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COMMUNICATION

Protonated rubyrin and C-Tips: co-carriers for the transport of guanosine 5′-monophosphate at neutral pH

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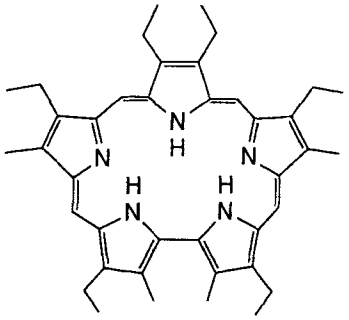
At neutral and near-neutral pH, rubyrin, a readily protonated, hexapyrrolic expanded porphyrin, and triisopropylsilyl-protected cytidine (C-Tips) act cooperatively to effect the efficient through-transport of guanosine 5′-monophosphate (GMP) in a model three-phase H_2O-CH_2Cl_2-H_2O bulk liquid membrane system.

Recently, we have become intrigued by the challenge of constructing molecular recognition based carriers for the through-membrane transport of nucleoside and nucleotide analogues.[1–3] Much of the impetus for this work derives from a desire to produce adjuvants for antiviral drug delivery. In particular, it is appreciated that carriers capable of enhancing the into-cell uptake of normally organic insoluble nucleotide drugs could be used to augment the clinical efficacy of agents, such as the anti-herpetic 9-(β-D-xylofuranosyl)guanine-5′-monophosphate (Xylo-GMP), that show promising antiviral activity *in vitro* but which are often inactive *in vivo*.[4] Such carriers could thus play an important role in the clinical practice of antiviral chemotherapy. Unfortunately, however, the design and performance criteria for such putative carriers are relatively severe: They would, for instance, be required to have proper lipophilicity (membrane solubility), show desirable selectivity (between, e.g., normal nucleotides and drugs), and demonstrate high transport enhancing activity at physiological pH. Not surprisingly, therefore no such carriers currently exist.

Previously,[2] we found that lipophilic, triisopropylsilyl (Tips)-protected, *nucleosides* (e.g., C-Tips, **3**), would act to enhance the transport of its corresponding complementary Watson-Crick nucleoside (i.e., guanosine, 4) at neutral pH in an Aq. I-CH_2Cl_2-Aq. II model membrane system. We also found[3] that certain expanded porphyrins, such as sapphyrin (**1**)[6] and its higher homolog rubyrin (**2**),[7] served as very efficient carriers for the through-CH_2Cl_2 liquid membrane



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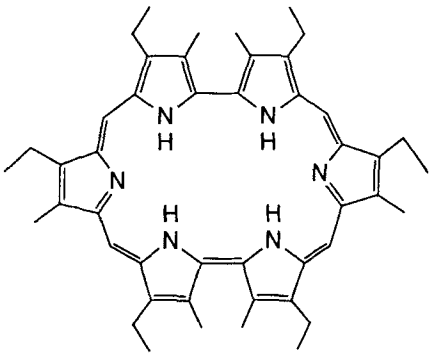

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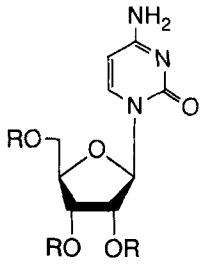

3. R = $Si(CH(CH_3)_2)_3$

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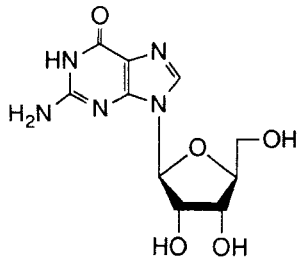

4. R = H
5. R = PO_3^{2-}

transport of phosphate-containing nucleotides, such as guanosine 5'-monophosphate (GMP, **5**), under acidic conditions (i.e., at pH < 4). Unfortunately, however, with these latter systems, little or no transport was observed at higher, more-neutral pH. Thus, the achievement of this critical goal (*viz.* nucleotide recognition and transport at phydsiologic pH) was left as an unsolved problem within the context of this approach to be a simple, but general, co-carrier based solution to this challenge.

