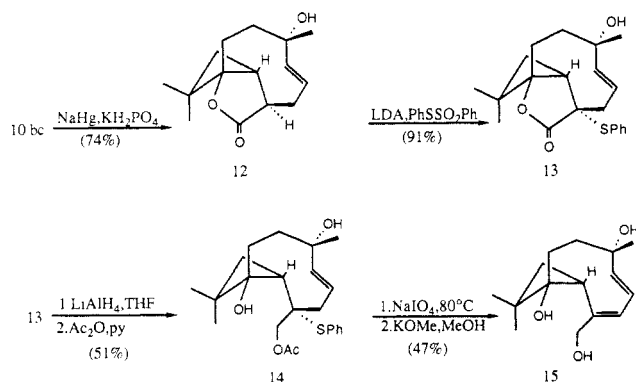




perature, 20 min, 58%) to the crystalline (*E,E*)-diene triol **15**, mp 112–114 °C.

The UV maximum of triol **15** ($\lambda_{\max}$ 237 nm, MeOH) and its 300 MHz ^1H NMR differed appreciably from those of the target molecule. However, when a dilute solution of **15** in CD_3OD was irradiated for 1 h by using a low-pressure UV source,¹² the isomerization monitored by NMR, chromatography, and recrystallization from EtOAc gave ca. 60% of colorless racemic punctaporonin B (**2**), mp 154–155 °C. The 300 MHz ^1H NMR spectra in both CDCl_3 and pyridine-*d*₅, the mass spectrum, the UV spectrum ($\lambda_{\max}$ 209 nm, MeOH, ϵ 6100), and the TLC behavior of this material were absolutely identical with the corresponding properties of natural punctaporonin B kindly supplied by Dr. J. P. Poyser (ICI Pharmaceuticals Division). Thus the first synthesis of ($\pm$)-**2** has been achieved in 13 steps from cyclobutanone **4**.¹³

Supplementary Material Available: Spectral data for **2**, **5**, **7**, **8**, **11**, **12**, **13**, and **15** (2 pages). Ordering information is given on any current masthead page.

(12) Cf. Shumate, K.; Fonken, G. J. *J. Am. Chem. Soc.* **1966**, *88*, 1073.

(13) Partial support of this research by a Fulbright Award (to I.K.) and by Grant CA-18846 from the National Cancer Institute, NIH, USPHS, is gratefully acknowledged.

The Peptide Way to Macrocyclic Bifunctional Chelating Agents: Synthesis of 2-(*p*-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic Acid and Study of Its Yttrium(III) Complex

Min K. Moi and Claude F. Meares*

Department of Chemistry, University of California
Davis, California 95616

Sally J. DeNardo

Departments of Internal Medicine and
Radiology, University of California, Davis
Medical Center, Sacramento, California 95817

Received March 21, 1988

Monoclonal antibody technology allows the specificity of an antibody for its antigen to be used in targeting cancer cells.¹ The conjugation of metals—particularly radionuclides such as ^{90}Y or ^{67}Cu —to monoclonal antibodies results in agents for radioimmunotherapy and other medical applications.^{2–4} Chelators that

