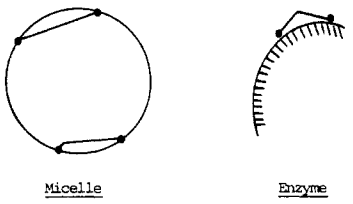

Figure 1.

oxide with 3.0 M KCl. Increasing the diacid concentration to 1.0 M (excess KOH in D_2O) does not change the coupling constant nor does increasing the temperature from 25 to 80 °C. Similarly, 50 mM **2** has a J = 3.8 Hz as the disalt (in acidic D_2O) or as the free amine (in ethanol). Apparently, the chains are fully extended, or nearly so, under all the preceding conditions.

One main reason for selecting **1** and **2** for our initial studies was to determine their behavior when bound to cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) micelles. Note that the fully extended chains of the bolaforms are too short to permit a radial disposition within the micellar host; a radial geometry would force one of the polar groups to reside within the supposedly dry interior. Nonetheless, **1** and **2** can still bind to micelles if they "loop" in order to place both head groups near the surface. Unexpectedly, however, the NMR data show that looping does *not* occur. When 0.03 M **1** in 0.10 M KOH was mixed with 0.09 M CTAB in D_2O, the coupling constant remains at 3.5 Hz. Elevating the concentration of **1** in the micellar CTAB has no effect on ^3J. Likewise, when unprotonated **2** (0.025 M) binds to 0.10 M SDS, the coupling constant also equals 3.5 Hz. Clearly, the two central linkages[12] of the adsorbed guest molecules are substantially *transoid*. Unless the outer C–C linkages possess kinks when the central ones do not (which is just the reverse of what is needed for a major loop), the bolaforms assume extended conformations within the micelles. This can occur only by "tangential" binding (Figure 1),[13] a phenomenon not incorporated into the classical Hartley "asterick" model. Nonradial binding is, however, consistent with "brush-heap" disorder and with the presence of fatty patches near the micelle surface where guests bind hydrophobically.[14]

There is no doubt that the dianionic bolaform **1** does indeed bind to the CTAB micelles. An Armstrong experiment[15] was carried out in which the bolaform was thin-layer chromatographed on a Brinkmann Polyamide-6 plate with 0.025 M aqueous CTAB as the mobile phase. The bolaform, moving with the CTAB front, displayed an R_f of 0.60 compared to 0.08 when pure water was the mobile phase. Partition coefficients, estimated from the R_f values,[15] indicate that >98% of **1** is micelle bound under NMR conditions. This is hardly surprising: amphiphiles of opposite charge are known to form tight complexes.[16,17] In the case of neutral **2** in SDS micelles, there is also no doubt of complete association because the diamine will not dissolve in water when SDS is absent.

Binding of bolaform **2** to trypsin (known to have a specificity pocket for lysine[18]) produced quite different results. Solutions containing 7.5×10^{-4} M bolaform and excess trypsin (20 mg/mL) at pD 6.0 were examined by ^{13}C NMR (9056 scans, 1612 sweep width, 8% dilabeled compound). The presence of the enzyme reduced ^3J from its solution value of 3.8 Hz to only 0.8 Hz, a value corresponding to a dihedral angle of about 70°. Competitive inhibition studies (carried out by measuring the enzyme-catalyzed hydrolysis rate of arginine methyl ester at pH 5.5 with the aid of a pH-stat) gave a K_i = 0.05 M. Under our NMR conditions, therefore, less than 10% of the bolaform is bound to the active site. Thus, the striking reduction in ^3J must be attributed to noncompetitive binding which induces a chain "kink" as depicted in Figure 1.

The di-^{13}C-labeled NMR method can be used to study chain folding in molecules attached to receptor sites, antibodies, membranes, and a host of other biologically important systems. We ourselves are currently synthesizing lipids that are di-^{13}C-labeled at several locations, and the results will be reported when the experiments are completed.

