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Phosphate anion chelation and base-pairing. Design of receptors and carriers for nucleotides and nucleotide analogues

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A rational approach to the development of effective carriers for the through-membrane transport of GMP (guanosine 5′-monophosphate) at neutral pH is described. The approach detailed is a ditopic one predicated on the use of nucleic acid–base 'nucleobase' subunits to provide stabilizing hydrogen bonding interactions and the use of expanded porphyrin anion binding subunits to provide phosphate chelation. Appropriate background studies along with the synthesis of a functioning state-of-the-art system are reported. In addition, the reasons for preparing such GMP transport systems are presented in full.

It has long been appreciated that a considerable number of ionic (e.g. phosphorylated) nucleotide analogues exhibit anti-viral activity in cell-free extracts, yet are inactive *in vivo* due to their inability to cross lipophilic cell membranes.[1,2] The anti-herpetic agent 9-[(2-hydroxyethoxy)methyl]-9H-guanine (acyclovir, **1**, see below), for example, is typical in that it is able to enter the cell only in its uncharged nucleoside-like form. Upon gaining entry to the cytoplasm it is phosphorylated, first by a viral-encoded enzyme, thymidine kinase, to give **2**, and then by relatively non-specific cellular enzymes to produce the active, ionic triphosphate nucleotide-like species **3**.[3] There it functions both as an inhibitor of the viral DNA polymerase and as a chain terminator for newly synthesized herpes simplex DNA. Many other potential anti-viral agents, including for instance the anti-HIV agent, 9-(β-D-xylofuranosyl)guanine (**4**), on the other hand, are not phosphorylated by a viral enzyme and are therefore largely or completely inactive.[4] If, however, the active monophosphorylated forms of these putative drugs (i.e. **5**) could somehow be transported into cells, it would suddenly become possible to fight viral infections with a large battery of otherwise inactive materials. These might include a number of the recently reported anti-viral phosphonate derivatives (e.g. **6** and **7**) that have documented anti-HSV and anti-HIV activity *in vitro*[5,6] or such simple species as the pyrophosphate derivatives PFA (**8**) and COMDP (**9**) that have demonstrated anti-HIV reverse transcriptase activity in cell-free media.[7] Thus, this capacity to effect specific transport into cells would greatly augment our ability to treat such debilitating diseases as herpes, hepatitis, measles, and AIDS.[8]

At present, no general set of nucleotide transport agents exists. In early work, Tabushi[9] was able to effect adenosine nucleotide transport (i.e. of **11**–**13**) using a lipophilic, diazabicyclooctane-derived quaternary amine system (**30**). However, this same system failed to mediate the transport of guanosine 5′-monophosphate (GMP, **16**) or other guanosine-derived nucleotides. Since then, considerable effort has been devoted to the generalized problem of nucleic acid–base 'nucleobase' recognition, and some very elegant binding systems are now known.*[10–56] These

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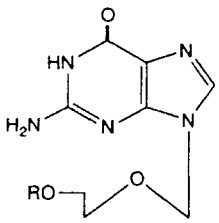
* For extensions of the approach in ref 10 that incorporate base recognition subunits see ref 11. For related work involving macromono- and macrobicyclic systems see ref 12. For earlier work from the group in ref 13 and extensions to sensor applications, see refs 14 and 15 respectively. For related approaches to the sensor development described in ref 15 see refs 31 and 55.

For examples of nucleotide/phosphate binding with simple acyclic polyamines see, e.g., ref 18.

For an example of a guanidinium-based approach to nucleotide sensor development see ref 31.

For HPLC separations achieved via nucleoside-type recognition see ref 44. For a report of nucleotide recognition achieved in aqueous media via the intermediacy of appropriate small peptides see ref 45. For an example of a ditopic approach to mononucleotide recognition formally related to ours see ref 56.



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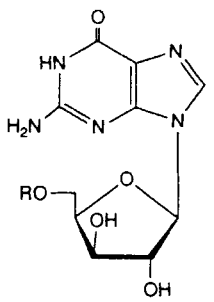
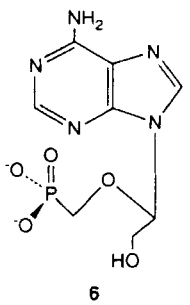
2. R = PO_3^{2-}
3. R = $PO_3PO_3PO_3^{4-}$



4. R = H
5. R = PO_3^{2-}



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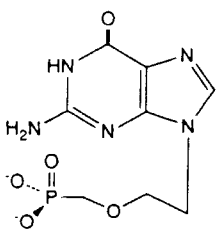

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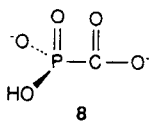

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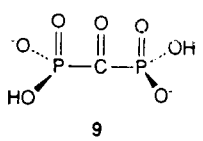

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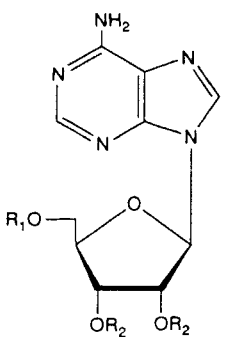
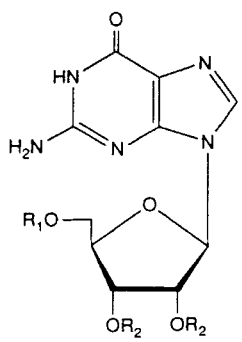
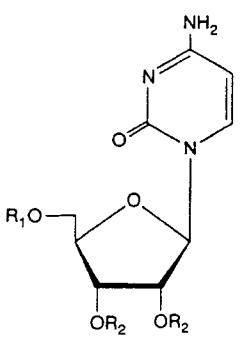


