Improved transport of nucleotide monophosphates by lipophilic phosphonium–nucleobase conjugates

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Lipophilic phosphonium–nucleobase conjugates 3 and 5 showed improved transport of AMP and GMP in comparison with lipophilic phosphonium salts, lipophilic complementary nucleobases, or a joint co-carrier system consisting of a lipophilic phosphonium salt and a nucleobase.

Nucleotide analogues which have potential antiviral activity *in vitro* cannot penetrate across lipophilic cell membranes due to their highly charged and hydrophilic nature.[1] Therefore, there has been increasing effort towards developing phosphate-binding receptors.[2] However, there are a few artificial carrier systems known which are capable of transporting phosphate-bearing nucleotides through organic liquid membranes.[3] Recently, lipophilic phosphonium salts were developed as carriers for 5′-AMP (AMP) and 5′-GMP (GMP) in our laboratory.[4] They showed better transport rates for AMP and GMP compared with transport by the structurally similar, lipophilic trioctylmethylammonium chloride.[3c,5] In addition, moderate rate enhancements were observed when a phosphonium salt and a lipophilic nucleoside were used together as joint co-carriers. We expected that lipophilic phosphonium salts connected covalently to a complementary nucleobase would display better transport rates for AMP and GMP in comparison with a joint co-carrier system. Here we report improved extraction and transport of AMP and GMP by lipophilic phosphonium–nucleobase conjugates **3** and **5**.†

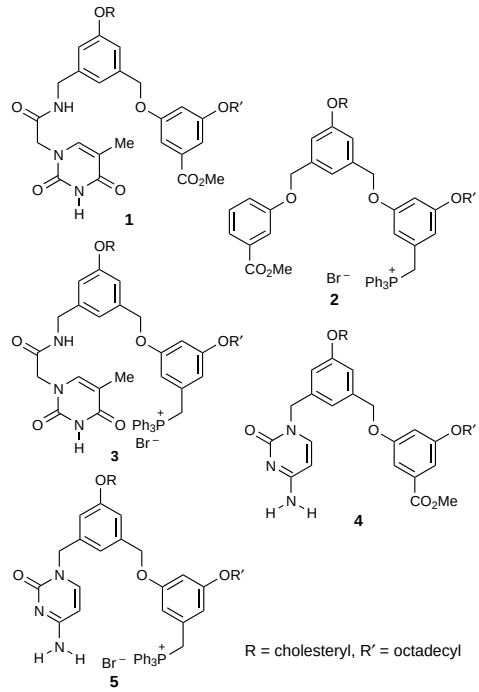


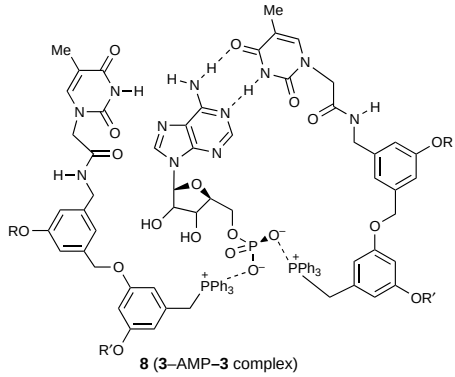
Table 1 shows extraction and transport data for AMP or GMP by lipophilic complementary nucleobases (**1** and **4**), a phosphonium salt (**2**) and phosphonium–nucleobase conjugates (**3** and **5**). There exist qualitative correlations between the extraction and the transport data. AMP (or GMP) is dissociated mostly into its monoanion (AMP^- or GMP^-) at pH 5, while only one third of AMP (or GMP) exists as AMP^- or GMP^- at pH 7. As shown in Table 1, the phosphonium carrier (**2**) exhibits a better transport rate for AMP and GMP at pH 5 than at pH 7 for entropic reasons.[4]

The phosphonium carrier is expected to mediate more efficient through-membrane transport of AMP or GMP with the aid of base-pairing in either an inter- or intra-molecular fashion.[3e,h,i,6] Previously we used an organic-soluble nucleobase as a co-carrier to improve through-membrane transport of nucleotide monophosphates by a phosphonium cation.[4]‡ Either at pH 5 or 7, in the presence of lipophilic uridine or cytidine as a co-carrier, moderate rate enhancements were observed in the case of AMP or GMP transport, respectively. To make more effective and selective carriers for AMP or GMP compared to the joint co-carrier system would require the construction of phosphonium–nucleobase conjugates, in which an adenine or guanine recognition unit is appended directly onto the phosphate-binding phosphonium centre. Compounds **3** and **5** extract and transport AMP or GMP, respectively, more efficiently than **2**, presumably because of base-pairing of the complementary nucleobase moiety of **3** or **5** with the nucleobase of AMP or GMP. In fact, the thymine (or cytosine)-bearing phosphonium carrier **3** (or **5**) was found to be much more effective for AMP (or GMP) transport, either at pH 5 or 7. As shown in Table 1, the receptor **3** displayed a higher transport rate for AMP (by a factor of 39 at pH 5 and 157 at pH 7) than the thymine-free phosphonium salt **2**. In the case of GMP transport, the cytosine-substituted phosphonium receptor **5** showed a similar rate enhancement (by a factor of 66 at pH 5 and 103 at pH 7) in comparison with the cytosine-free phosphonium salt **2**. However, control experiments (**5**/AMP and **3**/GMP) employing noncomplementary nucleobase–phosphonium conjugates led to decreased transport of AMP and GMP, respectively, compared to the complementary nucleobase–phosphonium carrier systems (**3**/AMP and **5**/GMP). This clearly shows that selective