We began the present study by investigating in greater detail the pH dependence of sapphyrin- and rubyrin-mediated transport. Here, we made use of our now-standard[2,3] 3-phase, Aq. I-CH_2Cl_2.Aq. II, Pressman-type model membrane system and chose to look at a substrate, *p*-toluenesulfonic acid (*p*-TsOH), that was expected to be far less complex in its ionization and transport characteristics than GMP (**5**) and its analogues. By looking at the pH dynamics of transport using this anion, we sought to derive a set of effective "transport pKa's" for these two expanded porphyrins. Typical results, determined in the case of sapphyrin **1**, are shown in Figure 1. These give values of 2.5 and 11 for the first and second pKa's of sapphyrin, respectively. Likewise, the first and second transport pKa's of rubyrin were found to be 3.7 and 10, respectively.

The larger effective first transport pKa for rubyrin is considered to be a reflection of the weaker electrostatic charge repulsion present within the core of this larger expanded porphyrin.[8] In both cases, however, the observed pKa's suggest that these two macrocycles, sapphyrin and rubyrin, in contrast to, e.g., octaethylporphyrin,[3] exist predominantly in their monoprotonated, singly charged (+1) forms at pH 7. By contrast, all the common acyclic nucleotide monophosphates are completely (or nearly completely) dissociated at neutral pH.[9] They thus exist pre-dominantly in their doubly charged (−2), dianionic forms.

The above considerations suggest that there is an inherent difficulty associated with using expanded porphyrins, such as **1** and **2**, to effect enhanced through-membrane transport of acyclic nucleotides at neutral pH: All the common acyclic nucleotide monophosphates are largely dissociated at neutral pH and, therefore, exist predominantly in their doubly charged (−2), dianionic forms.[9] Thus, in any putative 1:1 nucleotide-expanded porphyrin complex, the charge on the carrier and the substrate would simply not be the same. Thus, the requisite partitioning of the supramolecular macrocycle-nucleotide complex into

