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pH-Controlled Inclusion and Release of Oxyanions by Dendrimers Bearing Methyl Orange Moieties

Holger Stephan,*,† Hartmut Spies,† Bernd Johannsen,†
Christopher Kauffmann,‡ and Fritz Vögtle*,‡

Institut für Bioanorganische und Radiopharmazeutische Chemie, Forschungszentrum Rossendorf, 01314 Dresden, Germany, and Kekulé-Institut für Organische Chemie und Biochemie, Universität Bonn, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany

voegtle@uni-bonn.de

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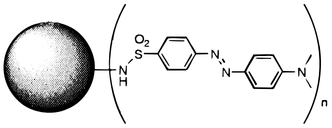
ABSTRACT



1-5

We report the synthesis of POPAM dendrimers, bearing up to 64 chromophores at their periphery. For these dendrimers, a radiotracer technique was used to study the liquid–liquid partition of pertechnetate and ^{14}C-labeled nucleotides in trichloromethane–aqueous systems. Inclusion and release of guest molecules can be controlled by changing the pH. The extraction efficacy increases with rising generation number.

The architecture of poly(propyleneamine) dendrimers (POPAM) makes them ideally suitable for accommodation of anionic substrates.[1] Lipophilic POPAM dendrimers are capable of extracting xanthene dyes as carboxylates.[2] The dyestuffs can be locked in a dendritic box.[3] Inclusion and release can also be governed by photoisomerization of azobenzene-functionalized dendrimers.[4]

Recently, we also showed that medically and biologically relevant anions such as pertechnetate, perrhenate, and the nucleotides ADP and ATP can be efficiently bound by urea-functionalized dendrimers.[5] In this case the binding behavior is principally determined by the protonation state of the tertiary amine groups in the interior of the dendrimer. To enhance the pH-sensitivity we now graft Methyl Orange units at the dendritic periphery (Scheme 1). In this paper we report the synthesis of these dendrimers up to generation 5 (G1-G5). This was accomplished by the reaction of the corresponding POPAM dendrimer with 4′-(dimethylamino)-azobenzene-4-sulfonic acid chloride (dabsyl chloride) and further purification by extraction of the organic phase with aqueous sodium sulfate and consecutive preparative thin-layer chromatography when appropriate (G4−G5). The compounds obtained were then applied to liquid−liquid partition[6] of $^{99}TcO_4^-$ and ^{14}C-labeled nucleotides AMP, ADP, and ATP to characterize the binding properties.

Methyl Orange dendrimers show a unique phase-transfer behavior with respect to pH changes. When the aqueous phase containing pertechnetate is immediately mixed with **3** (3rd generation) dissolved in trichloromethane, we found that the orange organic phase is entirely discolored, accompanied by formation of a reddish brown flaky precipitation at the aqueous phase in the pH range of 2.0 to 4.5 (Figure 1).

[†] Institut für Bioanorganische und Radiopharmazeutische Chemie.

[‡] Kekulé-Institut für Organische Chemie und Biochemie, Universität Bonn.

(1) (a) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. *Dendritic Molecules: Concepts, Syntheses, Perspectives*; VCH: Weinheim, Gemany, 1996. (b) Koper, G. J. M.; van Genderen, M. H. P.; Elissen-Román, C.; Baars, M. W. P. L.; Meijer, E. W.; Borkovec, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 6512. (c) Bosman, A. W.; Janssen, H. W.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665.

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(5) Stephan, H.; Spies, H.; Johannsen, B.; Klein, L.; Vögtle, F. *Chem. Commun.* **1999**, 1875.

