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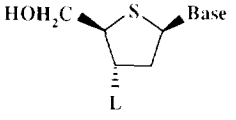
Synthesis and Antiviral Evaluation of Enantiomeric 2',3'-Dideoxy- and 2',3'-Didehydro-2',3'-dideoxy-4'-Thionucleosides.

Robert J. Young*, Sue Shaw-Ponter, Jamie B. Thomson[1], J. Allen Miller*, John G. Cumming[2], Ashley W. Pugh, and Peter Rider.

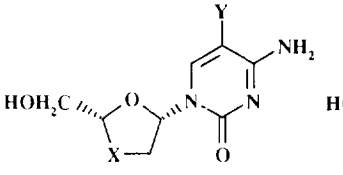
Department of Medicinal Chemistry, The Wellcome Foundation Ltd.,
Beckenham, Kent, BR3 3BS, United Kingdom.

Abstract: The synthesis and antiviral evaluation of 2',3'-dideoxy and 2',3'-didehydro-2',3'-dideoxy 4'-thionucleosides in both enantiomeric series are described. Enantiomeric 4-*O*-silylated-4-hydroxymethyl-4-thiobutyrolactones, produced in high yield from chiral glycidols, provided suitable chiral synthons: phenylselenation of these intermediates resulted in anomeric selectivity in nucleobase glycosidations. L-d4-cytidine analogues showed marked anti-HBV and anti-HIV activity.

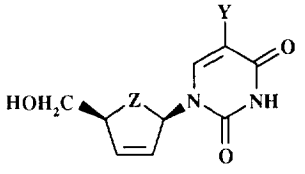
The search for new nucleoside analogues as potential chemotherapeutic agents to combat viral infections has produced numerous variations in the sugar portion of the molecule.[3] There had been, until recently, surprisingly little interest in compounds in which the ring oxygen had been replaced by sulphur,[4] largely due to difficulties in the large-scale synthesis of 4-thiopentofuranosides. However, recent synthetic developments have led to examples of 4'-thionucleosides with, *inter alia*, 2'-deoxy[5] (**1**), 2',3'-dideoxy[6] (**2**) and 2',3'-dideoxy-3'-*C*-hydroxymethyl[7] (**3**) substituents: several of these compounds have exhibited potent anti-viral activity. Nucleoside analogues with the unnatural or L-configuration, such as L-ddC,[8] 3TC[9] and FTC[10], which have shown marked anti-viral activity and, significantly, better selectivities than their corresponding D-enantiomers, have provided the stimulus for further work. In this paper we report on the high-yielding synthesis of both enantiomers in the pyrimidine 2',3'-dideoxy-4'-thio series from chiral thiolactones. This methodology was developed, through the incorporation of a 2-phenylselenyl ligand, to introduce anomeric selectivity which facilitated the first synthesis and antiviral evaluation of pyrimidine 2',3'-didehydro-2',3'-dideoxy-4'-thionucleosides (**4**), 4'-thio analogues of the recently licensed d4T,[11] with both D- and L- configurations.



