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Synthesis and Micellization of a Long-Chain β-Hydroxyalkyl *p*-Nitrophenyl Phosphate

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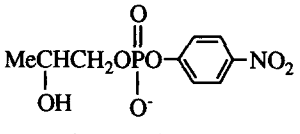
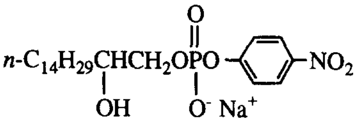
Abstract. β-Hydroxyhexadecyl *p*-nitrophenyl phosphate micellizes in water at ~4.5 x 10^{-5} M and is cleaved by added Eu^{3+} 7-11 times faster than its non-micellar β-hydroxypropyl analogue.

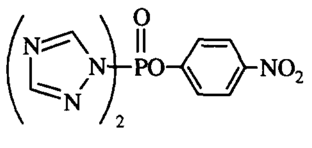
2-Hydroxypropyl *p*-nitrophenyl phosphate (**1**, HPNP)[1] has served as an entry-level RNA model in many studies of metal-mediated, hydroxyl-assisted phosphodiester hydrolysis.[2] In nature, however, phosphodiesters may be associated with membranes or other aggregates. For instance, phosphatidylinositol-specific phospholipase C,[3a-c] non-specific phospholipase C,[3d] and phospholipase A_2[3e] exhibit interfacial activation; *i.e.*, the preferential hydrolysis of aggregated substrates. Because the small-molecule substrate HPNP does not readily aggregate, we desired a long-chain analogue that would feature a dependence of phosphorolytic reactivity on substrate aggregation. Here we report the synthesis and preliminary aggregation/phosphorolysis studies of the first long-chain β–hydroxylalkyl activated phosphodiester substrate, 2-hydroxyhexadecyl *p*-nitrophenyl phosphate (**2**, HHNP).



1 (HPNP)



2 (HHNP)



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The literature procedure for preparation of **1** features a displacement reaction of *p*-nitrophenyl phosphate anion on propylene oxide in water at pH 8.[1] In our hands various attempts to extend this reaction to long-chain epoxides (including phase transfer methods) failed, due to mutual solubility problems between the hydrophilic anionic nucleophile and the hydrophobic substrate. Therefore, a multiple protecting group strategy (Scheme I) was adopted.[4]

The key problem in this synthesis of **2** is protection of the β-hydroxyl during phosphorylation of the primary hydroxyl, thus avoiding intramolecular displacement of *p*-nitrophenylate by the β-hydroxyl. At the same time, the β-protecting group must be labile to mild acid, because the use of strong acid or moderate base would lyse the *p*-nitrophenyl phosphate. The tetrahydropyranyl (THP) group proved to be an acceptable choice for β-OH protection.

As depicted in Scheme I, the primary hydroxyl of 1,2-hexadecane diol was selectively benzoylated via dioxastannane **3**,[5] affording β-hydroxybenzoate **4** in 84% overall yield from the diol. Next, **4** was protected

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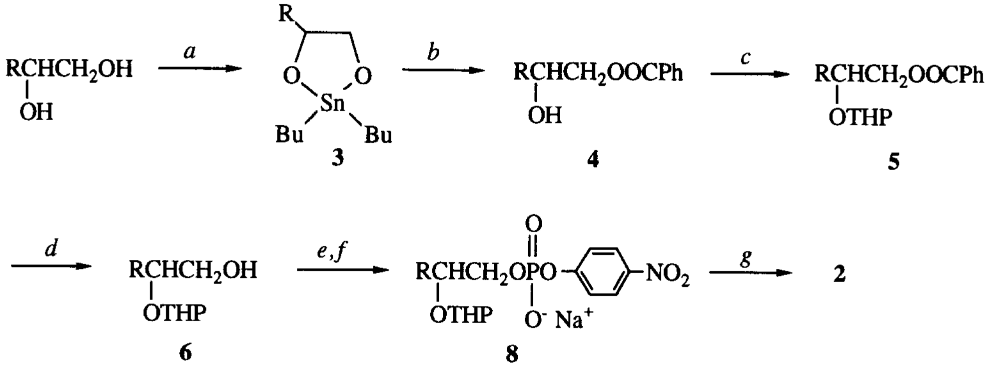
Scheme I (R = n-$C_{14}H_{29}$)



