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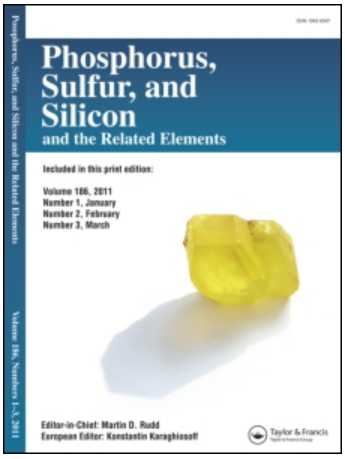

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
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To cite this Article Liu, Xiaohai , Zhang, Xiu-Rong and Blackburn, G. Michael(1999) '"Supercharged" Nucleotide Analogues Based On Halomethanetrisphosphonic Acids', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 541 — 544
To link to this Article: DOI: 10.1080/10426509908546301
URL: http://dx.doi.org/10.1080/10426509908546301

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Phosphorus, Sulfur and Silicon, 1999, Vol. 144-146, pp. 541-544
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Printed in Malaysia

"Supercharged" Nucleotide Analogues Based On Halomethanetrisphosphonic Acids

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Syntheses of two halomethanetrisphosphonic acids (**4b,c**) are described and pK_as for the second dissociation of the phosphonate moieties of three "supercharged" analogues of pyrophosphoric acid are given. New nucleotides based on these methanetrisphosphonic acids result from established Poulter and Moffatt-Khorana synthetic methodology leading to novel "supercharged" analogues of ADP and ATP.

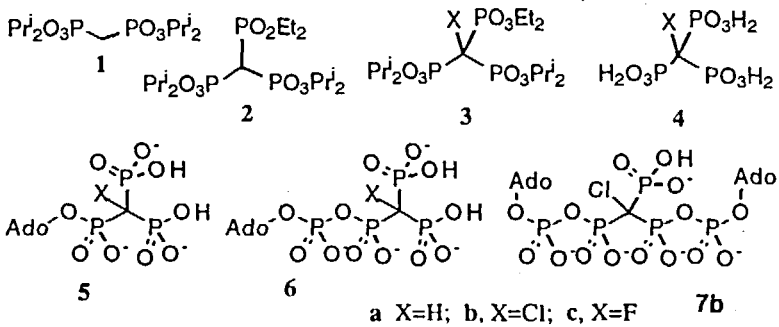
Keywords: methanetrisphosphonate; α-halotrisphosphonic acids; ADP analogs; ATP analogs

INTRODUCTION

Many of the functions of adenosine triphosphate, ATP, in cellular chemistry have been explored by nucleotide analogues containing phosphonate moieties stable to hydrolysis[1]. By contrast, the dinucleoside 5',5'''-polyphosphates, also components of all cells, have biological functions that are not yet well understood[2]. Wel[3] and others[4] have synthesised a variety of analogues of ATP and of diadenosine 5',5'''-polyphosphates made to resist specific or general enzymatic hydrolysis, of which some have shown promising therapeutic activity[5]. To access analogues of nucleotides with enhanced affinity for receptors[6] and better charge correlation with transition states for adenylate kinase and

other kinases[7], we have made new "supercharged" mimics of pyrophosphoric acid capable of introducing additional anionic charge relative to simple methylenebisphosphonates when built into ATP and Ap_4A analogs.

Methanetrisphosphonic acid **4** (X=H) and its α-chloro and α-fluoro derivatives fulfil this requirement because the introduction of a third ionizable phosphonate ($-PO_3H_2$) group into methylenebisphosphonic acid **1** (Pr^i=H) can deliver additional -ve charge at physiological pH. We have improved the published procedure[8] for the preparation of methanetrisphosphonate hexaesters to support syntheses of chloromethane- and fluoromethane-trisphosphonic acids[9].



a X=H; b, X=Cl; c, X=F

RESULTS

Tetraisopropyl methylenebisphosphonate **1** was reacted with 4 molar equivalents of diethyl chlorophosphite and sodium hexamethyldisilazane to drive the equilibrium towards product **2**. Intermediate **2** was unstable during acidic or basic work-up and readily decomposed to **1**. However oxidation of **2** with iodine in pyridine:THF:water gave **3a** in 72 % yield. Heating **3a** with TMSBr[10] in DCM overnight at reflux followed by solvolysis in the presence tri-*n*-butylamine gave methanetrisphosphonic acid as the tristri-*n*-butylammonium salt of acid **4a** in 98 % yield. The trisodium salt was obtained by precipitation from methanol solution using NaI solution in acetone.

