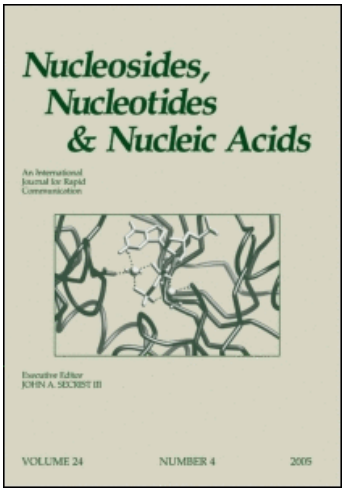


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of 5'-O-Aminothymidine and 5'-Deoxy-5'-Hydrazinothymidine, Novel Nucleoside Derivatives

Lawrence C. Davies[a]
[a] Department of Biochemical Pharmacology, Institute of Cancer Research, Sutton, Surrey, England

To cite this Article Davies, Lawrence C.(1985) 'Synthesis of 5'-O-Aminothymidine and 5'-Deoxy-5'-Hydrazinothymidine, Novel Nucleoside Derivatives', Nucleosides, Nucleotides and Nucleic Acids, 4: 3, 395 — 400
To link to this Article: DOI: 10.1080/07328318508056170
URL: http://dx.doi.org/10.1080/07328318508056170

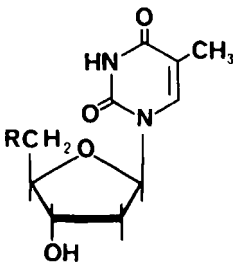
NUCLEOSIDES & NUCLEOTIDES, 4(3), 395-400 (1985)

SYNTHESIS OF 5'-O-AMINOTHYMIDINE AND 5'-DEOXY-5'-HYDRAZINOTHYMIDINE, NOVEL NUCLEOSIDE DERIVATIVES

Lawrence C. Davies
Department of Biochemical Pharmacology,
Institute of Cancer Research,
Sutton, Surrey, England.

ABSTRACT. Two novel nucleoside derivatives, 5'-O-aminothymidine and 5'-deoxy-5'-hydrazinothymidine were synthesised as potential thymidine kinase inhibitors. The former was made from N-hydroxyurethane and 5'-O-tosylthymidine and the latter from benzyl carbazate and 5'-O-tosylthymidine.

Our cancer chemotherapy programme has led us to be interested in developing inhibitors of the enzyme thymidine kinase. One of the best known competitive inhibitors of the enzyme is 5'-amino-5'-deoxythymidine[1] (1). We wished to examine the effect of extending the basic side chain by interposing an O or N atom and to this end we decided to make the 5'-O-amino and hydrazino compounds (2 and 3 respectively).



1. R = NH_2
2. R = ONH_2
3. R = $NHNH_2HCl$
4. R = $ONHCO_2Et$
5. R = $NHNHCO_2CH_2Ph$

No 5'-O-aminonucleosides have been reported and the only reference [2] to a 5'-hydrazinonucleoside, 5'-deoxy-5'-hydrazinoarabinosyladenosine, gives no experimental details

 0732-8311/85/0403-0395$3.50/0

or analytical data. We made 5'-O-aminothymidine by the condensation of the ethyl ester of potassium hydroxycarbamate with 5'-O-tosylthymidine followed by base hydrolysis of the urethane (13% overall yield). The mass spectral fragmentation pattern and the proton nmr spectrum confirmed the structure, the position of the 5'-CH_2 resonance confirming that the compound was an O-alkylhydroxylamine.

Four possible routes to the hydrazino compound were tried unsuccessfully: (a) Direct alkylation[3] of hydrazine by 5'-O-tosylthymidine; (b) Synthesis of an intermediate syndnone[4]; (c) Reaction of diethylazodicarboxylate[5] with 3'-O-tritylthymidine[6]; (d) Alkylation of benzalazine with 5'-O-tosylthymidine (cf. synthesis of methylhydrazine[7]). Use of hydroxylamine-O-sulphonic acid was not attempted because it is known that the 3-nitrogen would be readily aminated using this reagent.[8]

The hydrazine was eventually synthesised by a poorly exploited route; alkylation of benzyl carbazate by 5'-O-tosylthymidine followed by hydrogenolysis (of the carbazate group) (25% overall yield). Mass spectrometry, analysis and proton nmr confirmed the structure of 3. The position of the 5'-CH_2 resonance in the nmr showed that the hydrazine function was attached to this carbon. There is only one literature reference to the synthesis of a monoalkylcarbazate by the reaction of an unsubstituted carbazate at a tetrahedral carbon atom.[9]

Biological results with these compounds will be published elsewhere.