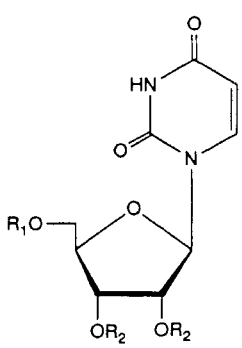
10. $R_1 = R_2 = H$
11. $R_1 = PO_3^{2-}$; $R_2 = H$
12. $R_1 = PO_3PO_3^{3-}$; $R_2 = H$
13. $R_1 = PO_3PO_3PO_3^{4-}$; $R_2 = H$
14. $R_1 = R_2 = Si(CH(CH_3)_2)_3$



15. $R_1 = R_2 = H$
16. $R_1 = PO_3^{2-}$; $R_2 = H$
17. $R_1 = PO_3PO_3^{3-}$; $R_2 = H$
18. $R_1 = PO_3PO_3PO_3^{4-}$; $R_2 = H$
19. $R_1 = R_2 = Si(CH(CH_3)_2)_3$



20. $R_1 = R_2 = H$
21. $R_1 = PO_3^{2-}$; $R_2 = H$
22. $R_1 = PO_3PO_3^{3-}$; $R_2 = H$
23. $R_1 = PO_3PO_3PO_3^{4-}$; $R_2 = H$
24. $R_1 = R_2 = Si(CH(CH_3)_2)_3$



25. $R_1 = R_2 = H$
26. $R_1 = PO_3^{2-}$; $R_2 = H$
27. $R_1 = PO_3PO_3^{3-}$; $R_2 = H$
28. $R_1 = PO_3PO_3PO_3^{4-}$; $R_2 = H$
29. $R_1 = R_2 = Si(CH(CH_3)_2)_3$

binding systems include various acyclic, macrocyclic, and macrobicyclic polyaza systems,[10-18] such as **31**, **32**, **33** and **34**, pioneered respectively by Lehn and Mertes,[10-12] Kimura,[13-15] Schmidtchen,[16] and Burrows,[17] the nucleotide-binding bis-intercalands of Lehn (e.g. **35**), [19,20-24] as well as guanidinium-based receptors [25-32] (e.g. **36**,[25] **37**[26] and **38**[27]), a class of anion-binding agents first developed by Lehn,[25] Schmidtchen[26] and de Mendoza,[27] and now recently elaborated by Rebek and collaborators,[28] to produce the beautiful adenine-adenine dinucleotide binding system **39**, and independently by Hamilton[29] and Anslyn,[30] to produce, respectively, the phosphodiester receptors **40** and **41**. Also of exceptional elegance are the rationally designed, and hence intellectually appealing, H-bonding receptors of Hamilton,[33] Wilcox,[35] Rebek,[36,*] Zimmerman,[21] Vögtle,[38] Rose,[39] Lindsey *et al.*[40] and Ogoshi and co-workers,[**,41] all of which have been shown to be effective for the chelation of neutral nucleobase- and/or nucleoside-derived substrates but which, without exception, have also all proved unsatisfactory for charged nucleotide recognition. Thus, in spite of an impressive rush of recent synthetic architectural elegance, and a fair amount of other related work in the field,† we are currently unaware of any synthetic system capable of effecting the recognition and through-membrane transport of guanosine-derived phosphate-bearing species (the ones, like **5**, that are in general both the least soluble in organic media and the most active as anti-virals) at neutral pH.‡ Nor are we aware of any rationally designed receptors which are 'tunable' for the selective complexation of a given nucleobase-derived system. The design and synthesis of such a system stands defined, therefore, as being an important, unsolved goal in the area of molecular recognition.

Our approach to nucleotide analogue transport derives from an appreciation that these compounds are divergent in structure: they contain both an ionic phosphate and a potentially hydrogen-bonding nucleobase linked together via some sort of cyclic or acyclic tether. We thus considered that an appropriately designed ditopic receptor, containing recognition units for both the charged phosphate and the nucleobase entiry, could be used to bind, neutralize, and transport such species through a membrane (Fig 1). Here, the thoughts were that the recognition of the nucleobase portion would have to be effected by hydrogen bonds as it is these interactions, rather than stacking ones, that would be expected to dominate in the non-polar biological membrane medium being targeted as the site for transport activity. In the same sort of way, it was thought that electrostatic forces would have to be used to effect recognition of the phosphate group since only by exploiting such neutralizing interactions would it be possible to induce the requisite organic solubility needed for transport.

At the commencement of this project, it was not appreciated that it would be the second of the above strategies that would prove the far more difficult to implement. As a result, initial work was largely concerned with developing methods for nucleobase recognition. Thus, in a first set of studies, begun some 3 years ago, we chose to use simple protonated polyamines for phosphate 'coordination' and focus the bulk of our attention towards the synthesis of elaborated receptors that would allow for nucleobase 'chelation' via complementary Watson–Crick type base-pairing interactions.[46-48] This choice of priorities led to the synthesis of the elaborated cytosine derivatives **42** and **43**.

Although quite successful in terms of achieving specific recognition (i.e. GMP was bound by **43** with high specificity in organic solvents such as DMSO; Fig 2), this approach failed to produce materials that were effective for enhancing through-membrane transport. The basic problem appeared to be that simple polyamines, even when highly substituted with organic 'spaghetti', could not be made sufficiently lipophilic to overcome the high intrinsic water solubility of the guanine component. However, this work was important as a first illustration of molecular recognition via base-pairing.[48] In addition, it provided a critical 'hint' that such interactions could be used to confer specificity in the design of yet-to-be-improved nucleotide receptors.

Further support for this latter hypothesis came from studies of the tri-isopropylsilyl (Tips) substituted (phosphate-free) nucleosides **14**, **19**, **24**, and **29**.[53] This work, which evolved out of efforts to obtain better solubilizing groups for receptors **42** and **43**, led to the interesting finding that efficient and selective through-membrane transport of non-charged

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* For related work see ref 37.

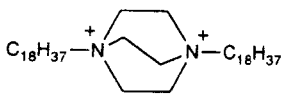
** For an extension of this approach that shows promise for binding non-nucleotide phosphate esters, see ref 42.