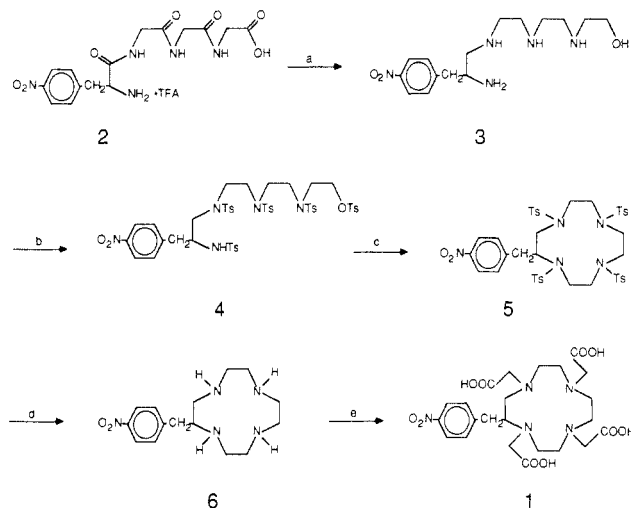


Figure 1. Synthesis of 2-(*p*-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid, **1**. Tetrapeptide [L]-NO₂Phe-Gly-Gly-Gly (**2**) was prepared by standard methods.¹¹ (a) Reflux with 17 equiv of $\text{BH}_3\cdot\text{THF}$, 31 h; 65% yield after silica gel chromatography. (b) Toluene-sulfonyl chloride (5 equiv) in $\text{CH}_3\text{CN}/\text{Et}_3\text{N}$, 8 h, room temperature; 49% yield after silica gel HPLC. (c) Cs_2CO_3 in DMF, 5 h, 60 °C; 79% yield after silica gel HPLC. (d) 96% H_2SO_4 , 16 equiv of $\text{C}_6\text{H}_5\text{OH}$, 48 h, 100 °C; 91% yield after C_{18} HPLC. (e) BrCH_2COOH (5 equiv), 3 h, pH 10, 70 °C; 58% yield after C_{18} HPLC.

can hold radiometals with high stability under physiological conditions are essential to avoid excessive radiation damage to nontarget cells.^{5,6}

Derivatives of polyazamacrocycles (bearing a C-substituted functional group for antibody attachment) can exhibit remarkable kinetic inertness; for example, the copper complex of the 14-membered 6-(*p*-nitrobenzyl)-1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid (nitrobenzyl-TETA) is very stable in human serum under physiological conditions, and a conjugate of this complex with a monoclonal antibody has tested well in tumor-bearing mice.^{3b,7} Also, the gadolinium complex of the 12-membered 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) is a stable, useful contrast agent for magnetic resonance imaging.⁸ Desreux and co-workers⁹ have shown that complexes of lanthanides with DOTA have formation constants that are several orders of magnitude higher than TETA; thus the 12-membered macrocycle is the favored target for binding trivalent yttrium.

Macrocyclic polyamines, the key precursors to macrocyclic bifunctional chelating agents, are synthesized by bimolecular cyclizations.¹⁰ Competition between polymerization and

(3) (a) Order, S. E.; Klein, J. L.; Lechner, P. K.; Frinke, J.; Lollo, C.; Carlo, D. J. *Int. J. Radiation Oncology Biol. Phys.* **1986**, *12*, 277. (b) Deshpande, S. V.; DeNardo, S. J.; Meares, C. F.; McCall, M. J.; Adams, G. P.; Moi, M. K.; DeNardo, G. L. *J. Nucl. Med.* **1988**, *29*, 217.

(4) (a) Halpern, S. E.; Hagan, P. L.; Garver, P. R.; Kozol, J. A.; Chen, A. W. N.; Frincke, J. M.; Bartholomew, R. M.; David, G. S.; Adams, T. H. *Cancer Res.* **1983**, *43*, 5347. (b) Hnatowich, D. J.; Layne, R. L.; Lantaigne, D.; Davis, M. A.; Griffin, T. W.; Doherty, P. W. *Science (Washington, D.C.)* **1983**, *220*, 613. (c) Pimm, M. V.; Perkins, A. C.; Baldwin, R. W. *Eur. J. Nucl. Med.* **1985**, *11*, 300.

(5) Klein, J. L.; Lechner, P. K.; Callahan, K. M.; Kopher, K. A.; Order, S. E. *Antibody, Immunoconjugates, and Radiopharmaceuticals* **1988**, *1*, 55.

(6) Meares, C. F.; Wensel, T. G. *Acc. Chem. Res.* **1984**, *17*, 202.