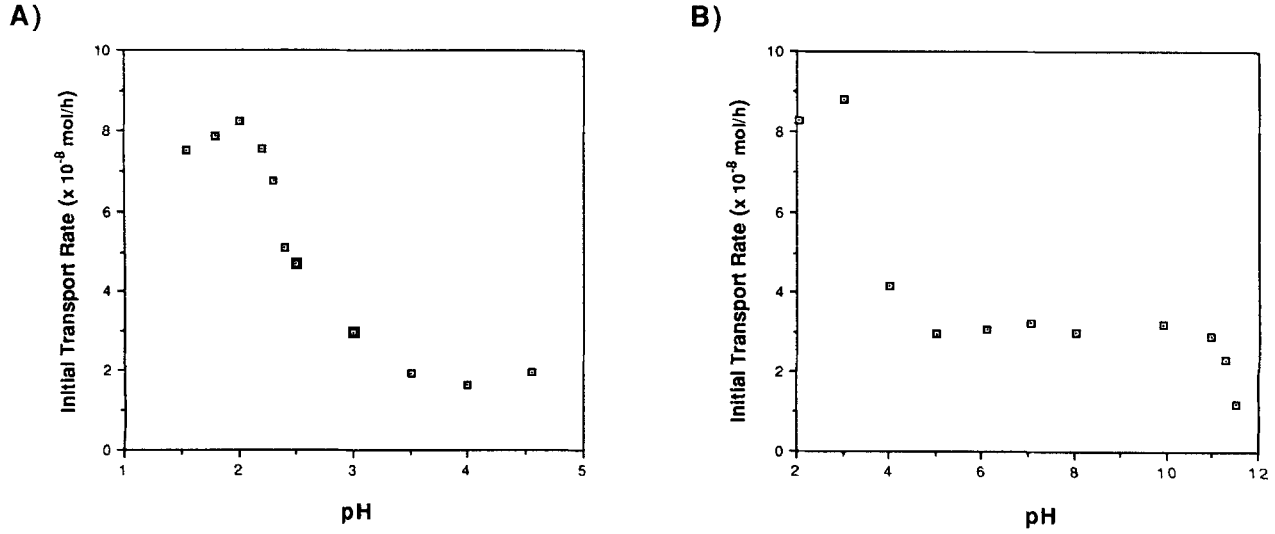

Figure 1 The effect of pH on the rate of through-membrane transport of *p*-toluenesulfonic acid effected by sapphyrin **1**. the system used was the Aq. I-CH_2Cl_2-Aq. II (Aq. = Aqueous) model membrane system described in references 2 and 3 of the text. Frame A. Transport condition: Aq. I: 50 mM *p*-toluenesulfonic acid, indicated initial pH obtained by the addition of NaOh; liquid membrane: CH_2Cl_2, 10^{-4} M in sapphyrin; Aq. II: pH 10 (NaOH). The initial transport rates plotted were calculated from the linear region of the various concentration vs. time curves. Error is within ±15%. Frame B. Same as Frame A but using a sapphyrin concentration of 4×10^{-4} M in the CH_2Cl_2 liquid membrane.

Table 1

Carrier	pH (*Aq. I*)	*Initial transport rate* 10^{-9} mol/(cm²·h)[a] *with C-Tips*[b]	*without C-Tips*	*Ref.*
rubyrin (**2**)[c]	3.0	56.0	0.74	this work
	4.0	ND[d]	0.48	this work
	5.0	0.80[e]	0.25	this work
	6.0	5.95	≪0.01	this work
	7.0[f]	0.70	≪0.01	this work
	7.2[g]	0.56	ND	this work
sapphyrin (**1**)	4.0	ND	1.87	3
	6.0	0.20	≪0.01	this work

[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. 3). Estimated errors are ±15%.
[b] 10 mM unless otherwise indicated.
[c] 0.1 mM in the organic (CH_2Cl_2) phase.
[d] "ND" indicates value was not determined or could not be measured.
[e] [C-Tips] = 1 mM.
[f] 100 mM sodium phosphate buffer.
[g] No buffer.
[h] 100 mM Tris-HCl buffer.

the organic, membrane phase would be precluded and/or severely restricted.

Our previous success with *nucleoside* transport at neutral pH,[2] however led us to consider that we might be able to overcome this seemingly insurmountable electrostatic mismatch difficulty. Specifically, we considered that the combination of an expanded porphyrin and an organic-soluble nucleic acid base ("nucleobase"), *together as joint co-carriers*, would allow for the specific and effective through-membrane transport of nucleotide monophosphate entities at neutral pH. Here, the key idea is that the expanded porphyrin would serve to effect phosphate neutralization whereas the added nucleobase would base-pair with the nucleic acid portion of the nucleotide thus "tying it up" so that deleterious hydrogen bonding interactions with water would be reduced.

To test the validity of the above hypothesis, we chose to investigate the effect of added C-Tips (**3**) on the rate of rubyrin-mediated GMP transport. In the absence of added C-Tips "cofactor", the rate of rubyrin-mediated GMP transport is not substantial at pH > 5 (Table 1). In fact, even at pH < 5, rubyrin, **2**, proved only moderately effective as a carrier. In the presence of C-Tips, however, substantial rubyrin-mediated rate enhancements were observed (Table 1). In fact, in the presence of C-Tips, rubyrin **2** was found to be an effective carrier even at neutral pH. A modest C-Tips enhancement effect was also seen in the case of sapphyrin-mediated transport. However, in this case the effect was not so large as in the case of rubyrin (Table 1).