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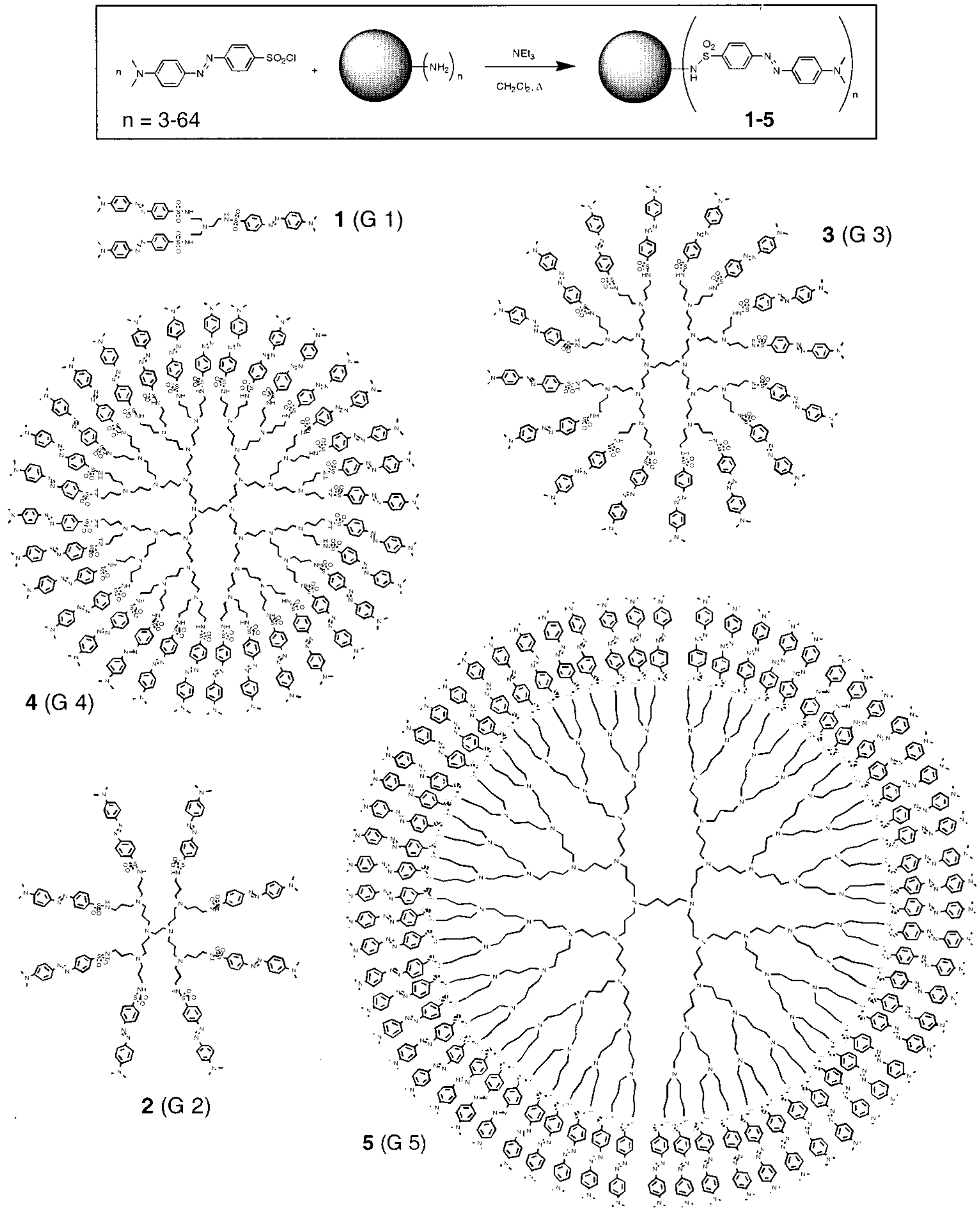
Scheme 1. Synthesis and Constitution of Methyl Orange POPAM Dendrimers G1−G5



Generation	1	2	3	4	5
No. of chromophores	3	8	16	32	64

This finding points out a strong interaction of the positively charged dendritic periphery with pertechnetate anions. Further increase of pH leads to the formation of a lipophilic complex of dendrimer **3** with pertechnetate which is completely soluble in trichloromethane. Obviously, the pertechnetate anion migrates into the inside of the dendrimer. Hence, TcO_4^- is bound by partially protonated tertiary amine groups of the core, and the anion is shielded by the hydrophobic periphery. Knowing the time dependency for the protonation of dansyl dendrimers,[7] we preequilibrated the Methyl Orange dendrimer **3** in a buffer solution for 4 h and then was added the potassium pertechnetate. In this case no precipitation was

