Oligosapphyrins: Receptors for the Recognition and Transport of Nucleotide Di- and Tri-phosphates

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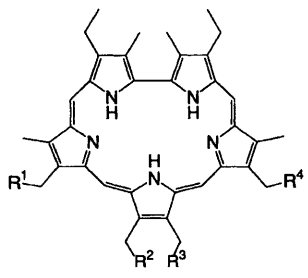
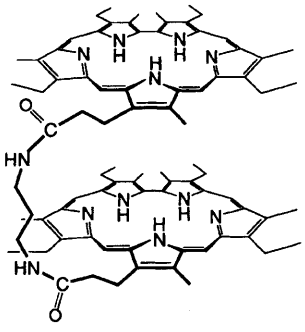
Covalently connected oligosapphyrins, in particular two trimers (linear and branched) and a tetramer, the syntheses of which are detailed, have been found to be effective receptors for the binding and through-model-membrane transport of nucleotide mono-, di- and tri-phosphates.

Anionic species, such as nucleotide mono-, di- and tri-phosphates, play important roles in biological processes. Recently, several nucleotide analogs exhibiting antiviral properties have been discovered.[1] Many of these, in spite of being active *in vitro*, are inactive *in vivo* due to an inability to cross hydrophobic cell membranes.[2] Catalysing the enhanced into-cell transport of these and other charged species through lipophilic media thus constitutes an important challenge for supramolecular and medicinal chemists. In response to this challenge, several classes of receptors capable of achieving the specific recognition of monophosphates have been developed over the last few years. However, many fewer receptors are known that are capable of binding and transporting higher order species, such as di- and tri-phosphates or oligonucleotides.[3] The reason for this is that near or full electrostatic neutralisation is generally required for the transport of these highly charged species and this, in turn, imposes much tougher requirements on the design of multidentate receptors.



1a $R^1 = R^4 = Me$; $R^2 = H$; $R^3 = CH_2CO_2H$

b $R^1 = R^4 = CH_2CONH(CH_2)_2NH_2$; $R^2 = R^3 = Me$



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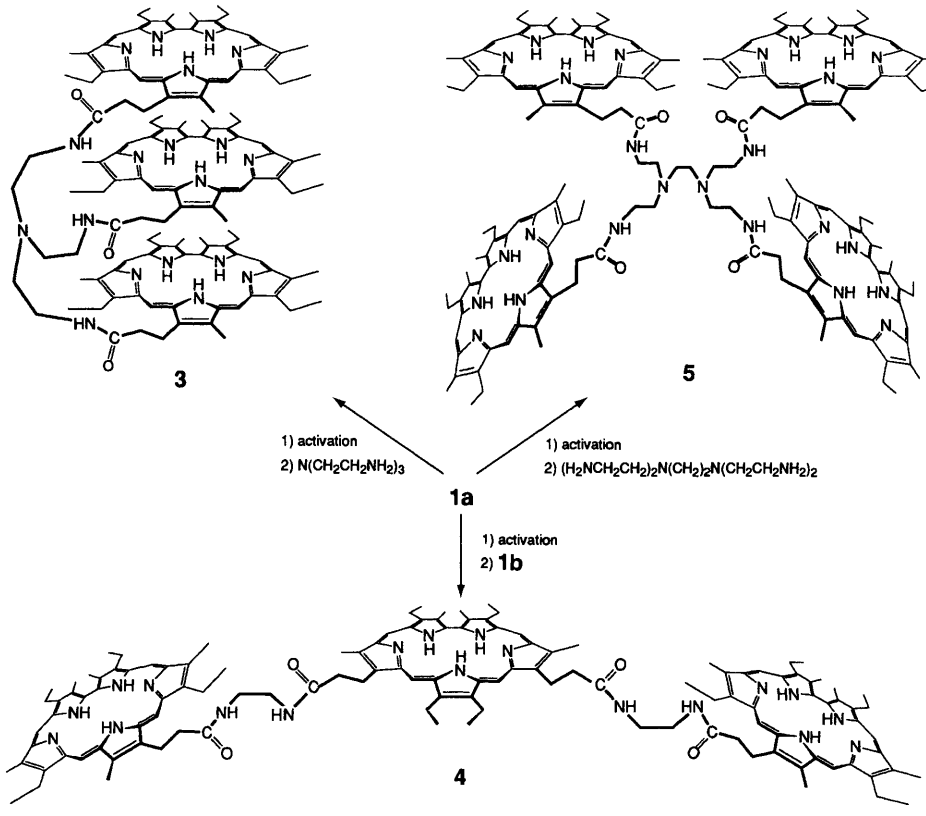
Recently we discovered that several expanded porphyrins, including sapphyrin (*e.g.* **1**), can function as specific receptors and carriers for various anions.[4] In particular, we found that monomeric sapphyrins are capable of binding phosphates and phosphonates, both simple[4b,d] and complex.[4a–c,e] We also found that the selective through-U-tube transport of GMP at neutral pH could be achieved using a ditopic sapphyrin–cytosine conjugate.[4a,b] Based on these findings, we considered it likely that *oligosapphyrins*, if prepared, would function as specific receptors and carriers for biologically important phosphorylated species such as mono-, di-, and tri-phosphates. Here we report the synthesis of several prototypical oligosapphyrins, namely systems **3**–**5**,† and show that these species do in fact bind and transport nucleotide mono-, di- and tri-phosphates under appropriate experimental conditions.