Acknowledgment. This work was supported by the National Institutes of Health. We thank Professor C. G. Trowbridge for his assistance with the enzyme kinetics.

(12) The NMR method examines only one of two identical C–C bonds in the center of the chain.
(13) Since we have no information on the outer C–C bonds, Figure 1 includes a micellized bolaform with a kink in this region.
(14) Menger, F. M. *Acc. Chem. Res.* **1979**, *12*, 111.
(15) Armstrong, D. W.; Stine, G. Y. *J. Am. Chem. Soc.* **1983**, *105*, 2962.
(16) Hoyer, H. W.; Marmo, A.; Zoellner, M. *J. Phys. Chem.* **1961**, *65*, 1804.
(17) Mukhayer, G. I.; Davis, S. S. *J. Colloid Interface Sci.* **1978**, *66*, 110.
(18) Zubay, G. "Biochemistry"; Addison-Wesley: New York, 1983; p 156.

Stereochemical Course of the Phosphoryl Transfer from Adenosine 5′-Diphosphate to Alcohols in Acetonitrile and the Possible Role of Monomeric Metaphosphate

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Following the initial proposal of Westheimer[1] and of Bunton[2] much evidence has been cited in support of the intermediacy of a monomeric metaphosphate in nucleophilic displacement reactions of monosubstituted phosphate esters.[3] However, recent stereochemical studies on the solvolysis of the monoanion of phenyl [^{16}O,^{17}O,^{18}O]phosphate and the dianion of 2,4-dinitrophenyl [^{16}O,^{17}O,^{18}O]phosphate in hydroxylic solvents have been shown to proceed with complete (within experimental error) inversion of configuration.[4] The previous evidence in favor of a metaphosphate intermediate can be reconciled with the stereochemical course of the reaction in terms of a preassociative mechanism, in which the intermediate is never "free" and indeed is only formed productively when the nucleophile is already preassociated in the encounter complex. Much of the direct evidence in favor of a free metaphosphate has come from reactions in aprotic solvents.[3,5] Pertinent to this study, Ramirez and co-workers[5] have demonstrated that aryl phosphate esters and the tri and tetra anions of ATP in acetonitrile will phosphorylate the hindered alcohol *tert*-butyl alcohol and they argue that this may be a good criterion for the participation of a metaphosphate. Despite the compelling evidence in favor of a metaphosphate-like intermediate the extent to which this can be freely solvated, particularly in aprotic solvent, is unclear. Utilizing isotopically chiral [β-^{16}O,^{17}O,^{18}O]adenosine 5′-diphosphate we have sought stereochemical information pertinent to this point.

We have shown that adenosine 5′-diphosphate tris(tetra-*n*-butylammonium) salt in dry acetonitrile phosphorylates alcohols under conditions analogous to those previously reported by Ramirez et al. for ATP.[5] *tert*-Butyl alcohol was phosphorylated at

(1) Butcher, W.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2420.
(2) Barnard, P. W. C.; Bunton, C. A.; Llewellyn, D. R.; Oldham, K. G.; Silver, B. L.; Vernon, C. A. *Chem. Ind. (London)* **1955**, 706.
(3) Di Sabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4400. Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415. Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209. Gorenstein, D. G.; *J. Am. Chem. Soc.* **1972**, *94*, 2523. Gorenstein, D. G.; Lee, Y.-G.; Kar, D. *J. Am. Chem. Soc.* **1977**, *99*, 2264. Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313. Rebek, J.; Gaviña, F.; Navaro, C. *J. Am. Chem. Soc.* **1978**, *100*, 8113.
(4) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 1438. Buchwald, S. L.; Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4911.
(5) Ramirez, F.; Maracek, J. F. *Tetrahedron* **1979**, *35*, 1581. Ramirez, F.; Maracek, J. F. *Tetrahedron* **1980**, *36*, 3151. Ramirez, F.; Maracek, J. F.; Yemul, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 1345. Ramirez, F.; Maracek, J. F.; Szamosi, J. *J. Org. Chem.* **1980**, *45*, 4748.

