Synthesis of three novel supercharged β,γ-methylene analogues of adenosine triphosphate

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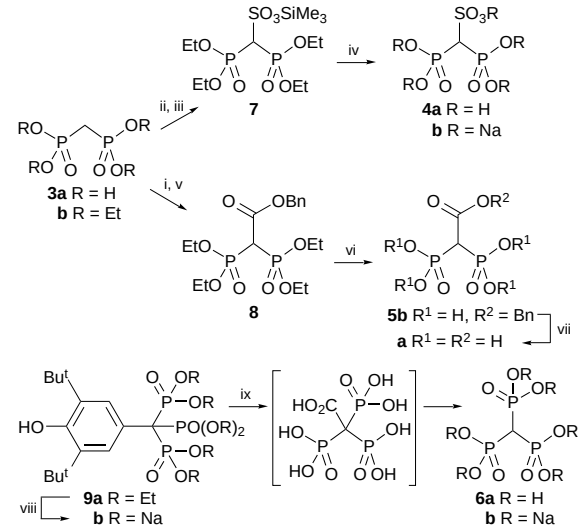
Three derivatives of methylenebisphosphonic acid containing an additional ionisable acidic function are incorporated into β,γ-bridged derivatives of adenosine triphosphate to give the first examples of supercharged nucleotide analogues 2a–c.

Adenosine triphosphate **1a** has multiple roles in cellular chemistry, among which its function as an energy source is predominant. However, a large variety of intra- and extracellular purinoceptors for adenine nucleotides is currently under intensive investigation and their affinities for nucleotides based on charge and metal ion-binding discrimination is likely to be of critical importance.[1] Also, the known higher affinity (*ca.* 1000-fold) of adenylate kinase for diadenosine 5′,5‴-pentaphosphate (K_d 40 nm) over the corresponding tetraphosphate[2] (K_d 20 μm) can be identified as arising from charge selectivity since the closed form of this enzyme has now been fully characterised in its stable ternary complex with AMP and β,γ-imino-ATP, which embraces six negative charges on phosphates in the reactive site without elongation of the polyphosphate structure.[3]

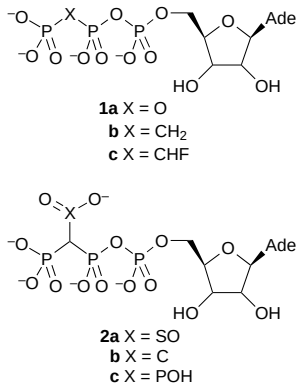
In previous studies, we have emphasised the significance of generating nucleotide analogues that are both isosteric and isopolar in character[4] with especial emphasis on the use of α-fluorophosphonates to ensure full ionisation of the phosphonate moiety.[5] We here describe supercharged analogues of nucleotides in which additional negative charge is provided without elongation of the polyphosphate chain. Such analogues **2** can be constructed by substituting the β,γ-methylene bridge of AMPPCP **1b** with an anionic function, *e.g.* SO_3^-, CO_2^-, or PO_3H^-. Such analogues will be especially important in the construction of bisubstrate analogues for kinases and related enzymes[2,6] where additional anionic charge cannot be accommodated within the phosphonate framework of simple isopolar nucleotide analogues.

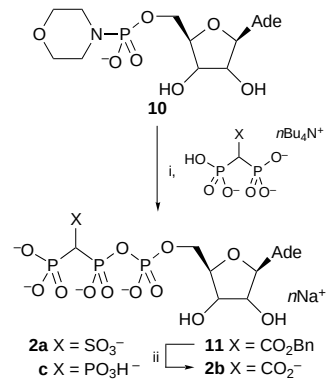
The preparation of such supercharged analogues of ATP and other nucleotides calls for convenient syntheses of derivatives of methylenebisphosphonic acid **3a** having an additional acidic OH function. We have identified 1,1-bis(phosphono)-methanesulfonic acid **4a**, 2,2-bis(phosphono)acetic acid **5a** and the known[7] methanetrisphosphonic acid **6a** as suitable polyanion equivalents to be incorporated into analogues of ATP and other nucleotides by standard methods. We here report syntheses of the first two of these bisphosphonates and an improved synthesis of **6a** along with their condensation with AMP to give three new supercharged analogues of ATP **2a-c**.

