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Thiouronium-Thymine Conjugate as a New Carrier for Selective Transport of 5'-AMP

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Abstract: Extraction and transport behaviors of the new 5'-AMP receptors which have thiouronium group and which have both thiouronium and thymine group were investigated. As expected, the sequence of transport rate was **1** > **3** ~ **2** > **4**. **1** showed the best transport ability, since it has both concave bis(thiouronium) binding site which can chelate phospate anion and covalently linked bis(thymine) moiety which can interact with adenine in 5'-AMP by hydrogen bonding involving both Watson-Crick and Hoogsteen recognition patterns. The less transport ability of **2** and **4** was assumed to be the result of disadvantage in complexation process entropically and enthalpically. The control experiment clearly showed that selective base-pairing contributed to increased transport.

Nucleotide analogues which show effective antiviral activity *in vitro* are completely inactive *in vivo*, since they cannot cross lipophilic cell membranes due to their highly charged and hydrophilic nature.[1,2] Therefore, tremendous efforts have been attended to the nucleotide recognition and transport.[3] In the nucleotide transport, major difficulty is how to extract water soluble phosphate moiety into non-polar organic media, so most researches have been focused on the phosphate binding and solubilization in non-polar organic solvents.[4,5] Urea and thiourea groups have been shown to bind dihydrogenphosphate selectively over various other anions.[5] Recently, Smith and co-workers demonstrated that the polarized urea by internal Lewis acid coordination showed improvements in binding affinities of up to 3.0 kcal/mol and can bind acetate more tightly compared to guanidinium cation because of enhanced acidity of urea NH residues and the generation of a strong host molecular dipole.[6]

Therefore we thought that the thiouronium group can function as a better phosphate binder and carrier compared to the thiourea group. In addition, supramolecular complex between thiouronium based receptor and phosphate anion guest is charge balanced and does not need an accompanying cation for transport, which is an energetically demanding process. For this reason, we developed thiouronium salts as a new anion receptor and carrier.[7] Here, we report extraction and transport behavior of 5'-AMP receptors (**1** ~ **4**, R = dodecyl).[8] Thiouronium salts were obtained by nucleophilic substitution of benzyl bromides with thioureas.[9]

The new receptors bind anions through hydrogen bonding and electrostatic interaction as guanidinium cation does. Furthermore, covalently linked complementary nucleobase (thymine) may contribute to improved binding ability. **2, 3,** and **4** were prepared as reference receptors to observe effect of the absence of thymine moiety and efficiency of the bis(thiouronium) group on the 5'-AMP recognition and transport.

The results of extraction and transport of 5'-AMP by the synthetic receptors are shown in Table 1. As expected, **1** and **2** displayed a higher transport rate for 5'-AMP than **3** (by a factor of 13 at pH 5.0 and 2 at pH

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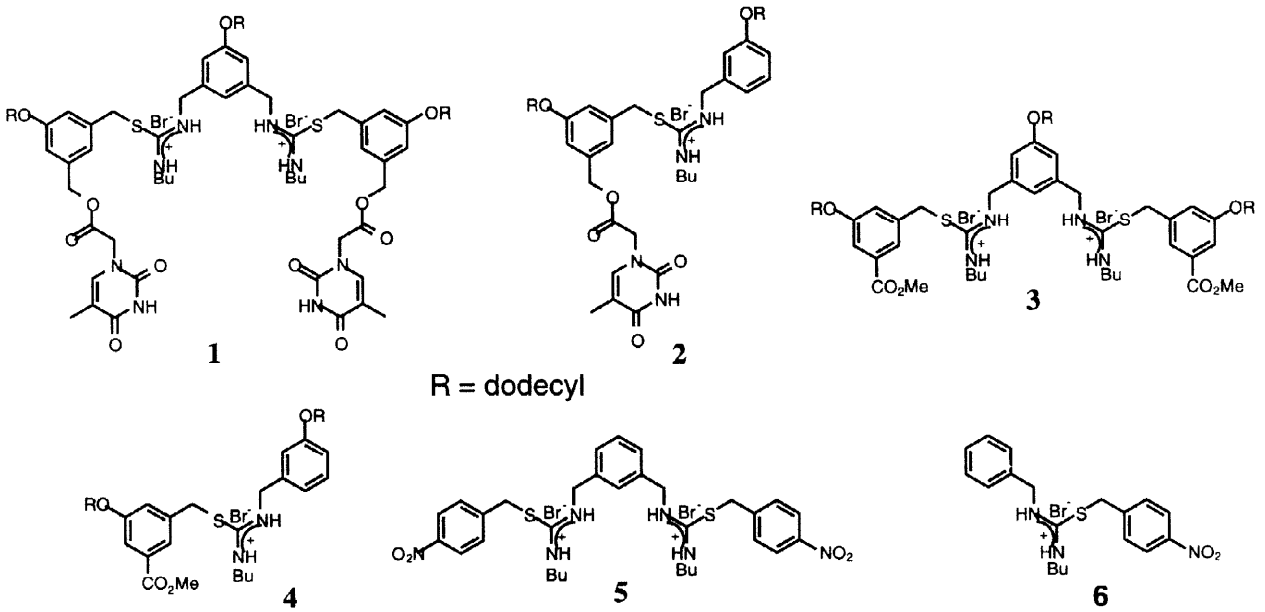