(*a*) Bu_2SnO, MeOH, refl. 2 h, 93%. (*b*) PhCOCl, Et_3N, MeOH, r.t., 10 min, 90%. (*c*) Dihydropyran, TsOH, CH_2Cl_2, r.t., 2 h, 70%. (*d*) NaOH, 1:6:6 $H_2O/MeOH/CH_2Cl_2$, r.t., 1.5 h, 76%. (*e*) **7**, CH_2Cl_2, r.t., N_2 atm., 2 h, 86%. (*f*) Amberlite IR-120 (plus) (Na^+ form), r.t., 1:1 $MeOH/CH_2Cl_2$, 91%.
(*g*) Amberlite IR-120 (plus) (pyridinium form), MeOH, r.t., 2 h, 51%.

with dihydropyran, giving doubly masked THP-ester **5** (mixture of diastereomers), which was saponified with NaOH to furnish the β-THP protected alcohol, **6**, in 53% yield from **4**. Phosphorylation of **6** with *p*-nitrophenylphosphorobis(1,2,4-triazolide), **7**,[6,7] gave a mixed sodium/triethylammonium salt of **8** (86% after chromatography on silica gel), which was converted (91%) to the pure Na salt, **8**, by ion exchange chromatography on Amberlite IR-120 (plus). Finally, cleavage of the THP by the mildly acidic pyridinium form of the Amberlite IR-120 (plus) ion exchange resin,[8] converted **8** into the desired **2** in 51% yield. The overall yield of **2** was 17-18% for 7 steps. Intermediates **4**, **5**, **6**, and **8**, as well as HHNP (**2**), were each characterized by NMR and elemental analysis.[9]

The micellization of HHNP in water was demonstrated in two ways. First we determined the dependence of the surface tension (γ) of aqueous solutions of HHNP (10 mM HEPES buffer, 10 mM KCl, pH 7, 25 °C) on the concentration of HHNP *cf.*, Figure 1. The obvious discontinuity in this correlation at [HHNP] = 4.5×10^{-5} M can be taken as the critical micelle concentration (CMC) of HHNP under the conditions of measurement.[10]

In a second approach, we measured rate constants for the Eu^{3+}-mediated cleavage of HHNP as a function of its concentration,[11,12] with the molar ratio of Eu^{3+}/HHNP held constant at 10:1. The results (Figure 2) reveal a sharp acceleration of HHNP cleavage commencing at $\sim 4 \times 10^{-5}$ M HHNP, in very good agreement with the CMC determined by surface tension. Rate constant enhancement at the onset of substrate micellization is commonly observed,[13] and, in this instance, is due to enhanced binding of Eu^{3+} to the anionic HHNP micelles with attendant concentration of the cations into the condensed reaction volume at the micellar surface. A similar effect operates in the Eu^{3+} cleavage of phosphodiester liposomes.[14] Note that the sigmoidal character of

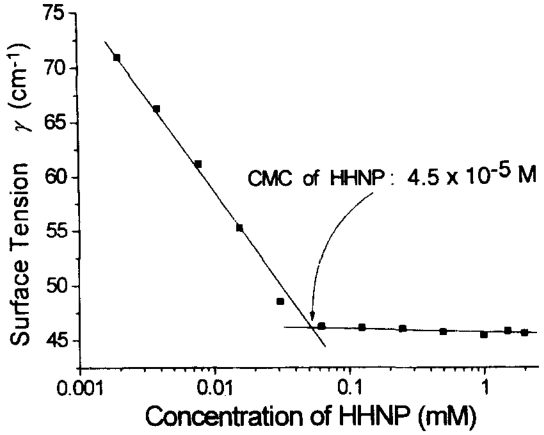


Figure 1. Surface tension (γ) vs. [HHNP]. See text for conditions.

