Pergamon

Tetrahedron Letters, Vol. 36, No. 44, pp. 8119-8122, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01691-0

Synthesis of Adenosine Cyclic 3',5'-Phosphorofluoridate (cAMP-F)

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Abstract: Work aimed at the chiral synthesis of both diastereomers of adenosine cyclic 3',5'-phosphorofluoridate, cAMP-F **7**, is described. Attempted debenzoylation of the intermediate $N^6,N^6,O^{2'}$-tribenzoyladenosine cyclic 3',5'-phosphorofluoridate **4** by ammonolysis resulted in cleavage of the P-F bond. Reaction of [Sp]-*S*-methyl adenosine 3',5'-cyclophosphorothioate **6** with AgF gives a mixture of the two diastereoisomers of the cyclic phosphorofluoridate **7** along with adenosine 2',3'-cyclic phosphate **8**. The conversion of **7** into **8** can be completed under remarkably mild conditions. Possible mechanisms for this unusual transformation are discussed.

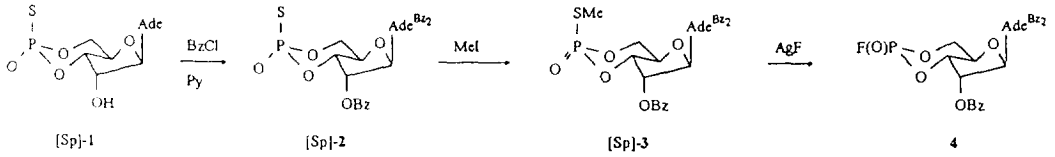
cAMP plays an important function as a signal molecule in hormone action and cell communication. Among many cAMP-dependent reactions, one most interesting observation has resulted from studies on the cAMP-induced activation of protein kinase. It is assumed that cAMP interacts with its receptor protein *via* electrostatic forces, such as ionic interactions, hydrogen bonds, and hydrophobic interactions.[1] Until recently, out of more than 600 derivatives of cAMP, only R_P-cAMPS and $cAMPS_2$ appeared to be antagonists of intracellular cAMP-dependent protein kinase. [Rp]-cAMPS blocks the activation of this enzyme, inactivates glycogen synthesis, and leads to decreased glycogenolysis.

The incorporation of fluorine into biomolecules has frequently resulted in changed biological properties.[2] Since both diastereomers of the phosphorofluoridate analogue of cAMP would be nonionic species, it was challenging to prepare adenosine cyclic 3',5'-phosphorofluoridate (cAMP-F) and to study its interactions with protein kinase. To date, cAMP-F has not been described in the chemical literature. As key substrates for the preparation of cAMP-F we decided to use both diastereomers of adenosine cyclic 3',5'-phosphorothioate (cAMPS, **1**) which we prepared in a stereocontrolled manner several years ago.[3] Taking into account the strong phosphophilic affinity of fluoride anion , we based our strategy on the conversion of the negatively charged phosphorothioate diester group in **1** into the neutral phosphorothiolate triester **2**, facilitating the substitution of thioalkyl group in **2** by silver fluoride. Another reason for the choice of cAMPS diastereomers as substrates is the expected stereospecificity of the PS→PF conversion; the availability of both pure diastereomers of cAMPS could lead to the stereospecific preparation of the [Rp]- and [Sp]- cAMP-F diastereomers.

Since the cAMPS diastereomers are poorly soluble in organic solvents, the 2'-hydroxy and N^6 amino groups were protected with benzoyl groups to enhance solubility. Then a benzene solution of [Sp]-$N^6,N^6,O^{2'}$-tribenzoyladenosine cyclic-3',5'-phosphorothioate (**2**, ^{31}P NMR 58.2 ppm in $CDCl_3$) was treated for 3 h with methyl iodide (1.2 molar equiv.). After solvent evaporation, crude [Sp]-$N^6,N^6,O^{2'}$-tribenzoyladenosine

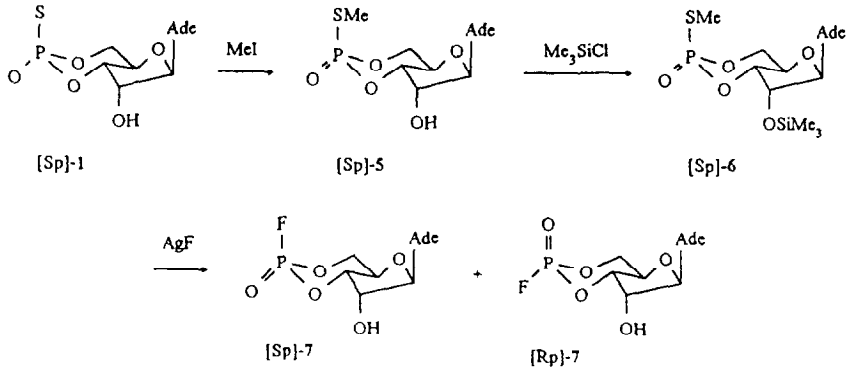
cyclic-3',5'-(*S*-methyl) phosphorothioate (**3**, ^{31}P NMR 25.0 ppm in C_6D_6) was dissolved in dry acetonitrile and then 25% aqueous solution[4] of AgF (1.2 molar equiv.) was added (Scheme 1).