(7) (a) Moi, M. K.; Meares, C. F.; McCall, M. J.; Cole, W. C.; DeNardo, S. J. *Anal. Biochem.* **1985**, *148*, 249. (b) Moi, M. K.; Yanuck, M.; Deshpande, S. V.; Hope, H.; DeNardo, S. J.; Meares, C. F. *Inorg. Chem.* **1987**, *26*, 3458.

(8) (a) Margerstadt, M.; Gansow, O. A.; Brechbiel, M. W.; Colcher, D.; Baltzer, L.; Knop, R. H.; Gorton, M. E.; Naegle, M. *Magn. Reson. Med.* **1986**, *3*, 808. (b) Runge, V. M.; Jacobson, S.; Wood, M. L.; Kaufman, D.; Adelman, L. S. *Radiology* **1988**, *166*, 835. (c) Bousquet, J. C.; Saini, S.; Stark, D. D.; Hahn, P. F.; Nigam, M.; Wittenberg, J.; Ferrucci, J. T. *Radiology* **1988**, *166*, 693.

(9) Loncin, M. F.; Desreux, J. F.; Merciny, E. *Inorg. Chem.* **1986**, *25*, 2646.

(1) DeNardo, S. J.; Peng, J. S.; Denardo, G. L.; Mills, S. L.; Epstein, A. L. *Nucl. Med. Biol.* **1986**, *13*, 303.

(2) DeNardo, S. J.; Jungerman, J. A.; DeNardo, G. L.; Lagunas-Solar, M. C.; Cole, W. C.; Meares, C. F. In *The Developing Role of Short-Lived Radionuclides in Nuclear Medical Practice*; Paras, P., Theissen, J. W., Eds.; US Department of Energy: Washington, DC, 1984.

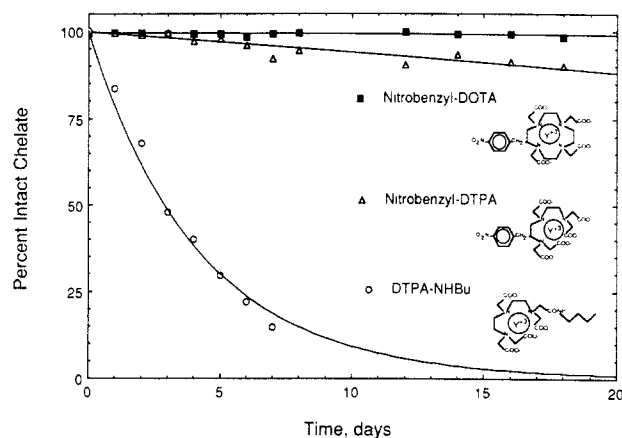


Figure 2. Loss of yttrium from metal chelates in serum. Data shown are the average of two independent experiments. In each case, the total ligand concentration was 10^{-7} M, while the total yttrium concentration was approximately 10^{-11} M. Serum was maintained sterile at 37 °C, pH 7.4, in an air/CO₂ atmosphere (approximately 95/5 by volume).

desired cyclization is a common problem; our efforts to form a 12-membered macrocycle with a *p*-nitrobenzyl sidechain by bimolecular cyclizations^{10c,k} gave unsatisfactory yields.

We have developed a new synthetic route to these macrocycles via peptide synthesis and intramolecular tosylamide ring closure. For polyazamacrocycles with nitrogens separated by two-carbon chains, peptides made from α -amino acids are readily accessible starting materials.¹¹ Treatment with borane converts peptides to linear polyamino alcohols, in which the original peptide backbone has been converted to a C-terminal alcohol, an N-terminal primary amine, and internal secondary amines (Figure 1, step a).¹² Treatment with *p*-toluenesulfonyl chloride produces a C-terminal tosyl ester, an N-terminal secondary tosylamide, and internal tertiary tosylamides (Figure 1, step b). Treatment with mild base converts the N-terminal tosylamide to a nucleophile, which displaces the C-terminal tosyl ester and thus forms a macrocyclic ring in high yield (Figure 1, step c). This intramolecular cyclization may be performed in very dilute solution, eliminating concerns about polymer formation.

