An Effective Chelating Agent for Labelling of Monoclonal Antibody with ^{212}Bi for α-Particle Mediated Radioimmunotherapy

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The ligand *N*-[2-amino-3-(*p*-isothiocyanatophenyl)propyl]-(±)-*trans*-1,2-diaminocyclohexane-*N,N',N''*-pentaacetic acid has been synthesized and linked to IgG and to monoclonal antibody B72.3, and labelled with ^{206}Bi and ^{212}Bi to demonstrate the *in vivo* stability of the label and its utility for ^{212}Bi-radioimmunotherapy.

When tumour-localizing monoclonal antibodies (mAb) are radiolabelled for use in therapy, it is essential that the radionuclide remain linked to the protein for at least five half-lives. Despite observations *in vitro* of remarkable tumour cell specific cytotoxicity of targeted ^{212}Bi-radioimmunoconjugates,[1] the almost instantaneous loss in circulation *in vivo* of ^{212}Bi (α 6.05 MeV; $t_{1/2}$ 60.6 min) from conventional diethylenetriaminepentaacetic acid (DTPA) anhydride chelators[2] linked to mAb has precluded their clinical use. Even ^{212}Bi–mAb studies in animal tumour model systems have been limited to investigations designed to avoid the circulatory system, one by using subcutaneous injection,[3] another by employing an intracavitary (i.p.) infusion.[4]

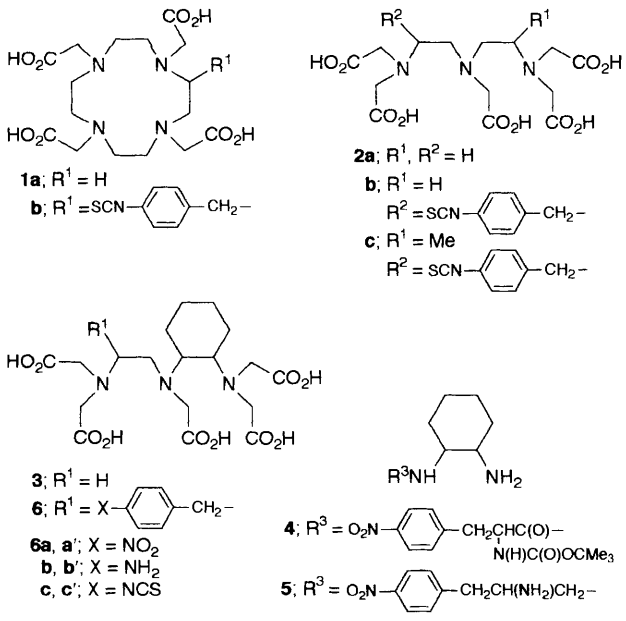
A less well appreciated chemical constraint on the chelator to be used is that formation of the labelled immunoconjugate should be rapid and efficient in order to effectively utilize carrier-free metal solutions and to minimize radiation damage.† Thus, we previously reported that a macrocyclic tetraazacyclododecane tetraacid (DOTA, **1a**) formed a kinetically inert Bi^{III} complex[5] and we showed that a C-functionalized DOTA derivative **1b**, when linked to mAb and labelled with ^{206}Bi, prevented the release *in vivo* of bismuth from the radioimmunoconjugate.[2] However, when we attempted to label mAb-linked **1b** with carrier-free $^{212}BiI_4^-$ according to established procedures,[1] radiochemical yields of less than a few percent were found. Inefficient labellings of DOTA derivatives with ^{90}Y have been investigated by Broan *et al.* who observed that divalent cations like Ca^{II} or Zn^{II} compete effectively for the ligand.[6]

In order to test the incorporation of Bi^{III} into the DOTA ligand in the absence of divalent metals, we measured the rate of reaction of BiI_4^- with DTPA **2a** and compared this rate with that for DOTA **1a**. Under similar reaction conditions, $[Bi]_T = 5.0 \times 10^{-6}$ mol dm^{-3}, $[L] = 5.0 \times 10^{-5}$ mol dm^{-3}, 0.1 mol dm^{-3} NaI, pH 4.0, *T* 25 °C, the half-life for formation of the $Bi(DTPA)^{2-}$ was 0.06 s whereas $Bi(DOTA)^-$ formed more than 10^4 times more slowly. These measurements, together with the suggestion that the DOTA ligand may be immunogenic,[7] required us to design an improved ligand for ^{212}Bi-immunotherapy.

In considering new chelators, we recalled that the substituted DTPA's **2b,c** we studied earlier, while not useful for Bi^{III} *in vivo*, increased in stability in circulation with the addition of methyl groups on the carbon backbone.[2] We also noted that alkaline earth cation DTPA complexes are markedly less stable than those of DOTA and so could compete less well with trivalent metals in labelling reactions. We therefore synthesized the ligand cyclohexyl DTPA‡ (CyDTPA), **3** to

† We and others have reported that exposure of mAb to ^{212}Bi for 20–30 min labelling reaction time does not in itself compromise the immunoprotein.[1,3,4]

‡ The CyDTPA ligand was prepared by using CBZ-glycine in place of the protected *p*-nitrophenylalanine in the synthesis described for **6**.



increase backbone substitution and complex rigidity and found the half-life for reaction of BiI_4^- to [Bi(CyDTPA)$^{2-}$] to be 0.27 s under the reaction conditions described above.

