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4′-Methyloxycarbamyl-3′-deoxy-5-methyluridine; Synthesis of a Novel Nucleoside Analogue

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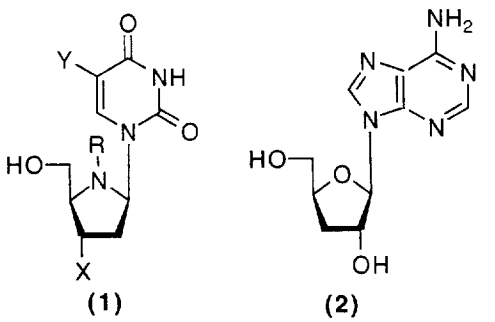
Abstract: The preparation of 4′-methyloxycarbamyl-3′-deoxythymidine from chiral amino acid precursors is described. The route chosen employs a hitherto unreported electrochemical oxidation of a suitably protected derivative of trans-4-hydroxy-L-proline, to obtain the key intermediate compound. Conventional condensation methodology is then used to arrive at the target nucleoside

The recent upsurge of interest in novel nucleoside analogues having a modified heterocyclic sugar unit, has led to the development of a number of novel nucleoside analogues in which the furanose ring oxygen is replaced with a protected secondary amine function (Figure 1)(**1**)[1,2,3,4]. Nucleosides of this type have considerable scope for modification by varying the groups carried by the nitrogen atom, and may possess interesting biological activity. Here we report the first synthesis of this class of nucleoside in which the oxygen atom of a 3'-deoxyribose nucleoside is replaced by a protected secondary amine function (methyloxycarbamyl unit). Naturally occurring 3'-deoxyribose nucleosides, such as cordycepin (**2**),[5,6] have been shown to have significant biological activity[7,8,9]. There is also increasing interest in the preparation of oligomeric 3'-deoxynucleosides[10] which mimic the structures of naturally occurring 5'-2' linked oligonucleotides. To date there has been only one example of a nucleoside analogue containing a modified 3'-deoxyribose sugar unit[11]. As a result of this we were interested in preparing a 4'-amino-3'-deoxyribose nucleoside analogue with potential biological activity.



(1) (2)

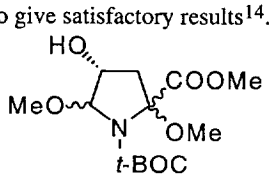
Figure 1

Previously, we and other groups have reported the synthesis of 4'-amino-sugar nucleosides using common amino acids as starting materials[1, 2, 4]. This approach again seemed suitable, however the routes described by ourselves and others to obtain 4'-amino-2',3'-dideoxyribose nucleoside analogues, in which L-pyroglutamic acid was converted to an α-methoxy pyrrolidine and then coupled to a nucleoside base, did not appear to offer a simple entry to the 4'-amino-3'-deoxyribose nucleosides. An alternative route to a suitably derivatised pyrrolidine, appeared to be through the anodic oxidation of a proline derivative. The electrochemical oxidation of prolines has been well studied[12], and generally gives good yields of the desired α-methoxy pyrrolidines. To produce a pyrrolidine suitable for coupling to a nucleoside base to give a 4'-amino-3'-deoxy ribose nucleoside, oxidation of *trans*-4-hydroxy-L-proline was necessary. The oxidation of this naturally occurring amino acid has been less well studied. However Thaning and Wistrand[13] have reported the oxidation of an *N*-acetyl derivative of *trans*-4-hydroxy-L-proline and Barrett and Pilipauskas[14] have published results on the electrochemical oxidation of a variety of derivatives of *cis*-4-hydroxy-L-proline. Their findings encouraged us to attempt this approach.

The electrochemical method used[13,14] was the constant current method. This procedure uses an easy-to-construct undivided cell in which the oxidation reactions take place at the anode but as both electrodes are in contact with the solution of substrate, other reactions may occur. Also, because the potential is not controlled, further oxidative processes are possible and thus any chemoselectivity and regioselectivity desired in the oxidative process must be as a result of stabilising groups built into the molecule at, or adjacent to, the sites at which the electrochemical process is to take place.

The anodic oxidation of proline derivatives is a two-electron process in which initially a single electron is removed from the nitrogen lone pair, giving a radical cation. Following this, the second lone pair electron is abstracted, causing the electrons in a C-H bond on the adjacent methylene group to migrate to the vacant nitrogen lone pair orbital, resulting in the concomitant loss of a proton. The resulting iminium ion is stabilised by the protecting group on the nitrogen and reacts rapidly with a nucleophile, usually the solvent.