† For recent examples of literature on the interactions of metal centres with phosphate portion(s) of mono- and polynucleotides see refs 57 and 58. For an example of nucleoside recognition achieved via metal chelation see ref 57(e).

For an example of enhanced nucleotide transport effected using metal salts see ref 58.

There is voluminous literature associated with polynucleotide-to-polynucleotide interactions in aqueous media. For leading and recent references see ref 59.

‡ In fact the total number of receptor systems capable of even recognizing nucleotides or other phosphate-bearing species in organic media is extremely limited; those we know are cited in refs 9, 27–30, 42, 48, 54, 56 and 58, with the two that function as carriers in refs 54 and 58. Systems that function in aqueous or other polar protic (i.e. non-membrane-like) environments are more numerous; cf. refs 10–19, 25, 26, 31, 32, 45, 55 and 59.



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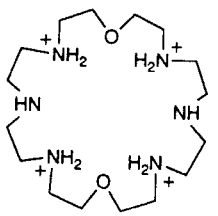

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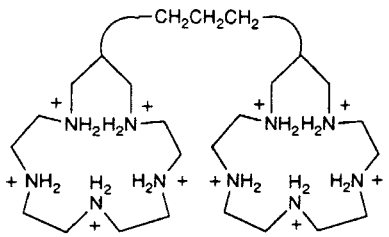

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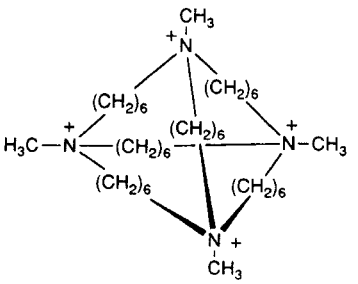

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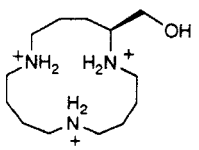

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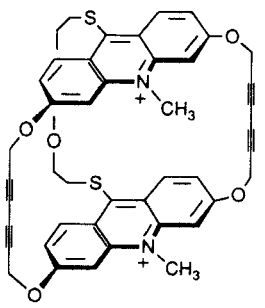

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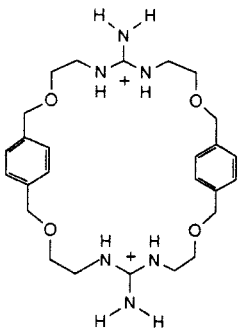

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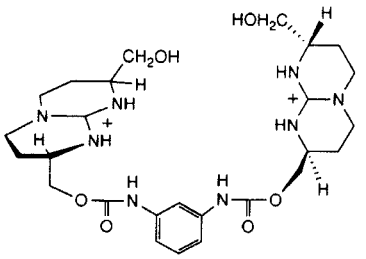

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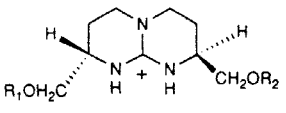
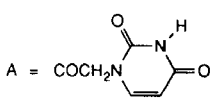
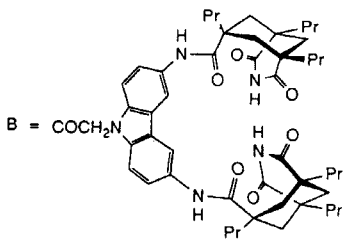






38. R_1 = 2-naphthoyl; R_2 = A
39. $R_1 = R_2$ = B

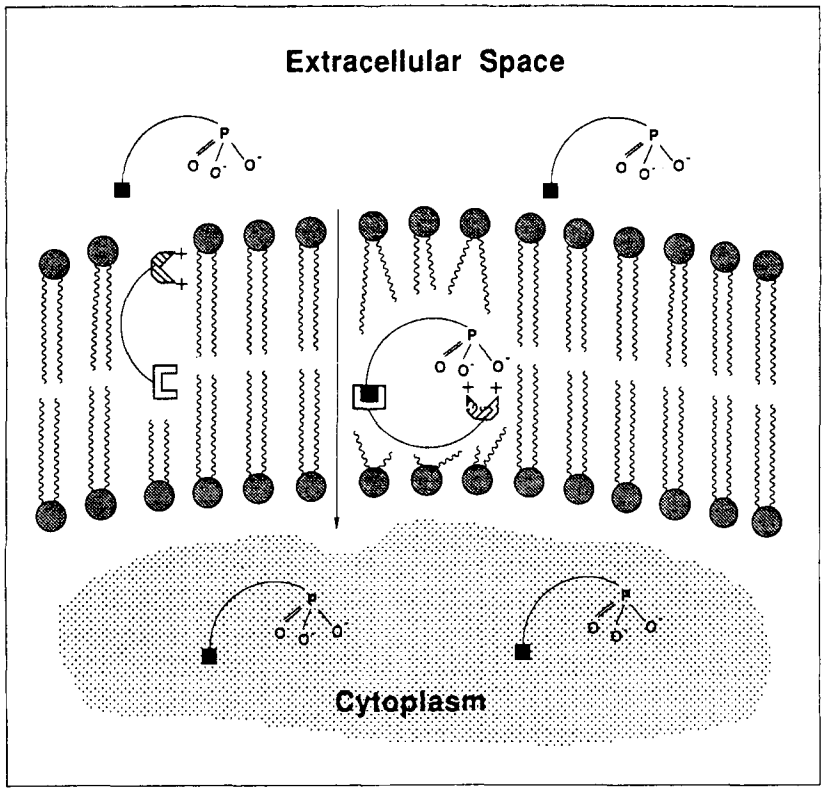
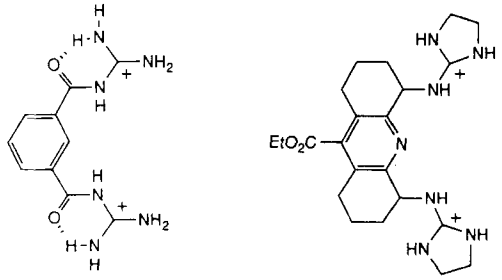


Figure 1 Schematic representation of proposed rational approach to nucleotide carriers.