SCHEME 1



The ^{31}P nmr spectrum of this reaction mixture, recorded after 3 h stirring at room temperature, showed loss of the signal corresponding to substrate **3**, replaced by a doublet at -7.20 ppm, with direct spin-spin coupling constant $^1J_{PF}$=925 Hz.[5] Evaporation of solvent left a solid whose analysis by FAB-MS confirmed the elemental composition of N^6,N^6,$O^{2'}$-tribenzoyladenosine cyclic-3',5'-phosphorofluoridate [**4**, cAMP$^{Bz\text{-}3}$-F, FAB$^-$ MS 642 (M-1)]. However, attempts at removal of the benzoyl protecting groups from **4** by treatment with concentrated aqueous ammonia led to rapid hydrolysis of the phosphorofluoridate. The ^{31}P NMR spectrum of the reaction mixture recorded 30 min. after introduction of conc. aq. ammonia to a solution of **4** (a time inadequate for removal of benzoyl groups)[6] showed only traces of phosphorofluoridate while an unexpected signal resonating at 19.4 ppm had appeared. The appearance of a singlet excluded the presence of a direct P-F bond in this obscure product. Such a course of events forced us to modify the synthetic route (Scheme 1) to avoid final deprotection with ammonia. Therefore, we alkylated [Sp]-cAMPS (**1**) by means of CH_3I (1.2 M equiv., 3 h) in benzene-methanol (9:1) solution. Subsequently, we protected the 2'-hydroxy function of **5** (^{31}P NMR 24.1 ppm in CD_3CN) using Me_3SiCl/pyridine (Scheme 2).

SCHEME 2



After removal of the solvent, the crude **6** (^{31}PNMR 24.5 ppm in CD_3CN) was dissolved in acetonitrile/pyridine (9:1) and then AgF (1.2 molar equiv.) was added. The reaction was maintained for 3 h with stirring at room temperature. A yellow unidentified precipitate was filtered off and the ^{31}P NMR

spectrum of the supernatant was recorded (Figure 1).

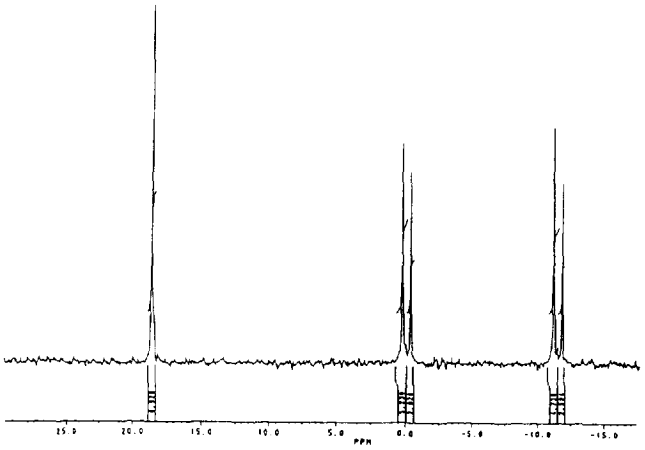


Fig. 1. ^{31}P NMR spectrum of reaction mixture between [Sp]-**6** and AgF

Two doublets corresponding to the two diastereomers of **7** and resonating at -6.0 and -6.1 ppm, $^{1}J_{PF}$ 929 and 932 Hz (in C_6D_5N, ratio 2:1, respectively), indicated that the process of replacement of the thioalkyl group by fluoride is not stereospecific, since pure [Sp]-**1** was used as the substrate. One reason for P-epimerisation[7] might result from the presence of pyridine added to the reaction medium for enhancement of the solubility of substrate **6**. It should be mentioned that, according to the synthetic strategy presented in Scheme 2, we expected that AgF could perform a double function: replacement of -SMe ligand at phosphorus by fluoride and also removal of the protecting trimethylsilyl group from the 2'-hydroxy position. Surprisingly, the ^{31}P NMR spectrum revealed an additional slow formation of a compound resonating at 19.4 ppm. After evaporation of solvents under reduced pressure, part of the crude reaction mixture was dissolved in a mixture of CD_3CN and triethylammonium bicarbonate buffer (pH=7, 1:1 v/v). The slow disappearance of doublets corresponding to **7** was observed, accompanied by increase of intensity of signal at 19.4 ppm. A second portion of crude **7** was dissolved in CD_3CN/triethylammonium bicarbonate buffer (pH=9). ^{31}P NMR assay indicated that after 30 min only traces of **7** were present and the predominant product showed ^{31}P resonance at 19.4 ppm. Addition of the solution containing crude **7** to CD_3CN/ammonium acetate buffer (pH=4) slows down the process responsible for P-F bond disappearance. Even so, after 20 h no traces of compounds containing a P-F bond could be detected.

