3.27 (m, 6H), 3.76 (s, 2H), 4.23 (t, 1H, $J = 7.4$ Hz), 7.51 (d, 2H, $J = 8.5$ Hz), 8.25 (d, 2H, $J = 8.6$ Hz). R_f (MeOH): 0.15. MS ($M+H^+$): 310.

Copyright © 2005 John Wiley & Sons, Ltd.

1-(4-Nitrobenzyl)triethylenetetraamine **15**

Synthesis was identical to compound **5**, starting with 0.16 g of compound **14** (0.51 mmol), 5.1 ml of BH_3/THF 1 M (5.1 mmol). Compound **15** (0.115 g, 79%) was obtained as a yellow oil.

^{1}H NMR ($CDCl_3$) (δ, ppm): 1.67 (s, 6H), 2.67–2.78 (m, 10H), 2.80–3.45 (m, 2H), 3.67 (t, 1H, $^3J = 7.5$ Hz), 7.37 (d, 2H, $J = 8.7$ Hz), 8.17 (d, 2H, $J = 8.7$ Hz). MS ($M + H^+$): 282.

1-(4-Nitrobenzyl)-triethylenetetraaminepentaacetyl tertiobutyl ester **16**

Synthesis was identical to compound **6**, starting with 0.45 g of compound **15** (1.6 mmol), 0.29 g of potassium iodide (1.76 mmol), 2.4 g of 1,8-*bis*-(dimethylamino)naphthalene (11.20 mmol). Compound **16** was obtained as a yellow oil (1.2 g, 78%).

^{1}H NMR ($CDCl_3$) (δ, ppm): 1.43 (s, 18H), 1.50 (s, 36H), 2.70–2.88 (m, 12H), 3.27–3.46 (m, 1H), 3.46 (s, 12H), 7.48 (d, 2H, $J = 8.82$ Hz), 8.11 (d, 2H, $J = 8.82$ Hz). R_f (AcOEt/$CHCl_3$ 1/1): 0.5. MS ($M + H^+$): 966.

1-(4-Aminobenzyl)-triethylenetetraaminepentaacetyl tertiobutyl ester **17**

Catalytic hydrogenation of 0.5 g of compound **6** (0.52 mmol) in 25 ml of MeOH was carried out over 50 mg of 10% Pd/C (0.1eq.; w/w) at atmospheric pressure. After 3 h, the catalyst was filtered off (Celite) and the filtrate was removed under reduced pressure. Compound **17** (0.48 g, 100%) was obtained as an orange powder.

^{1}H NMR ($CDCl_3$) (δ, ppm): 1.42 (s, 18H), 1.47 (s, 36H), 2.68–2.85 (m, 14H), 3.27–3.51 (m, 1H), 3.38 (s, 12H), 6.60 (d, 2H, $J = 8.2$ Hz), 6.99 (d, 2H, $J = 8.2$ Hz). MS ($M + H^+$): 936.

1-(4-Aminobenzyl)-triethylenetetraaminepentaacetic acid **18**

To 0.45 g of compound **17** (4.81 mmol) dissolved in 2 ml of acetone was added 5 ml of HCl 6 M. After stirring for 16 h at room temperature, the reaction mixture was dried under vacuum, to obtain a white solid (0.375 g, 100%).

^{1}H NMR (D_2O) (δ, ppm): 3.0–3.4 (m, 13H), 3.92 (s, 12H), 7.40 (m, 4H).

1-(4-Isothiocyanatobenzyl)-triethylenetetraaminepentaacetic acid **19**

Synthesis was identical to compound **9**, starting with 20 μl of thiophosgene (0.27 mmol) and 4.2 mg of **18** (5.3 μmol) in 1 ml of HCl 0.5 M. Compound **9** (4.4 mg, 98%) was obtained as a pale yellow solid.

MS ($M + H^+$): 642. HPLC: solvent A: TFA 0.1%, Solvent B: CH_3CN, flow rate: 1 ml/min, gradient: 0–5 mn 100%A isocratic, 5–35 mn 100%A to 100%B. Retention time: 19.1 min. IR: 2100 cm^{-1} (SCN).

Copyright © 2005 John Wiley & Sons, Ltd.

Radiolabelling and serum media stability

The control solution contained, 5 µl of $^{153}SmCl_3$ (0.370 nmol) and 595 µl of citrate buffer (0.02 M, pH5). For the radiolabelling, 5 µl of $^{153}SmCl_3$ (0.370 nmol), 550 µl of a solution at 135 nmol/ml of the two different chelating agents in a citrate buffer (0.02 M, pH5) and 45 µl of citrate buffer (0.02 M, pH5) were stirred for 1 h at room temperature.