7.0) and **4** (by a factor of 2 at pH 5.0 and 7 at pH 7.0), respectively. This result led us to consider that thymine moiety in **1** and **2** would base-pair with adenine in 5'-AMP so that deleterious hydrogen bonding interaction with water would be reduced. Bis(thiouronium) salts **1** and **3** transported 5'-AMP faster than mono(thiouronium) salts **2** (by a factor of 17 at pH 5.0 and 5 at pH 7.0) and **4** (by a factor of 2.5 at pH 5.0 and 14 at pH 7.0), respectively. The less transport ability of mono(thiouronium) salts (**2** and **4**) than that of bis(thiouronium) salts (**1** and **3**) was assumed to be the result of disadvantage in complexation process for entropic and enthalpic reasons in spite of using double concentration of the carriers (**2** and **4**) in the transport process. From pK_a values, 5'-AMP is dissociated into monoanionic AMP^- at pH 5.0 and mostly into dianionic

Table 1. Extraction and transport of 5'-AMP and 5'-GMP by synthetic receptors

Carrier		Extraction (%)[a]		Transport rate (10^{-8} M/h·cm)[b]	
		5'-AMP	5'-GMP	5'-AMP	5'-GMP
1	pH 5.0	9.9	9.1	15.1	2.4
1	pH 7.0	11.7	8.8	5.9	~2[d]
2	pH 5.0	7.3	2.4	0.9	0.7
2	pH 7.0	9.3	7.5	1.3	ND[c]
3	pH 5.0	7.1	4.3	1.2	0.6
3	pH 7.0	8.3	3.7	2.7	0.7
4	pH 5.0	5.3	4.2	0.5	~1[d]
4	pH 7.0	7.0	5.8	~0.2[d]	ND[c]

a. In the case of **1** and **3** [Carrier] = 5 mM, [Guest] = 0.1 mM, in the case of **2** and **4** [Carrier] = 10 mM, [Guest] = 0.1 mM.
b. In the case of **1** and **3**, Guest 0.1 M, H_2O, 4 mL/Carrier 1.0×10^{-3} M, $CHCl_3$, 8 mL/NaBr 2.5×10^{-2} M, H_2O, 4mL, in case of **2** and **4**, Guest 0.1 M, H_2O, 4 mL/Carrier 2.0×10^{-3} M, $CHCl_3$, 8 mL/NaBr 2.5×10^{-2} M, H_2O, 4mL.
c. Not determined.
d. Detectable, but cannot be determined accurately.

AMP^{2-} at pH 7.0. Considering this, **2** and **4** presumably formed enthalpically less favorable 1:1 complex or entropically less favorable 2:1 complex at pH 5.0 and entropically less favorable 2:1 complex at pH 7.0 with 5'-

AMP. Comparison of **1** with the other receptors illustrated that two thymine subunits in **1** would be the beneficiary of two different kinds of hydrogen bonding interactions, involving both Watson-Crick and Hoogsteen recognition patterns.