When *tert*-butoxycarbonyl was used to protect the nitrogen, although some of the desired compound was produced (34%), a significant amount of the pyrrolidine (**10**) (Figure 2) was present (data not shown). By-products of this type have previously been reported[13]. When the functionality on the molecule was reduced to a minimum (**11**) (Figure 2) and was subjected to electrolysis, no over oxidised product was observed, which confirmed the source of the problem and the expected product (**12**) could be isolated. It has recently been argued that higher yields and improved regioselectivity can be achieved if smaller nitrogen protecting groups are used. This was rationalised partially as a steric effect which helps the substrate to approach the electrode surface with the correct orientation. The methyl carbamate protecting group was found to give satisfactory results[14].



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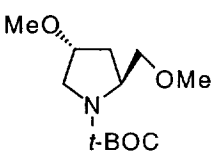

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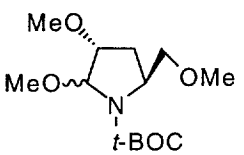
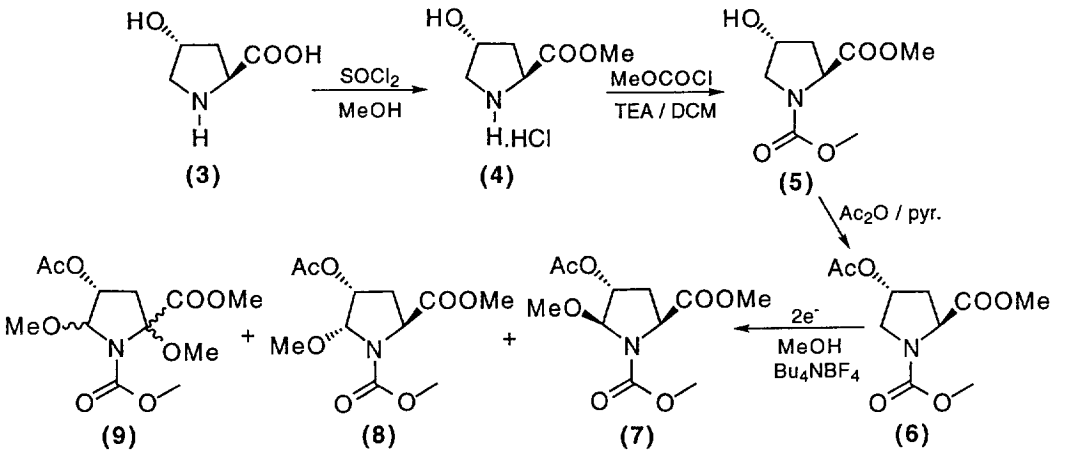


(12)

Figure 2

Thus, starting from trans-4-hydroxy-L-proline (**3**) (Scheme 1), treatment with thionyl chloride in methanol afforded the ester (**4**). This was then converted to the methyl carbamate (**5**) by treatment with methyl chloroformate in the presence of triethylamine. Protection of the C-4 hydroxyl group gave the protected proline derivative (**6**). The proline was then subjected to electrochemical oxidation in methanol at ambient temperature with tetrabutylammonium tetrafluoroborate as a supporting electrolyte. After passing a current of 0.6-0.7 A for a period of 10 hours, t.l.c. suggested that no starting material remained and the reaction was stopped.