(**1**) L = OH
(**2**) L = H
(**3**) L = CH_2OH

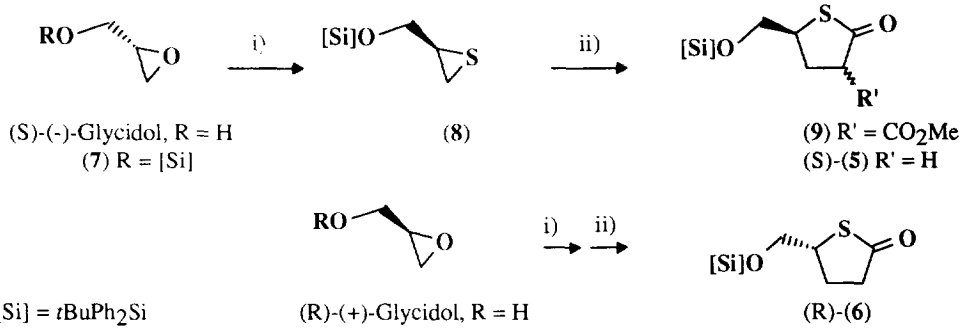


L-ddC X = CH_2, Y = H
3TC X = S, Y = H
FTC X = S, Y = F



d4T Z = O, Y = Me
(**4**) Z = S

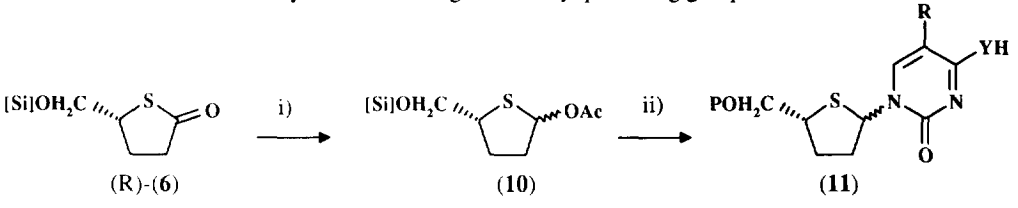
The enantiomeric thiolactones (**5**) and (**6**),[12] the key chiral intermediates in our synthesis, were prepared as follows: (S)-(-)-glycidol was converted to its *tert*butyldiphenylsilyl ether (**7**)[13] and reacted with thiourea in methanol[14] to give the (R)-thiirane (**8**) with clean inversion of configuration.[15] (Scheme 1). The conversion of (**7**) to the (4S)-2-methoxycarbonyl thiolactone (**9**) was achieved by reaction with dimethyl malonate and sodium hexamethyldisilazide in refluxing thf; careful control of concentration minimised side-products due to thiirane polymerisation.[16] After demethoxycarbonylation, a short column was employed, as the only purification step, to produce >100 g lots of (**5**) in 65% overall yield, with enantiomeric excesses between 82 to 91%, as measured by chiral hplc.[17] Similarly, (R)-(+)-glycidol was converted to the (R)-lactone (**6**).



i) [Si]Cl, DMAP, imidazole, DCM; then $(H_2N)_2CS$, MeOH;
ii) $CH_2(CO_2Me)_2$, $NaN(TMS)_2$, thf, reflux; then DMSO, brine, 160 °C.

Scheme 1

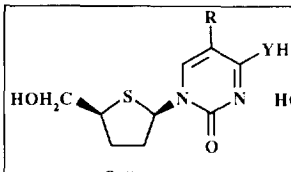
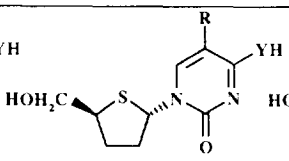
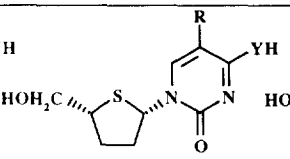
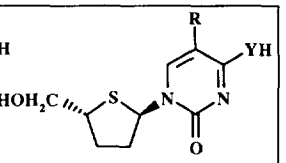
Either thiolactone [exemplified in Scheme 2 by (R)-(**6**)] was converted to the corresponding thiolactol acetates (**10**) by reduction with diisobutylaluminium hydride in toluene at -78 °C and subsequent acetylation in 90% yield (Scheme 2). Glycosylations were achieved, in a similar fashion to the published procedures,[6] by reaction of these acetates with the bis(trimethylsilyl)-derivatives of a number of substituted uracils and cytosines, in the presence of tin(IV) chloride in acetonitrile, forming the 5'-*O*-silylated nucleosides (**11**, P = [Si]) as a mixture of anomers (~1:1 ratio[18]), which could be separated at this stage by flash chromatography. Better yields were obtained for cytidine glycosylations by using potassium nonaflate, trimethylsilyl chloride and hexamethyldisilazide. The enantiomeric deblocked nucleosides (**11**, P = H), with both anomeric configurations as listed in Table 1, were obtained by fluoride cleavage of the silyl protecting group.[19]



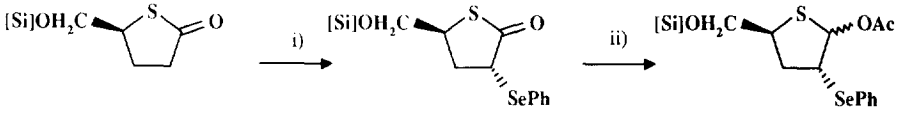
i) DIBALH, PhMe; then Ac_2O, DMAP, DCM.
ii) TMS_2Uracil, $SnCl_4$, MeCN or $C_4F_9SO_3K$, TMSCl, HMDS, MeCN; then Et_4NF, MeOH/thf.