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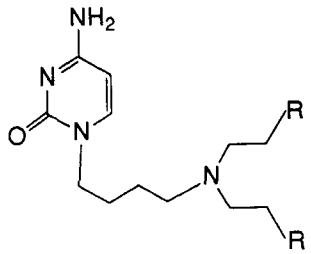


42. R = H
43. R = $N(CH_2CH_3)_2$

nucleoside analogues could be achieved by using the complementary Tips derivatives as carriers.[53] Not surprisingly, however, these same Tips derivatives proved completely ineffective as transport agents for the analogous phosphate-containing nucleotide derivatives. Thus, while reconfirming the viability of the base-pairing approach to selective nucleotide recognition, this work served to highlight further the need for an organic, soluble, neutralizing, phosphate-binding group. These experiments also served to explain why no general solution to the nucleotide transport problem had been reported in the literature: the difficulty lay not so much in achieving nucleobase recognition as in phosphate group solubilization. In fact, it began to become clear to us at this point that if we were to solve the problem of developing usable adjuvants for anti-viral drug delivery, it would be necessary to learn how to extract water-soluble phosphate derivatives into very non-polar organic media. In other words, it suddenly began to dawn on us (as well as others[60]) that it was this anion binding problem, rather than simple nucleobase recognition, that represented the real challenge in the anti-viral adjuvant development field.

To our delight, we have recently found what appears to be an interesting and effective phosphate binding and solubilizing group. Specifically, we have found that the diprotonated form (H_5Sap^{2+}) of sapphyrin **44**,[61] which exists at pH $\leqslant$ 3.5, in marked contrast to its far better studied tetrapyrrolic analogues (e.g. octaethylporphyrin **47**), acts as an effective carrier for the efficient through-transport of phosphate-

containing compounds, including GMP (**16**), adenosine 5′-monophosphate (AMP, **11**), and acyclovir monophosphate (**2**) in a simple $H_2O—CH_2Cl_2—H_2O$ three-phase Pressman-type[62] U-tube type model membrane system (Fig 3 and Table 1).[54]

This result is of considerable importance. Not only does it establish a new direction in porphyrin-related research (one in which large pyrrole-containing materials, the so-called 'expanded porphyrins',[63] are used as anion binding receptors rather than as simple cation chelating agents), but it also serves to show, in marked contrast to the results of Lehn,[10-12,81-83] Schmidtchen,[16,26,87] Kimura,[13,14] and other pioneers in the anion chelation field,[10d,14,16b,64] that high binding affinity and net charge neutralization need not be mutually inconsistent. This critical result, which reflects the fact that the expanded porphyrins contain many neutral hydrogen bond donors, provided the critical augury that led us to consider that it should be possible to obtain viable carriers for nucleotide drug delivery that function under real, physiological conditions. In other words, even though initial nucleotide transport experiments effected with **44** 'worked' only at pH $\leqslant$3.5 and were completely unselective with regard to choice of nucleobase XMP^{2-} (X = A, C, G, or U), the fact that we saw any transport at all was considered to represent a major 'victory' in the phosphate chelation area; above all, it showed we were on the right track.

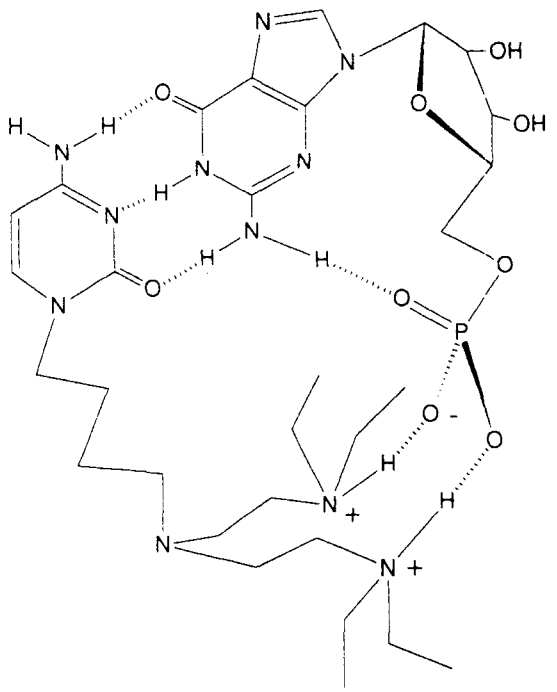
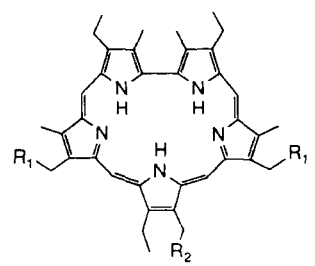


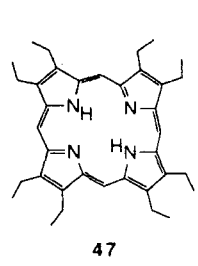
Figure 2 Proposed structure for supramolecular complex formed between receptor **43** and GMP.

Left undefined by the above phenomenological findings was the question of just how phosphate itself or various phosphate ester anions (e.g. nucleotides) could be interacting with the protonated forms of sapphyrin. The definition of such interactions, however, was clearly considered important both in terms of explaining what had already been observed and in terms of the first step towards preparing expanded porphyrin-based nucleotide transport systems that would function at neutral pH. Thus, considerable effort in recent months has been devoted to the problem of obtaining single crystal X-ray structures of phosphate-protonated sapphyrin complexes. So far, two such structures have been obtained (J.L. Sessler, H. Furuta, V. Král and V. Lynch, unpublished results). In Figures 4 and 5 can be seen the 2:1 complex between monobasic phenylphosphate and diprotonated sapphyrin and the 1:1 complex formed from the reaction of phosphoric acid with sapphyrin, respectively. In both cases, it is obvious from inspection that the anionic phosphate is held in place by highly specific hydrogen bonds involving one of three (or four) possible chelating phosphate oxygen atoms. Also obvious, but perhaps less expected, is that anywhere between two and five hydrogen bonds will apparently suffice to effect phosphate-to-sapphyrin ligation in the crystalline phase.