Treatment of the methanetrisphosphonate ester **3a** with NaOCl solution[11] gave chloromethanetrisphosphonate ester **3b** in 97 % yield. Deesterification was achieved by heating with TMSBr and tributylamine in DCM overnight in 98 % yield. Perchloryl fluoride at -78 °C in the presence of NaHMDS converted **3a** into fluoromethanetrisphosphonate

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ester **3c** in 77 % yield. Fluoromethanetrisphosphonic acid **4c** was obtained quantitatively by deprotection of the hexaester under conditions as above.

pK_as of methanetrisphosphonic acid analogues[6] were measured under physiological conditions. All three methane-trisphosphonic acid analogues are clearly "supercharged" at pH 7.0 with methane-, chloromethane-, and fluoromethane-trisphosphonic acids having at least one more negative charge than pyrophosphate at pH 7.0 (Table). Note that α-carboxymethylenebisphosphonic acid[6] has a charge of 3.3 minus at pH 7.0.

TABLE Ionisation constants for polyphosphonic acids and nucleotide analogs determined at 3.5 < pH < 10.5 at 37 °C and 0.152 M NaCl.

Entry		pK_a4	pK_a5	pK_a6	Net charge (pH 7.0)
$O(PO_3H_2)_2$	(PP_i)	9.4	*6.6*†	-	2.72
$HO_2CCH(PO_3H_2)_2$	[9]	7.24	10.11	-	3.35
$HO_3SH(PO_3H_2)_2$	[9]	6.61	10.57	-	3.71
$HC(PO_3H_2)_3$	**4a**	6.46	9.90	-	3.77
$ClC(PO_3H_2)_3$	**4b**	5.92	9.08	-	3.92
$FlC(PO_3H_2)_3$	**4c**	5.77	8.86	-	3.95
AdoOPOP	(ADP)	*6.35*†		-	2.81
$AdoOPCHP_2$	**5a**	7.48	-	-	3.25
AdoOPCH(SO_3H)P		8.64			3.02
AdoOPOPOP	(ATP)	6.58	-	-	3.73
$AdoOPOPCHP_2$	**6a**	na	7.50	-	4.25
$AdoOPOPCClP_2$	**6b**	na	7.05	10.34	4.47
$AdoOPOPCFP_2$	**6c**	na	6.69	10.13	4.68
$(AdoOPOP)_2CClP$	**7b**	na	na	8.87	5.02

na - The strongly acidic dissociation constants were off-scale for measurement by titration. pH titration curves were deconvoluted for overlapping pK_a values using a programme written for an AppleMacintosh™ computer. †pK_a3. ΔpK_a error ± 0.05

We have previously described[6] the incorporation of the analog **4a** of pyrophosphoric acid into ATP analog **6a**. We here present data from the incorporation of **4a** into an analog of ADP and of **4a,b,c** into analogs of ATP along with the novel use of the C3 symmetry branch point of such methanetrisphosphonic acids to create new adenosine nucleotide analogs related to the transition state for adenylate kinase.

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Methanetrisphosphonic acid **4a** was readily incorporated into ADP analogs **5a** by Poulter's method[12] and acids **4a,b,c** into analogs of ATP **6a,b,c** by Khorana's phosphoromorpholidate method[13]. Both of the primary phosphonic acid functions in the analog **6b** provided sites for further adenylation by a modification to Khorana's procedure in the synthesis of the novel Ap_4A analog **7b**. pK_a values for these species (Table) clearly show the benefit of α-halogenation for increasing the acidity of the second dissociation constants of phosphonic acids. These "supercharged" analogs of ADP and ATP can have up to one additional negative charge at pH 7 and also have enhanced ligation potential for metal ions. Biological experiments using these analogs are in progress.

ACKNOWLEDGMENTS We gratefully acknowledge financial support for this work by the BBSRC (ROPA/MOLO4558).

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