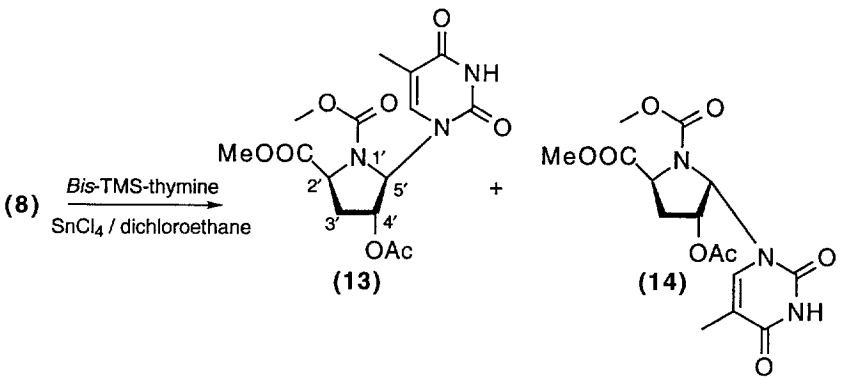


Scheme 1

Chromatographic analysis of the product initially indicated that two products were present. The more mobile of the two was separated by column chromatography, and was shown by ^{1}H NMR spectroscopy to be the desired product (**7**). Closer examination of the less mobile material revealed that it consisted of two distinct compounds. These were separated by careful column chromatography, the more mobile component of the mixture proved to be the diastereoisomer of the product initially isolated (**8**), the remainder was identified as an overoxidised species (**9**) of a type observed previously by Thaning and Wistrand.

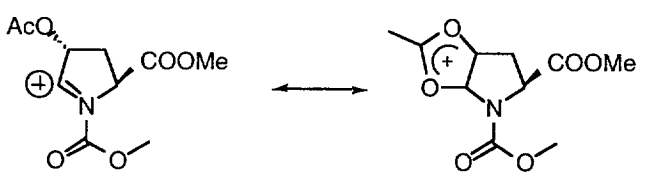
The more mobile of the two diastereoisomers of the pyrrolidine was tentatively assigned the 5-*S* configuration on the basis of a small ^{1}H NMR coupling constant observed for the C-5 proton, and was obtained in 31% yield. The 5-*R* isomer was isolated in 7% yield. The overall yield of 51% obtained by Barrett for the oxidation of the *cis*-isomer of the methyl carbamate suggests that there is increased steric hindrance in the *trans*-isomer, which hinders the approach of the substrate to the anode surface. This may also contribute to the poorer regioselectivity observed, as demonstrated by the significant amount of overoxidised material produced.

Conversion of the α-methoxy pyrrolidine (**7**) to a 4'-methoxycarbamyl-3'-deoxythymidine analogue (Scheme 2) was achieved by treatment with *bis*-2, 4-trimethylsilyloxy-5-methylpyrimidine in the presence of stannic chloride. The desired nucleoside analogue was obtained in a rather low yield as a mixture of diastereoisomers (**13, 14**). Separation of the two isomers by column chromatography gave the β–anomer (**13**) in 19% yield and the α–anomer (**14**) in 7% yield.



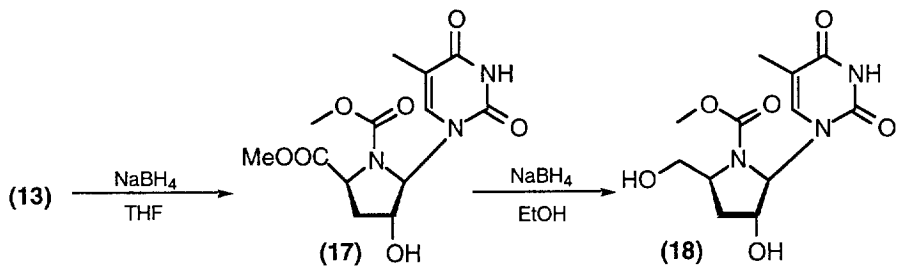
Scheme 2

In five membered rings, it is difficult to assign configuration based on the magnitude of vicinal coupling constants. However, in closely related experiments, Thaning and Wistrand[13] have conclusively shown that no racemization at C-4′ occurs. Also, from double irradiation experiments, it was shown that the β-anomer does not show any vicinal coupling between the protons on C-5′ and C-4′, whereas the α-anomer shows a *J* value of between 4-5 Hz. A similar result was obtained with compounds (**13**) and (**14**) and so their stereochemistry has been assigned accordingly. Further support for the assignment of the more abundant isomer as the β-anomer came from the observation of a large downfield chemical shift (1ppm) for the C-6 proton of the base, possibly arising from the transannular interaction between the proton and the C-2′ ester group which is clearly not possible for the α-anomer. It has previously been noted[13] that although the formation of a C-C bond at C-5′ is possible, it is formed with low stereoselectivity. Under the conditions of condensation, it was shown that the methoxy group at C-2′ would isomerise and this then reacts with the nucleophile through a common intermediate cation. Although substitution exclusively trans to a β-acetate group has been observed for the acyliminium ions[16], presumably due to neighbouring group effect, this clearly is only one determinant feature in this present condensation reaction, but is in agreement with previous results[13], which indicate some participation of the acetyl group (Figure 3) in the stabilisation of the iminium ion (**15**) formed on treatment of the pyrrolidine with stannic chloride. The acetyloxonium ion (**16**) thus formed, favours attack of the nucleophile at one face to give the observed preponderance of the β-anomer.



