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# AN APPROACH TO LIPOPHILIC NUCLEOTIDE PHOSPHATE ANALOGS. SYNTHESIS OF A LIPOPHILIC ISOSTERE OF ATP.

Renee M. Otoski and Craig S. Wilcox*
Department of Chemistry - University of Pittsburgh
Pittsburgh, PA 15260

*Abstract. The tertiary structures of $Mg^{+2}$ complexes of ATP and ADP can be imitated by other, more lipophilic atomic arrangements. A design rationale is presented and a synthetic approach to such lipophilic ATP isosteres is described.*

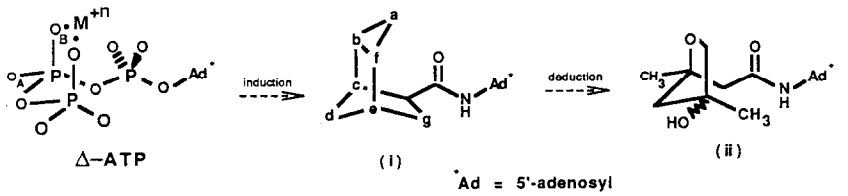
While nucleo*sides* do play a role in metabolic regulation[1], it is the various nucleo*tides* (including monophosphate, diphosphate and triphosphate derivatives of the nucleosides) which are most commonplace and essential to cellular and viral growth and reproduction.[2] Many, if not most, nucleoside analogs are active only following phosphorylation by an endogenous kinase.[3] It is commonly believed that nucleotide analogs which bear charged phosphate groups are (in the absence of an active transport mechanism) unlikely to efficiently penetrate cell membranes. It has been suggested that uncharged and weakly charged molecules will interact only weakly with a binding site appropriate for a "highly charged" substrate. The urgent need for new antiviral agents demands that this latter assumption be carefully examined.

There are several observations which contradict the idea that "low charge" substrates will not bind to phosphate binding sites: Tunicamycin, a potent inhibitor of dolichylpyrophosphoryl-GlcNAc synthesis, is neutral and yet binds to a site which accommodates a tetraanionic transition state.[4] Other "low charge" antibiotics are also believed to mimic charged natural metabolites.[5] In water, binding of a charged substrate is opposed by the favorable solvation energies of the substrate; solvophobic forces will enhance binding of a less charged substrate. Phosphate binding catalytic sites contain fewer ionic groups than phosphate binding regulatory sites.[6]

If a neutral phosphate mimic is to succeed as a component of a bis-substrate analog or nucleotide analog it must not occupy the "enzyme-essential" volume of the active site.[7] For this reason, neutral nucleotide mimics should be preorganized to match in detail the bound shape (the "tertiary structure") of the natural nucleotide (or nucleotide-metal complex) substrate. Information regarding the structure of metal complexes of ATP has been available for many years and more data continue to appear.[8,9] There are two diastereomeric $\beta,\gamma$-bidentate magnesium complexes of ATP (Chart 1) and enzymes discriminate between these two forms.[10] We report here the preparation of a furan-2-acetic acid derivative **9a-d**. The synthesis illustrates the principle of preparing mimics of the tertiary structure

of nucleotides and nucleotide complexes and validates the utility of 5'-amino-5'-deoxyadenosine as a precursor to such mimics.[11]

A family of ATP analogs may be represented by the generalized structure "i". The letters a-g represent sites which might be occupied by carbon, nitrogen, oxygen, or phosphorus atoms. Monocyclic structures (wherein atoms b-f, a-f, or c-d-e-g are used), or [2.2.1] (atoms b-g) and [3.2.1] (atoms a-g) bicyclic systems may be created. When a specific binding site is known, polar or non-polar substituents can be attached to exocyclic sites to enhance the binding of the analog. A simple specific example of this general analog is "ii", a branched furan-2-acetic acid amide. The analog is obviously far less ionic and more lipophilic than the parent phosphate, but three oxygen atoms are present in the analog and modeling studies indicate that these oxygen atoms are positioned in an array similar to three of the oxygen atoms in ATP. The mimic could therefore be selective toward ATP binding sites which contain hydrogen bond donors directed toward those three oxygen atoms. Because the analog is designed to mimic the shape of ATP, it can fit in a cavity adequate for ATP and the tertiary alcohol can serve as a connecting link for creating bis-substrate based kinase inhibitors. Control of the tertiary structure of the phosphate mimic lessens any ambiguities concerning either the shape of the bound mimic or the relative positions of the two components of a bis-substrate analog derived from this mimic.

