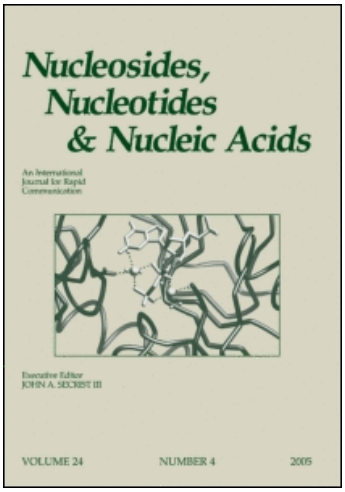

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SYNTHESIS OF 2',5'-DIDEOXY-ADENOSINE-3'-MONOPHOSPHATE DERIVATIVES AS ALLOSTERIC INHIBITORS OF ADENYLYL CYCLASE.

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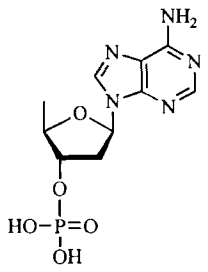
Abstract : The synthesis of a fluorescent and a lipophilic conjugate of 2',5'-dideoxy-3'-AMP, an allosteric inhibitor of adenylyl cyclase, has been devised.

Adenosine-3':5'-cyclic monophosphate (cAMP) is the second messenger involved in signal transduction for numerous neurotransmitters and hormones. The cAMP pathway can be regulated pharmacologically by many drugs that are of particular value in the treatment of many diseases, and therefore there is much current interest in identifying new agents acting on this pathway. Regulation of this pathway can be achieved through changes in the activities of cAMP-phosphodiesterases[1], cAMP-dependent protein kinases[2], or adenylyl cyclases. Adenylyl cyclases are a family of enzymes that catalyze the formation of cAMP from adenosine-5'-triphosphate (ATP). Whereas numerous drugs have been developed that act on the cyclic nucleotide phosphodiesterases, those that act directly on adenylyl cyclases have been less well explored. Adenylyl cyclases is potently and directly inhibited by analogs of adenosine, via an allosteric domain referred to as the "P"-site.[3] 2',5'-Dideoxy-3'-AMP is by far the most potent inhibitor of the "P"-site known at the present time (IC_{50}= 0.1 μM).[4] This paper describes the synthesis of a fluorescent and a lipophilic conjugate of 2',5'-dideoxy-3'-AMP as tools for the study of the structure of the "P"-site and its physiological relevance.



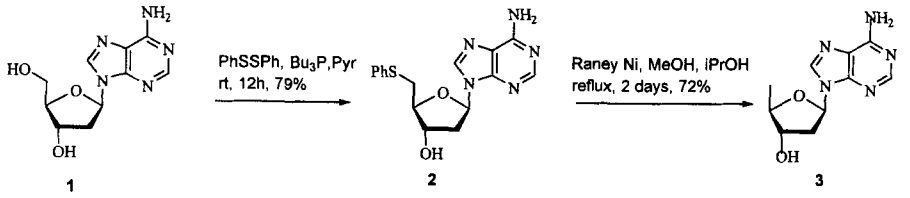
2',5'-Dideoxy-3'-AMP

2',5'-Dideoxyadenosine **3** is classically prepared by chlorination of 2'-deoxyadenosine **1** with thionyl chloride in hexamethylphosphoramide[5] followed by dechlorination with tributyltin hydride.[6] As we needed the nucleoside **3** in large quantities and as we wanted to avoid the use of the toxic reagents and solvent of the previous method, we prepared this compound by another route. 2'-Deoxyadenosine **1** was converted to the 5'-phenylthio derivative **2** by modification of a known procedure.[7] Desulfurization of **2** by Raney nickel[8] afforded 2',5'-dideoxyadenosine **3** in 72% yield (Scheme I).

The fluorescent derivative **6** has been prepared as a probe for the structural study of the "P"-site of adenylyl cyclase. Resonance energy transfer can be used to estimate the distance between this probe and tryptophan residues of the enzyme. Its synthesis was achieved through the one-pot formation of phosphotriester **5** via the chemoselective O-phosphorylation of N-unprotected 2',5'-dideoxyadenosine **3** using the methodology of the alcohol activation developed by Noyori and coll.[9] (Scheme II).