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(6) Liquid−liquid extraction studies were performed at 25 ± 1 °C in 2 cm^3 microcentrifuge tubes by mechanical shaking. The phase ratio $V_{(org)}$:$V_{(w)}$ was 1:1 (0.5 cm^3 each); the shaking period was 30 min. The extraction equilibrium was achieved during this period. All samples were centrifuged after extraction. The anion concentration in both phases was determined radiometrically using the β-radiation measurements of $^{99}TcO_4^-$ (Amersham) and ^{14}C-labeled nucleotides (NEN Life Science Products) in a liquid scintillation counter (LS 6000 LL, Beckman). The aqueous solution was adjusted using 0.05 mol•dm^3 NaOAc/HCl (pH 2.0−5.2), 2-[*N*-morpholino]-ethanesulfonic acid (MES)/NaOH (5.4−6.5), and *N*-[2-hydroxyethyl]-piperazine-*N*′-[2-ethanesulfonic acid] (HEPES)/NaOH buffer (7.0−8.0).

(7) Vögtle, F.; Gestermann, S.; Kauffmann, C.; Ceroni, P.; Vicinelli, V.; De Cola, L.; Balzani, V. *J. Am. Chem. Soc.* **1999**, *121*, 12161.

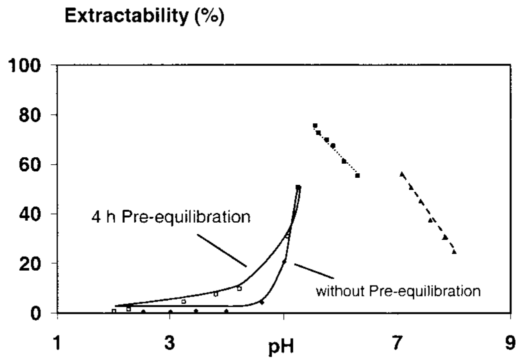

Figure 1. Extractability of pertechnetate with dendrimer **3** as a function of pH: $[KTcO_4] = 1 \times 10^{-4}$ mol·dm^{-3}; $[\mathbf{3}] = 1 \times 10^{-4}$ mol·dm^{-3} in trichloromethane; pH = 2.0−5.5 (NaOAc/HCl buffer: —); pH = 5.4−6.5 (MES/NaOH buffer: ···); pH = 7.0−8.0 (HEPES/NaOH buffer: − −).

observed.[8] Apparently, after preequilibration, the tertiary amine groups in the interior of the dendrimer are protonated, and consequently the phase distribution behavior is altered. The extraction of pertechnetate slightly increases in the pH range between 2 and 3.5 where the Methyl Orange units should be mainly protonated.[9] Most likely, due to the electrostatic repulsion of the positively charged periphery, complete protonation of tertiary amine groups in the interior of these dendrimers may be prevented. After deprotonation of the Methyl Orange moieties (pH > 4.5), a sharp increase of pertechnetate extraction was obtained. As a result of a rising pH, the color of the organic phase changes from deep to pale orange. Also, the increasing formation of the pertechnetate anion[11] under the experimentally chosen conditions could be responsible for the rise of extractability, but that may play only a minor role, because at pH 2 $HTcO_4$ should be 98% dissociated. A controlled release of pertechnetate from the dendrimer can be achieved by graduated deprotonation of the tertiary amino groups with further increase of pH (Figure 1).[13]

Regarding the complexation of pertechnetate in a solution obtained from a ^{99}Mo−^{99m}Tc generator,[14] the interaction of TcO_4^- with dendrimer **3** was investigated in isotonic sodium chloride. As shown in Figure 2, a linear relationship between the distribution ratio[15] D_{TcO_4} and the dendrimer concentration is obtained. The slope of the line in the log D_{TcO_4}−log c_3 diagram is significantly lower than 1, indicating that one dendrimer molecule binds some pertechnetate anions.[16]

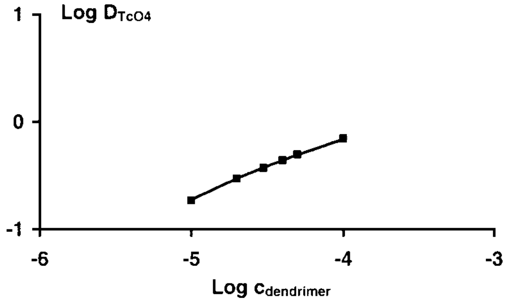

Figure 2. Extraction of pertechnetate with dendrimer **3** as a function of dendrimer concentration: $[KTcO_4] = 1 \times 10^{-5}$ mol·dm^{-3}, [NaCl] = 0.9%, [dendrimer] = 1×10^{-5} to 0.1×10^{-6} mol·dm^{-3} in trichloromethane.