1,1-Bis(phosphono)methanesulfonic acid **4a** was prepared by trimethylsilylation[8] of tetraethyl methylenebisphosphonate **3b** and treatment of its anion with trimethylsilyl chlorosulfate in THF at room temperature. Without further purification, the product **7** was treated with an excess of bromotrimethylsilane in CH_2Cl_2 in the presence of triethylamine and solvolysed in MeOH to give the pentakis(triethylammonium) salt of 1,1-bis-(phosphono)methanesulfonic acid in 83% yield. This was converted into the free acid by ion exchange chromatography (Dowex-50, H^+) (Scheme 1).



Scheme 1 *Reagents and conditions*: i, LiBu, THF, −78 °C, then Me_3SiCl, −78 to 0 °C; ii, LiBu, THF, −78 °C; $Me_3SiOSO_2CF_3$, −30 °C; iii, Me_3SiOSO_2Cl; iv, Me_3SiBr, CH_2Cl_2, Et_3N; v, BnOCOCl, THF; vi, Me_3SiBr, CH_2Cl_2; vii, Pd/C, H_2, MeOH; viii, Me_3SiBr, CH_2Cl_2, then Na_2CO_3 (aq); ix, H_2O_2, F_3CCO_2H





Scheme 2 *Reagents and conditions*: i, pyridine, 20 °C, 12 h, camphorsulfonic acid; ii, Pd–$BaSO_4$, H_2O

Table 1 NMR and mass spectral data for ATP and various β,γ-bridged nucleotide analogues

Compound	^{31}P NMR data[a]					^{1}H NMR data (P_2CHX)[a]		HRMS data[b]	
	δ_α	δ_β	δ_γ	$^2J_{\alpha\beta}$	$^2J_{\beta\gamma}$	δ_H	$^2J_{PH}$	Found $[M + 1]^+$	Calc.
ATP[c] **1a**	−10.21	−20.7	−5.0	18.8	19.5	—	—	—	—
AMPPCHFP[c] **1c**	−10.48	+2.42	+8.58	29.0	15.0	5.26	13.3	—	—
AMPPC(S)P **2a**	−10.097	+1.788	+7.788	25.4	2.1	3.90	19.8, 21.6	695.8931	695.8908
AMPPC(C)P **2b**	−10.217	+4.589	+11.160	27.3	9.4	3.49	20.0	659.9249	659.9238
AMPPC(P)P **2c**	−9.415	+7.848	+13.225	26.3	8.8	2.85	21.3	695.9012	695.9003
AMPPCP[c] **1b**	−10.35	+14.36	+12.14	26.8	7.9	2.38	19.4	—	—

[a] NMR at 161.97 MHz (phosphorus) and 400 MHz (proton) in D_2O at pH 7.8. [b] Positive ion FAB-MS data for pentasodium salt $[M + 5Na - 4H]^+$. [c] Ref. 5.

Tetraethyl lithiotrimethylsilylmethylenebisphosphonate (*v.s.*) was reacted with benzyl chloroformate in anhydrous THF to give tetraethyl benzyloxycarbonylmethylenebisphosphonate **8** (43% yield). Treatment of ester **8** with bromotrimethylsilane[9] and methanolysis generated the free phosphonic acid **5b**, which was debenzylated by hydrogenolysis of its cyclohexylamine salt (Pd/C–H_2–MeOH) to give the tris(cyclohexyl)ammonium salt of **5a** (Scheme 1).

Hexaethyl (3,5-di-*tert*-butyl-4-hydroxyphenyl)methanetrisphosphonate[10] **9a** was transesterified with excess bromotrimethylsilane in CH_2Cl_2, the solvent evaporated and the residue treated with aqueous sodium carbonate, and the pH adjusted to 11 with sodium hydroxide. The sodium salt **9b** was precipitated by addition of MeOH and added to a mixture of hydrogen peroxide (8%, 30 volume strength) in trifluoroacetic acid and the reaction mixture quenched with EtOH and concentrated *in vacuo* to give the hexasodium salt of methanetrisphosphonic acid **6b**. This was converted into the free acid **6a** by ion exchange (Dowex-50, H^+) in 28% overall yield. It proved identical in spectroscopic properties to that described by Gross.[7] Evidently, the intermediate 2,2,2-trisphosphonoethanoic acid decarboxylates spontaneously.