The above structural results suggest that sapphyrin and other similar expanded porphyrins (such as, e.g.,



44. $R_1 = R_2 = CH_3$
45. $R_1 = CH_3$; $R_2 = CH_2CO_2H$
46. $R_1 = CH_2CH_2OH$; $R_2 = CH_3$



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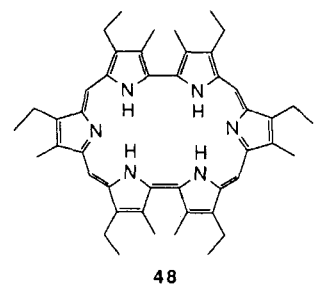

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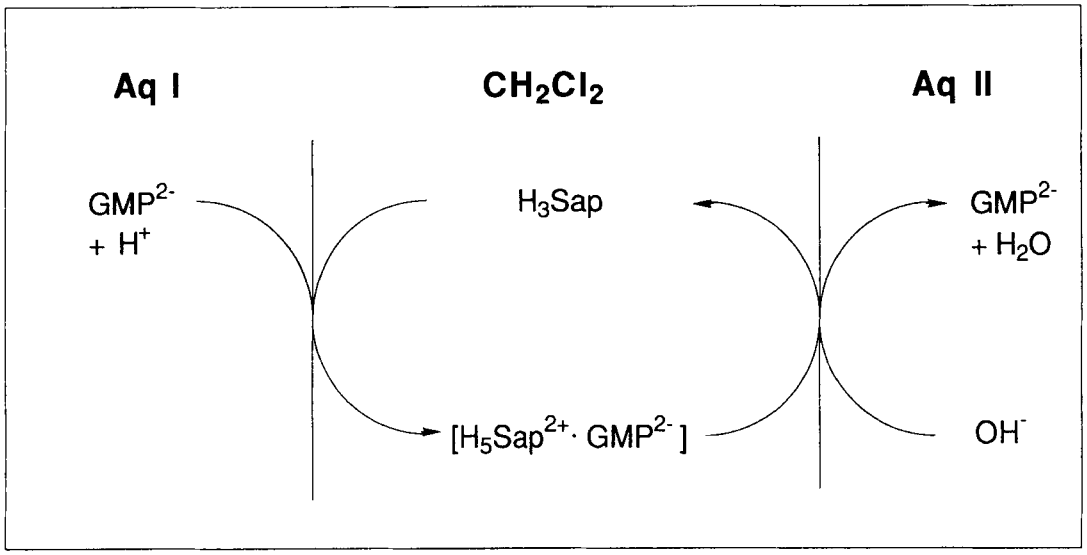


Figure 3 Schematic representation of GMP transport effected under synport conditions using sapphyrin **44** as the hydrophobic phosphate-binding carrier.

Table 1 Guanosine 5'-monophosphate transport effected using actaethylporphyrin and various expanded porphyrins as carriers

		Initial transport rate[a] $\times 10^{-9}$ mol/cm^2 · h		
Carrier	*pH (Aq I)*	*With C-Tips*[b]	*Without C-Tips*	*Reference*
Rubyrin (**48**)[c]	3.0	56.0	0.74	66
	4.0	ND	0.48	66
	5.0	0.80[d]	0.25	66
	6.0	5.95	≪0.01	66
	7.0[e]	0.70	≪0.01[f]	66
	7.2[g]	0.56	ND	66
Sapphyrin (**44**)[c]	3.0	ND	51.9	54
	4.0	ND	1.87	54
	6.0	0.20	≪0.01	66
Octaethylporphyrin (**47**)	2.0	ND	≪0.01	54
	6.0	≪0.01	≪0.01	66, 68

[a] An Aq I—CH_2Cl_2—Aq II (Aq = Aqueous) model membrane system was used for these studies (see text). Initial transport rates were calculated from the linear region of the various (Aq II) concentration vs. time curves (see ref. 53). Estimated errors are ± 15%. [b] 10 mM unless otherwise indicated. [c] 0.1 mM in the organic (CH_2Cl_2) phase. [d] [C-Tips] = 1 mM. [e] 100 mM sodium phosphate buffer. [f] No buffer. [g] 100 mM Tris-HCl buffer. ND, not determined.

rubyrin **48**[65]) might be quite diverse in terms of their phosphate anion binding capability in solution as well as in the solid state. In particular, these solid state results led us to consider that the monoprotonated form of sapphyrin, which bears protons on four of the five pyrrolic nitrogens, and which is the dominant form between *ca.* pH 3.5 and 11,[66] might be capable of interacting with monoanionic phosphate entities at or near neutral pH. To the extent this proved true, it would be expected that sapphyrins, such as **44**, should be very effective for the through-liquid-membrane-transport of c-AMP and other nucleotide entities that remain monoanionic at neutral pH. In addition, it might also be expected that sapphyrin and its congener would display a high affinity for DNA and other phosphate-bearing oligonucleotides. In particular, it is recognized that an expanded porphyrin approach could be used not only to bind to 'normal' DNA but also to achieve delivery of anti-sense DNA drugs.