Using a nonlinear regression program[17] for calculation of the extraction constant, the best fit was found for the 1:4 complexation (dendrimer:pertechnetate). This finding is confirmed by a $c_{TcO_4(org)}$ vs $c_{TcO_4(total)}/c_{denrimer}$ plot which shows a marked flattening after 1:4 complex formation (cf. Supporting Information). These dendrimers are very promising in view of the development of radiopharmaceuticals having a high specific activity.

Extractabilities of pertechnetate and the nucleotides AMP, ADP, and ATP at pH = 5.4 (Methyl Orange units are deprotonated) with dendrimers **2**−**5**[18] are summarized in Figure 3. A dendritic effect is clearly visible.[19] As expected, the extractability of pertechnetate is better compared to the more hydrophilic nucleotides.

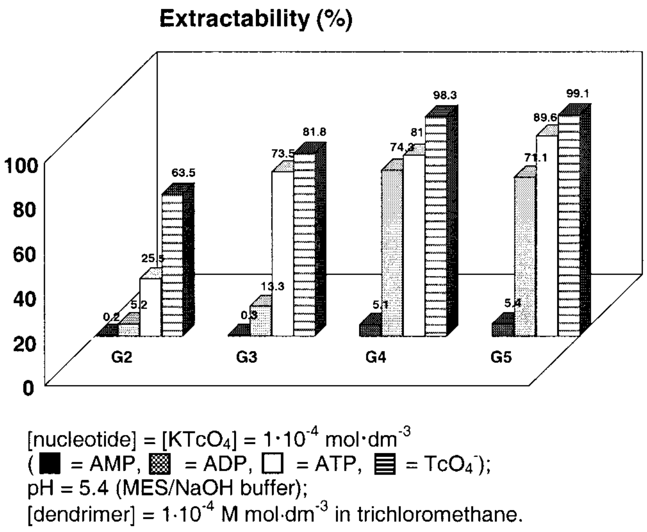

Figure 3. Extractability of nucleotides and pertechnetate with dendrimers **2**−**5**.

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[8] The formation of red droplets insoluble in both the organic and aqueous phases is observed in the buffer system at a dendrimer concentration of ≥ 10^{-4} M. Probably oligomeric aggregates are formed.[21]

[9] pK_a(Methyl Orange) = 3.46.[10]

[10] *Handbook of Chemistry and Physics*, 78th ed; Lide, D. R., Frederikse, H. P. R., Eds.; CRC Press: Boca Raton, FL, 1997.

[11] pK_a($HTcO_4$) = 0.30.[12]

[12] Rulfs, C. L.; Hirsch, R. F.; Pacer, R. A. *Nature* **1963**, *199*, 66.

[13] The extraction protocol shows no difference for non- and preequilibration.

[14] Stephan, H.; Berger, R.; Spies, H.; Johannsen, B.; Schmidtchen, F. P. *J. Radioanal. Nucl. Chem.* **1999**, *242*, 399.

[15] Distribution ratio D_{TcO_4} is defined as the quotient of the concentration of pertechnetate in the organic and aqueous phases.

[16] Gloe, K.; Mühl, P. *Isotopenpraxis* **1979**, *15*, 236.

[17] Petrich, M.; Beyer, L.; Gloe, K.; Mühl, P. *Anal. Chim. Acta* **1990**, *228*, 229.

To sum up, Methyl Orange dendrimers offer a new approach for controlled inclusion and release of anionic substrates. By variation of the basicity of the dye introduced, the pH window can be tailored. Furthermore, dendrimers possessing some bidendate Congo Red or Chrysamine analoges[20] seem to be very promising in view of highly efficient amyloid plaque targeting molecules.

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Supporting Information Available: Experimental procedures and full characterization for compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Under the experimental conditions, the chosen low-generation dendrimer **1** (1st generation) is not capable of extracting anions of the type investigated here.

(19) In the pH range of 5.4−7.4 the extraction efficacy is mainly dependent on the generation number of POPAM dendrimers. Further investigations with functionalized dendrimers show that functional groups (azobenzene, *tert*-butylbenzene, dimethoxybenzene, naphthalene, dansyl) grafted at the dendrimer periphery play a less important role.

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