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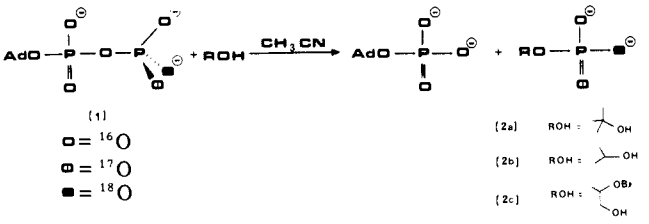
Scheme I. Phosphoryl Transfer Reactions of the Trianion of Adenosine 5′-Diphosphate



the same rate as the less hindered secondary alcohol isopropyl alcohol to give **2a** and **2b**, respectively. Furthermore, the primary alcohol 2-*O*-benzyl-(*S*)-propane-1,2-diol was phosphorylated at a rate comparable to this to give **2c**, (Scheme I).

Adenosine 5′-[β-(*S*)-^{16}O,^{17}O,^{18}O]diphosphate (**1**) was synthesized by a route analogous to that reported for [γ-^{16}O,^{17}O,^{18}O]ATP.[6] The absolute configuration at phosphorus follows from the synthesis and was independently established.[7] Using adenosine 5′-[β-(*S*)-^{16}O,^{17}O,^{18}O]diphosphate (**1**) we have determined the stereochemical course of the phosphoryl transfer to 2-*O*-benzyl-(*S*)-propane-1,2-diol in acetonitrile.[8] 1-[^{16}O,^{17}O,^{18}O]Phosphopropane-1,2-diol obtained from **2c** by hydrogenolysis was subjected to stereochemical analysis,[9] and the resulting ^{31}P NMR spectrum is shown in Figure 1A. *The configuration at phosphorus was found to be racemic within experimental error.*[10] Control experiments confirmed the following: (i) adenosine 5′-[β-(*S*)-^{16}O,^{17}O,^{18}O]diphosphate (**1**) reisolated from the reaction had not suffered significant racemization;[7,8] (ii) 2-*O*-benzyl 1-[(*R*)-^{16}O,^{17}O,^{18}O]phosphopropane-1,2-diol[11] did not racemize under the conditions of the phosphoryl transfer (Figure 1B). Since both the starting material and the product are configurationally stable under the reaction conditions, *the observed racemization must arise during the phosphoryl transfer step.*

A phosphoryl transfer reaction proceeding with racemization of configuration would accord with a reaction involving a relatively free metaphosphate intermediate which can be trapped from either side. An alternative explanation, without invoking a free metaphosphate, would involve a transient phosphoryl transfer to an alternative acceptor, which would most likely be the solvent acetonitrile.[12] Assuming this transfer proceeds by a preassociative mechanism, in order to account for a *racemic* product, the adduct between acetonitrile and the metaphosphate must undergo multiple phosphoryl transfer reactions with other solvent molecules before being trapped by the alcohol.[13] The stereochemical experiment