At present, the available evidence seems consistent with the above promise. For instance, sapphyrin **44** is highly effective as a carrier for c-AMP at neutral pH, serving to effect through-membrane transport at rates exceeding 4 $\times 10^{-8}$ mol/cm^2 · h in our standard[53] Aq I—CH_2Cl_2—Aq II liquid model membrane system (H. Furuta and J. L. Sessler, unpublished results). Furthermore, in preliminary work, it has been found that a sapphyrin–iron EDTA conjugate is highly

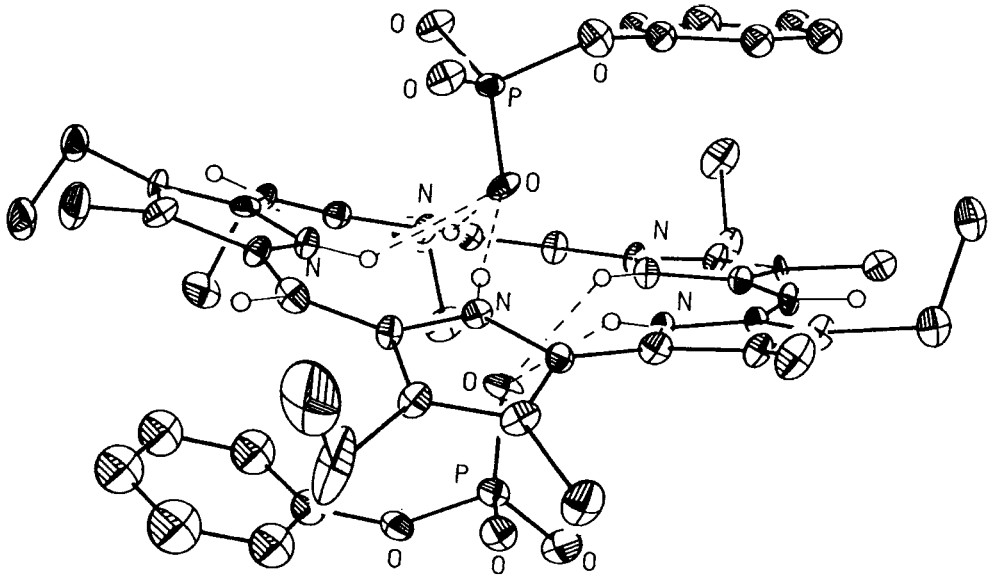


Figure 4 Single crystal X-ray structure of the bis($C_6H_5PO_3H^-$) salt of H_5Sap^{2+} (diprotonated **44**). In addition to the two phosphate anions shown, there is a third, fully protonated phenylphosphate molecule found in the unit cell. It is not involved in chelation to the macrocycle but is involved in hydrogen bonding interactions within the crystal lattice.

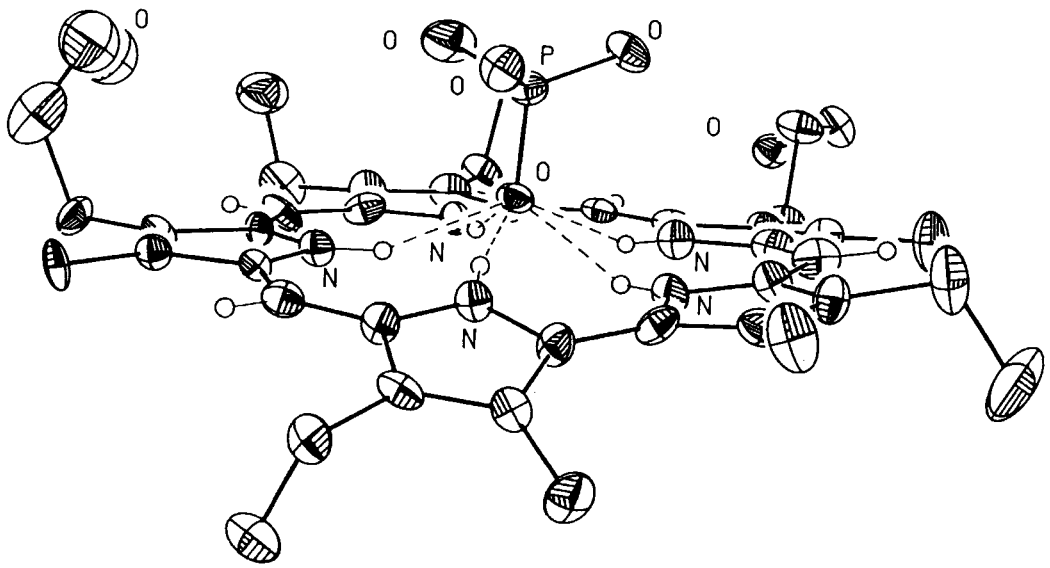


Figure 5 Single crystal X-ray structure of the phosphoric acid salt of diprotonated, bis-hydroxypropyl-substituted sapphyrin **46**. In addition to the monobasic phosphate anion shown, there is a second, monobasic phosphate anion in the unit cell. There is also one molecule of fully protonated phosphoric acid. Neither of these are involved in chelation to the macrocycle. Both, however, are involved in hydrogen bonding interactions within the crystal lattice.

effective for the cleavage of supercoiled DNA (B. Iverson, K. Schreder and J. L. Sessler, unpublished results).