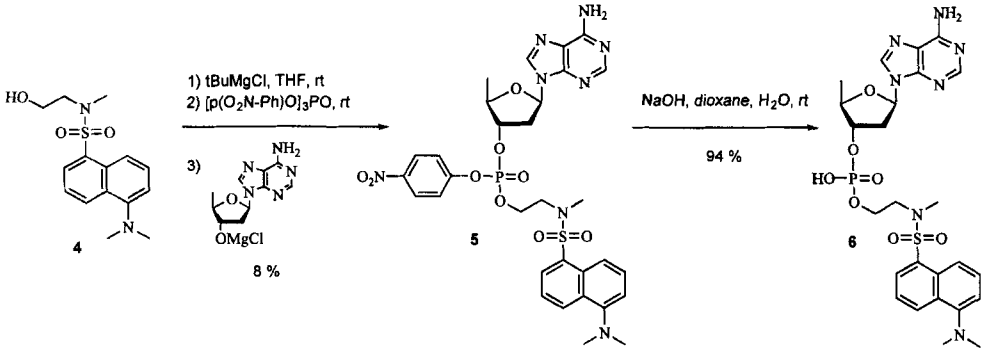
N-methyl-2-aminoethanol was treated with dansyl chloride[10] and the resulting alcohol **4** was sequentially treated with equimolar amounts of *tert*-butylmagnesium chloride, tris(*p*-nitrophenyl) phosphate, and the alkoxide of **3**. The phosphotriester **5** was deprotected by alkaline hydrolysis.[11] The fluorescent probe **6** exhibited a excitation maximum at 240 nm in ethanol and an emission maximum at 510 nm. This fluorescence is almost totally quenched in aqueous medium. This medium dependance can be used to study the environment of the "P"-site. The transfer of fluorescence of this probe with the tryptophan residues of the adenylyl cyclase will be studied and reported elsewhere. When tested for the inhibition of rat brain adenylyl cyclase it exhibited an IC_{50}= 3 μM. The potency of this inhibition will allow the use of **6** in fluorescence studies of the enzyme.

One of the major problems encountered in the use of nucleotides or oligonucleotides on intact cells is the low permeability of cell membranes to such charged molecules. The increase in cellular uptake of this class of compounds by their conjugation with cholesterol is well known.[12-15] The preparation of the cholesteryl conjugate **8** was similar to that of the fluorescent probe **6** (Scheme III). The phosphotriester **7** was obtained in 54 % yield by a one-pot reaction of the nucleotide **3** with one equivalent of *tert*-butylmagnesium chloride and subsequently with one equivalent of magnesium chloride cholesterate. The phosphotriester **7** was deprotected by alkaline hydrolysis.[11]



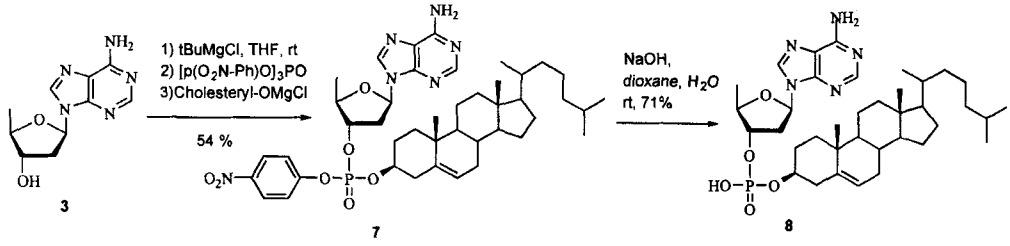
1 2 3

Scheme I



4 5 6

Scheme II



3 7 8

Scheme III

The cholesteryl conjugate **8** is being studied as a potential prodrug in studies of "P"-site-induced differentiation in cultured preadipocytes.[16] Results from those experiments will be reported elsewhere.

EXPERIMENTAL SECTION

5'-(Phenylthio)-2'-deoxyadenosine (2).

To a suspension of 2'-deoxyadenosine (**1**) (30.14 g, 120 mmol) in 350 ml of pyridine were added diphenyl disulphide (32.75 g, 150 mmol) and tri-*n*-butylphosphine (37.5 ml, 150 mmol). The reaction mixture was allowed to stir overnight at room temperature. Methanol (200 ml) was added, the mixture was stirred for 15 min and then concentrated in vacuo. The residue was washed with hexane and then with toluene. The precipitate was recrystallized from water to give the title compound **2** (32.54 g, 79%).