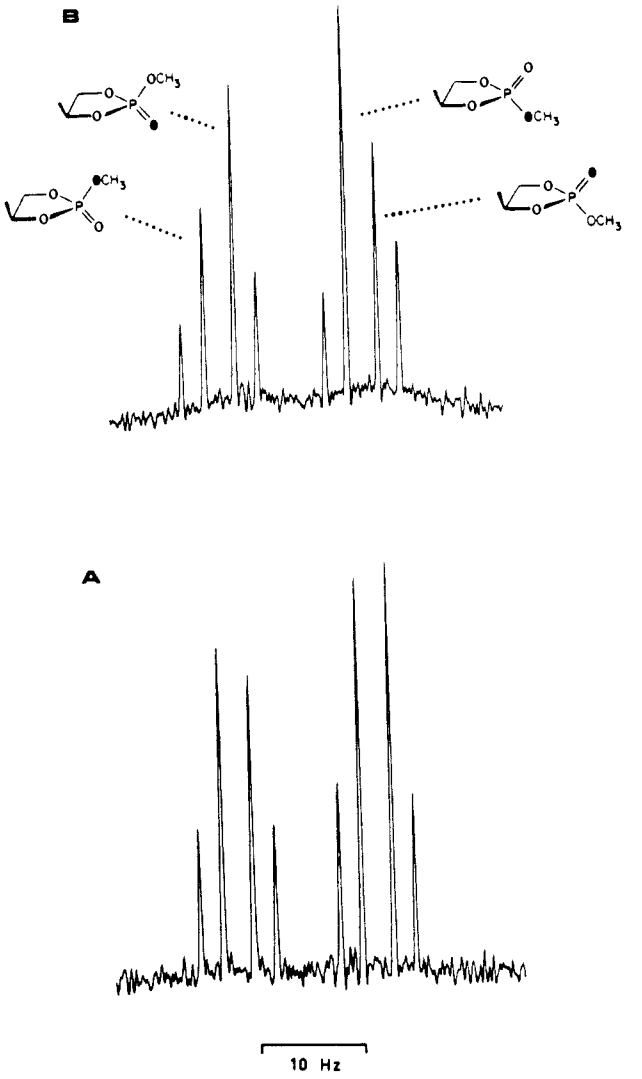


Figure 1. ^{31}P NMR spectra (scale 10 Hz) of (A) the product from in-line ring closure and methylation of 1-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol obtained from the phosphoryl transfer reaction from [β-(*S*)-^{16}O,^{17}O,^{18}O]adenosine 5′-diphosphate (**1**) to 2-*O*-benzyl-(*S*)-propane-1,2-diol in acetonitrile and (B) the product from in-line ring closure and methylation of 1-[^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol obtained from 2-*O*-benzyl-1-[(*R*)-^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol that had been subjected to the same experimental conditions of the phosphoryl transfer reaction. The spectra were obtained on a Bruker AM-300 instrument at 121.5 MHz with a deuterium lock and broad band decoupling: spectral width 1300 Hz; acquisition time, 6.21 s; pulse width, 10 μs; number of transients 8000; Gaussian multiplication (Gaussian broadening 0.1 Hz, line broadening −0.3 Hz); Fourier transformed in 32 K.

alone cannot easily distinquish between these alternatives. However, our recent study of the stereochemical course of phosphoryl transfer from a P^1,P^1-disubstituted pyrophosphate derivative to 2-*O*-benzyl-(*S*)-propane-1,2-diol in *dichloromethane* also showed extensive racemization.[14] Presumably dichloromethane would be less likely to participate directly in phosphoryl transfer reactions than acetonitrile. Also, recently Knowles et al.[15] have carried out similar investigation of the stereochemical

(6) Blättler, W. A.; Knowles, J. R. *Biochemistry* **1979**, *18*, 3927.

(7) Adenosine 5′-[β-^{16}O,^{17}O,^{18}O]diphosphate reisolated from the reaction (see ref 8) was incubated with adenylated kinase and hexokinase in the presence of D-glucose. Since both phosphoryl transfer steps occur with inversion of configuration at phosphorus, the resulting glucose 6-[^{16}O,^{17}O,^{18}O]phosphate has the same absolute configuration at phosphorus as the β-chiral ADP (Sheu, K.-F. R.; Richard, J. P.; Frey, P. A. *Biochemistry* **1979**, *18*, 5548. Lowe, G.; Potter, B. V. L. *Biochem. J.* **1981**, *199*, 227). Stereochemical analysis by the literature procedure (Jarvest, R. L.; Lowe, G.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **1981**, 3186) showed that the adenosine 5′-[β-^{16}O,^{17}O,^{18}O]diphosphate was ≥90% S_p and that no significant racemization occurred during the phosphoryl transfer reaction.