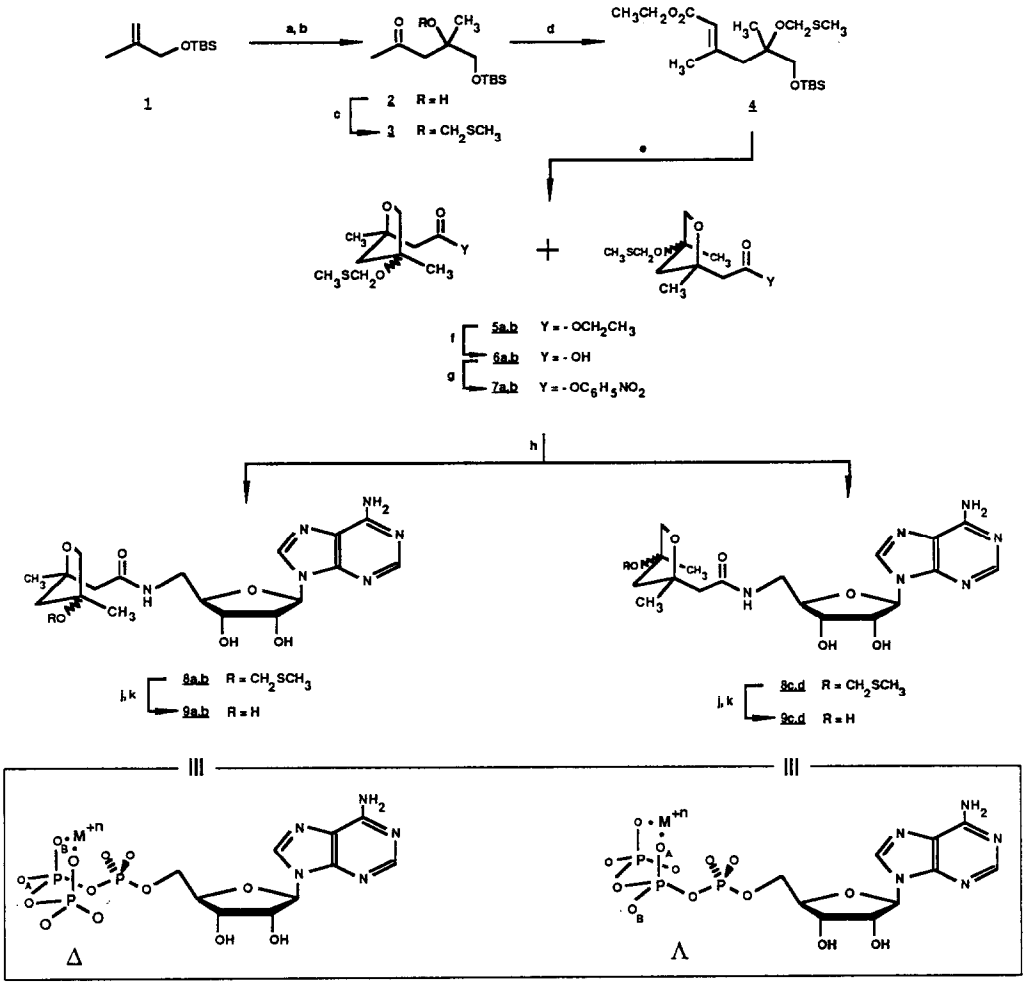



A simple approach to ATP analog "ii" is outlined in Chart 1. Cycloaddition of the nitrile oxide (generated *in situ*) with olefin **1** provided the 2-isoxazoline in 75% yield after flash chromatography (25% EtOAc/Skelly B). Reduction and hydrolysis of the cycloadduct afforded the β–hydroxy ketone **2** in 75% yield.[12,13]

