STEREOSPECIFIC SYNTHESIS OF ADENOSINE CYCLIC 3',5'-R_P- AND S_P-PHOSPHORO-[^{35}S]THIOATES

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SUMMARY

Stereospecific reaction of both diastereoisomers of $O^{2'},N^6,N^6$-tribenzoyladenosine cyclic 3',5'-phosphoranilidates (1) with sodium hydride and carbon [^{35}S]disulphide gave, after removal of the protective groups, the desired R_P- and S_P-diastereoisomers of [^{35}S]cAMPS (2).

Key Words: Sulphur-35, Nucleoside Cyclic 3',5'-phosphorothioates, Diastereoisomers, Stereospecific synthesis, Absolute configuration.

INTRODUCTION

The stereospecific enzymatic synthesis of both diastereoisomers of adenosine 5'-(α-thio)triphosphates (ATPαS)[1,2], assignment of the absolute configuration at phosphorus [3,4] and chemical stereospecific synthesis of R_P- and S_P-diastereoisomers of adenosine cyclic 3',5'-phosphorothioates (2, cAMPS)[5] allowed to perform the studies on the mechanism of action of bacterial[6] and mammalian[7] adenylate cyclases. Besides these enzymes, cAMP-phosphodiesterases from beef-heart[8,9] and baker's yeast[9] and cAMP-dependent protein kinases[10,11] were also studied by means of cAMPS diastereoisomers.

In this paper we present the synthesis of diastereoisomers of cAMPS of known configuration labelled with [^{35}S]. We believe that an easy access to [^{35}S]cAMPS diastereoisomers will broaden the scope of further applications of

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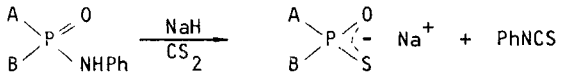
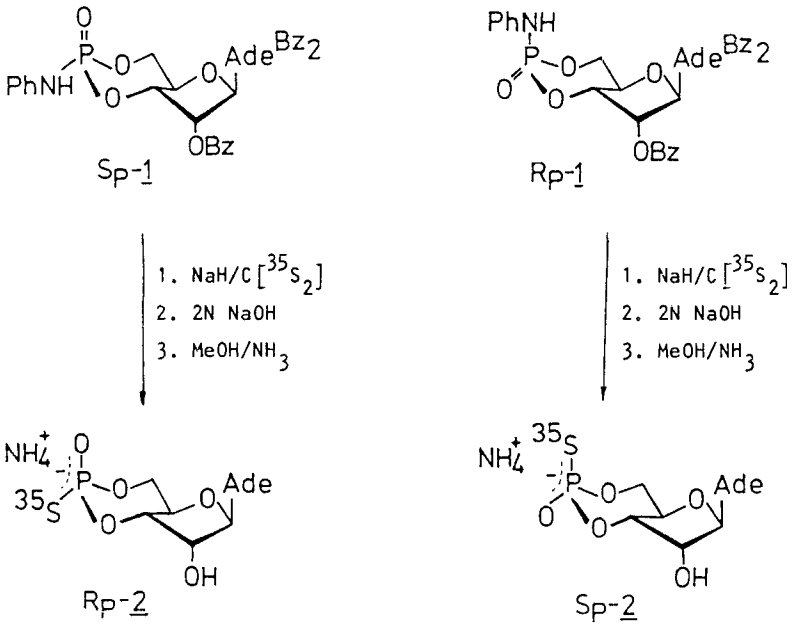
the phosphorothioate analogues of cAMP in the studies on the role of this last compound in the regulation of cellular mechanisms [12]. The procedure presented in this work follows that reported earlier for the synthesis of unlabelled cAMPS diastereoisomers [5] with few exceptions: i/ the scale of experiment is much lower, ii/ the excess of $C[^{35}S_2]$ is much lower than that used in the preparation of unlabelled cAMPS. The starting compounds, $O^{2'},N^6,N^6$-tribenzoyladenosine cyclic R_p- and S_p-phosphoranilidates (1) were obtained according to the method described earlier [5,13]. It should be pointed out, that according to the equation describing the stoichiometry of the Wadsworth-Emmons Reaction[14]



as a second product radioactive $PhNC[^{35}S]$ was obtained.

The overall procedure is depicted in Scheme 1.

SCHEME 1.



EXPERIMENTAL

Into the solution of R_P-1 (13.9 mg, 19.5 μmol) in dimethoxyethane (0.4 mL, dried over $LiAlH_4$) sodium hydride (2.4 mg, 50% dispersion in mineral oil) was added and this mixture was stirred with magnetic stirrer under atmosphere of dry argon for 2 hr at room temperature. Then carbon [^{35}S]disulphide (2 μL, 33 μmol, 17.5 MBq/mmol [15]) was added and stirring of reaction mixture under argon was continued for 5 hr. Then unlabelled CS_2 (4 μL) was added into reaction mixture and stirring was continued for further 2 hr. An excess of C[$^{35}S_2$] was removed by distillation and into the reaction mixture pentane (0.75 mL) was added. The solid precipitate was filtered off and dissolved in ethanol (0.2 mL). To this solution water (30 μL) followed by 2N NaOH (15 μL) were added. After 5 min small amount of Dowex 50Wx8 pyridinium form was added, ion-exchange resin was filtered off and solvents were removed under reduced pressure. The solid residue was dissolved in methanol saturated with ammonia (3 mL) and this solution was left for 24 hr at room temperature. After this period of time the solution was concentrated under reduced pressure to the volume ca. 0.2 mL and the product was isolated on preparative TLC (Kiesegel 60G, 0.25 mm, developing system i-PrOH-aqNH_3-H_2O, 7:1:1). The product S_P-2 was extracted from silica gel with MeOH. Ammonium adenosine cyclic 3',5'-S_P-phosphoro[^{35}S]thioate (S_P-2) was obtained in 75% yield (5.3 mg, 8.1 MBq/mmol, radiochemical purity 99.5% [17]). Radiochemical yield, as calculated on the starting carbon[^{35}S]disulphide, was 20.8%. This product according to TLC, was identical with genuine sample of unlabelled S_P-cAMPS [5] (Kieselgel 60G, 0.25 mm, developing system as above, R_f 0.57). Analogous procedure, applied to S_P-1 (10.5 mg, 14.7 μmol) gave R_P-2 in 70% yield (3.5 mg, 8.4 MBq/mmol, radiochemical purity 99.2%, radiochemical yield 15%). Chromatographic mobility of R_P-cAMPS under conditions specified *vide supra* is identical with that of S_P-cAMPS [5].

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