(8) Phosphoryl transfer from adenosine 5′-[β-(*S*)-^{16}O,^{17}O,^{18}O]diphosphate tris(tetrabutylammonium) salt (**1**) (100 mM) was carried out in acetonitrile/2-*O*-benzyl-(*S*)-propane-1,2-diol (1:1; v/v) at ca. 70 °C for 24 h.

(9) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1980**, *102*, 6601.

(10) The expected ^{31}P NMR pattern can be calculated on the basis of the normal assumptions taking into account (i) isotopic enrichment at each of the sites and (ii) enantiomeric excess of the 2-*O*-benzyl-(*S*)-propane-1,2-diol. The slight residual slope on the stereochemically informative peaks (2nd,3rd and 6th,7th) *if real* would suggest that there is a slight excess of the S_p configuration, which would arise from phosphoryl transfer occurring with *retention* of configuration.

(11) 2-*O*-Benzyl 1-[(*R*)-^{16}O,^{17}O,^{18}O]phosphopropane-1,2-diol was synthesized by the literature procedure (Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 2558. Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Bockhoff, F. M.; McLafferty, F. W.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 4323) and subjected to the same experimental conditions required to bring about the phosphoryl transfer reaction.

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(13) The possibility of phosphoryl transfer reactions to other phosphoryl residues can be excluded since no significant levels of tri and higher polyphosphates were observed by NMR or ion-exchange chromatography. Furthermore, intermolecular phosphoryl transfer to the phosphate of AMP or ADP has been exclude by results of PIX analysis reported in the following paper. The tentative observation of a very small excess of *retention* of configuration (ref 10) may favor the suggestion that acetonitrile participates in the reaction since the excess reaction would then arise from the slight predominance of immediate capture of the CH_3CN^+–PO_3^{2-} by the alcohol.

(14) Cullis, P. M.; Rous, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 6721.

(15) Friedman, J. M.; Knowles, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 6126.

course of phosphoryl transfer from phenyl phosphate to *tert*-butyl alcohol in acetonitrile. This reaction was shown to proceed with almost complete racemization, in agreement with the results of this present study.

In the accompanying paper, the results of positional isotope exchange experiments complementary to the stereochemical study reported here are discussed. It is argued that the data would most support a preassociative stepwise mechanism for the phosphoryl transfer from ADP to alcohols in acetonitrile.

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Positional Isotope Exchange in Adenosine 5′-[β-$^{18}O_4$]Diphosphate and the Possible Role of Monomeric Metaphosphate

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Since monomeric metaphosphate (PO_3^-) was first postulated as an intermediate in the hydrolysis of monosubstituted phosphates 30 years ago,[1] much evidence has accumulated which is consistent with its intervention.[2] However, stereochemical analysis of the products formed when phenyl, 2,4-dinitrophenyl, and creatine [^{16}O,^{17}O,^{18}O]-(*R*)-phosphate were solvolysed in aqueous methanol under conditions where the intermediacy of monomeric metaphosphate had been invoked[2] showed that phosphoryl transfer proceeds with inversion.[3] Likewise, in the Conant-Swan fragmentation of 1,2-dibromo-2-phenylethyl[^{16}O,^{17}O,^{18}O]-(*R*)-phosphonate dianion, a reaction also considered to proceed by way of a metaphosphate intermediate,[4] phosphoryl transfer to an alcohol acceptor was also found to proceed with inversion.[5] The apparent conflict between the kinetic[2] and stereochemical evidence[3,5] can be reconciled, however, in terms of a preassociative mechanism.[6] In an attempt to provide a more sensitive probe of events occurring within the solvent cage during phosphoryl transfer reactions we have undertaken positional isotope exchange experiments with adenosine 5′-[β-$^{18}O_4$]diphosphate, prepared from adenosine 5′-phosphomorpholidate and mono(tri-*n*-butylammonium) [$^{18}O_4$]phosphate.[7]

The pK_a values of the diphosphate moiety of ADP are <1, 3.9, and 6.4.[8] [β-$^{18}O_4$]ADP was incubated in tris-HCl aqueous buffer (20 mM), in the presence of (a) $MgCl_2$ (0.5 M) and (b) EDTA (10 mM) at pH 5, 6, 7, 8, and 9 for 3 weeks at 20 °C, by which time approximately 20% [β-$^{18}O_4$]ADP had been hydrolyzed to [^{18}O]AMP and [$^{18}O_3$]P_i. The high-resolution ^{31}P NMR spectrum (101.232 MHz) of the recovered [$^{18}O_4$]ADP showed no evidence of positional ^{18}O exchange between the P_β-O-P_α bridge and the nonbridging sites at P_α in either experiment.

