Lipophilic Phosphonium and Cytidine as Co-carriers for the Transport of Guanosine 5′-Monophosphate at Neutral pH

J. Chem. Research (S), 1998, 304–305
J. Chem. Research (M), 1998, 1290–1296

Sang Bok Lee, Hyunah Choo and Jong-In Hong*

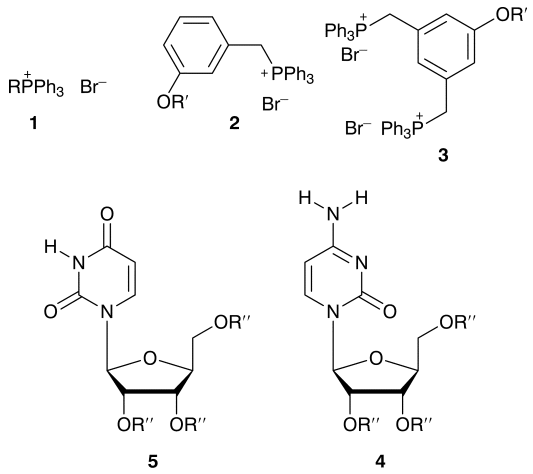
Department of Chemistry, Seoul National University, Seoul 151-742, Korea

The efficient through-membrane transport of GMP at neutral pH in a model three-phase H_2O–$CHCl_3$–H_2O bulk liquid membrane system was achieved when a lipophilic phosphonium and *tert*-butyldimethylsilyl (TBDMS)-protected cytidine were used together as joint co-carriers.

A large number of phosphorylated nucleotide analogues that have been shown to exhibit potential antiviral activity in cell-free extracts are inactive *in vivo* owing to their inability to penetrate lipophilic cell membranes.[1] Therefore, there has been increasing effort to construct receptor-based carriers.[2] However, there are a few artificial carrier systems known which are capable of transporting phosphate-bearing nucleotides through organic liquid membranes.[3] Simple lipophilic ammonium ions bearing long hydrocarbon chains allow phosphate anion extraction into an organic phase and render liquid membranes permeable to the anion by an anion exchange process.[3a–c,f,g] However, structurally similar, lipophilic phosphonium ions have seldom been adopted as anion carriers.[3k,4] In this paper we wish to report efficient extraction and transport of 5′-GMP (guanosine 5′-monophosphate) at neutral pH by a combination of a triphenylalkyl phosphonium cation (**2** or **3**) and lipophilic, TBDMS-protected cytidine (**4**) as a co-carrier.

Transport experiments were performed using the standard U tube methodology.[3d,5] The transport rate was monitored at 260 nm by the initial appearance of GMP in the receiving phase. In comparison with trioctylmethylammonium chloride ($TOMA^+Cl^-$),[3c,6] **1** (Fig. 1) shows a relatively better extractability and transport rate for simple organic anions (carboxylate, sulfonate and phenolate) and highly hydrophilic nucleotide monophosphates (AMP and GMP), which is presumably owing to the more lipophilic character of the phosphonium salt. Comparing a series of organic anions having similar molecular sizes [*p*-$CH_3C_6H_4SO_3^-$, *p*-$O_2NC_6H_4COO^-$, *o*,*o*′,*p*-$(O_2N)_3C_6H_2O^-$], the anion carrier **1** exhibited a moderate transport selectivity: carboxylate > sulfonate > picrate. This transport trend of **1** is similar to that of a TOMA phase-transfer reagent.

The monophosphonium salt **2** and the diphosphonium salt **3** were synthesized to test the possibility of the selective transport of the monobasic or dibasic form of GMP. There exist qualitative correlations between the extraction and the transport data, as shown in Table 1. GMP is dissociated mostly into its monoanionic form (GMP^-) at pH 5, while it exists predominantly in its doubly charged, dianionic, form at neutral pH. A concentration-dependent extraction study has provided valuable information on the mode of interaction between the monophosphonium carier **2** (or diphosphonium carrier **3**) and GMP. When aqueous solutions containing varying amounts of GMP were shaken with a chloroform solution of **2** (1.0×10^{-5} mol dm^{-3}), the maximum extractability of GMP was 92% at pH 5 and 55% at pH 7. This result supports the assertion that **2** undergoes formation of a 1:1 carrier **2**-GMP complex (**6**) at pH 5 and a 2:1 carrier **2**-GMP complex (**7**) at pH 7 in the organic phase (Fig. 2). Similar concentration-dependent extraction studies in the case of the diphosphonium carrier **3** supports the formation of a 1:2 carrier **3**-GMP complex (**8**) at pH 5 and a 1:1 carrier **3**-GMP complex (**9**) at pH 7 in the organic phase (Fig. 2). Since formation of the bimolecular complex **6** between **2** and the monobasic form of GMP is entropically more favourable than formation of the termolecular complex **7** between two molecules of **2** and the dibasic form of GMP, **2** transports GMP more efficiently at pH 5 than at pH 7. Carrier **3** extracts and transports more efficiently than **2**, presumably because of the higher effective molarity of the phosphonium ion in **3**. Especially, the transport rate for GMP by **3** at pH 7 is better than that by **2** for entropic reasons: formation of the bimolecular complex **9** is entropically more favourable than formation of the termolecular complex **7**.



