Lipophilic urea-functionalized dendrimers as efficient carriers for oxyanions†‡

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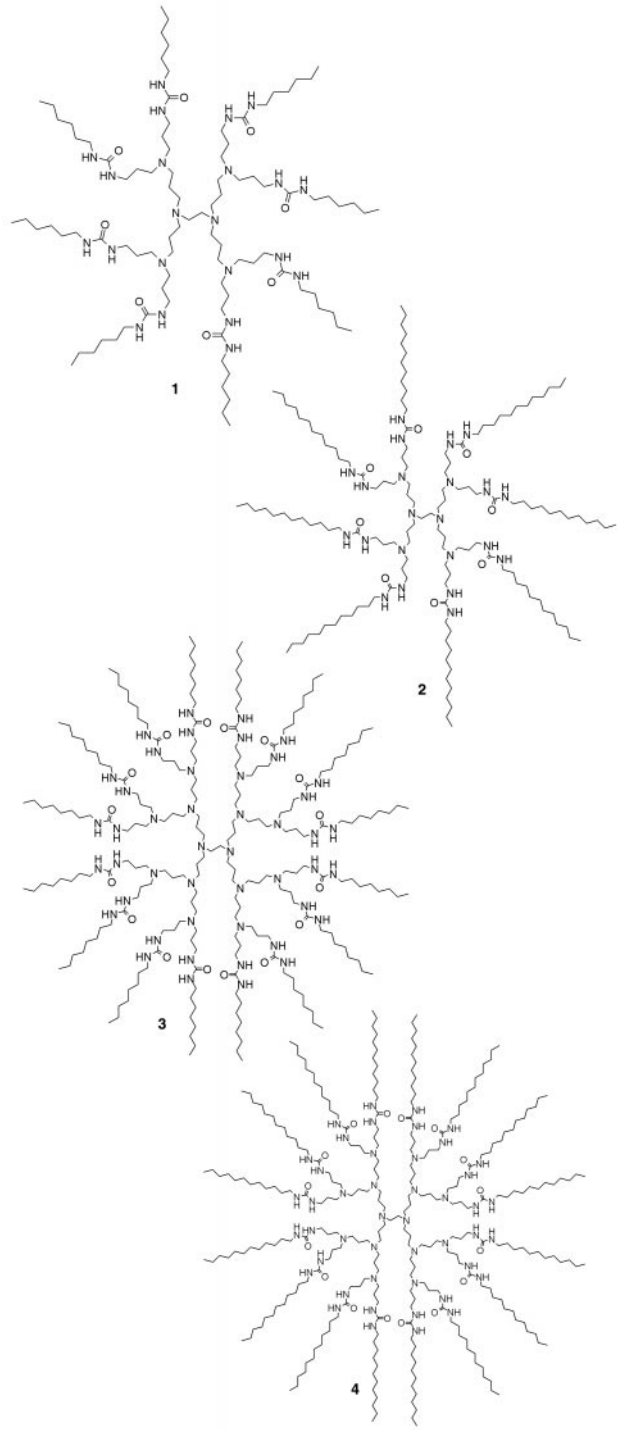
Urea-functionalized dendrimers are prepared which show very efficient phase transfer, in particular of the diagnostically relevant anions pertechnetate, perrhenate and ATP; the extractability rates are evaluated quantitatively by tracer methods; their pH dependancy allows controlled release of guest molecules from the dendrimer host.

Due to their unique topology and unusual guest-binding behaviour, dendrimers are promising reagents for use in diagnostic imaging and therapy.[1] Commercially available compounds such as poly(aminoamide) and poly(propylenimine) dendrimers have been investigated with this application in mind. At physiological pH more than 50% of the amino groups of such dendrimers are protonated, resulting in a polycation.[2] Consequently these are capable of binding anions. We are especially interested in the efficient binding of the tetrahedral oxoanion pertechnetate as a new approach to labelling organic compounds.[3] In this context dendrimers are desired having a hydrophobic periphery in order to shield the anion from hydrophilic attack. A convenient way to obtain this is reaction of the primary amino groups with alkyl and aryl isocyanates giving lipophilic polyurea-functionalized dendrimers.[4] Urea, as hydrogen bond donating moiety, is also able to stabilize the complexed anion.[5] Knowing the interaction of dendrimers with phosphate groups in oligonucleotides,[6] DNA[7] and other guests[8] we have also investigated the binding of the nucleotides AMP, ADP and ATP with the polyurea dendrimers prepared.