Although encouraging, the above results beg the question, however, of whether or not one might be able to use an expanded porphyrin approach to effect the through-membrane transport of such nominally dianionic entities as GMP and other simple nucleotide phosphate monoesters. Here, of course, it is to be appreciated from the literature the second phosphate-based pKa of GMP (and analogues AMP, UMP, etc.) falls at approximately 6.7.[67] Thus, under normal conditions it is the dianionic (dibasic) form of this mononucleotide that will be the dominant one at or near neutral pH. This means that to achieve effective through-membrane transport of such doubly negative entities, one must either (1) use receptors that remain doubly cationic at neutral pH or (2) find some way

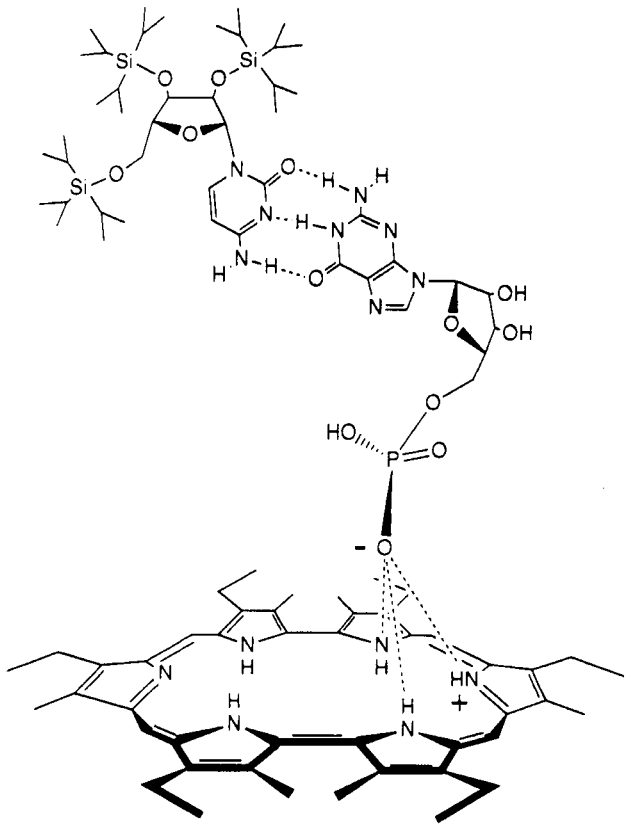


Figure 6 Schematic representation of the possible neutral, ternary transport complex formed from rubyrin **48**, C-Tips, and GMP.

of increasing the effective pKa of GMP and congeners such that the monobasic (−1) forms dominate at neutral pH. In either case, the critical requirement of charge neutralization would be met.* This, in turn, should make effective through-membrane transport possible.

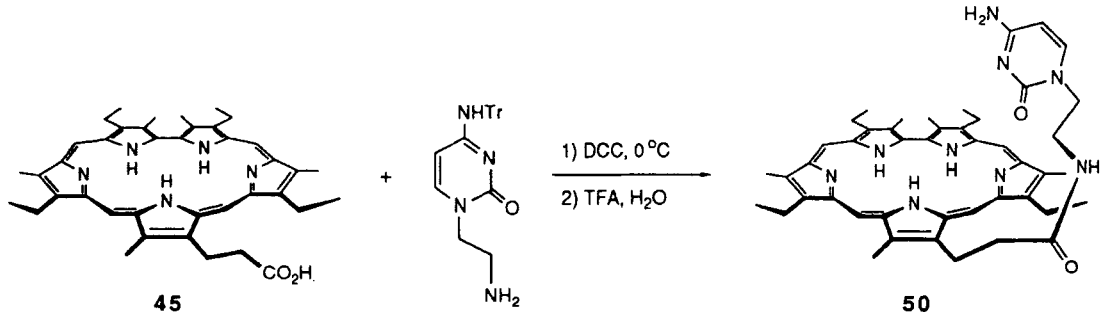
To get started on testing the above ideas, transport experiments were carried out using rubyrin **48** as a potential carrier.[66] Here, the thoughts were that this system, being bigger than sapphyrin, would be more amenable to double protonation (because of reduced electrostatic repulsion) at or near neutral pH. As it turned out, it was found, using our standard H_2O—CH_2Cl_2—H_2O Pressman-type[62] U-tube liquid membrane model system, that this system by itself was not all that effective as a GMP carrier near neutral pH (Table 1).[66] Addition of C-Tips, however, as a co-carrier led to a dramatic increase in the rubyrin-mediated GMP transport rate. In fact, the magnitude of this enhancement proved so significant that efficient transport was observed even at neutral pH (i.e. at a pH value above that associated with the second phosphate-based deprotonation constant of GMP!). In addition, this enhancement effect was found to be completely selective: under the conditions of the experiment (i.e. at neutral pH), only GMP is transported; no through-transport of either CMP or AMP is observed.

Taken together, the above results are considered consistent with the formation of a neutral rubyrin–C-Tips–GMP ternary complex such as that represented schematically in Figure 6. Here, the critical concept being presented is the idea that the formation of a base-pairing stabilized complex such as that depicted would serve to augment the effective concentration of the monobasic form of GMP (i.e. GMP^-) at the aqueous–organic interface. Thus, this Figure (and the results from whence it derives) sets the stage for the development of yet-to-be-improved receptors. In particular, it embodies *inter alia* the suggestion that at the highly effective local nucleobase concentration that would be achieved by tethering directly a nucleobase subunit to an expanded porphyrin, even systems such as sapphyrin **44**, that are otherwise ineffective as carriers at pH 7 (because they are only monoprotonated), could be used to effect the transport of phosphate monoesters: the transport would simply involve the recognition and stabilization of the normally equilibrium-unfavorable monobasic, monoanionic form of GMP or its analogues.

Based on the above considerations, it was considered worthwhile to construct the cytosine-bearing sapphyrin nucleobase conjugate **50** (Scheme I).*,[68] Much to our delight, this new state-of-the-art system was found to be surprisingly effective for the neutral regime, through-membrane transport of GMP. As can be discerned from the data in Table 2, not only does this new system effect the through-membrane transport of GMP at neutral pH, but also it does so with remarkable specificity. In the best of cases, the relative rate enhancements for GMP over CMP and AMP are 100:1 and 11:1, respectively.[68] At neutral pH, these same relative selectivities can be as high as *ca.* 45:1 and 10:1, respectively.[68] To achieve this level of specificity, however, and to provide a high overall flux, a strongly basic receiving phase, Aq II, must be used. Presumably, this latter requirement just reflects the fact that deprotonation of the sapphyrin is necessary for rapid substrate release at Aq II. Nonetheless, it is clear that this system works. The schematic representation given in Figure 8, is designed to show how. As in the case of rubyrin **48** above,[66] the key idea is that appropriate stabilizing interactions allow

*Charge neutralization as a requirement for efficient carrier-mediated transport is known as Fick's first law (see ref 62, p. 78).