Scheme 2

Table 1: ***D and L 2',3'-dideoxy-4'-thio-Pyrimidine Nucleosides Synthesised.***

			
β-D	**α-D**	**β-L**	**α-L**
Uridines, Y = O,	***Uridines***, Y = O,	***Uridines***, Y = O,	***Uridines***, Y = O,
R = H, Me	R = H, Me	R = H, Me	R = H, Me
R = Et, Br (Mixed α/β)			
Cytidines, Y = NH	***Cytidine*** Y = NH	***Cytidines***, Y = NH	***Cytidine***, Y = NH
R = H	R = H,	R = H	R = H
R = F (Mixed α/β)		R = F, I (Mixed α/β)	

Thiolactones (**5**) and (**6**) provided a useful entry to 2',3'-didehydro-2',3'-dideoxy-4'-thionucleosides, a new class of compounds, using methodology developed in the corresponding oxa-series.[20] Thus *O*-silyl-(S)-thiolactone (**5**) was phenylselenated at the 2 position, *via* the *in situ* generated trimethylsilyl keteneacetal, which produced better diastereofacial selectivity than with the 4-oxa analogue.[20c] This selectivity was further improved by optimising the quenching conditions; adding saturated aqueous sodium sulphate to the reaction mixture maintained at -78 °C produced (**12**) with at least 15:1 α- to β-face selectivity.[21] Reduction and acetylation of (**12**) was achieved as described for (**10**), which furnished the desired glycosylating agent (**13**), reproducibly in >75% yield from the thiolactone (**5**) (Scheme 3).



(**5**) (**12**) (**13**)

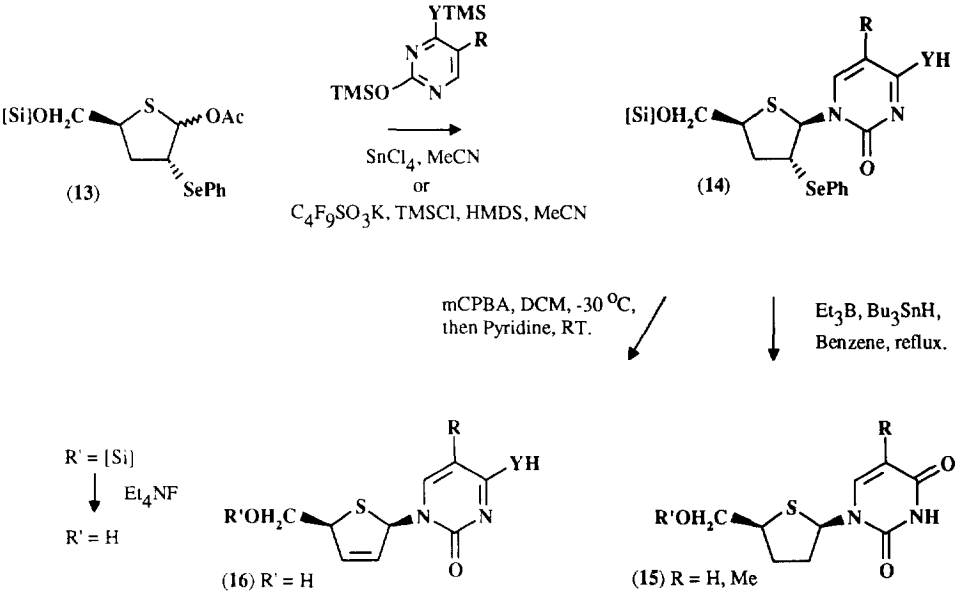
i) $LiN(TMS)_2$, thf; then TMS-Cl, then PhSeBr, all *in situ* at -78 °C,
ii) DIBALH, PhMe; then Ac_2O, DMAP, DCM.

Scheme 3

Coupling of (**13**) with bis-*O*-silylated uracil-derived bases, in the presence of tin(IV) chloride in acetonitrile, furnished the glycosylated products (**14**) (Scheme 4) in 50 to 90 % yield with 15:1 or better selectivity for the desired β-anomer, yielding pure β-product after flash chromatography. The literature has seen some debate as to the origins of similar directed glycosylations.[22] We have observed that when cytosine was glycosylated under our preferred conditions, using potassium nonaflate / trimethylsilyl chloride / hexamethyldisilazide, β-selectivity was maintained, perhaps demonstrating a minor influence of tin co-ordination on the directing effect in our system.