We report the synthesis and complexation behaviour *via* liquid–liquid extraction of various generations of these new monodisperse urea-functionalized dendrimers. Various isocyanates (hexyl, octyl, dodecyl and phenyl isocyanate) dissolved in CH_2Cl_2 [9 mmol for second generation dendrimer-$(NH_2)_8$,[9] 17 mmol for third generation dendrimer-$(NH_2)_{16}$[9]] were slowly dropped into a solution of 1 mmol poly(propylenimine) dendrimer in 150 ml of CH_2Cl_2. After stirring for 48 h at 25 °C the solvent was partially evaporated. Upon adding 40 ml of light petroleum to the remaining solution, the urea-functionalized dendrimers were precipitated. Crude products were filtered and washed with light pertroleum. The pure compounds were isolated as colourless solids in 90% yield.§

The alkyl urea-functionalized dendrimers **1**–**4** are soluble in organic solvents like $CHCl_3$ and CH_2Cl_2, a prerequisite for studying the complexation behaviour by liquid–liquid extraction.¶ On the other hand, dendrimers having phenyl urea units at the periphery are completely insoluble in these diluents. Accordingly, we have only examined the alkyl urea dendrimers. These dendritic hosts are capable of extracting pertechnetate with remarkable efficacy (Fig. 1). As expected the extractability is enhanced with decreasing pH accompanied by a higher state of protonation. Also the increasing number of potential binding sites leads to an improvement of pertechnetate extraction (generation 3 > generation 2). Introduction of hexyl and octyl



† Part of this work was presented as a poster contribution at the Seminar 'Functional Supramolecular Systems', Frankfurt (Main), Germany, June 1999.

‡ MALDI-TOF data for **3**, and **3**·$(ATP)_5$·$(H_2O)_x$ are available from the RSC web site, see http://www.rsc.org/suppdata/cc/1999/1875/

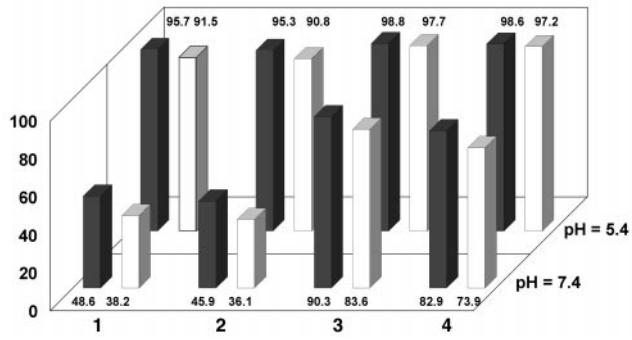


Fig. 1 Extractability of pertechnetate (grey columns) and perrhenate (white columns) with dendrimers **1**–**4**; $[KTcO_4] = [NH_4ReO_4] = 1 \times 10^{-4}$ mol dm^{-3}, pH = 5.4 (MES/NaOH buffer), pH 7.4 (HEPES/NaOH buffer), [dendrimer] $= 1 \times 10^{-3}$ mol dm^{-3} in $CHCl_3$.

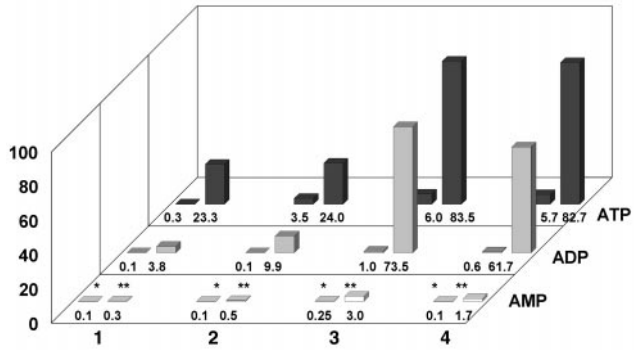


Fig. 2 Extractability of nucleotides with dendrimers **1**–**4**; [nucleotide] = 1×10^{-4} mol dm^{-3}, * = pH 7.4 (HEPES/NaOH buffer), ** = pH 5.4 (MES/NaOH buffer), [dendrimer] = 1×10^{-3} mol dm^{-3} in $CHCl_3$.

