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^{99m}TECHNETIUM LABELLING OF ANTIBODIES USING METHYLPHOSPHONYLATED, BIFUNCTIONAL CYCLAMS AS CHELATORS

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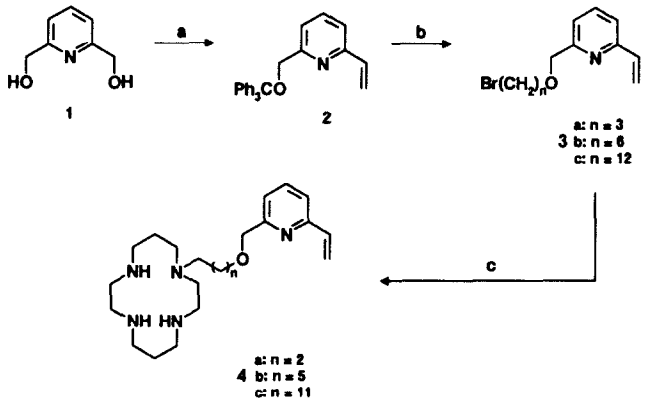
Abstract: Cyclam based bifunctional chelators are used for antibody labelling. The antibody conjugate 7, containing the unmodified macrocycle, shows only slow complexation kinetics. The introduction of methylphosphonic acid groups into the cyclam (9) significantly enhances the complexation kinetics.

In today's medicine a variety of imaging techniques are well established. Most of them primarily show morphological changes. In contrast scintigraphy, a highly sensitive γ-radiation based technique, focuses on the visualisation of physiological changes. Immunosczintigraphy[2] combines this principle with the high specificity of antibodies and uses it e. g. for tumor imaging. The most favourite γ-emitter for clinical applications is [^{99m}Tc]technetium. It emits only γ-radiation, is available from a generator, has a low radiation energy and a short half live of only six hours.

In order to overcome the limitations of direct antibody labelling[3] with ^{99m}Tc which is limited to certain classes of antibodies we have developed a generally applicable principle for the labelling of antibodies and other diagnostically relevant compounds[4].

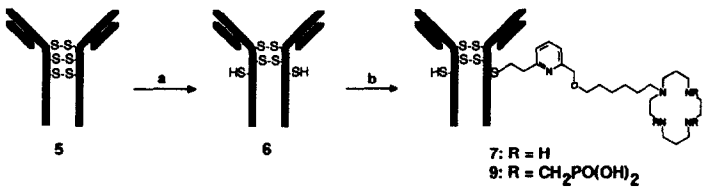
Polyazamacrocycles are well known chelators for cations[5]. Especially 1,4,8,11-tetraazacyclotetradecan (cyclam) forms thermodynamically stable complexes with ^{99m}Tc which is required for medicinal applications. We decided to use a nitrogen atom of the cyclam to link the macrocycle to an antibody. In the past a variety of principles for linking macrocycles to proteins have been developed[6]. We have chosen the vinylpyridin group which has been shown[7] to selectively react with thiolgroups to covalently link the cyclam to the antibody. Free

thiol groups at the surface of antibodies can selectively be generated with 3-mercapto-1,2-propandiol[8] without major alteration of their tertiary structure and biological activity.



Reagents and conditions: a: 1) $ClCPh_3$, Pyridine, DMAP, 1h RT, then 0,5h 60°C, 74%; 2) MnO_2, Dichloromethan; 6h RT, 77%; 3) $Ph_3PCH_3Br/NaNH_2$, THF, 4h RT, 75%; b: 1) HCl-gas, Dichloromethan, 0,5h RT, 95%; 2) Dibromopropan (a), Dibromohexan (b) or Dibromododecan (c), KOH, THF, 6h RT, 51-63%; c: 1,4,8,11-Tetraazacyclotetradecan, KOH, dioxan, 6h reflux, 72-78%.

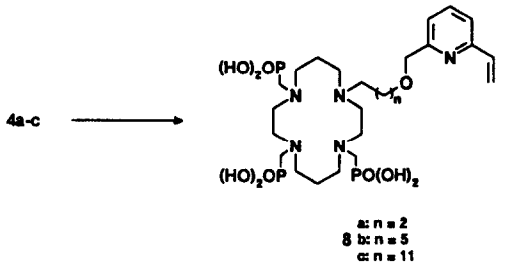
The synthesis of the linker starts with selective tritylation of 2,6-di-(hydroxymethyl)-pyridin **1** followed by manganese dioxide oxidation and a Wittig reaction generating the vinylpyridine **2**. Acidic cleavage of the trityl group and subsequent alkylation with dibromopropane, dibromohexane or dibromododecane led to **3a-c** having hydrophobic spacers with different length. Selective monoalkylation of cyclam with **3a-c** results in the desired bifunctional chelators **4a-c**.



Reagents and conditions: a: 3-Mercapto-1,2-propandiol; b: 4b or 8b (excess).

The human immunoglobulin Beriglobin® S[9] **5**, the antibody used in this study, is treated with 3-mercapto-1,2-propandiol to generate **6** which has an average of 7 free thiol groups on its surface. These react with **4b**[10] forming an average of 5.5 thioether linkages to the antibody conjugate **7**. Treatment of **7** with tin-(II)-citrate and sodium [^{99m}Tc]pertechnetate-(VII) under

usual conditions[11] yields 90% labelling of the antibody conjugate 7 after about 3 hours (figure 1, □).Complexation times around 3 hours are unacceptable considering the short half life of ^{99m}Tc and a convenient clinical application.