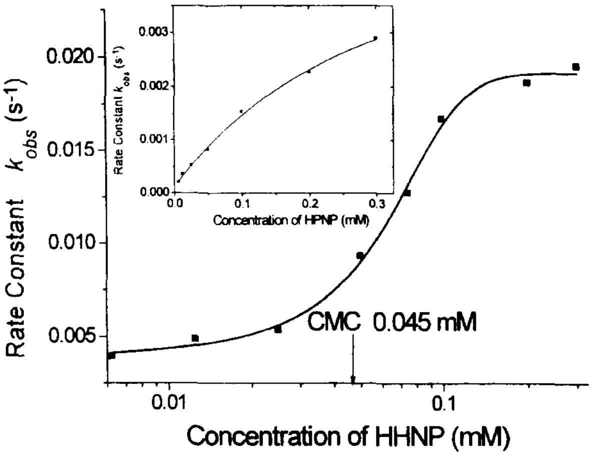


Figure 2. k_{obs}, s^{-1}, for the Eu^{3+}-mediated cleavage of HHNP as a function of [HHNP].[11] Inset: An analogous plot for HPNP.

the HHNP correlation, which features an inflection point at the CMC, differs from the more typical "saturation" behavior (1:1 P-O$^-$/Eu^{3+} binding) displayed by (non-micellar) HPNP and Eu^{3+} (*cf.*, inset of Figure 2).

The extent of micellar catalysis in the Eu^{3+} cleavage of HHNP can be estimated by comparing the observed rate constants to those for the cleavage of HPNP under similar conditions. At [substrate] ~ 0.3 mM,[15] where k_{HHNP} is maximal, k_{HHNP}/k_{HPNP} = 0.0195 s^{-1}/0.00289 s^{-1} = 6.7. At lower concentrations, the micellar advantage is more pronounced; *i.e.*, at [substrate] = 0.1 mM,[15] k_{HHNP}/k_{HPNP} ~ 11. Detailed studies of the lanthanide cleavages of HHNP and other micellar phosphodiesters will appear in due course.

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(7) Reagent **7** was prepared by the reaction of *p*-nitrophenyl phosphorodichloridate with 1,2,4-triazole in Et_3N/CH_2Cl_2 (r.t., 10 min). Direct phosphorylation of **6** with the dichloridate was difficult to control, gave low yields of **8**, and products derived from the HCl-cleavage of the THP-protecting group followed by intramolecular β-OH attack on the PNP-phosphate. Sodium cations were introduced during the work-up (aqueous $NaHCO_3$ wash).

(8) This method is a combination of related usages of pyridinium *p*-toluenesulfonate [Miyashita, M.; Yoshikoshi, A.; Grieco, P.A. *J. Org. Chem.* **1977**, *42*, 3772] and acidic ion exchange resins [Bongini, A.; Cardillo, G.; Orena, M.; Sandri, S. *Synthesis* **1979**, 618].

(9) ^1H NMR of **2** (200 MHz, CD_3OD, δ): 8.1 8.2, 7.4, 7.5 (AB q, 4H, Ar), 3.8 – 4.0 (m, 2H, CH_2O), 3.6 – 3.8 (m, 1H, *CH*OH), 1.2 – 1.7 (m, 26 H, $Me(CH_2)_{13}$), 0.9 (t, 3H, Me). Anal. C,H,N,P.

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(11) Conditions: pH 7.0, 0.01 M KCl, 37 °C. The release of *p*-nitrophenylate was spectroscopically monitored at 400 nm.

(12) For lanthanide cleavages of HPNP (**1**), see references 2c,d,i. For leading references to cleavages of related phosphodiester substrates, see Roigk, A.; Hettich, R.; Schneider, H-J. *Inorg. Chem.* **1998**, *37*, 751.

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(15) $[Eu^{3+}] = 10[\text{substrate}]$.