The supercharged ATP analogues **2a**–**c** were prepared by condensation of the appropriate *n*-butylammonium salts of the free acids **4a**, **5b** and **6a** with adenosine 5′-phosphoromorpholidate[11] **10**. The free acids **4a**, **5b** and **6a** were treated with tetrabutylammonium hydroxide to pH 8.9, and the resulting tetrabutylammonium salts lyophilised repeatedly from anhydrous pyridine. The resulting dry salts were each reacted with **10** in anhydrous pyridine at room temperature to give the three nucleotide analogues as their tetrabutylammonium salts, purified by DEAE Sephadex chromatography (triethylammonium hydrogen carbonate gradient, pH 7.8). Promotion by 10-camphorsulfonic acid proved very effective for these condensations. The resulting triethylammonium salt products were dissolved in MeOH, from which their sodium salts were precipitated by addition of sodium iodide in propanone to give β,γ-sulfonatomethylene adenosine 5′-triphosphate **2a** (80% yield), β,γ-benzyloxycarbonylmethylene adenosine 5′-triphosphate **11** (75% yield) and β,γ-phosphonomethylene adenosine 5′-triphosphate **2c** (25% yield) as their sodium salts. Catalytic hydrogenolysis (Pd–$BaSO_4$–H_2) of **11** removed the benzyl ester function to give β,γ-carboxymethylene adenosine 5′-triphosphate **2b** as its pentasodium salt (61% yield) (Scheme 2).

All three ATP analogues **2a**–**c** were fully characterised by reverse phase high performance liquid chromatography (HPLC), high resolution MS, and ^{1}H and ^{31}P NMR (Table 1). Their phosphorus NMR spectra show AMX spectra for **2a** and **2b** and an AMX_2 spectrum for **2c.** The magnitude of the $^2J_{\alpha\beta}$ coupling constants is typical of ATP and its analogues while that for $^2J_{\beta\gamma}$ shows some variation, as is seen for other β,γ-bridged ATP species[5] (Table 1). The two supercharged analogues **2a**,**b** are necessarily mixtures of epimers with respect to the chiral centre in the β,γ-bridge though they are not resolved by HPLC (C-18 reverse phase MeCN–MeOH). The difference between the epimers is manifest most clearly in the ^{31}P chemical shift for P_α for sulfonic acid **2a** where the signals for P_α in the two isomers are separated by 0.03 ppm. For all of the other phosphorus resonances, this diastereoisomeric separation appears to be not significantly greater than the phosphorus NMR line width (approx. 0.01 ppm). The proton NMR of the adenosine moieties of **2a**–**c** were unexceptional, while the chemical shifts of the methine proton in the β,γ-bridge lie between those for AMPPCP and AMPPCHFP, directly reflecting the increasing electronegativity of the functions: $PO_3H^- < CO_2^- < SO_3^-$ (Table 1). Accordingly, we see a clear need to generate supercharged nucleotides with this methine proton replaced by fluorine and such syntheses are in progress.

The positive ion FAB-MS spectra show a homologous series of peaks of mass increment 22 and having correct accurate masses for $[M + xH^+ + yNa^+]$ where $(x + y = 1)$. The sulfonic acid **2a** showed three to six sodium ions, the carboxylic acid **2b** zero to five sodium ions, and the trisphosphonate **2c** from three to six sodium ions. These data clearly reflect the strong cation binding potential of these nucleotide analogues and data on metal ion and proton affinities will be described elsewhere.

The spectroscopic properties of β,γ-sulfonatomethylene adenosine 5′-triphosphate **2a** most closely approximate to those of β,γ-fluoromethylene adenosine 5′-triphosphate, while it has the added feature of an extra negative charge on the molecule. Syntheses of the corresponding analogues of ADP are in progress and the affinities of various enzymes and receptors for analogues **2a**–**c** are being determined.

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