EXPERIMENTAL

Nmr spectra were determined in DMSO-d_6 with TMS as internal standard using a Perkin-Elmer R12B spectrometer.

Chemical ionisation mass spectra were determined on a VG 7070H at 50eV with methane as reagent gas. Melting points were determined on a Kofler Block and are uncorrected. Thin layer chromatograms were run on fluorescent silica (Merck 5735) with spot location by uv light and by the cysteine/sulphuric acid reagent.[10] Preparative HPLC was carried out on Merck Kieselgel 60 (Art 15111) using a Jobin Yvon Chromatospac Prep 10 coupled to a Cecil 212A uv monitor operating at 254nm. Dimethyl acetamide (DMA) was dried by storage over activated 4A molecular sieves.

5'-O-Thymidinylurethane (4)

Hydroxycarbamic acid ethyl ester (1.05g, 10mmol) was added to a solution of KOH (0.5g, 9mmol) in AR ethanol (40ml). 5'-O-Tosylthymidine [11] (1.60g, 4.0mmol) was then added and the mixture refluxed for 2 hours. After cooling in ice the heavy white precipitate was filtered off and the filtrate evaporated to dryness. The residue contained two main components (tlc) which were separated on preparative HPLC (CH_2Cl_2-EtOH; 94:6), the faster running component being the desired urethane and the slower running component O2-ethylthymidine[12]. The urethane crystallised from ethyl acetate (50ml) giving white crystals (290mg) which were pure on tlc (CH_2Cl_2-EtOH; 9:1) mp 163-4°C. A second crop gave 40mg of pure crystals. Total yield 330mg (25%).

Analysis: $C_{13}H_{19}N_3O_7$ requires C 47.4, H 5.8, N 12.8; found C 47.2, H 5.5, N 12.3.

'H-Nmr: 1.20 (3H,t,ethylCH$_3$), 1.85 (3H,d,5-CH$_3$), 2.10 (2H,q,2'-CH$_2$), 3.95 (3H,bs,4'-CH + 5'-CH$_2$), 4.10 (2H,q,ethylCH$_2$), 4.2 (1H,m,3'-CH), 5.35 (1H,d,3'-OH), 6.20 (1H,t,1'-CH), 7.60 (1H,d,6-CH), 10.4 (1H,s,amideNH), 11.25 (1H,bs,3-NH).

5'-O-Aminothymidine (2)

The urethane (4) (100mg, 0.3mmol) was dissolved in MeOH-NNaOH (1:3, 3ml) which was then heated at 60°C for six hours. After cooling the solution was passed through an AG 50W-X4 Dowex column (pyridinium form; 17cm x 1.8cm) which was eluted first with MeOH-H_2O (1:3; 20ml) and then with pyridine-water (9:1). The fractions were combined and evaporated to dryness in vacuo. The residue was dissolved in boiling methanol (10ml); crystals appeared on cooling. More crystals were formed when the volume was reduced in vacuo. All the crystals were collected giving 41mg (52%) of colourless crystals, mp 192-195°C. On a tlc plate the product gave a red colour with the picryl chloride spray for hydroxylamines[13].

Analysis: $C_{10}H_{15}N_3O_5$ requires C 46.7, H 5.9, N 16.3; found C 46.8, H 6.0, N 16.3.

'H-Nmr: 1.80 (3H,d,5-CH_3), 2.1 (2H,q,2'-CH_2), 3.6-4.0 (3H,c,4'-CH + 5'-CH_2) 4.2 (1H,m,3'-CH), 5.25 (1H,d,3'-OH), 5.95-6.35 (3H,c,1'-CH + ONH_2), 7.50 (1H,d,6-CH), 11.2 (1H,s,3-NH).

Mass spectrum m/e 258 $(M+1)^+$, 243 $(M-NH+1)^+$, 132 $(Sugar+1)^+$, 127 $(base+1)^+$.

5'-Benzylcarbazyl-5'-deoxythymidine (5)

Benzyl carbazate (1.64g, 10mmol) and 5'-O-tosylthymidine (0.80g, 2.0mmol) were dissolved in dry DMA (20ml). Some activated 4A molecular sieves were added and the solution heated with stirring at 110°C for 2 days. The cooled solution was filtered and the solvent evaporated in vacuo at 40°C. The residue was dissolved in ethanol (10ml) and the solution slowly formed a crystalline mass. The product was recrystallised from ethanol (50ml) and gave

330mg (42%) of white crystals, which were pure on tlc (CH_2Cl_2-EtOH; 9:1), mp 200-210°C.

