

Improved transport of nucleotide monophosphates by lipophilic phosphonium–nucleobase conjugates

Yong-Gyu Jung, Woon-Seok Yeo, Sang Bok Lee and Jong-In Hong*

Department of Chemistry, Seoul National University, Seoul 151-742, Korea

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 triethylmethylammonium 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**.[†]

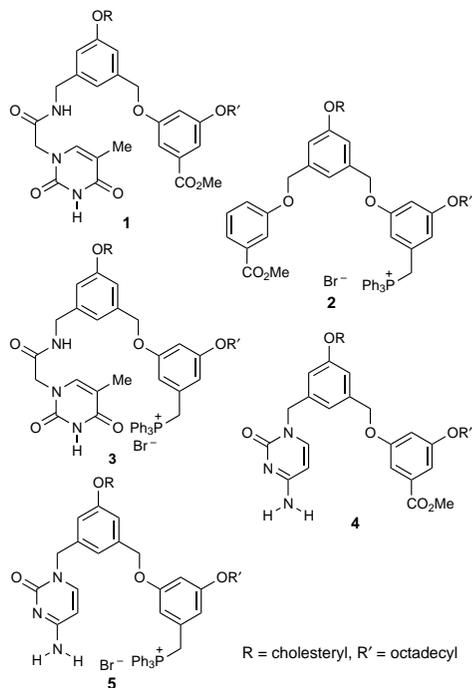


Table 1 shows extraction and transport data for AMP or GMP by lipophilic complementary nucleobases (**1** and **4**), a phosphonium salt (**2**) and phosphonium–nucleobase conjugates (**3** and **5**). There exist qualitative correlations between the

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**

| Carrier | Guest | Extraction (%) ^a | | Transport rate/ 10 ⁻⁹ mol h ⁻¹ cm ^{-2b} | |
|----------|-------|-----------------------------|----------|---|----------|
| | | pH 5.0 | pH 7.0 | pH 5.0 | pH 7.0 |
| 1 | AMP | <i>c</i> | <i>c</i> | <i>c</i> | <i>c</i> |
| 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 | <i>c</i> | <i>c</i> |
| 2 | GMP | 13 | 8 | 0.08 | 0.04 |
| 5 | GMP | 33 | 33 | 5.3 | 4.1 |
| 3 | GMP | 13 | 15 | <i>c</i> | 0.06 |

^a [Carrier] = 1 × 10⁻² M in CHCl₃, [Guest] = 1 × 10⁻⁴ M in deionized water. ^b Source phase: [AMP] (or [GMP]) = 0.05 M in H₂O. Receiving phase: [NaBr] = 0.025 M in H₂O. Organic phase: [carrier] = 0.001 M in CHCl₃. ^c Not detected. ^d No transport observed after 24 h.

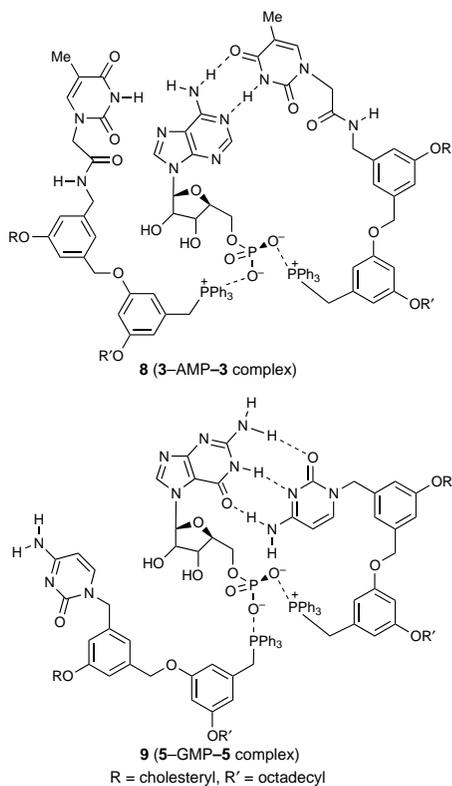


Fig. 1 Possible structures for the transport complexes **8** and **9** at pH 7

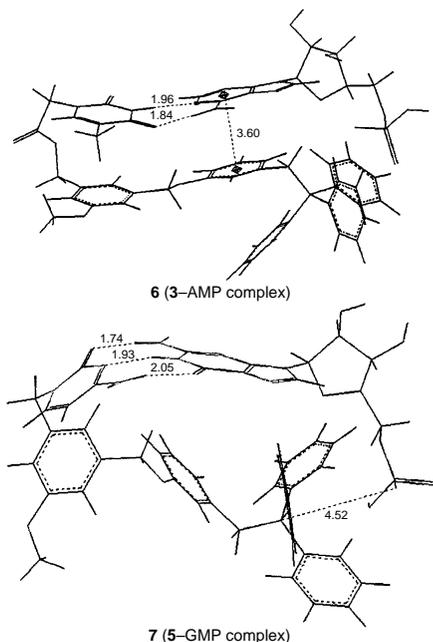


Fig. 2 Energy-minimized structures of the possible transport complexes **6** and **7** at pH 5 (DISCOVER 95.0 with CVFF force field)

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-3** or **5-GMP-5**) 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 \S 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.

We thank Ministry of Education (BSRI-96-3416), OCRC, and KOSEF (Grant No. 961-0302-008-2) for financial support.

Footnotes

* E-mail: jihong@plaza.snu.ac.kr

† 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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Received in Cambridge, UK, 23rd January 1997; Com. 7/00527J