Three New β,γ-Methylene Analogues of Adenosine Triphosphate

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Summary The preparation and physical properties of β,γ-dichloromethylene, -fluoromethylene, and -difluoromethylene analogues of adenosine triphosphate, ATP, are described.

ISOSTERIC analogues of nucleotides have proved valuable for the investigation of many enzyme-catalysed processes.[1,2] Particular success has been achieved with 5′-adenylyl imidobisphosphonate, AMPPNP,[3] and 5′-adenylyl methylenebisphosphonate, AMPPCP,[4] analogues of adenosine 5′-triphosphate, ATP. Neither of these analogues is ideal. The former suffers more rapid hydrolysis than ATP in acidic solution because of the lability of the P–N–P linkage.[2] The latter suffers from the general limitations common to phosphonate analogues of biological phosphates, which can be attributed largely to the exchange of an electronegative oxygen ligand for an electropositive methylene substituent at a phosphoryl centre.[5]

The second design feature of these compounds is to replace the β,γ-oxygen atom with a group of matchihg electronegativity and apicophilicity,[11] especially important for processes involving P^{γ}–O bond cleavage in ATP.[5] A useful indication of the appropriate response of the β- and γ-phosphorus atoms to the increased group electronegativities for CCl_2, CHF, and CF_2 functions is seen in the upfield change in the ^{31}P resonances for P^{β} and P^{γ} (Table), though it must be appreciated that there is no simple correlation to be made.[10]

The n.m.r. data also show that there is an unusual degree of electronic interaction between the β- and γ-phosphorus atoms in the difluoromethylene analogue (**3c**)

TABLE. ^{31}P N.m.r. and pK_a data for ATP and some analogues.[10]

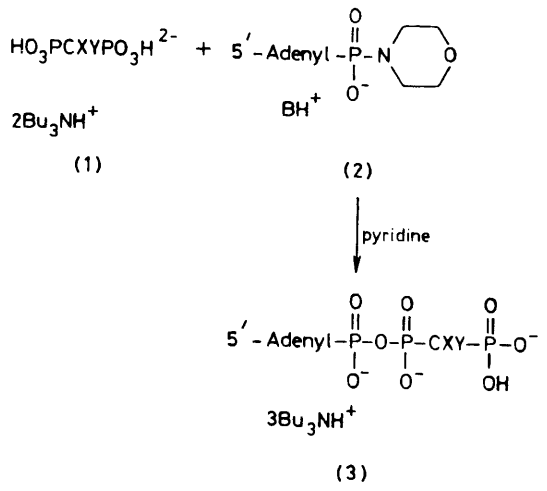
Compound	pK_a[4]	$\delta\alpha$	$\delta\beta$	$\delta\gamma$	$^2J_{\alpha\beta}$	$^2J_{\beta\gamma}$
AMPPCP	8·4	−10·35	+14·37	+12·14	26·0	8·5
AMPPNP	7·7	−9·66	−6·19	+0·32	20·75	3·67
$AMPPCCl_2P$ (**3a**)	7·0	−10·16	+2·45	+8·55	30·5	17·7
AMPPCHFP (**3b**)	7·4	−10·48	+2·42	+8·58	28	16
$AMPPCF_2P$ (**3c**)	6·7	−10·35	−2·20	+4·20	32	57
ATP	7·1	−10·21	−20·74	−4·99	18·9	19·5

^{31}P N.m.r. data obtained at 161·9 MHz in H_2O solution at pH 10 using a Brucker WH400 instrument. Chemical shifts are quoted relative to external 85% phosphoric acid in p.p.m., downfield shifts positive, coupling constants in Hz.

It thus appeared that a better ATP analogue should have a stable P–C–P linkage in place of the scissile P^{β}–O–P^{γ} unit and should be at once an *isosteric* and an *isopolar* analogue of the prototype. Several lines of analysis suggest that this can be achieved by the use of α-halogenomethylenephosphonates,[5] especially employing α-fluorination of phosphonates.[6] We here report the synthesis and certain physical characteristics of three ATP analogues in which the β- and γ-phosphorus atoms are linked by dichloromethylene, fluoromethylene, and difluoromethylene groups respectively.

Dichloromethylenebisphosphonic acid,[7] fluoromethylenebisphosphonic acid,[8] and difluoromethylenebisphosphonic acid[8] were separately converted into their bistributylammonium salts (**1a**, **b**, **c** respectively) and brought into reaction in anhydrous pyridine with adenosine 5′-monophosphoromorpholidate, as its 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt (**2**),[9] in the usual way (Scheme).[4,9] The nucleotide products were purified as their bistrialkylammonium salts (**3a**—**c**) by means of ion-exchange and gel filtration chromatography and characterised fully by elemental and spectroscopic analysis.

The first objective of halogen-substitution in the β,γ-methylene group is to approximate the fourth phosphate ionisation constant from its depressed value in AMPPCP close to that of ATP. As the data show (Table) this is nicely achieved for the dichloromethylene (**3a**) and the fluoromethylene (**3b**) analogues while the difluoromethylene analogue (**3c**) appears to be even more acidic than ATP.



SCHEME

a, X = Y = Cl; **b**, X = F, Y = H; **c**, X = Y = F.
BH^+ = 4-Morpholine-*N*,*N*′-dicyclohexylcarboxamidinium

as shown by the large value of 57 Hz for the $^2J_{\beta\gamma}$ coupling constant, which is unusual for two phosphorus atoms joined by a saturated carbon bridge.[12]

Lastly, the ^{19}F n.m.r. spectra show that the two fluorine environments are almost magnetically equivalent both in the difluoromethylene compound (**3c**) (δ −118·6 p.p.m.)†

† ^{19}F Chemical shifts relative to external $CFCl_3$.

and in the mixture of diastereoisomeric monofluoromethylene compounds (**3b**) (δ −218·2 p.p.m.). It is expected that this feature may prove advantageous in the investigation of the interaction of these ATP analogues with enzymes, now in progress.

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