(**15**) **Figure 3** (**16**)

Final conversion of the ester to the target nucleoside (Scheme 3) was to have been by selective reduction of the C-2 ester and then deprotection of 2'-hydroxyl group. However, on treatment of the ester (**13**) under mild reducing conditions ($NaBH_4$, THF)[15], the result was the selective removal of the acetyl group to give the nucleoside ester (**17**). Under more vigorous reducing conditions ($NaBH_4$, EtOH)[15], the C-2 ester was reduced to give the desired nucleoside analogue (**18**).



Scheme 3

Compounds 5-8, 13, 14, 17 and 18 were tested for activity against herpes simplex virus type 1, herpes simplex virus type 2, varicella zoster virus, human immunodeficiency virus and human cytomegalovirus. No activity or toxicity was found for any of the compounds below a concentration of 100 micromolar.

EXPERIMENTAL

Thin layer chromatography was performed on precoated aluminium backed t.l.c plates (Silica gel 60 F_{254}) supplied by E.Merck A.G. Glass chromatography columns were slurry packed with 70-250 mesh Silica gel and eluted under pressure. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared spectra were acquired on a Perkin-Elmer 1600 series Fourier transform infrared spectrometer. NMR spectra were recorded on a Bruker AC300 (300MHz) spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured on a Kratos MS-80 mass spectrometer with DS-55 data system or a Kratos MS580RF mass spectrometer. High resolution mass spectrum measurements and elemental analyses were made at the Wellcome Foundation Laboratories, Beckenham, Kent.UV spectra were recorded on a Perkin-Elmer 552 spectrometer with samples prepared in spectroscopic ethanol.

(2*S*, 4*R*)-(-)-4-Hydroxy-2-pyrrolidinecarboxylic acid methyl ester hydrochloride salt (4).

Thionyl chloride (1.7 g, 15.2 mmol) was added *via* a dropping funnel to a cooled, stirred suspension of (2*S*, 4*R*)-(-) 4-hydroxyproline (**3**) (2.0 g, 15.2 mmol) in dry methanol (50 mL). After the addition of thionyl chloride was complete, the resutling solution was stirred at ambient temperature for 4 hours. The solution was then concentrated under vacuum to give a white solid which was repeatedly co-evaporated with dichloromethane to remove all traces of thionyl chloride. The pure product was obtained as a white solid

(yield= 2.76 g, 100%). $[\alpha]_D^{20}$= -27.4°(c=0.19, MeOH); IR (nujol) 4328, 4256, 3380, 3322, 2698, 2600, 2419, 1742, 1592, 1284, 1247, 1181, 1075, 1049, 1026, 957, 903, 721 cm^{-1}; ^1H NMR (300 MHz, d_6-DMSO) δ 10.10(2H, s, br, NH_2^+), 5.46(1H, s, br, OH), 4.46(1H, m, H4), 4.42(1H, m, H2), 3.75(3H, s, CH_3O), 3.35(1H, dd, *J*=4.5, 12.5 Hz, H5), 3.06(1H, dd, *J*=5.0, 12.5 Hz, H5), 2.26-1.98(2H, m, H3); ^{13}C NMR (d_6-DMSO) δ 170.3(carbonyl), 169.1(carbonyl), 68.6(C4), 68.5(C4), 57.7(C2), 57.5(C2), 38.8(C3), 37.3(C3); MS (CI) *m/z* 146(M^+).