Reagents and conditions: 1) 2N HCl, lyophilise; Diethylphosphite, Para-formaldehyde, Acetonitrile, 3Å-molecular sieves, HCl-gas, 7h reflux; 2) 5N HCl, 7h reflux.

In order to optimise the chelation kinetics we introduced phosphonic acids which are well established ^{99m}Tc chelators. E. g. methylenediphosphonic acids are used as diagnostics for bone cancer[12]. The introduction of methylphosphonic acid units turned out to be quite tricky[13]. Finally we have been able to obtain the desired trimethylphosphonic acids **6a-c** in 75% yield[14]. The corresponding free acids 8a-c are generated by acidic hydrolysis. The reaction of 8b with the activated antibody 6 leads to the thioether 9 (4 thioethers in average per Ab). ^{99m}Tc labelling yielded 95% complexation in only 30 minutes (figure 1, ■).

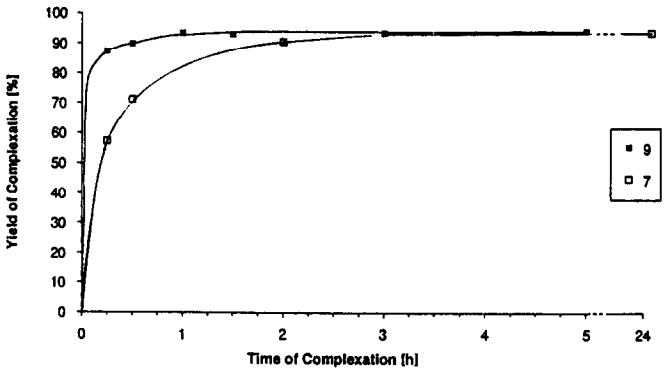


Figure 1: ^{99m}Tc labelling of the antibody conjugates 7 and 9.

These results clearly demonstrate the advantage of the N-methylphosphonic acid residues. 8a-c have proven to be superior bifunctional chelators with rapid complexation kinetics and high thermodynamic stability. With regards to antibody labelling the conjugate 9 fulfils the requirements for a potential clinical application. 8a-c are currently under further investigation for the labelling of other antibodies and molecules of diagnostic value.

Literature:

1) Currently at: E. Merck, 64271 Darmstadt, Germany.

2) Magerstädt, M.; Hachmann, H. J.; Kuhlmann, L.; Seidel, L. *Chemiker-Zeitung* **114**, *1990*, 123.

3) Hnatowich, D. J.; Mardirossian, G.; Ruschowski, M.; Fogarasi, M.; Virzi, F.; Winnard, P. *J. Nucl. Med.* **34**, *1993*, 109.

4) For related approaches see for example: a) Franz, J.; Volkert, W. A.; Barefield, E. K.; Holmes, R. A. *Nucl. Med. Biol.* **14**, *1987*, 569; b) Frizberg, A.R.; Abrams, P. G.; Baumier, P. L.; Kasina, S.; Morgan, A. C. Jr.; Rao, T. N.; Reno, J. M.; Sanderson, J. A.; Srinivasan, A.; Wilbur, D. S.; Vanderheyden, J. L. *Proc. Natl. Acad. Sci.* **85**, *1988*, 4025; c) Baidoo, K. E.; Lever, S. Z. *Bioconjugate Chem.* **1**, *1990*, 132; d) Lister-James, J.; Weber, R. W.; Boutin, R.; Nedelmann, M. A.; Dean, R. T. *J. Nucl. Med.* **30**, *1989*, 793.

5) For a recent review see: Izatt, R. M.; Pawlak, K.; Bradshaw, J. S. *Chem. Rev.* **91**, *1991*, 1721.

6) Parker, D. *Chem. Soc. Rev.* **19**, *1990*, 271.

7) a) Hancock, R. D.;Martell, A. E. *Chem. Rev.* **89**, *1989*, 1875; b) Morphy, J. R.; Parker, D.; Kataky, R.; Eaton, M. A. W.; Millican, A. T.; Alexander, R.; Harrison, A.; Walker, C. *J. Chem. Soc., Perkin Trans. 2* *1990*, 573.

8) Zhang, Z. M.; Ballinger, J. R.; Sheldon, K.; Boxen, I. *Int. J. Nucl. Med. Biol.* **19**, *1992*, 607.

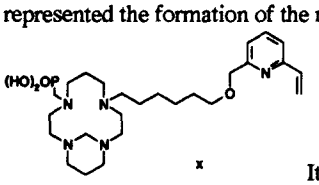
9) The human immunoglobulin was used as a model for monoclonal antibodies because it was easily available in larger quantities.

10) **4b** was selected to be used with Beriglobin® S in this study.

11) For details of the chelation procedure see: Kuhlmann, L.; Pütter, D. EP 0498333.

12) Reiners, C. *Der Nuclearmediziner* **16**, *1993*, 5.

13) There are several side reactions that can occur: Moisture in the reaction mixture results in the formation of large quantities of hydroxymethylphosphonic acid. A bigger problem represented the formation of the methylenbridged cyclam like for example **x**.



x

Its formation can be avoided by using the hydrochlorides of **4a-c** as starting material. Furthermore the yields can be increased by adding catalytic amounts of HCl-gas.

14) For related compounds see: Broan, C. J.; Cole, E.; Jankowski, K. J.; Parker, D.; Pulukkody, K.; Boyce, B. A.; Beeley, N. R. A.; Millar, K.; Millican, A. T. *Synthesis* *1992*, 63.

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