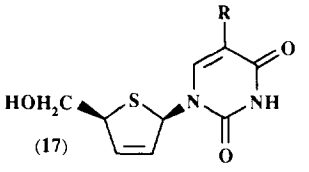
The 2'-selenyl functionality was removed either reductively with tributyltin hydride,[23] or *via* selective selenium oxidation with *m*CPBA,[24] then elimination. The reductive deselenation, which gave the 2',3'-dideoxy

uridine and thymidine analogues (**15**) with anomeric selectivity, worked well on 100 mg scale in 70% yield, but was of little utility on larger quantities. Indeed, a gram scale reaction gave <15% yield of the required uridine contaminated by ~20% of the α-anomer, a consequence of partial scrambling of the all β-substrate. In contrast, oxidative elimination of the selenyl functionality worked well and was amenable to scale-up. This introduced, regiospecifically, the olefinic functionality of the novel 2',3'-didehydro-2',3'-dideoxy analogues (**16**) in >70% isolated yield. The silyl protecting group was removed in typically >85% yield with tetraethylammonium fluoride in thf-methanol. The products (**17**) were conveniently handled as lyophilised solids after purification on silica flash columns, eluted with gradients of methanolic ammonia in chloroform. The enantiomeric compounds (**18**), with the unnatural or L-configuration, were similarly synthesised from the (R)-thiolactone (**6**) (Table 2).



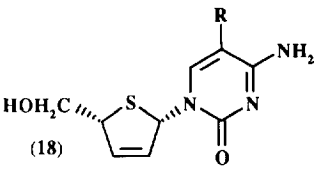
Scheme 4

Table 2: ***D and L 2',3'-didehydro-2',3'-dideoxy-4'-thio-Pyrimidine Nucleosides Synthesised.***



β-D-d4 (as uridine)

Uridines, R = H, Me, Et
Cytidines, R = H, F



β-L-d4 (as cytidine)

Uridines, R = H, Me
Cytidines, R = H, F

All reported compounds were screened *in vitro* for antiviral activity against the following viruses: HIV (HeLa CD4 cells), HSV-1 (Vero), HSV-2 (Vero), VZV (MRC5), HCMV (MRC5), Influenza (MDCK), and HBV (Hep G2/P5A). Marked inhibition of the replication of Human Immunodeficiency and Hepatitis B viruses was observed with the L-d4-cytidine analogues (Table 3), with no associated toxicity. The activity appears to reside in this unnaturally-configured series and the low levels of activity observed with the D-d4C compounds are probably due to ~7% of the enantiomer present. No other activity was seen up to 100 μM concentrations.

Table 3: In vitro *Antiviral Evaluation of Cytidine Analogues: representative IC_{50} figures in μM.*

	β-D-Configuration				β-L-Configuration			
2',3' Substitution	dd	dd	d4	d4	dd	dd	d4	d4
5-Substituent	H	F	H	F	H	F	H	F
HIV-(HeLa CD4)	38	>50	23	<50	>50	>50	0.8	0.4
HBV-(HepG2)	>100	>100	15	>50	>100	>100	0.8	3.5
$CC_{ID}50$ (Vero)	>500	>500	>500	>500	>500	>500	>500	>500

Acknowledgements.