R = octadecyl, R′ = dodecyl, R″ = TBDMS

Fig. 1 Components for the transport of guanosine 5′-monophosphate

Table 1 Extraction and transport of GMP by synthetic carriers **2** and **3** and co-carriers **4** and **5**

Carrier	Extraction (%)[a]		Transport rate[b]/ 10^{-10} mol h^{-1} cm^{-2}	
	pH 5.0	pH 7.0	pH 5.0	pH 7.0
2	7	4	0.72	0.40
2+**4**	38	25	13.5	5.5
2+**5**	10	5	1.50	0.73
3	10	8	0.96	0.92
3+**4**	45	41	15.0	25.7
3+**5**	15	10	1.55	1.80

[a] **2** or **3** 0.010 mol dm^{-3}/$CHCl_3$ (5 ml) GMP 1.0×10^{-4} mol dm^{-3}/H_2O, pH 5.0 and 7.0. [b] Source phase: GMP 1.0×10^{-1} mol dm^{-3}/H_2O (the specified pH values were adjusted by addition of a small amount of HCl or NaOH solution). Receiving phase: NaBr 2.5×10^{-2} mol dm^{-3}/H_2O (4 ml) (pH 5.0 and 7.0). Organic phase: **2** or **3** 2.5×10^{-3} mol dm^{-3}/$CHCl_3$ (8 ml); [**4**] (or [**5**]) = 1.25×10^{-1} mol dm^{-3}.

*To receive any correspondence (*e-mail:* jihong@plaza.snu.ac.kr).

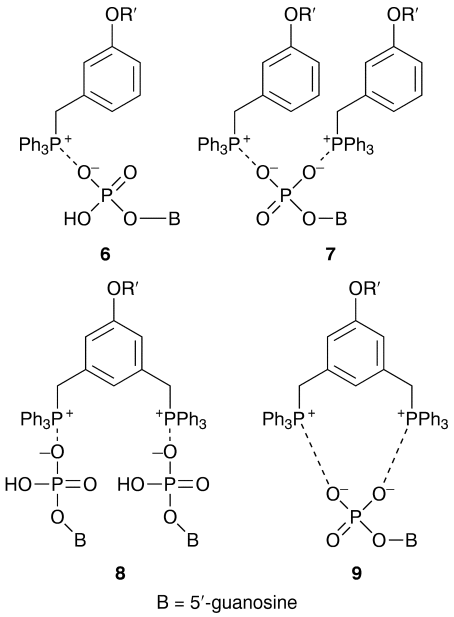


Fig. 2 Proposed structures for the transport complexes (**6**–**9**)

Our system is expected to mediate more efficient through-membrane transport of GMP with the aid of base-pairing in either inter- or intra-molecular fashion.[3e,h,i,7] (A lipophilic cytidine-substituted phosphonium carrier showed improved transport of GMP in comparison with the joint co-carrier system.[3k]) To effect better through-membrane transport of nucleotide monophosphates by a phosphonium cation, an organic-soluble nucleobase was used as a co-carrier.[7] Phosphonium cations (**2** and **3**) and lipophilic cytidine (**4**) function as a cooperative binary carrier system[7] in which the nucleoside is expected to undergo novel hydrogen bonding with the nucleobase portion of GMP as shown in Fig. 3.

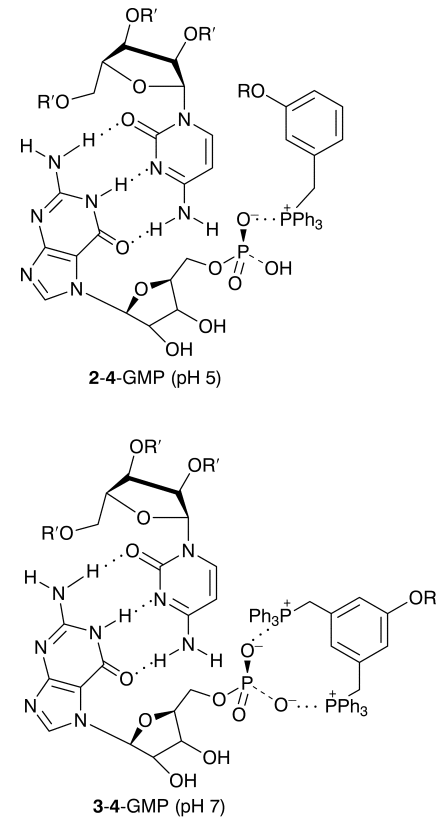


Fig. 3 Plausible ternary transport complexes formed from **2** (or **3**), **4** and GMP