Analysis: $C_{18}H_{22}N_4O_6$ requires C 55.1, H 5.7, N 14.3; found C 55.2, H 5.7, N 14.2.

'H-Nmr: 1.82 (3H,s,5-CH_3), 2.1 (2H,t,2'-CH_2), 3.0 (2H,t,5'-CH_2), 3.65-3.95 (1H,m,4'-CH), 4.1-4.4 (1H,m,3'-CH), 4.6-4.9 (1H,m,5'-CH_2NH), 5.07 (2H,s,benzylCH_2), 5.1 (1H,d,3'-OH), 6.15 (1H,t,1'-CH), 7.35 (5H,s,C_6H_5), 7.6 (1H,s,6-CH), 8.5-8.65 (1H,bs,CONH), 11.25 (1H,s,3-NH).

5'-Deoxy-5'-Hydrazinothymidine Hydrochloride (3)

Carbazate (5) (130mg, 0.33mmol) was dissolved in AR MeOH-conc. HCl (98:2; 5ml). Palladium-charcoal (5%; 25mg) was added to the turbid solution, the catalyst having been first slurried with a little ethanol, and hydrogen was then passed over the mixture for 2 hours. The catalyst was filtered off and the filtrate evaporated to dryness in vacuo. The residue was dissolved in boiling ethanol (20ml), filtered and the solution evaporated to 5-10ml and then heated until clear. When cool, ethyl acetate was added until some white solid was deposited. The mixture was stored in the fridge for several days and then centrifuged yielding a pale yellow amorphous powder (36mg), pure on tlc (nPrOH-2N aqNH_3; 7:3). More ethyl acetate was added to the supernatant yielding a further 25mg of product as a white precipitate, pure on tlc (nPrOH/2NaqNH_3; 7:3); total yield (hydrochloride salt, monohydrate) 61mg (59%).

Analysis: $C_{10}H_{16}N_4O_4.H_2O.HCl$ requires C 38.7, H 6.2, N 18.0; found C 38.7, H 6.5, N 18.1.

'H-Nmr: 1.82 (3H,d,5-CH_3), 2.15 (2H,t,2'-CH_2), 3.15 (2H,d,5'-CH_2), 3.7-4.05 (1H,m,4'-CH), 4.1-4.4 (1H,m,3'-CH),

6.15 (1H,t,1'-CH), 5.9-7.0 (7H,bs,3'-OH,$NHNH_2$, HCl, H_2O), 7.55 (1H,d,6-CH), 11.25 (1H,s,3-NH).

Mass spectrum m/e 257 $(M+1)^+$, 131 $(M+1-base)^+$, 127 $(base+1)^+$.

ACKNOWLEDGEMENTS

This investigation was supported by Medical Research Council/Cancer Research Campaign grants. I am indebted to Dr. T.R. Jones for suggesting the synthesis of the hydrazine and to Mr. M. Baker for making the mass spectral measurements.

REFERENCES

1. Neenan, J.P., Rohde, W. J. Med. Chem. 1973, 16, 580.
2. Maskell, T.H. Ann. N.Y. Acad. Sci. 1977, 284, 81.
3. Westphal O. Chem. Ber. 1941, 74, 759.
4. Stewart, F.H.C. Chem. Revs. 1964, 64, 129.
5. Dow, R.L., Kelly, R.C., Schletter, L., Wierenga W., Synth. Commun. 1981, 11, 43.
6. Davies, L.C., Farmer, P.B., Jarman, M., Stock, J.A. Synthesis 1980, 75.
7. Hatt, H.H. Org. Synth. Collective Vol. II, 1943, p. 395.
8. Wallace, R.G., Aldrichimica Acta, 1980, 13, 3.
9. Barraclough, P., Caldwell, A.G., Harris, J.C., Whittaker, N. J. Chem. Soc. Perkin 1, 1981, 2096.
10. Buchanan, J.G. Nature, 1951, 168, 1091.
11. Reist, E.J., Benitez, A., Goodman, L. J. Org. Chem. 1964, 24, 554.
12. Kusmierek, J.T., Singer, B. Nucleic Acids Res. 1976, 3, 989.
13. "Dyeing Reagents for Thin Layer and Paper Chromatography", E. Merck, Darmstadt, No. 234.

Received October 29, 1984