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References and Notes

1. Present Address, School of Chemistry, University of St Andrews, St Andrews, Fife, KY16 9ST, Scotland.
2. Present Address, University Chemistry Laboratory, Lensfield Road, Cambridge, CB2 1EW, United Kingdom.
3. The most recent reviews of antiviral targeted nucleosides: Huryn, D.M., and Okabe, M., *Chem. Rev.*, **1992**, *92*, 1745-1768; De Clercq, E., *Nucleosides and Nucleotides.*, **1994**, *13*, 1271-1295.
4. Wnuk, S.F., *Tetrahedron Report No. 343, Tetrahedron*, **1993**, *49*, 9877-9936.
5. a) Secrist, J.A. III, Riggs, R.M., Tiwari, K.N., and Montgomery, J.A., *J. Med. Chem.*, **1991**, *34*, 2361-2366; b) Dyson, M.R., Coe, P.L., and Walker, R.T., *J. Chem Soc., Chem. Commun.*, **1991**, 741-743; c) Dyson, M.R., Coe, P.L., and Walker, R.T., *J. Med Chem.*, **1991**, *34*, 2782-2786; d) Uenishi, J., Motoyama, M., Nishiyama, Y., and Wakabayashi., S., *J. Chem Soc., Chem. Commun.*, **1991**, 1421-1422; Uenishi, J., Motoyama, M., and Takahashi., K., *Tetrahedron Asymmetry*, **1994**, *5*, 101-106.
6. Secrist, J.A. III, Riggs, R.M., Tiwari, K.N., and Montgomery, J.A., *J. Med. Chem.*, **1992**, *35*, 533-538.
7. a) Brånalt, J., Kvarnström, I., Niklasson, G., Svensson, S.C.T., Classon, B., and Samuelsson, B., *J. Org. Chem.*, **1994**, *59*, 1783-1788; b) Brånalt, J., Kvarnström, I., Svensson, S.C.T., Classon, B., and Samuelsson, B., *J. Org. Chem.*, **1994**, *59*, 4430-4432; c) Mann, J., Tench, A.J., Weymouth-Wilson, A., Shaw-Ponter, S., and Young R.J., *J. Chem Soc., Perkin Trans. 1*, **1995**, 677-681.
8. Lin, T.-S., Luo, M.-Z., Liu, M.-C., Pai, S.B., Dutschman, G.E., Cheng, Y.-C., *J. Med. Chem.*, **1994**, *37*, 798-803.
9. Soudeyns, H., Yao, X.-J., Gao, Q., Belleau, B., Kraus, J.-L., Nguyen-Ba, N., Spira, B., and Wainberg, M.A., *Antimicrob. Agents Chemother.*, **1991**, *35*, 1386-1390; Schinazi, R.F., Chu, C.K., Peck, A., McMillan, A., Mathis, R., Cannon, D., Jeong, L-S., Beach, J.W., Choi, W.-B., Yeola, S., and Liotta, D.C., *Antimicrob. Agents Chemother.*, **1992**, *36*, 672-676.

10. Schinazi, R.F., McMillan, A., Cannon, D., Mathis, R., Lloyd, R., Peck, A., Sommadossi, J.-P., St Clair, M., Wilson, J., Furman, P.A., Painter, G., Choi, W.-B., and Liotta, D.C., *Antimicrob. Agents Chemother.*, **1992**, *36*, 2423-2431.

11. Mansuri, M.M., Starrett, J.E., Jr., Hitchcock, M.J.M., Sterzycki, R.Z., Brankovan, V., Lin, T.-S., August, E.M., Prusoff, W.H., Sommadossi, J.-P., and Martin, J.C., *J. Med. Chem.*, **1989**, *32*, 461-466.

12. Whilst this manuscript was in preparation, the synthesis of both enantiomers of the dd-4'-thio-cytidine analogue were reported using the same intermediate lactones (**5**) and (**6**), but produced by a different route: Rassu, G., Spanu, P., Zanardi F., and Casiraghi, G., *Tetrahedron Lett*, **1995**, *36*, 1941-1944.

13. Gao, Y., Hanson, R.M, Klunder, J.M., Ko, S.Y., Masamune, H., and Sharpless, K.B., *J. Am. Chem. Soc.*, **1987**, *109*, 5765-5780.

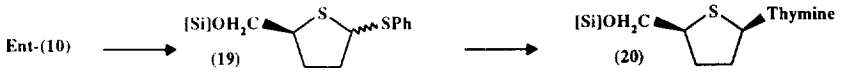
14. Pedersen, R.L., Liu, K.-C., Rutan, J.F., Chen, L., and Wong, C.-H., *J. Org. Chem.*, **1990**, *55*, 4897-4901.

15. All new compounds gave satisfactory spectroscopic and microanalytical data.

16. Taguchi, Y., and Suhara, Y., *Bull. Chem. Soc. Jpn.*, **1986**, *59*, 2321-2324. Optimum conditions: dimethyl malonate (64.1 g, 0.486 mol) was reacted with sodium bis(trimethylsilyl)amide (0.445 mol) in 2.5 l of thf at room temperature, thiirane (**8**, 132.8 g, 0.405 mol) was added to this in 2 l of thf and the solution was refluxed for 80 h.