Compounds **2**–**5** were specifically chosen as synthetic targets because they contain sapphyrin 'building blocks' connected by flexible spacers. Here, the thinking was that the sapphyrin



Scheme 1

subunits would provide the basic phosphate chelation needed for binding, while the flexible linking chains would allow for the kind of generalised conformational mobility required to accommodate a range of substrates. Conformational flexibility is an inherent feature of many enzymes and enables them to accommodate more than one substrate.[5] The use of flexible spacers, it was thought, would also impart one other advantage: It would facilitate the synthetic incorporation of 'extra' stabilising interactions. At neutral pH, these 'extra' stabilising interactions could include, for instance, hydrogen bonding interactions involving the spacer amide subunits,[6] as well as, in the case of receptors **3** and **5**, more direct electrostatic attractions associated with the protonated tertiary amine 'linchpins'.

The synthetic strategy used to prepare oligosapphyrins **3–5** is one based on multiple amide bond formation (Scheme 1). Specifically, a 30% excess of the activated form of sapphyrin mono acid **1a**[4a] (*i.e.* as acid chloride, acylimidazole, mixed anhydride or activated ester) was reacted with the corresponding amine-bearing component. In this way, the branched sapphyrin trimer **3** was obtained directly from **1a** and tris(2-aminoethyl)amine. Using a similar approach, **1a** was converted into the linear trimer **4**. In this case, however, rather than using a commercially available polyamine, a bisaminosapphyrin derivative **1b**‡ was used as the critical amino component. Finally, by reacting this same key activated precursor (*viz.* **1a**) with *N,N,N′,N′*-tetrakis(2-aminoethyl)ethylenediamine,[7] it proved possible to generate the branched sapphyrin tetramer **5**. In all cases, the resulting oligomeric sapphyrin products were purified chromatographically on silica gel to give final isolated yields of 50–80%.§

Table 1 Binding constants for the complexation of adenosine di- and triphosphates to oligosapphyrins **3–5** in water–methanol (1 : 1) mixtures under neutral conditions[a]

	K_a (dm^3 mol^{-1})		
Receptor	ADP	ATP	K_a (ADP)/K_a (ATP)
3	2200	5000	0.44
4	3600	1900	1.89
5	4000	6800	0.59

[a] Measurements were made at 293 K using solutions that were constant in receptor concentration [10^{-6} mol dm^{-3} in water–methanol (1 : 1), pH = 7]. To these solutions were added aliquots of the particular substrate under study [as 10^{-3} mol dm^{-3} solutions in water–methanol (1 : 1), adjusted to pH = 7 by the addition of NaOH] while the decrease in Soret band intensity at 420 nm was monitored spectroscopically. Errors in individual K_a values are less than 15%.

Visible spectroscopic analyses carried out in dichloromethane and methanol revealed that compounds **2–5** are aggregated in these solvents: Two Soret maxima, at λ_{max} = 426 and 450 nm and 422 and 441 nm, are observed in dichloromethane and methanol, respectively. Here, the high and low energy transitions correspond to the aggregated (*i.e.* monomeric, but 'folded over') and completely non-aggregated forms, respectively.[4f,¶] Addition of anionic species, in particular phosphates, to solutions of oligosapphyrins **2–5** in methanol causes the Soret-like band with the higher wavelength maximum to increase in intensity at the expense of the one at lower wavelength. This kind of behavior is seen throughout the series **2–5** and is attributed to deaggregation of an 'internally stacked', or 'folded over', macrocyclic species as the result of anion chelation.‖ Thus, by following the decrease in relative absorbance at *ca.* 420 nm as a function of substrate-to-receptor ratio, it proved possible to determine association constants (K_a), accurate at least in a relative sense, for the formation of 1 : 1 complexes between receptors **3–5** and ADP or ATP.** Relevant results are summarised in Table 1 and are found, at least in terms of absolute K_a values, to reflect a fairly high level of absolute receptor-to-substrate binding affinity.