However, control experiments (extraction and transport of 5'-GMP with synthetic receptors) employing noncomplementary nucleobase-thiouronium conjugates led to decreased transport, compared to the complementary nucleobase-thiouronium conjugate systems. This clearly shows that selective base-pairing contributes to increased transport rate. The fact that guanine in 5'-GMP is less lipophilic than adenine in 5'-AMP explains the less transport ability of **3** and **4** for 5'-GMP than for 5'-AMP.[10]

A simple bis(thiouronium) receptor (**5**) turned out to bind dihydrogen phosphate more strongly (^{1}H NMR titration showed K_a to be 1,080 M^{-1} in DMSO-d_6 and UV titration to be 34,000 M^{-1} in 1,2-dichloroethane) than a mono(thiouronium) receptor (**6**, K_a in DMSO-d_6 = 340 M^{-1}, K_a in 1,2-dichloroethane = 16,000 M^{-1}).[11] Previous report on the phosphate binding by bis(thiourea) group[5] and transport data and association constants indicated the actual mode of binding between **1** and 5'-AMP as shown in Figure 1, which was also judged from a CPK model. Unfortunately, cocrystal of **1** and 5'-AMP which can support this conclusion wasn't obtained.

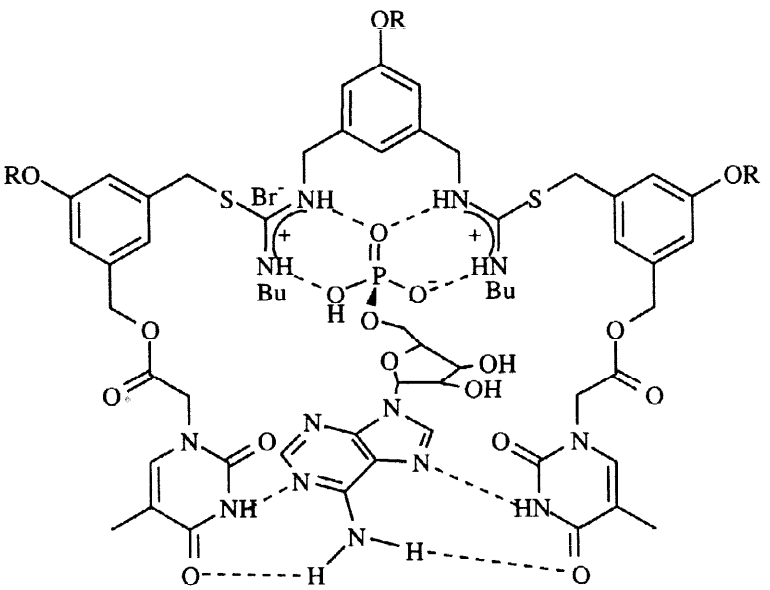

Figure 1. Proposed structure of the major transport complex formed from **1** and 5'-AMP at pH 5.

In summary, we have shown that the thiouronium salt could function as a phosphate anion receptor and the transport of normally organic-insoluble 5'-AMP could be improved by using appropriately designed lipophilic nucleobase (thymine)-substituted thiouronium carrier. We are presently investigating binding property of thiouronium salts toward various anions (phosphates, phosphonates, carboxylates and sulfonates).[11]

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8. Selected spectral data for **1**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (s, 2H), 7.02-6.70 (m, 9H), 5.12 (s, 4H), 4.63 (bs, 8H), 4.56 (s, 4H), 3.91 (t, *J* = 13.0 Hz, 4H), 3.82 (br, 2H), 3.46 (t, *J* = 14.8 Hz, 4H), 1.85 (s, 6H), 1.77-0.80 (m, 83H, alkyl *H*); MS (MALDI) 1490 (M^{2+}-2). **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30-6.75 (m, 8H), 5.15 (s, 2H), 4.67 (s, 4H), 4.59(s, 2H), 3.90 (m, 4H), 3.46 (br, 2H), 1.90 (s, 3H), 1.77-0.80 (m, 53H, alkyl *H*) ; FAB MS m/z 877 (M^+). **3**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 2H), 7.48 (s, 2H), 7.21 (s, 2H), 7.20-6.70 (m, 3H), 4.74 (bs, 4H), 4.61 (bs, 4H), 3.97 (t, *J* = 12.7 Hz, 4H), 3.81 (s, 6H), 3.65 (br, 2H), 3.50 (br, 4H), 1.80-0.80 (m, 83H, alkyl *H*); FAB MS 1216(M^{2+}-1). **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60-6.75 (m, 7H), 4.48 (s, 2H), 4.09 (s, 2H), 3.96(m, 4H), 3.91(s, 3H), 3.28 (t, *J* = 13.8 Hz, 2H), 1.81-0.80 (m, 53H, alkyl *H*) ; FAB MS m/z 739 (M^+).
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