The product resonating at 19.4 ppm was isolated and analyzed by means of ^{1}H NMR and was identified as adenosine cyclic-2',3'-phosphate (**8**) by comparison with a genuine sample. Interestingly, during our earlier studies on the reaction of adenosine cyclic-3',5'-phosphorothioates with styrene oxide, the same cyclic-2',3'-phosphate had been identified as the major product, while a similar reaction between thymidine cyclic-3',5'-phosphorothioate and styrene oxide led to expected thymidine cyclic-3',5'-phosphate.[8] The lack of stability of monoalkylphosphoromonofluoridates in presence of a vicinal hydroxyl group in ribonucleosides has been reported in elegant studies of Sund and Chattopadhyaya.[9] We therefore suggest that the low hydrolytic stability of **7** and the conversion **7**→**8** is caused by the presence of a 2'-unprotected hydroxyl function. In order to check this hypothesis, we prepared 2'-deoxycytidine cyclic-3',5'-phosphorofluoridate (**9**) and monitored its stability in aqueous solution. We have used a diastereomeric mixture of 2'-deoxycytidine cyclic-3',5'-phosphorothioates (**10**, [Sp]:[Rp]=2:1).[1] Using the process depicted in Scheme 1, only a single

diastereomer of 2'-deoxycytidine cyclic 3',5'-phosphorofluoridate [**9**, ^{31}P NMR -7.9 ppm, $^{1}J_{PF}$ 950 Hz, in $CDCl_3$, FAB^- MS 306 (M-1)] was obtained. Crude **9** was dissolved in D_2O and conc. ammonia added. ^{31}P NMR spectra, recorded after 24 h show that this product does not undergo hydrolytic decomposition. This result provides experimental evidence that the easy hydrolysis of **7** involves some type of anchimeric assistance from the 2'-hydroxyl group of the ribonucleoside. Because our proposed enzymatic studies using **7** would require it to have considerable stability in aqueous solution, further studies on the synthesis, conformational and configurational analysis of **7** have been set aside for the time being.

The mechanism of the conversion of **7** into **8** remains obscure. Direct nucleophilic participation of the 2'-hydroxyl group to displace fluoride ion from phosphorus might be deemed to proceed *via* a tricyclic [6.5.5] system to give a 2',3',5'-bicyclic AMP intermediate. This would call for a bridgehead phosphorus and a *trans* ring junction between the two five membered rings. To test this hypothesis, we have computed the strain energy of the putative 2',3',5'-tri-*O*-adenosine bicyclic tetraoxyfluorophosphorane by means of "Sybyl".[10] The structure minimised after 10^4 iterations and has a net energy of 93.3 kcal mol^{-1}, which is comprised of 82.1 kcal bond angle strain plus 14.7 kcal torsion strain, offset by -3.5 kcal for favourable van der Waals interactions. The P-O bond lengths in the pentaco-ordinate species are all close to 1.6 Å and its geometry is much closer to that of a square planar pyramid than to a trigonal bipyramid. That result is independent of the relative location of the F and O^- ligands at phosphorus. We therefore believe that this species is most unlikely to participate as an intermediate in the transformation **7**→**8**. A simpler, alternative process might involve S_N2 cleavage of the 5'-C-O-(P) bond by water, aided by hydrogen bond participation of the 2'-OH group. The resulting adenosine 3'-*O*-phosphorofluoridate **11** would be expected to show rapid ring closure to give **8** with loss of HF. However, we should not at this stage rule out the possibility that the marked difference in the stability between **7** and its deoxycytidine counterpart could be a consequence either of participation of the adenine ring or of a conformational difference between the ribo- and deoxyribo-furanose rings. Further studies designed to elucidate this mechanism are in progress.

Acknowledgments: This project was financially assisted by The State Committee of Scientific Research, Grant No.500-02-01-5 (to J.B.) and a British Council travel grant (to G.M.B.).

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(*Received in UK* 27 *July* 1995; *revised* 5 *September* 1995; *accepted* 8 *September* 1995)