Further support for the proposed receptor–substrate interactions came from ^{1}H and ^{31}P NMR experiments. Here, large (in some cases up to 2 ppm) changes in the nucleotide proton chemical shifts were recorded upon complexation by oligosapphyrins. Further, the ^{31}P signals of the phosphorylated substrates were found to be shifted to a higher field as the result, presumably, of exposure to the strong diamagnetic sapphyrin ring current.††

In previous studies we found that simple monomeric sapphyrins[4b] and nucleobase-substituted sapphyrin conjugates[4a–c] can function as efficient carriers for the transport of nucleotide monophosphates across bulk liquid membrane. In these same studies, however, we also found that transport efficiency is strongly pH-dependent. In the case of an unmodified sapphyrin, for instance, the upper pH limit for detectable transport is 5.5. Even after attaching a complementary nucleobase recognition subunit, the effective transport rates drop off dramatically as neutral pH is approached. By contrast, the oligosapphyrins **3–5**, which are expected to be multiply protonated at neutral pH,‡‡ are effective carriers for nucleotide mono-, di-, and tri-phosphate species at neutral pH. Sapphyrin trimers **3** and **4**, for instance, were found to be very efficient carriers for the through-bulk-membrane transport of nucleotide diphosphates (Table 2, Fig. 1).§§ These same species, however, failed to act as good carriers for nucleotide triphosphates. On the other hand, tetramer **5** was found to be a highly effective carrier for all of the studied phosphorylated species (Fig. 1). Interestingly, for this carrier and its simpler congeners, **2–4**, adenosine-derived nucleotides (*i.e.* AXP; X = M, D, T) were

Table 2 Initial nucleotide di- and tri-phosphate transport rates (k_t) for carriers **3–5**[a,b,c]

		k_t (10^{-9} mol cm^{-2} h^{-1})							
Carrier[d]	Aq. II	ADP	CDP	GDP	UDP	ATP	CTP	GTP	UTP
3	H_2O	4.1	0.7	2.3	1.0	<0.01	<0.01	<0.01	<0.01
	1 mmol dm^{-3} NaOH	66.3	9.2	4.2	10.7	4.4	0.3	0.3	0.9
4[e]	1 mmol dm^{-3} NaOH	52.3	n.d.[f]	31.7	13.2	17.4	n.d.[f]	n.d.[f]	n.d.[f]
5	H_2O	1.3	0.3	0.4	0.5	0.6	0.2	0.1	0.3
	1 mmol dm^{-3} NaOH	14.8	1.6	1.8	3.4	7.0	0.9	0.3	2.2

[a] Transport experiments were performed as described in ref. 4*a,b* under conditions of competitive transport. The initial rate values given for ADP, CDP, GDP and UDP transport are thus derived from experiments involving equimolar mixtures of these four nucleotides (5 mmol dm^{-3} each) in the initial aqueous phase, Aq. I. Likewise, those given for ATP, CTP, GTP and UTP are from studies involving equimolar mixtures of these latter four species (again 5 mmol dm^{-3} each in Aq. I). For most carrier/receiving phase permutations, the initial rates of transport for at least two substrates were measured independently; in all cases the rate values obtained were found to be *ca.* 30% higher than those obtained under conditions of competitive transport. [b] Values are averages from two separate experimental runs; estimated errors are ±15%. [c] The initial aqueous phase, Aq. I, was in all cases adjusted to pH = 7.2 by the additions of NaOH. [d] 0.1 mmol dm^{-3} in dichloromethane (as free-base form). [e] 5% MeOH in CH_2Cl_2 was used as the organic phase to prevent carrier precipitation. [f] n.d. = Not determined due to carrier precipitation.