It was in our interest to transform the carbonyl of **2** to a α,β–unsaturated ester derivative without protecting the tertiary alcohol. Under Horner-Emmons conditions, β–hydroxyketone **2** underwent a retro-aldol reaction in which two olefinic products (derived from the TBS-protected hydroxyacetone) were isolated. Our attentions therefore turned to the use of the Peterson olefination reaction [14]. Although the reaction of the anion of ethyl(trimethylsilyl)acetate with **2** did not give an adequate yield of the desired adduct, similar treatment of a protected derivative of **2**, methylthiomethyl ether **3**, was successful. Compound **4** was obtained as a mixture of geometric isomers (1:2.3 cis:trans). This mixture **4** could be purified either by preparative thin layer chromatography (10% EtOAc/Skelly B) or flash chromatography ( 25% EtOAc/Skelly B) to give 95% and 75% yields respectively.

Deprotection of the TBS group and cyclization of the mixture of α,β–unsaturated esters **4** were accomplished in one step ($Bu_4NF$, THF, 25 °C). These conditions afforded a (1.8:1.0) mixture of diastereomeric esters **5a,b**. The diastereomers were separated from side products arising from β-elimination by flash chromatography (25% EtOAc/Skelly B). Hydrolysis of **5a,b** with lithium hydroxide to the carboxylic acids **6a,b**, followed by the preparation of the p-nitrophenyl esters[15] provided **7a,b** in a 86% yield. The coupling reaction of **7a,b** with the p-TSA salt of 5'-amino-5'-deoxyadenosine to form the amide bond resulted in a 48% yield of **8a-d** after flash chromatography (10% MeOH/$MeCl_2$).

Chart One. Preparation of four diastereomeric ATP analogs.



(a) PhNCO, $EtNO_2$, $Et_3N$, $C_6H_6$, 25°C; (b) W-2 Raney Ni, $H_2$, HOAc, 90% aq. EtOH, 25°C; (c) $Me_2SO/(Ac)_2O$, 25°C; (d) 1.06 equiv. of LDA then 1.2 equiv. of $(CH_3)_3SiCH_2CO_2Et$/-78°C for 15 min.,then 1.0 equiv. of **3**, warm to -10°C over 2 hrs.; (e) $Bu_4NF$, THF, 25°C; (f) LiOH, 4:1 MeOH/$H_2O$, 25°C; (g) p-$NO_2C_6H_4OH$, DCC, $CH_2Cl_2$, 50°C; (h) 5'-amino-5'-deoxyadenosine (p-TSA salt), $Et_3N$, DMF, 25°C; (i) $HgCl_2$, 4:1 $CH_3CN/H_2O$; (j) Dowex 1-X8 ion exchange resin ( $OH^-$), MeOH.

Removal[16] of the methylthiomethyl group followed by purification by flash chromatography (20% MeOH/$MeCl_2$) provided a 38% yield of **9a-d** as their p-TSA salts. Finally, exchange of the p-toluene sulfonate ion for hydroxide ion by stirring the salts of **9a-d** over ion exchange resin in methanol (Dowex 1X-8; $OH^-$ form) provided pure **9a-d**.

*Conclusion.* These methods can provide gram quantities of the analogs **9a-d**. The results described here illustrate a rationale for the design of conformationally restricted triphosphate mimics and, by extension, bis-substrate analogs. Conformational control assures that "enzyme-essential" volume will not be occupied by these mimics. The amide functional group is a practical choice for attaching phosphate mimics to adenosine and (presumably) other nucleosides.

The *in vitro* biological activities (if any) of these analogs are now being evaluated.[17] The discovery of specific protein binding interactions involving these prototypical analogs was not our major goal. The objectives of this study were to (1) explore allowed synthetic methodology within the context of phosphate analog synthesis and (2) demonstrate the idea of designing phosphate analogs based on analysis of the tertiary structure of the enzyme bound nucleotide or nucleotide-metal complex. The next stage of the project is to examine the structure of known active sites and to design and synthesize a kinase transition state analog which will be complementary to that site.

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