In fact, at either pH 5 or pH 7, in the presence of lipophilic cytidine (**4**) as a co-carrier, moderate rate and extractability enhancements were observed as expected (Table 1). 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 led to better GMP transport as can be seen from Table 1. The combination of **2** and **4** displays a better transport rate at pH 5 than at neutral pH. In contrast, in the presence of **4**, **3** was found to be a more effective carrier at neutral pH than at pH 5, presumably owing to the entropically more favourable termolecular complex formation at neutral pH, as shown in Fig. 3. However, a control experiment employing a noncomplementary nucleobase (TBDMS-protected uridine, **5**) and a phosphonium salt (**2** or **3**) led to decreased transport of GMP compared with the complementary nucleobase–phosphonium joint co-carrier system. This indicates that selective base pairing contributes to increased transport. A lipophilic cytidine itself is not able to transport GMP, which shows the importance of phosphate anion solubilization by a phosphonium cation in $CHCl_3$ for GMP transport.[3k]

In conclusion, we have demonstrated that the efficient transport of a normally organic-insoluble GMP at neutral pH can be achieved by using appropriately designed lipophilic cytidine–phosphonium joint co-carriers.[7]

We thank OCRC and KOSEF (Grant No. 961-0302-008-2) for financial support.

Techniques used: ^1H and ^{13}C NMR, MS

References: 8

Received, 29th July 1997; Accepted, 5th January 1998
Paper E/7/05484J

References

1 *Nucleotide Analogues as Antiviral Agents*, ed. J. C. Martin, American Chemical Society, Washington, D.C., 1989.
2 M. W. Hosseini, A. J. Blacker and J.-M. Lehn, *J. Am. Chem. Soc.*, 1990, **112**, 3896; I. Tabushi, J.-i. Imuta, N. Seko and Y. Kobuke, *J. Am. Chem. Soc.*, 1978, **100**, 6287; B. L. Iverson, K. Shreder, V. Kral and J. L. Sessler, *J. Am. Chem. Soc.*, 1993, **115**, 11022; A. Galan, E. Pueyo, A. Salmeron and J. de Mendoza, *Tetrahedron Lett.*, 1991, **32**, 1827.
3 (*a*) I. Tabushi, Y. Kobuke and J.-i. Imuta, *J. Am. Chem. Soc.*, 1980, **102**, 1744; (*b*) I. Tabushi, Y. Kobuke and J.-i. Imuta, *J. Am. Chem. Soc.*, 1981, **103**, 6152; (*c*) K. Maruyama, H. Tsukube and T. Araki, *J. Am. Chem. Soc.*, 1982, **104**, 3197; (*d*) H. Furuta, M. J. Cyr and J. L. Sessler, *J. Am. Chem. Soc.*, 1991, **113**, 6677; (*e*) V. Kral, J. L. Sessler and H. Furuta, *J. Am. Chem. Soc.*, 1992, **114**, 8704; (*f*) F. Diederich and T. Li, *J. Org. Chem.*, 1992, **57**, 3449; (*g*) T. Li, S. J. Krasne, B. Persson, H. R. Kaback and F. Diederich, *J. Org. Chem.*, 1993, **58**, 380; (*h*) J. L. Sessler, H. Furuta and V. Kral, *Supramol. Chem.*, 1993, **1**, 209, and references therein; (*i*) V. Kral and J. L. Sessler, *Tetrahedron*, 1995, **51**, 539; (*j*) C. Andreu, A. Galan, K. Kobiro, J. de Mendoza, T. K. Park, J. Rebek, A. Salmeron and N. Usman, *J. Am. Chem. Soc.*, 1994, **116**, 5501; (*k*) Y.-G. Jung, W.-S. Yeo, S. B. Lee and J.-I. Hong, *Chem. Commun.*, 1997, 1061.
4 Ion-selective electrodes based on the phosphonium salt: M. Geissler and R. Kunze, *Anal. Chim. Acta*, 1986, **189**, 245.
5 G. T. Morin, M.-F. Paugam, M. P. Hughes and B. D. Smith, *J. Org. Chem.*, 1994, **59**, 2724.
6 J.-P. Behr and J.-M. Lehn, *J. Am. Chem. Soc.*, 1973, **95**, 6108; H. Tsukube, *J. Chem. Soc., Perkin Trans. 1*, 1983, 29.
7 H. Furuta, T. Morishima, V. Kral and J. L. Sessler, *Supramol. Chem.*, 1993, **3**, 5.