(2*S*, 4*R*)-(-)-*N*-Methyloxycarbonyl-4-hydroxypyrrolidine-2-carboxylic acid methyl ester (5).
To a suspension of (2*S*, 4*R*)-(-)-4-hydroxy-2-pyrrolidinecarboxylic acid methyl ester hydrochloride salt (**4**) (7.42 g, 40.8 mmol) in dry dichloromethane (100 mL) was added triethylamine (8.25 g, 81.6 mmol) with cooling and stirring on an ice bath. Methyl chloroformate (3.86 g, 40.8 mmol) was then added dropwise to the cooled solution. The mixture was then allowed to warm to room temperature and was stirred for a further 12 hours. The solution was then washed with dilute HCl and brine before concentrating under vacuum to give a solid residue. Acetone was added to the residue, and the solid filtered off. Concentration of the filtrate under vacuum gave a clear oil which was chromatographed on a silica gel column with 80:20 ethyl acetate-hexane, to give the title compound as an oil (yield= 6.90 g, 83%). $[\alpha]_D^{25}$= -81.8°(c=0.2, MeOH); IR (nujol) 3491, 2603, 2496, 2359, 1750, 1672, 1344, 1319, 1285, 1190, 1167, 1131, 1085, 1004, 962, 942, 882, 834, 773 cm^{-1};^1H NMR (300 MHz, $CDCl_3$) δ 4.53(1H, m, H4), 4.49(1H, m, H2), 3.74, 3.72(3H, 2s, $NCOOCH_3$), 3.71, 3.69(3H, 2s, $COOCH_3$), 3.65, 3.54(2H, m, H5), 2.32(1H, s, br, OH), 2.25(1H, s, br, H3), 2.10(1H, m, H3); ^{13}C NMR ($CDCl_3$) δ 172.1($NCOOCH_3$, carbonyl), 69.9(C4), 68.9(C4), 58.2(C2), 57.8(C2), 55.2(C5), 54.2(C5), 53.0(CH_3O), 52.9(CH_3O), 39.1(C3), 38.8(C3); MS (CI) *m/z* 204$(M+H)^+$, 221$(M+NH_3)^+$; Found C;48.34, H;6.55, N;6.80 $C_8H_{13}NO_5$ requires C;47.29, H;6.45, N;6.90.

(2*S*, 4*R*)-(-)-*N*-Methyloxycarbonyl-4-acetoxy-2-pyrrolidinecarboxylic acid methyl ester (6).
(2*S*, 4*R*)-(-)-*N*-methyloxycarbonyl-4-hydroxy-2-pyrrolidinecarboxylic acid methyl ester (**5**) (6.80 g, 33.5 mmol) in dry pyridine (50 mL) was teated with acetic anhydride (17.08 g, 167.3 mmol) at ambient temperature. The solution was stirred for 18 hours before concentrating under vacuum and co-evaporating the residue obtained with toluene to remove all traces of acetic anhydride. Purification by column chromatography gave the title compound as an oil (yield= 6.20 g, 76%). $[\alpha]_D^{25}$= -59.4°(c=0.30, MeOH); IR (neat) 3524, 2998, 2956, 2886, 2361, 1741, 1708, 1545, 1391, 1206, 1129, 1068, 1019, 966, 907, 879, 773 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.80(1H, m, H4), 4.47(2H, m, H2), 3.80, 3.79(3H, 2s, $NCOOCH_3$), 3.74, 3.72(3H, 2s, $COOCH_3$), 3.73-3.59(2H, m, H5), 2.43(1H, m, H3), 2.22(1H, m, H3), 2.08(3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 172.1($NCOOCH_3$, carbonyl), 170.1($COOCH_3$, carbonyl), 145.2($OCOCH_3$, carbonyl), 72.6(C4), 71.7(C4), 57.8(C2), 57.5(C2), 52.7(CH_3O), 52.5(CH_3O), 52.4(C5), 52.1(C5), 36.6(C3), 35.6(C3), 20.9(CH_3O), MS (CI) *m/z* 246$(M+H)^+$.

(2*S*, 4*R*)-*N*-Methyloxycarbonyl-4-acetoxy-5-methoxy-2-pyrrolidinecarboxylic acid methyl ester (7, 8).
(2*S*, 4*R*)-(-)-*N*-methyloxycarbonyl-4-acetoxy-2-pyrrolidinecarboxylic acid methyl ester (**6**) (6.2 g, 25.4 mmol) was dissolved in methanol (100 mL). Tetrabutylammonium tetrafluoroborate (0.5 g) was added to the methanolic solution and the mixture electrolysed at 0.6-0.7 A (platinum anode-nickel cathode) for 10 hours.