*To the best of our knowledge compound **50** is the first example of a nucleic acid derivative ('nucleobase') conjugated to an expanded porphyrin. Such conjugates, however, are known in the porphyrin series; see for instance ref 69, also refs 47, 50, 51 and 52.



Scheme I

Table 2 Initial nucleotide transport rates obtained using conjugate 50 as carrier[68]

Carrier[a]	pH[b] Aq I	Aq II	k_T CMP	k_T GMP (10^{-9} mol/cm^2·h)[c]	k_T AMP	k_G/k_A	k_G/k_C
50	6.15	H_2O	0.1200	12.000	1.5700	7.6	100
50	6.70	H_2O	0.0670	2.870	0.3300	8.7	43
50	7.05	H_2O	0.0005	0.011	0.0010	11.0	22
50	6.15	NaOH[d]	0.5400	14.200	5.2000	2.7	26
50	6.70	NaOH[d]	0.3000	12.300	2.8200	4.4	41
50	7.05	NaOH[d]	0.1600	7.080	0.7400	9.6	44
None	6.15[e]	H_2O	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	ND[f]	ND[f]
44	7.00	H_2O	$<10^{-4}$	$<10^{-4}$	0.0002		
44	7.00	NaOH[d]	$<10^{-4}$	$<10^{-4}$	0.0040		

[a] 0.1 mM in dichloromethane. [b] The source phase, Aq I, contained a 1:1:1 ratio of AMP, CMP and GMP, each at a 10 mM. [c] Transport experiments were performed in a manner similar to those reported in ref 53. Initial transport rates were calculated from the linear region of concentration vs. time curves (cf. supplementary material). Values reported are the average of three independent measurements; estimated error <5%. [d] NaOH concentration was 10 mM. [e] Contained 1:1:1:1 AMP, CMP, GMP, UMP, each at 10 mM. [f] Not determined.

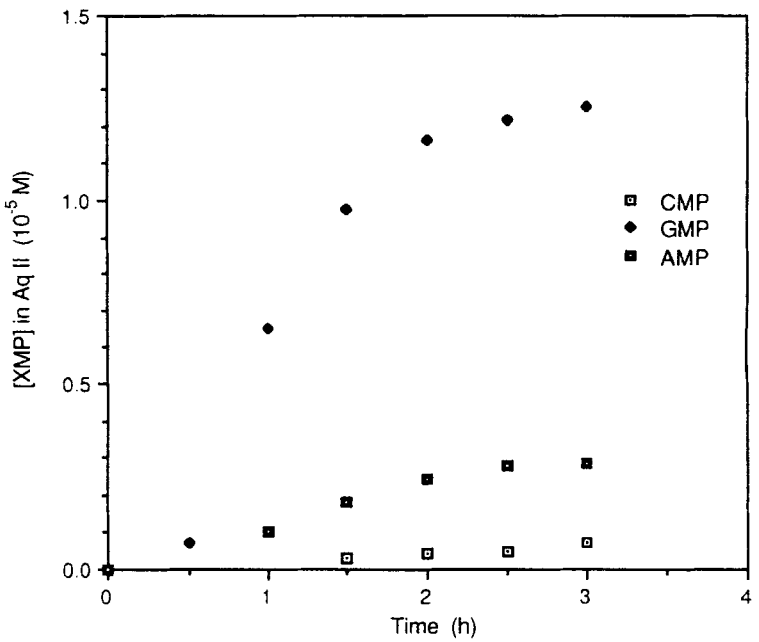


Figure 7 Time course of AMP, CMP, and GMP transport through a liquid dichloromethane membrane effected using carrier **50** (0.1 mM in CH_2Cl_2). Conditions: Aq I, 10 mM (for each) in AMP, CMP, and GMP; pH 7.05. Receiving phase (Aq II): 10 mM NaOH.

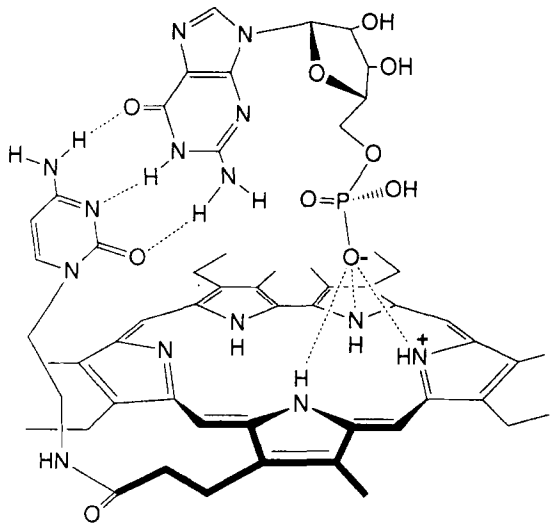


Figure 8 Possible structure for the proposed supramolecular complex formed between conjugate **50** and monobasic GMP.

the monobasic form of GMP (GMP^-) to be recognized and transported in an overall neutral way.

To the best of our knowledge, compound **50** is the first carrier complex to be reported that is able to effect the selective through-membrane transport of a given, i.e. chosen, nucleotide at neutral pH. It therefore not only meets the stringent design requirements embodied in the introductory sections of this paper, but also provides a prototype for the design of related, equally sophisticated, receptor systems. Thus, current work is focused both on the biological testing of system **50** (as an *in vitro* carrier for nucleotide drug delivery) and on the synthesis of related systems wherein expanded porphyrins are appended to other nucleobase derivatives such as, e.g. those derived from adenine or guanine. The results of these studies will be reported in due course.

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