For linkage to mAb, we prepared *N*-[2-amino-3-(*p*-isothiocyanatophenyl)propyl]-(±)-*trans*-1,2-diaminocyclohexane-N,N′,N″-pentaacetic acid **6c,c′**. Reaction of *trans*-1,2-diaminocyclohexane with the *N*-hydroxysuccinimidyl ester of *N*-*t*-Boc-*p*-nitrophenylalanine[8] (*t*-Boc = *tert*-*N*-butoxycarbonyl) generated amide **4**. The carbamate was removed with dioxane saturated with HCl(g) and the amide reduced with diborane–tetrahydrofuran (THF) to produce triamine **5**. Alkylation of **5** with *tert*-butyl bromoacetate[9] followed by treatment with anhydrous trifluoroacetic acid provided two pairs of enantiomers **6a,a′**.

Preparative HPLC separation of the diastereoisomers was effected by employing a linear gradient of 0.5 mol dm^{-3} aqueous triethylammonium acetate to methanol and a C_{18} reverse phase column. The separated ligands were further purified by anion-exchange chromatography (AG1X8, 200–400 mesh, chloroacetate form).§ Hydrogenation of the nitro group (10% Pd/C) and reaction of the anilines **6b,b′** with thiophosgene was used to prepare the isothiocyanate derivatives **6c,c′** which were linked to mAb–B72.3[10] by well-defined methods.[11]

After labelling with ^{206}Bi ($t_{1/2}$ = 6.24 days), a γ-ray tracer for the short-lived ^{212}Bi, the **6c,c′**–B72.3 radioimmunoconjugates were separately injected intraperitoneal (i.p.) into athymic mice. The accretion of the radionuclide in the kidney was compared in tissue distribution studies with that exhibited by similarly labelled B72.3 radioimmunoconjugates formed with ligands **2b,c** and with ^{125}I–B72.3 and to results obtained by intravenous (i.v.) injection in the earlier study.[2]¶ When ligand **2b** was used, within one hour after injection, kidney levels rose to 25.7% of the injected dose per gram of tissue (% ID g^{-1}) and remained above this level for 6 h. Similarly, if **2c** was employed, kidney levels rose to 18.6% ID g^{-1} at 1 h and stayed high over 6 h. The labelled macrocycle immunoconjugate ^{206}Bi–**1b**–B72.3 showed kidney levels of 10.6% ID g^{-1} and 11.3% ID g^{-1} at 1 h and 4 h after injection. Levels of ^{125}I and those for ^{206}Bi complexed by ligands **6c,c′** were statistically identical over 6 h, did not rise above 6.88% ID g^{-1} in the kidney at 1 h and the ^{206}Bi level remained below 9.25% ID g^{-1} at 6 h. The close parallel between ^{125}I levels and those for the ^{206}Bi levels seen for immunoconjugates formed with ligands **6c,c′** indicates that the bismuth label remains with the antibody for at least six hours, *i.e.* six half-lives of ^{212}Bi. Liver levels for ^{206}Bi immunoconjugates with **6c,c′** were normal and paralleled those for ^{125}I in rising from 2–7% ID g^{-1} from 0.5 to 3 h post injection and dropping slightly thereafter as the antibody label cleared the blood.

Immunoconjugates of bovine IgG with **6c** were then tested for effectiveness in labelling immunoproteins with ~3 mCi of ^{224}Ra generator[12,13] produced $^{212}BiI_4^-$ in 0.1 mol dm^{-3} HI. The solution was neutralized to pH 4 with 2 mol dm^{-3} acetic acid and reacted with 450 μg (45 μl) of IgG–**6c** conjugate in 0.02 mol dm^{-3} 2-morpholinoethanesulphonic acid (MES)–0.15 mol dm^{-3} NaCl solution for 20 min. After purification, 1 mCi (400 μg) of labelled protein was recovered. Decay corrected, the radiochemical yield was *ca.* 60%. These amounts of ^{212}Bi when used to label mAb–103a–**6c** immunoconjugate were sufficient to perform a study of ^{212}Bi targeting and radioimmunotherapy by i.v. injection in the Rauscher leukaemia model system.[14–16]

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§ All compounds gave satisfactory NMR (^{13}C and ^{1}H) and mass spectral data. HPLC, t_R = 11.59, 12.48 min for **6a,a′**, respectively. Satisfactory chemical analyses were found for **6a,a′**.

¶ Since any free bismuth in circulation is rapidly cleared to the kidney, the levels of ^{206}Bi radiolabel found there in excess of those of ^{125}I–mAb in blood may be used to quantitate the amount of ^{206}Bi released from the chelate immunoconjugate. See refs. 2, 4.