After this time, the solution was concentrated under vacuum and the residue partitioned between diethyl ether and water. The ether layer was separated, dried over magnesium sulfate and filtered. The filtrate was concentrated under vacuum to give a yellow oil. Column chromatography of the oil, initially with 70:30 ethyl acetate-hexane, allowed isolation of the more mobile of the two diastereomeric products (2*S*, 5*S*-isomer) (yield= 2.14 g, 31%). Careful chromatography of the remaining material (1:1 ethyl acetate-hexane) afforded the 2*S*, 5*R*-isomer (yield= 0.46 g, 7%). **(2*S*, 5*S*)-isomer(7):** $[\alpha]_D^{25}$= -34.5°(c=0.25, MeOH); IR (neat) 3628, 2998, 2957, 2838, 2361, 1715, 1447, 1375, 1303, 1235, 1201, 1129, 1093, 1052, 1019, 959, 976, 876, 775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.44-5.19(1H, m, H5), 5.07(1H, m, H4), 4.61-4.30(1H, m, H2), 3.78, 3.77, 3.74, 3.73, 3.72(6H, 5s, $NCOOCH_3$, $COOCH_3$), 3.48, 3, 41(3H, 2s, CH_3O), 2.37(2H, m, H3), 2.09, 2.03(3H, 2s, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 171.2($NCOOCH_3$, carbonyl), 171.1($NCOOCH_3$, carbonyl), 169.9($COOCH_3$, carbonyl), 169.8($COOCH_3$, carbonyl), 156.2(CH_3CO, carbonyl), 91.8(C5), 91.2(C5), 86.4(C5), 85.9(C5), 76.1(C4), 75.3(C4), 71.3(C4), 70.5(C4), 58.2(C2), 58.0(C2), 57.7(C2), 57.4(C2), 55.6(CH_3O), 55.2(CH_3O), 53.0(CH_3O), 52.5(CH_3O), 52.3(CH_3O), 33.6(C3), 32.5(C3), 31.5(C3), 30.5(C3), 20.9(CH_3CO), 20.6(CH_3O); MS (CI) *m/z* 276$(M+H)^+$, 244$(M-CH_3O)^+$; HRMS (EI) calcd. for $C_{11}H_{17}NO_7$ (M^+) 275.1005 found (M^+) 275.1014.

(2*S*, 5*R*)-isomer (8): $[\alpha]_D^{25}$= +21.7°(c=0.31, MeOH); IR (neat) 3628, 2955, 2840, 2361, 1738, 1525, 1446, 1371, 1300, 1244, 1124, 1089, 1022, 982, 776, 731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.29(1H, m, H5), 5.15-4.96(1H, m, H4), 4.00(1H, m, H2), 3.83, 3.81, 3.78(3H, 3s, $NCOOCH_3$), 3.76, 3.73, 3.69(3H, 3s, $COOCH_3$), 3.49, 3.47, 3.43, 3.39, 3.34, 3.32(3H, 6s, CH_3O), 2.55(1H, m, H3), 2.36(1H, m, H3), 2.13, 2.12, 2.10, 2.08(3H, 4s, CH_3CO); ^{13}C NMR ($CDCl_3$) δ 170.1(NCO, carbonyl), 153.9($COOCH_3$, carbonyl), 96.7(C5), 93.9(C5), 71.2(C4), 53.9(CH_3O), 52.9(CH_3O), 52.4(C2), 52.3(CH_3O), 43.2(C3), 38.4(C3), 21.6(CH_3O), 20.8(CH_3O); MS (CI) m/z 276$(M+H)^+$; HRMS (EI) calcd. for $C_{11}H_{17}NO_7$ $(M)^+$ 275.1005 found $(M)^+$ 275.0957.

(2*S*, 4*R*)-*N*-*tert*-butyloxycarbonyl-2-methoxymethyl-4,5-dimethoxypyrrolidine (12)