17. Data for (S)-(**5**): ^{1}H NMR spectrum ($CDCl_3$) δ_H, 1.05 (9H, s, CMe_3), 2.05 to 2.55 (4H, m, H-2/3), 3.80 (2H, m, H-5), 4.05 (1H, m, H-4), 7.40 to 7.70 (10H, m, Ar-H); I.R. ν_{max} (neat film) 1700 cm^{-1}; Mass spectrum (m/z) (FAB+) 371 ($M+H^+$, 5%), 313 (M+H-CMe_3, 80); CD Spectrum (EtOH) 231 (+1.2) nm (ee measured as 86%); Microanalysis, found: C, 67.73; H, 6.94; $C_{21}H_{26}O_2SSi$ requires C, 68.06; H, 7.07%; hplc, Chiracel OD (cellulose tris-3,5-dimethyl carbamate), eluted 9:1 hexane:isopropanol; Retention times 12.9 min (S)-(**5**) and 16.1 min (R)-(**6**).

18. Anomeric selectivity was later achieved by using conditions adapted from those of Sujino, K., and Sugimura, H., *Synlett.*, **1992**, 553-555. Treatment of (**10**) with $PhSTMS/BF_3.OEt_2$ in dichloroethane gave the required phenyl thioglycoside (**19**); coupling of this with bis(trimethylsilyl)thymine in dichloromethane with *N*-bromosuccinimide activation gave (**20**), in an unoptimised 70% yield, but with an anomer ratio of 2.6:1 β:α.



19. Data on these compounds was consistent with the D-dd examples published.[6] Circular Dichroism spectra of enantiomeric pairs showed traces consistent with other 4'-thionucleosides, notably the characteristic positive and negative regions of the spectra are reversed with respect to those found with analogous 4'-oxa nucleosides. For dd-4'-thio-uridine (EtOH), β-D, 259.5 (-5.8) and 225.0 (+5.6) nm; β-L-, 259.5 (+5.8) and 225.0 (-5.6) nm.

20. a) Kawakami, H., Ebata, T., Koseki, K., Matsushita, H., Naoi, Y., and Itoh, K, *Chemistry Lett.*, **1990**, 1459-1462; b) Liotta, D., and Wilson, L.J., *Tetrahedron Lett.*, **1990**, *31*, 1815-1818; c) Chu, C.K., Babu, J.R., Beach, J.W., Ahn, S.K., Huang, H., Jeong, L.S., and Lee, S.J., *J. Org. Chem.*, **1990**, *55*, 1418-1420.

21. Using an ammonium chloride quench at -78 °C or warming to room temperature induced some epimerisation at C-2, resulting in an α:β ratio as low as 5:1. These epimers were readily distinguished by the shifts of the two 3-protons in the ^{1}H NMR spectrum: α-selenium (*anti* to hydroxymethyl) δ_H, 2.35 ppm (2H, m), β-selenium (*syn* to hydroxymethyl) δ_H, 2.10 and 2.60 ppm (each 1H, ddd).

22. Kawakami, H., Ebata, T., Koseki, K., Matsumoto, K., Matsushita, H., Naoi, Y., and Itoh, K, *Heterocycles*, **1991**, *29*, 2451-2470 and cited references.

23. Nozaki, K., Oshima, K., Utimoto, K., *Tetrahedron Lett.*, **1988**, *29*, 6125-6126

24. Toru, T., Kanefusa, T., and Maekawa, E., *Tetrahedron Lett.*, **1986**, *27*, 1583-1586.

25. Data for L-d4-4'-thiocytidine, hygroscopic solid; ^{1}H NMR spectrum (DMSO-d_6) δ_H, 3.58 (2H, m, 5'-H), 4.24 (1H, m, 4'-H), 5.04 (1H, t, OH), 5.75 (1H, d, H-5), 5.84 and 6.24 (1H, ddd, 2'/3'-H), 6.83 (1H, m, 1'-H), 7.05 (2H, brs, NH_2), 7.04 (1H, d, H-6); I.R. ν_{max} (KBr) 3449, 3377, 3 117, 1636, 1603 and 1497 cm^{-1}; Mass spectrum (m/z) (FAB+) 226 ($M+H^+$, 24%), 112 (Cytosine+H^+, 100%); CD Spectrum (EtOH) 278 (+12.9), 230 (-5.6) and 205 (-6.1) nm (ee of precursor lactone 86%); Microanalysis, found: C, 43.97; H, 5.33; N, 16.93, S, 13.04%; $C_9H_{11}N_3O_2S.1.17H_2O$ requires C, 43.88; H, 5.46, N, 17.06; S, 13.01%.

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