A variety of control experiments, effected using different triisopropylsilyl-protected nucleosides (adenosine-, uridine, and guanosine-Tips), were also carried out. In no cases was evidence obtained that indicated any kind of enhancement affect (initial rate $\ll 0.001 \times 10^{-9}$ mol/cm²·h). Moreover, as reported earlier,[2] C-Tips, by itself, was found to be completely ineffective as a carrier for GMP at neutral pH.

Taken together, therefore, the present experimental results are considered as being consistent with a cooperative, supramolecular effect involving locus-specific recognition of GMP by both C-Tips and monoprotonated rubyrin. Such dual recognition could serve to make organic soluble an overall monoanionic, $[(\text{C-Tips})\text{-}(\text{rubyrin})^{+}\text{-}(\text{GMP})^{2-}]^{-}$, ternary complex. Or, as shown schematically in Figure 2, it may provide a driving force sufficient to favor the formation of an overall, organic soluble, neutral, $[(\text{C-Tips})\text{-}(\text{rubyrin})^{+}\text{-}(\text{GMP})^{-}]$, ternary complex in the membrane phase. Such neutral complex formation, presumbly, would be accomplished by shifting the effective pKa_2 of GMP at the Aq. I-CH_2Cl_2 interface so as to make the mono-basic form less acidic. Although the data do not allow a strict distinction between these two limiting mechanistic possibilities, we currently favour the latter.

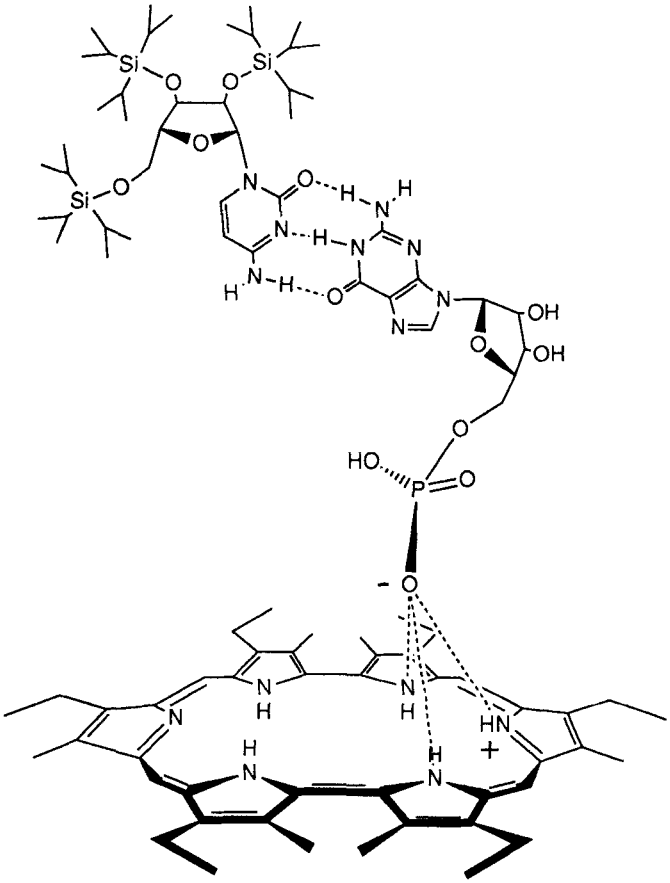

Figure 2 A schematic representation of the possible neutral, ternary transport complex formed from rubyrin, C-Tips, and GMP. For further explanation, see text.

It best accounts for the observed drop off in transport rate observed as a function of increasing pH.

In conclusion, we have observed GMP transport at near physiological pH using an appropriate combination of phosphate and nucleobase recognition units. These results, this provide experimental support for the logical prediction[10] that appropriately designed synthetic expanded porphyrin-nucleobase conjugates could serve to bind and transport mononucleotides efficiently under neutral pH, *in vivo* conditions. We are currently exploring this possibility.[11]

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