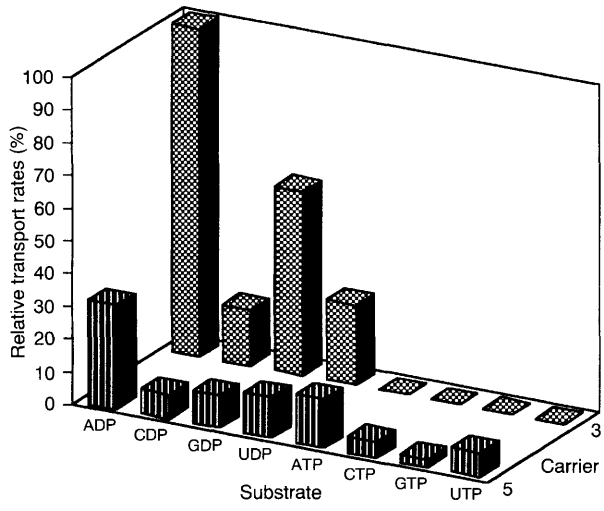


Fig. 1 Relative rates (%) of nucleotide di- and tri-phosphate transport using oligosapphyrins **3** and **5** as carriers (Table 2, entries 1 and 4)

always found to be transported with greater efficiency than those derived from uridine, cytosine or guanine. This selectivity, although not yet fully understood, has been observed previously in the case of other systems.[3f,h,8]

Taken *in toto*, the evidence presented here supports the proposal that oligosapphyrins can indeed function as effective receptors for the recognition and transport of nucleotide mono-, di-, and tri-phosphates at or near neutral pH. This could augur well for the eventual design of adjuvants for antiviral chemotherapy, physiologically compatible carriers, capable of effecting the into-cell transport of phosphorylated antiviral agents *in vivo*.

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Footnotes

† The synthesis of dimer **2** has already been published.[4f]

‡ Compound **1b** was prepared by coupling the known sapphyrin bis acid, 3,12,13,22-tetraethyl-8,17-bis(carboxyethyl)-2,7,18,23-tetramethylsapphyrin,[4a] with $H_2N(CH_2)_2NH(Boc)$ followed by cleavage of the Boc protective groups by TFA : CH_2Cl_2 (1 : 1).

§ Satisfactory spectroscopic and analytical data were obtained for compounds **3–5**.

¶ The postulated folding/non-folding equilibrium could also be followed using ^1H NMR spectroscopy. For example, both the pattern and the chemical shift (up to *ca.* 0.48 ppm) of methine protons of dimer **2** were different when the spectra of **2** were taken in $CDCl_3$ or, alternatively, in CD_3OD. This indicates that the ratio of aggregated to non-aggregated conformations of **2** is different in these intrinsically different solvents.

‖ The associations followed in this way are rigorously bimolecular in nature: Dilution experiments, carried out in methanol, show that the monitored ratios in Soret maxima for receptors **2–5** is independent of the concentration of these species (±6%) over 10^{-7}–10^{-5} mol dm^{-3}. On the other hand, when a more polar water–methanol (1 : 1) mixture was used as solvent, only one band (at roughly 420 nm) was observed for all four compounds **2–5**. This latter finding is, of course, consistent with a model wherein the 'folded over' or internally aggregated forms are stabilised by an increase in solvent polarity.

** These spectroscopic titrations were carried out in methanol–water mixtures (1 : 1), adjusted to pH = 7, with aliquots of the substrates (10^{-3} mol dm^{-3} in this same solvent mixture) being added to fixed (10^{-6} mol dm^{-3}) initial concentrations of the receptors in question. Association constants and the stoichiometric compositions of the complexes were determined by standard nonlinear curve-fitting protocols: K. A. Connors, *Binding Constants*, Wiley, New York, 1987. For a complete discussion, see supplementary material to ref. 4*f*.

†† For example, the phosphorus atom signal of AMP underwent a *ca.* 2.4 ppm shift when 1 equiv. of **2** was added to a solution of AMP in D_2O. Likewise, the P_α and P_β signals of ADP shifted 3.21 and 3.25 ppm upfield, respectively upon addition of 3 equiv. of **2** to a solution of ADP in a D_2O–MeOH (1 : 1) mixture.

‡‡ The central cores of monomeric sapphyrins, such as **1**, are monoprotonated at neutral pH.[4]

§§ Nucleotide monophosphate transport can be effected at pH = 7 using dimer **2** as the carrier. For instance, with Aq. I containing 5 mmol dm^{-3} each of AMP, CMP, and GMP and the other conditions as the first entry of Table 2, initial transport rates (10^{-9} mol cm^{-2} h^{-1}) of 3.7, 0.1, and 0.2 are observed for these three substrates, respectively. However, importantly, no enhanced transport of either di- or tri-phosphate species could be induced using this carrier system ($k_t < 10^{-11}$ mol cm^{-2} h^{-1}).

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