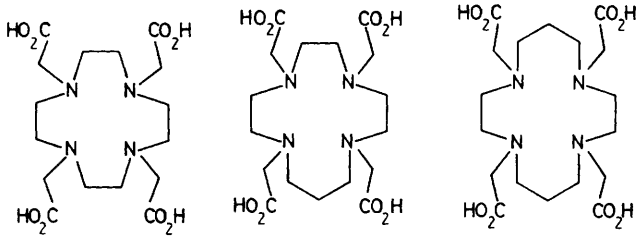
Synthesis of a Kinetically Stable Yttrium-90 Labelled Macrocycle–Antibody Conjugate

Jonathan P. L. Cox,[a] Karl J. Jankowski,[a] Ritu Kataky,[a] David Parker,[a]* Nigel R. A. Beeley,[b] Byron A. Boyce,[b] Michael A. W. Eaton,[b] Kenneth Millar,[b] Andrew T. Millican,[b] Alice Harrison,[c] and Carole Walker[c]

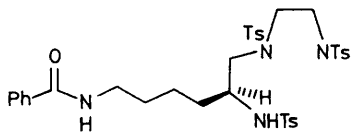
[a] *Department of Chemistry, University of Durham, South Road, Durham DH1 3LE, U.K.*
[b] *Celltech Ltd., 216 Bath Road, Slough SL1 4EN, U.K.*
[c] *M.R.C. Radiobiology Unit, Harwell, Didcot OX11 0RD, U.K.*

Lysine is a precursor for the synthesis of an aminobutyl *C*-functionalised tetra-azacyclododecane tetra-acid which has been linked to B72.3 antibody and may be labelled with ^{90}Y to form a kinetically stable complex of potential use in radioimmunotherapy.

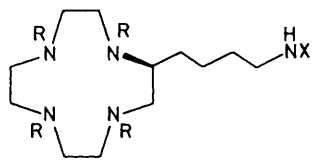
A limiting feature in the use of radiolabelled monoclonal antibodies has been the premature release of varying amounts of the radionuclide from the antibody. In radioimmunotherapy, where the aim is to deliver a sterilising dose of radiation selectively to the tumour stem cells, the deposition of β^--emitting isotopes in non-tumour tissue can induce lethal radiotoxic effects *e.g.* myelosuppression. Ytrrium-90 is an attractive isotope for therapy ($t_{1/2}$ = 64 h, E_{max} = 2.25 MeV, range = 3.9 mm),[1,2] but its clinical use will be very limited because of bone-marrow toxicity, resulting from acid-promoted release of ^{90}Y from an antibody-linked chelate, such as diethylenetriaminepenta-acetic acid (DTPA).[3,4] The use of functionalised macrocyclic ligands which tend to be more resistant to acid-promoted dissociation has led to some very encouraging results with ^{67}Cu[5,6] and ^{111}In.[7] The solution stability and labelling efficiency of the octadentate macrocyclic ligands (**1**), (**2**), and (**3**) was examined, in the light of the tendency of yttrium to form octadentate complexes.[8,9] Stability constants for the formation of 1:1 complexes of yttrium with (**1**), (**2**), and (**3**) were measured by careful, slow potentiometric titrations followed by data analysis using SUPERQUAD,[10] {log K (298 K, I = 0.1 [NMe_4NO_3]): [(**1**)·Y] = 24.9; [(**2**)·Y] = 19.6; [(**3**)·Y] = 16.3}. The rate of dissociation of yttrium from preformed complexes of (**1**) and (**2**) at pH 3.6 (OAc^- buffer), measured by scavenging free ligand with excess Ni^{2+}, gave values of $t_{1/2}$ of the order of 5 min for [(**2**)·Y], 1 h for [(**Y**)·DTPA] but with [Y·(**1**)] no dissociation could be detected after three months. The forward rate of ^{90}Y binding was measured semi-quantitatively using h.p.l.c. radiometry, examining ligand concentrations in the range 20 to 100 μM. Both (**2**), (**3**), and DTPA bound yttrium inefficiently (pH 5.8, 1 h, 310 K, 1 M OAc^-, ligand concentration = 100 μM) but ligand (**1**) gave a 98% radiolabelling yield under these conditions.† Furthermore, when the [Y·(**1**)]$^-$ complex was examined in mice, it was observed that the complex cleared from the body rapidly and after 24 h the amount of activity in the liver and spleen averaged only 0.004 and 0.005% respectively of the injected dose. More critically, the amount of ^{90}Y in the bone and bone-marrow (48 h) was too low to measure. Evidently, the yttrium complex of (**1**) is stable *in vivo*, and not just in serum (pH 7.4).[11] Accordingly, in order to link a *C*-functionalised derivative of (**1**) to the antibody the synthesis of (**8**) was undertaken. Co-condensation of (**4**), derived from 2*S*-lysine as described earlier,[7] with $TsN(CH_2CH_2OTs)_2$ (Ts = p-$OSO_2C_6H_4Me$) in dimethylformamide (DMF) in the presence of Cs_2CO_3 gave the macrocycle (**5**) (72%).‡ This was detosylated under reductive conditions [Li/NH_3/MeOH tetrahedrofuran (THF)] to yield (**6**) (61%) which was separated from the small amount of the corresponding *N*-benzyl compound which formed (10%) by h.p.l.c. Alkylation of (**6**) with bromoacetic acid (pH 10) yielded (**7**) which was purified by anion exchange h.p.l.c. (52%), and acid hydrolysis (6 M HCl, 18 h) afforded (**8**) quantitatively. Conversion of (**8**) to the thiol-sensitive intermediate (**9**) was effected as described previously[7] and conjugation to a thiol-functionalised B72.3 antibody was achieved under standard conditions.[5] The evaluation of the practicability of using this macrocycle–antibody conjugate for tumour therapy *in vivo* is being actively pursued.



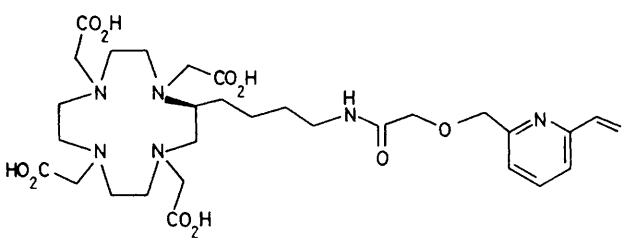
(**1**) (**2**) (**3**)



(**4**)



(**5**) R = Ts = p-$MeC_6H_4SO_2$, X = COPh
(**6**) R = H, X = COPh
(**7**) R = CH_2CO_2H, X = COPh
(**8**) R = CH_2CO_2H, X = H



(**9**)

† This result is equivalent to the labelling of 1.2 mg of an immunoglobulin with one macrocycle per antibody with 380 mCi of ^{90}Y.

‡ New compounds gave spectroscopic (^1H, ^{13}C, i.r., *m/z*) and chromatographic (h.p.l.c.) analyses in accord with the proposed structures. The synthesis of (**8**) is amenable to scale-up on the gram scale, and is versatile in permitting selective functionalisation at N-10.

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