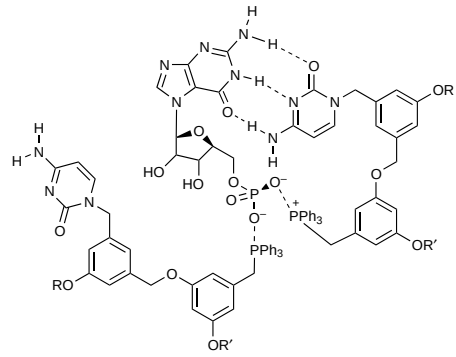
Table 1 Extraction and transport of AMP and GMP by **1**–**5**

		Extraction (%)[a]		Transport rate/ 10^{-9} mol h^{-1} cm^{-2}[b]	
Carrier	Guest	pH 5.0	pH 7.0	pH 5.0	pH 7.0
1	AMP	[c]	[c]	[c]	[c]
2	AMP	7.4	6.3	0.14	0.03
3	AMP	10	9	5.4	4.7
5	AMP	12	7	0.5[d]	0.2
4	GMP	6	2	[c]	[c]
2	GMP	13	8	0.08	0.04
5	GMP	33	33	5.3	4.1
3	GMP	13	15	[c]	0.06

[a] [Carrier] = 1×10^{-2} M in $CHCl_3$, [Guest] = 1×10^{-4} M in deionized water. [b] Source phase: [AMP] (or [GMP]) = 0.05 M in H_2O. Receiving phase: [NaBr] = 0.025 M in H_2O. Organic phase: [carrier] = 0.001 M in $CHCl_3$. [c] Not detected. [d] No transport observed after 24 h.



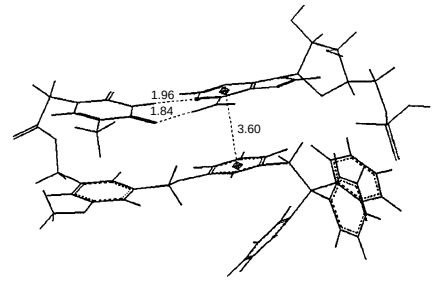
8 (**3**–AMP–**3** complex)



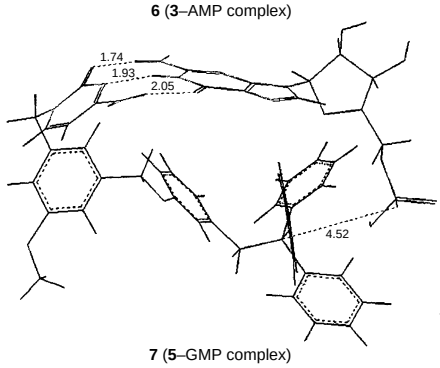
9 (**5**–GMP–**5** complex)

R = cholesteryl, R′ = octadecyl

Fig. 1 Possible structures for the transport complexes **8** and **9** at pH 7



6 (**3**–AMP complex)



7 (**5**–GMP complex)

Fig. 2 Energy-minimized structures of the possible transport complexes **6** and **7** at pH 5 (DISCOVER 95.0 with CVFF force field)

base pairing contributes to increased transport. The fact that a phosphonium-free nucleobase **1** or **4** doesn't transport AMP or GMP at all indicates the importance of the phosphate group solubilization in $CHCl_3$ for transport of nucleotide monophosphates.

A concentration-dependent extraction study supports possible structures for the neutral supramolecular transport complexes between the phosphonium–nucleobase conjugate carriers (**3** or **5**) and nucleotide monophosphates, as depicted in Figs. 1 and 2. When aqueous solutions containing varying amounts of AMP (or GMP) were shaken with a chloroform solution of **3** or **5** (1.0×10^{-5} M), the maximum extractability of AMP (or GMP) was 105% (or 119%) at pH 5 and 54% (or 49%) at pH 7, respectively. This result supports the suggestion that **3** (or **5**) undergoes formation of a 1 : 1 complex (**3**–AMP or **5**–GMP) at pH 5 (Fig. 2) and a 2 : 1 complex (**3**–AMP–**3** or **5**–GMP–**5**) at pH 7 in the organic phase (Fig. 1). Since formation of the bimolecular complex **6** (or **7**) between **3** (or **5**) and the monobasic forms of AMP (or GMP) is entropically more favourable than that of the termolecular complex **8** (or **9**) between two molecules of **3** (or **5**) and the dibasic form of AMP (or GMP), **3** (or **5**) transports either AMP or GMP slightly more efficiently at pH 5 than at pH 7.

Computer-generated structures§ of the possible transport complexes **6** and **7** at pH 5 are shown in Fig. 2. While in the case of **6** hydrogen-bonding (base-pairing), electrostatic interactions, and aromatic stacking interactions are clearly visible, hydrogen-bonding and electrostatic interactions are shown to be operative in the case of **7**.

In summary, we have showed that the transport of normally organic-insoluble AMP or GMP can be improved by using appropriately designed lipophilic nucleobase-substituted phosphonium carriers.

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Footnotes

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† All new compounds gave satisfactory spectroscopic and analytical data.

‡ A joint co-carrier system showed a better transport rate than the phosphonium carrier, by a factor of 2–3, when an equimolar amount of the phosphonium salt and the nucleobase was used. Increasing the concentration of the co-carrier (nucleobase) led to better transport of the corresponding nucleotide monophosphate (ref. 4).

§ The energy-minimization (CVFF force field) with conjugate gradient algorithm was performed with DISCOVER 95.0 of MSI (1995) on a Silicon Graphics INDY workstation.

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