residues on the periphery of the dendrimers leads to higher distribution ratios in comparison with the dodecyl analogue. This finding is probably due to steric effects. The same trend of influence on extraction is observed for perrhenate, which is similar in size and other properties to pertechnetate. However, the efficacy is slightly decreased compared to pertechnetate extraction. This fact is also found for bimetallic cyclotriveratrylene[10] and bicyclic guanidinium hosts.[11] Differences of charge density and hydration between these two anions is probably responsible for the observed extraction behaviour.

The dendrimers investigated show also the ability to extract the highly hydrophilic nucleotide anions AMP, ADP and ATP with remarkable graduation (Fig. 2). The extraction of these anions is strongly influenced by the state of protonation and the number of binding sites in comparison to the more hydrophobic anions pertechnetate and perrhenate. The extractability of the nucleotides is enhanced with increasing number of phosphate groups, giving the order ATP > ADP > AMP. This trend meets our expectations and clearly points toward the participation of all phosphate groups in binding with the dendrimers.

As the loading capacity of the organic phase is limited, at higher concentrations of nucleotides a third phase‖ is formed during extraction. ^{31}P NMR clearly indicates that ATP is enriched in this phase. In order to obtain information about the stoichiometry of the complex extracted we utilized mass spectrometry.** Surprisingly, the MALDI-TOF mass spectrum of this complex shows one peak at *m*/*z* 6992.5, pointing to some ATP molecules being attached to the dendrimer. Considering that ATP remains hydrated†† after extraction a complex $\mathbf{3}\cdot(ATP)_5\cdot(H_2O)_x$ (with $x \approx 15$) can be postulated.

In conclusion, easily accessible urea-functionalized dendrimers are capable of binding anionic guests and transferring them to the organic phase (Table 1). The improvement of the stability of the pertechnetate complexes appears promising, and may lead to novel imaging agents. Furthermore higher generations of lipophilic dendrimers possessing anion affecting groups like (thio)ureas, amides and sulfonamides should give unusual

Table 1 Extractabilities of selected anions guests by hosts **1**–**4**

		Host–guest complex concentration/mol dm^3			
pH	Guest	**1**	**2**	**3**	**4**
7.4	$[ReO_4]^-$	38.2	36.1	83.6	73.9
	$[TcO_4]^-$	48.5	45.9	90.3	82.9
	AMP	0.1	0.1	0.25	0.1
	ADP	0.1	0.1	1.0	0.6
	ATP	0.2	3.5	6.0	5.7
5.4	$[ReO_4]^-$	91.5	90.8	97.7	97.2
	$[TcO_4]^-$	95.7	95.3	98.8	98.6
	AMP	0.3	0.5	3.0	1.7
	ADP	3.8	9.9	73.5	61.7
	ATP	23.3	24.0	83.5	82.7

complexation and distribution properties for biologically relevant guests such as oligonucleotides and nucleic acids. Another concept is the use of poly (Lewis acid) hosts, recently realized for ferrocenyl dendrimers.[12]

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Notes and references

§ Compounds were fully characterised by ^{1}H and ^{13}C NMR spectroscopy and MALDI-TOF mass spectrometry.

¶ 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 β-radiation measurement of $^{99}TcO_4^-$, $^{188}ReO_4^-$ (Amersham) and ^{14}C-labelled nucleotides (NEN Life Science Products) in a liquid scintillation counter (Tricarb 2500, Canberra-Packard). The aqueous solution was adjusted using 0.05 mol dm^3 and HEPES/NaOH buffer.

‖ Extraction of ATP (2 cm^3, 5×10^{-2} mol dm^{-3}) with **3** (1 cm^3, 5×10^{-3} mol dm^{-3} in $CHCl_3$) leads to the formation of a third phase.

** For this purpose ATP (2 cm^3, 5×10^{-3} mol dm^{-3}) is extracted from aqueous solution into $CHCl_3$ using **3** (1 cm^3, 5×10^{-3} mol dm^{-3}).

†† The ATP applied has a hydration state of 7.3.

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