The inclusion of peptide synthesis allows one to vary sidechains on the ring conveniently by selecting the appropriate amino acids as building blocks. Loss of enantiomeric purity is unlikely under the reaction conditions involved.^{10g} Amino acids such as glycine, β -alanine, and γ -aminobutyric acid are potential sources of 2-, 3-, and 4-carbon chains between the nitrogens in the macrocycle.

Synthesis of the title compound **1** is illustrated in Figure 1. The ⁸⁸Y(III) complex of octadentate ligand **1** (7 mM) forms in a few hours at room temperature in 0.1 M ammonium acetate, pH 5. Under the same conditions, the ⁸⁸Y(III) complexes of 14-membered nitrobenzyl-TETA or 16-membered nitrobenzyl-HETA¹³ do not form in significant yields.

(10) (a) Newkome, G. R.; Pappalardo, S.; Gupta, V. K.; Fronczek, F. R. *J. Org. Chem.* **1983**, *48*, 4848. (b) Martin, A. E.; Bulkowski, J. E. *J. Org. Chem.* **1982**, *47*, 415. (c) Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268. (d) Dietrich, B.; Hosseini, M. W.; Lehn, J.-M.; Sessions, R. B. *Helv. Chim. Acta* **1983**, *66*, 1262. (e) Buttafava, A.; Fabbri, L.; Perotti, A.; Poggi, A.; Seghi, B. *Inorg. Chem.* **1986**, *25*, 1456. (f) Sabatini, L.; Fabbri, L. *Inorg. Chem.* **1979**, *18*, 438. (g) Marecek, J. F.; Burrows, C. J. *Tetrahedron Lett.* **1986**, *27*, 5943. (h) Hediger, M.; Kaden, T. A. *J. Chem. Soc., Chem. Commun.* **1978**, *14*. (i) Smith, W. L.; Ekstrand, J. D.; Raymond, K. N. *J. Am. Chem. Soc.* **1978**, *100*, 3539. (j) Vellacio, F.; Punzar, R. V.; Kemp, D. S. *Tetrahedron Lett.* **1977**, *6*, 547. (k) Barefield, E. K.; Wagner, F.; Hodges, K. D. *Inorg. Chem.* **1976**, *15*, 1370. (l) Tabushi, I.; Fujiyoshi, M. *Heterocycles* **1977**, *7*, 851. (m) Tabushi, I.; Taniguchi, Y.; Koto, H. *Tetrahedron Lett.* **1977**, *12*, 1049. (n) Kimura, E.; Koike, T.; Takahashi, M. *J. Chem. Soc., Chem. Commun.* **1985**, 385. (o) Muller, F. R.; Handel, H. *Tetrahedron Lett.* **1982**, *23*, 2769. (p) Izatt, R. M.; Christensen, J. J. *Synthesis of Macrocycles*; Wiley-Interscience: New York, 1987. (q) Vriesema, B. K.; Buter, J.; Kellogg, R. M. *J. Org. Chem.* **1984**, *49*, 110.

(11) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*; Pierce Chem. Co.: New York, 1984.

(12) Meares, C. F.; Rice, L. S. *Biochemistry* **1981**, *20*, 610.

(13) 3-(*p*-nitrobenzyl)-1,5,9,13-tetraazacyclohexadecane-*N,N',N'',N'''*-tetraacetic acid, prepared by the same methods used for *p*-nitrobenzyl-TETA.⁷