^{1}H NMR (DMSO d6) δ 2.26-2.35 (m, 1H, H-2'α); 2.92-3.02 (m, 1H, H-2'β); 3.22-3.42 (m, 3H, H-4' and H-5'); 3.97 (m, 1H, H-3'); 5.48 (d, 1H, 3'-OH); 6.37 (t, 1H, H-1'); 7.13-7.41 (s, 7H, NH_2 and H-Ph); 8.17 (s, 1H, H-2); 8.34 (s, 1H, H-8).

2',5'-Dideoxyadenosine (3).

The sulfide **2** (6.87 g, 20 mmol), dissolved in a mixture of methanol/isopropanol (1/1) (60 ml), was stirred under reflux in the presence of Raney nickel (10 g) for 2 days. The organic solvent was decanted and the catalyst was washed with two portions of hot methanol (200 ml). The solvent of the combined organic extracts was evaporated in vacuo to give the crude product which was recrystallized in water affording **3** (3.39 g, 72%).

^{1}H NMR ($CDCl_3$) δ 1.25 (s, 3H, H-5'); 2.20-2.35 (m, 1H, H-2'α); 2.81-2.86 (m, 1H, H-2'β); 3.90 (m, 1H, H-4'); 4.20 (m, 1H, H-3'); 5.32 (d, 1H, 3'-OH); 6.28 (m, 1H, H-1'); 7.27 (s, 2H, NH_2); 8.15 (s, 1H, H-2); 8.30 (s, 1H, H-8).

N-(5-dimethylamino-1-naphthalenesulfonamido)-N-methyl-2-ethanolamine (4).

A solution of dansyl chloride (1.08 g, 4 mmol) in acetone (60 ml) was added to a solution of N-methyl-2-ethanolamine (0.30 g, 4 mmol) in 2% $NaHCO_3$ solution (40 ml) and stirred overnight at room temperature. Acetone was removed in vacuo and the medium was extracted twice with 30 ml of EtOAc. The combined organic extracts were washed with

brine and dried over $MgSO_4$. Removal of solvent in vacuo afforded **4** as a yellow-green fluorescent paste (1.20 g, 97%).

^1H NMR ($CDCl_3$) δ 2.32 (t, 1H, OH), 2.87 (s, 6H, $N(CH_3)_2$); 2.93 (s, 3H, $SO_2N(CH_3)$; 3.33 (t, 2H, CH_2); 3.73 (m, 2H, CH_2); 7.17 (d, 1H); 7.53 (q, 2H); 8.16 (d, 1H); 8.33 (d, 1H); 8.55 (d, 1H).

4-Nitrophenyl[N-(5-dimethylamino-1-naphthalenesulfonamido)-N-methyl]-2-aminoethyl 2', 5'-dideoxyadenosine 3'-phosphate (5).

To a solution of the dansyl derivative **4** (0.93 g, 3 mmol) in THF (30 ml) at 15°C was added dropwise a 1.0 M solution of *tert*-butyl magnesium chloride (3 ml, 3 mmol) in THF. After stirring for 5 min, tris-(*p*-nitrophenyl) phosphate (1.38 g, 3 mmol) was added and stirring was continued for 90 min under argon. The resulting solution was subjected directly to the next step (one-pot linkage of the nucleoside) without further treatment. A 1.0 M solution of *tert*-butyl magnesium chloride (3 ml, 3 mmol) in THF was added dropwise to a suspension of 2',5'-dideoxyadenosine **3** (0.71 g, 3 mmol) in THF (50 ml). After stirring 5 min, the solution of the phosphorylated adduct, prepared as above, was added dropwise to this mixture in 30 min. The mixture was stirred at room temperature for 4 h. It was then concentrated in vacuo, 50 ml of water was added, and this solution was extracted 3 times with 30 ml of EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a gum which was subjected to flash chromatography on silica gel (EtOAc/EtOH, 90/10), affording the protected adduct **5** (0.18 g, 8%).