Then 250 µl of each solution was added to 5 ml of human serum and incubated at 37°C, under stirring. At different time intervals (0, 1, 4, 24, and 144 h), 500 µl of each serum media were purified on a PD-10 column and eluted with 10 ml of PBS. Twenty tubes of 0.5 ml were collected for each time and their activity counted on a γ counter.

Conclusion

We have described the synthesis of a novel bifunctional chelating agent, the 3-(4-isothiocyanatobenzyl)TTHA that can be easily linked to any vector biomolecules. The synthesis of its position isomer, the 1-(4-isothiocyanato-benzyl)TTHA was also improved. Unlike the ^{153}Sm-1-(4-aminobenzyl)TTHA complex, the ^{153}Sm-3-(4-aminobenzyl)TTHA complex showed remarkable serum media stability. Future animal studies will be carried out to confirm the interest of this new bifunctional chelating agent for *in vivo* applications in radioimmunotherapy.

Acknowledgements

We thank Schering-Cis Bio International for supplying the $^{153}SmCl_3$ solution. We also want to thank 'La Ligue départementale Contre le Cancer de Vendée' for a doctoral grant.

References

1. Goldenberg DM. *J Nucl Med* 1983; **24**: 360–362.
2. Couturier O, Faivre-Chauvet A, Filippovich IV, Thedrez P, Saï-Maurel C, Bardiès M, Mishra AK, Gauvrit M, Blain G, Apostolidis C, Molinet R, Abbé JC, Bataille R, Wijdenes J, Chatal JF, Chérel M. *Clin Cancer Res* 1999; **5**: 3165–3170.
3. Chatal JF, Peltier P, Bardies M, Chetanneau A, Resche I, Mahe M, Charbonnel B. *Med Nucl Imag Fonct Metabol* 1993; **17**: 81–85.
4. Barbet J, Kraeber-Bodere F, Vuillez JP, Gautherot E, Rouvier E, Chatal JF. *Cancer Biother Radiopharm* 1999; **14**(3): 153–166.
5. Rosegrough SF. *Q J Nucl Med* 1996; **40**: 234–251.
6. Bardies M, Chatal JF. *Phys Med Biol* 1994; **39**(6): 961–981.
7. Moi MK, Meares CF. *J Am Chem Soc* 1998; **110**: 6266–6267.
8. Ouadi A, Loussouarn A, Remaud P, Morandeau L, Apostolidis C, Musikas C, Faivre-Chauvet A, Gestin JF. *Tetrahedron Lett* 2000; **41**: 7207–7209.
9. Brechbiel MW, Gansow OA. *J Chem Soc Perkin Trans* 1992; **I**: 1173–1178, 2740.

Copyright © 2005 John Wiley & Sons, Ltd.

10. Achour B, Costa J, Delgado R, Garrigues E, Geraldea CF, Korber N, Nepveu F, Prata MI. *Inorg Chem* 1998; **37**: 2729–2740.
11. Loussouarn A, Faivre-Chauvet A, Chatal JF, Gestin JF. *Mater Sci Forum* 1999; **315–317**: 262–267.
12. Loussouarn A, Ouadi A, Morandeau L, Remaud P, Giles R, Gestin JF, Webb J. *Tetrahedron Lett* 2003; **44**: 3539–3541.
13. Ouadi A, Loussouarn A, Morandeau L, Remaud P, Faivre-Chauvet A, Webb J, Gestin JF. *Europ J Med Chem* 2004; **39**(5): 467–472.
14. Ouadi A, Bultel K, De France-Robert A, Loussouarn A, Morandeau L, Gestin JF. *Tetrahedron Lett* 2004; **45**(7): 1395–1397.
15. Bhargava KK, Zhang ZY, Palestro CJ, Acharya SA. *J Protein Chem* 1999; **18**(7): 761–770.
16. Corson DT, Meares CF. *Bioconjugate Chem* 2000; **11**: 292–299.
17. Brechbiel MW, Gansow OA. *J Chem Soc Perkin Trans* 1992; **I**: 1173–1178.
18. Morandeau L, Benoist E, Loussouarn A, Ouadi A, Lesaeac P, Mougin M, Faivre-Chauvet A, Le Boterff J, Chatal JF, Barbet J, Gestin JF. *Bioconjugate Chem* 2005; **16**: 184–193.

Copyright © 2005 John Wiley & Sons, Ltd.