The stability of a metal complex under physiological conditions may be assessed by dissolving the complex at high dilution in sterile human serum at 37 °C, pH 7.4, and measuring the rate of transfer of the metal from the complex to serum proteins over the course of several days.¹⁴ A comparison of Y(III) complexes of **1** and other ligands which have recently been reported to form complexes with Y(III) or In(III) appropriate for use in living systems^{15,16} is shown in Figure 2. At 10^{-7} M, the noncyclic octadentate ligand 1-(*p*-nitrobenzyl)diethylenetriaminepentaacetate loses Y(III) with a pseudo-first-order rate constant $k = 0.006$ day⁻¹. An analogue of the ligand formed by reaction of DTPA cyclic anhydride with a lysine residue, ⁸⁸yttrium diethylenetriaminepentaacetate-mobutylamide, loses Y(III) 45 times faster, having $k = 0.27$ day⁻¹. Incubation of the ⁸⁸Y(III) complex of **1** for 18 days results in loss of so little Y(III) from the complex—less than 0.5%—that the rate of loss cannot be measured under these conditions. This remarkable stability appears to be superior to a number of metal chelates now being tested in living systems.¹⁴⁻¹⁷ Further progress in this area depends on such stability.

Acknowledgment. Supported by research Grant CA 16861 from the National Institutes of Health (CFM) and by Grant DE F903-84ER60233 from the Department of Energy (S.J.D.). We thank Majid Akhavan for helpful suggestions and Daniel Jones for the high-resolution mass spectra.

Supplementary Material Available: Experimental details for the synthesis of the title compound **1** and yttrium complex studies in serum (3 pages). Ordering information is given on any current masthead page.

(14) Yeh, S. M.; Meares, C. F.; Goodwin, D. A. *J. Radioanal. Chem.* **1979**, *53*, 327.

(15) (a) Hnatowich, D. J.; Virzi, F.; Doherty, P. W. *J. Nucl. Med.* **1985**, *26*, 503. (b) Vaughan, A. T. M.; Keeling, A.; Yankuba, S. C. S. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 803. (c) Washburn, L. C.; Sun, T. T. Hwa; Crook, J. E.; Byrd, B. L.; Carlton, J. E.; Hung, Y. W.; Stepkowski, Z. S. *Nucl. Med. Biol.* **1986**, *13*, 453. (d) Anderson-Berg, W. T.; Squire, R. A.; Strand, M. *Cancer Res.* **1987**, *47*, 1905.

(16) Brechbiel, M. W.; Gansow, O. A.; Atcher, R. W.; Schlom, J.; Esteban, J.; Simpson, D. E.; Colcher, D. *Inorg. Chem.* **1986**, *25*, 2772.

(17) Cole, W. C.; DeNardo, S. J.; Meares, C. F.; McCall, M. J.; DeNardo, G. L.; Epstein, A. L.; O'Brien, H. A.; Moi, M. K. *J. Nucl. Med.* **1987**, *28*, 83.

Highly Sensitive Flash Photolysis with Optical Waveguides: Photodeposition of Ag onto Particulate TiO₂ from Solution

Kiminori Itoh* and Akira Fujishima

Department of Synthetic Chemistry, Faculty of Engineering, The University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113, Japan

Received May 23, 1988

Typical difficulties in the optical monitoring of photoreactions taking place at semiconductor surfaces are as follows: small optical density changes at flat surfaces and large light scattering in opaque particulate systems. In order to overcome these difficulties, we introduced optical waveguides (OWG's) and carried out flash photolysis measurements on OWG systems for the first time.¹

It is well known that Ag⁺ ions deposit onto TiO₂ surfaces in a metal form under UV illumination.² Since the deposited metallic Ag absorbs visible light, the deposition process can be monitored optically. Thus, photodeposition of Ag⁺ from aqueous solution onto particulate TiO₂ was used here as a model system.

(1) A part of this work was presented at the 195th American Chemical Society Meeting at Toronto, Canada, 1988.

(2) For example: Nishimoto, S.; Ohtani, B.; Kajiwa, H.; Kagiya, T. *J. Chem. Soc., Faraday Trans. 1* **1983**, *79*, 2685. Baba, R.; Konda, R.; Fujishima, A.; Honda, K. *Chem. Lett.* **1986**, 1307.