^1H NMR ($CDCl_3$) δ 1.43 (d, 3H, H-5'); 2.65-2.85 (m, 1H, H-2'α); 2.85 (s, 6H, $N(CH_3)_2$); 2.86 (s, 3H, $SO_2N(CH_3)$), 3.1-3.3 (m, 1H, H-2'β); 3.59 (t, 2H, N-CH_2); 4.25-4.5 (m, 3H, CH_2-O-P and H-4'); 5.17 (m, 1H, H-3'); 6.33 (m, 1H, H-1'); 7.16 (d, 1H, H-dansyl); 7.25 (s, 2H, NH_2); 7.41 (d, 2H, H-Ph-NO_2); 7.48-7.56 (m, 2H, H-dansyl); 7.96 (s, 1H, H-2); 8.12 (m, 1H, H-dansyl); 8.15- 8.31 (m, 4H, H-dansyl, H-Ph-NO_2 and H-8).

Triethylammonium [N-(5-dimethylamino-1-naphthalenesulfonamido)-N-methyl]-2-aminoethyl-2',5'-dideoxyadenosine 3'-phosphate (6).

To a solution of the phosphate triester **5** (0.11 g, 0.15 mmol) in dioxane (4 ml) was added a 1 M solution of NaOH (0.4 ml). The reaction mixture was allowed to stir overnight at

room temperature. Then it was concentrated in vacuo, diluted with 50 ml of water, neutralized with a 1 M solution of HCl, washed twice with EtOAc (20 ml), and subjected to DEAE-Sephadex column chromatography eluting with a 0.01-1 M triethylammonium bicarbonate linear gradient. The appropriate fractions were pooled and desalted by repeated evaporations of portions of MeOH under vacuum to afford 0.10 g (94 %) of **6** as a green glass. No impurities were noted in **6** on ion exchange HPLC.

^{1}H NMR ($CDCl_3$) δ 1.17-1.39 (m, 12H, H-Et_3N and H-5'); 2.70-3.10 (m, 17H, H-2', $N(CH_3)_2$, $SO_2N(CH_3)$, and H-Et_3N); 3.48 (t, 2H, N-CH_2); 4.03-4.10 (m, 2H, CH_2-O-P); 4.30 (m, 1H, H-4'); 4.69 (m, 1H, H-3'); 6.31-6.37 (m, 3H, H-1' and NH_2); 7.13 (d, 1H, H-dansyl); 7.44-7.54 (q, 2H, H-dansyl); 7.97 (s, 1H, H-2); 8.09 (d, 1H, H-dansyl); 8.26 (s, 1H, H-8); 8.28 (d, 1H, H-dansyl); 8.49 (d, 1H, H-dansyl).

Cholesteryl 4-Nitrophenyl 2', 5'-dideoxyadenosine 3'-phosphate (7).

A 1.0 M solution of *tert*-butyl magnesium chloride (5 ml, 5 mmol) in THF was added dropwise to a suspension of 2',5'-dideoxyadenosine (**3**) (1.18 g, 5 mmol) in THF (50 ml). After stirring for 10 min, tris-(*p*-nitrophenyl) phosphate (2.31 g, 5 mmol) was added, and stirring was continued for 90 min under argon. The resulting solution was subjected directly to the next step (one-pot linkage of the nucleoside) without further treatment. To a solution of cholesterol (1.93 g, 5 mmol) in THF (20 ml) a 1.0 M solution of *tert*-butyl magnesium chloride (5 ml, 5 mmol) in THF was added dropwise at room temperature. After stirring 15 min the solution of the phosphorylated adduct, prepared as above, was added dropwise to this mixture in 30 min. The mixture was stirred at room temperature for 3 h and then concentrated in vacuo, diluted with 150 ml of EtOAc, and washed with a 0.5 M solution of $NaHCO_3$ (150 ml). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a gum which was subjected to flash chromatography (CH_2Cl_2/acetone, 95/5, followed by CH_2Cl_2/EtOH, 95/5) affording the adduct **7** (2.23 g, 54%).

^{1}H NMR ($CDCl_3$) δ 0.65-3.07 (m, H-2', H-5' and H-cholesteryl); 4.22-4.37 (m, 2H, CH-O-P and H-4'); 5.08 (m, 1H, H-3'); 5.38 (s, 1H, H-6 cholesteryl); 6.17 (broad s, 2H, NH_2); 6.30 (m, 1H, H-1'); 7.39 (d, 2H, H-Ph); 7.92 (s, 1H, H-2); 8.25 (d, 2H, H-Ph); 8.31 (s, 1H, H-8).

Sodium cholesteryl 2', 5'-dideoxyadenosine 3'-phosphate (8).