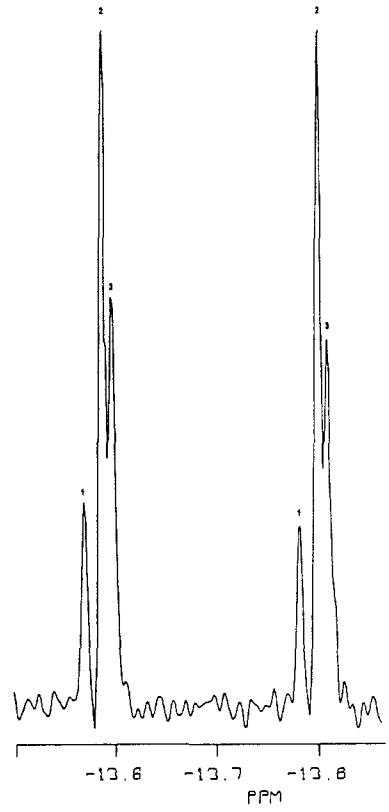

Figure 1. ^{31}P NMR spectrum (101.232 MHz) of P_α of the recovered isotopically labeled ADP after incubation of [β-$^{18}O_4$]ADP (87 atom % ^{18}O per site) in acetonitrile at 70 °C for 48 h. Assignments are 1, all species unlabeled at P_α; 2, all species with ^{18}O in P_α-O-P_β bridge; 3, all species with ^{18}O in nonbridging sites at P_α. The chemical shift reference is trimethyl phosphate.

Ramirez et al. have shown that ATP and ADP phosphorylated hindered alcohols (Pr-*i*-OH and Bu-*t*-OH) when incubated in acetonitrile at 70 °C and have proposed the intermediacy of monomeric metaphosphate.[9] [β-$^{18}O_4$]ADP tris(tetra-*n*-butylammonium) salt was incubated alone in dry acetonitrile at 70 °C in a drybox and after 2 days the four components present were separated by HPLC and identified as AMP, ADP, adenosine 2′,5′-biphosphate (pAp), and adenosine 2′-phospho-5′-diphosphate (ppAp). High-resolution ^{31}P NMR spectroscopy showed the AMP to be singly ^{18}O labeled and the pAp and ppAp to be triply ^{18}O labeled at the 2′-phosphate group. The high-resolution ^{31}P NMR spectrum of the recovered ADP (and the ppAp) showed that extensive ^{18}O exchange from the P_β-O-P_α bridge to the nonbridging site at P_α had occurred (Figure 1).

The possibility that the positional isotope exchange had occurred by $P_\beta{}^{18}O_3$ transfer from [β-$^{18}O_4$]ADP to [^{18}O]AMP formed in situ was excluded by a control experiment in which [$^{18}O_2$]AMP was incubated with unlabeled ADP (as their tetra-*n*-butylammonium salts) in acetonitrile at 70 °C for 12 h by which time about 60% of the ADP had been converted to other products. The recovered ADP remained completely unlabeled demonstrating that ADP does not transfer $P_\beta O_3$ to the phosphate moiety of AMP, presumably owing to electrostatic repulsion.

Failure to observe positional isotope exchange in [β-$^{18}O_4$]ADP (or its Mg^{2+} complex) after incubation as its di- and trianion in aqueous solution is consistent with a preassociative concerted

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(7) Wehrli, W. E.; Verheyden, D. L. M.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 2265-2277.
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