To a solution of the phosphate triester **7** (0.66 g, 0.8 mmol) in methanol (25 ml) was added a 1.0 M solution of NaOH (3.2 ml). The reaction mixture was allowed to stir overnight at room temperature. Then it was concentrated in vacuo. The precipitate was washed successively with a 1.0 M solution of HCl, EtOH, EtOAc, NaOH 0.1 M, water and EtOH to give **8** (0.28 g, 71%).

To get this salt in solution one equivalent of tetrabutylammonium fluoride was added: ^{1}H NMR ($CDCl_3$) δ 0.65-3.07 (m, H-2', H-5', nBu_4N and H-cholesteryl); 4.15- 4.24 (m, 1H, H-4'); 4.25-4.33 (m, 1H, CH-O-P); 4.82-4.85 (m, 1H, H-3'); 5.37 (s, 1H, H-6 cholesteryl); 5.83 (broad s, 2H, NH_2); 6.30 (m, 1H, H-1'); 7.91 (s, 1H, H-2) ; 8.31 (s, 1H, H-8).

No impurity was detected by ^{1}H NMR and based on FAB mass spectroscopy, the sample was free from species that would suppress the ion beam. FAB-MS $(M+H)^+$at *m/z* 684.

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REFERENCES

1. Beavo, J.A.; Reisfsnyder, D.D. *Trends Pharmacol. Sci.*, **1990**, *11*, 150.
2. Murray, Kenneth J.; Warrington, Brian H.; In: *Comprehensive Medicinal Chemistry* Sammes P.G., Ed.; Pergamon, 1990; Vol. 2, Chapter 8.7., pp 531.
3. Johnson, R.A.; Yeung S.-M.H.; Bushfield, M.; Stübner, D.; Shoshani, I. In: *Role of adenosine and adenine nucleotides in the biological systems*, Imai, S.; Nakazawa, M., Eds.; Elsevier: Amsterdam, 1990, pp 43-55.
4. Johnson, R.A.; Yeung S.-M.H.; Stübner, D.; Bushfield, M.; Shoshani, I. *Mol. Pharmacol.* **1989**, *35*, 681-688.
5. Beacham III, L.M. *J. Org. Chem.* **1979**, *44*, 3100-3101.
6. Wang, Y.; Hogenkamp, H.P.C.; Long, R.A.; Revankar, G.R.; Robins, R.K. *Carbohydr. Res.*, **1977**, *59*, 449-457.

7. Nakagawa, I.; Aki, K.; Hata, T.*J. Chem. Soc., Perkin Trans. I* **1983**, 1315-1318.
8. Gross, B.H.; Dodson, B.K.; Goins, T.L.; Williams, J.O., Jr. *Abstract of Papers*, 207th National Meeting of the American Chemical Society, San-Diego, CA; American Chemical Society: Washington, DC, 1994; ORGN 230.
9. Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y. *J. Org. Chem.* **1993**, *58*, 373-379.
10. Tanizawa, K.; Nakano, M.; Kanaoka, Y. *Bioorg. Chem.* **1987**, 50-58.
11. van Boom, J.H.; Burgers, P.M.J.; van Deursen, P.H.; Arentzen, R.; Reese, C.B. *Tetrahedron Lett.* **1974**, 3785-3788.
12. Letsinger, R.L.; Zhang, G.; Sun, D.K.; Ikeuchi, T.; Sarin, P.S. *Proc. Natl. Acad. Sci. USA*, **1989**, 6553-6556.
13. Krieg, A.M.; Tonkinson, J.; Matson, S.; Zhao, Q.; Saxon, M.; Zhang, L.-M.; Bhanja, U.; Yakubov, L.; Stein, C.A. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 1048-1052.
14. Boutorin, A.S.; Gus'kova, L.V.; Ivanova, E.M.; Kobetz, N.D.; Zarytova, V.F.; Ryte, A.S.; Yurchenko, L.V.; Vlassov, V.V., *FEBS Lett.*, **1989**, *254*, 129-132.
15. Stein, C.A; Pal, R.; De Vico, A.L.; Hoke, G.; Mumbauer, S.; Kinsler, O.; Sarngadharan, M.G.; Letsinger, R.L. *Biochemistry* **1991**, *30*, 2439-2444.
16. Johnson, R.A., Maghareie, H., Ibrahimi, A., and Abumrad, N. *Can. J. Physiol. Pharmacol.* **1994**, *72 (Suppl. 1)*, 511

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