(2*S*, 4*R*)-N-*tert*-butyloxycarbonyl-2-methoxymethyl-4-methoxypyrrolidine **(11)** (0.7g, 2.8mmol) in methanol (100ml) was placed in an electrochemical cell. Tetrabutylammonium tetrafluoroborate (1.5g) was added and a current of 1.8-2.0A passed through the solution, at a temperature of 35-40°C, for a total of 4 hours. The solution was then concentrated under vacuum and the residue obtained partitioned between diethyl ether and water. Separation of the ether layer, followed by drying and concentration under vacuum gave the crude product as an oil. Column chromatography (70:30 hexane-ethyl acetate) and pooling of the appropriate fractions, yielded the title compound as a colourless oil (yield= 36%). ^1H NMR (300MHz, $CDCl_3$) δ 5,32(1H, m, br, H5), 4.98-4.75(1H, m, H4), 3.90(1H, m, H2), 3.71(3H, CH_3O), 3.48, 3.44(3H, 2s, CH_3O), 3.40(1H, m, CH_2OCH_3), 3.36, 3.34(3H, 2s, CH_3O), 3.32(1H, m, CH_2OCH_3), 2.53(1H, m, H3), 2.47(1H, m, H3), 1.50, 1.48, 1.47, (9H, 3s, *t*-BOC); ^{13}C ($CDCl_3$) δ 171.2(*t*-BOC, carbonyl), 171.0(*t*-BOC, carbonyl), 103.0(C5), 84.3(C4), 80.6(Cq*t*-BOC), 78.3(CH_3O), 67.3(CH_2O), 65.9(CH_2O), 59.7(C2), 58.8(C2), 55.9(CH_3O), 55.4(CH_3O), 51.9(CH_3O), 36.4(C3), 34.8(C3), 28.2(CH_3, *t*-BOC); MS (CI) *m/z* 276 $(M+H)^+$, 246$(M-OCH_3)^+$; Found C; 56.50, H; 8.76, N; 5.41 $C_{13}H_{25}NO_5$ requires C; 56.70, H; 9.15, N; 5.09.

1-[2*S*, 4*R*) -*N*-Methyloxycarbonyl-4-acetoxy-2-pyrrolidin-5-ylcarboxylic acid methyl ester]-5-methyluracil (13, 14).

To freshly prepared *bis*-2,4-trimethylsilyloxy-5-methylpyrimidine (4.04 mmol) under an atmosphere of nitrogen, was added a solution of (2*S*, 4*R*, 5*S*)-*N*-methyloxycarbonyl-4-acetoxy-2-pyrrolidinecarboxylic acid methyl ester (**7**) (1.0 g, 3.67 mmol) in dichloroethane (5 mL). The solution was cooled to 0°C on an ice bath with stirring before adding stannic chloride (1.16 g, 4.44 mmol) slowly from a syringe. The mixture was then allowed to warm to ambient temperature, with stirring continued for 3 hours. The reaction was then diluted with dichloromethane and the solution poured into aqueous $NaHCO_3$. The organic layer was separated and washed with brine, before drying over magnesium sulfate. The filtrate was concentrated under vacuum to give an orange oil. Thin layer chromatography (95:5 dichloromethane-ethanol) revealed the product to be a mixture of two isomers which were separated by column chromatography (95:5 dichloromethane-ethanol) and isolated as white foams (Rf=0.46, 2*S*, 4*R*, 5*R*-isomer, yield= 0.26 g, 19%; Rf=0.40, 2*S*, 4*R*, 5*S*-isomer, yield= 0.10 g, 7%). **(2*S*, 4*R*, 5*R*)-isomer (β-anomer) (13):** $[\alpha]_D^{25}$= -3.8°(c=0.06, MeOH); IR (nujol) 3189, 2361, 1748, 1692, 1216, 1136, 1054, 1016, 779, 725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.76-8.30(1H, m, br, N^3H), 8.19, 8.02(1H, 2s, H6), 6.05(1H, m, H5'), 5.26(1H, m, H4'), 4.57(1H, m, H2'), 3.95, 3.94, 3.84(3H, 3s, $NCOOCH_3$), 3.78, 3.76(3H, 2s, $COOCH_3$), 2.70-1.90(2H, m, H3'), 2.12(3H, s, CH_3CO), 1.97(3H, s, 5-CH_3); ^{13}C NMR ($CDCl_3$) δ 172.4($NCOOCH_3$, carbonyl), 169.8($COOCH_3$, carbonyl), 163.7(C4), 154.9(CH_3CO, carbonyl), 150.4(C2), 135.0(C6), 111.1(C5), 76.5(C5'), 58.9(C2'), 53.9(CH_3O), 52.9(CH_3O), 33.9(C3'), 32.9(C3'), 20.85(CH_3CO), 12.7(CH_3); MS (CI) *m/z* 370(M+H)$^+$; UV λ_{max}= 268 nm (ε= 9736); HRMS (EI) calcd. for $C_{15}H_{19}N_3O_8$ $(M)^+$ 369.1172 found $(M)^+$ 369.1137.

(2*S*, 4*R*, 5*S*)-isomer (α-anomer) (14): $[\alpha]_D^{25}$= +44.1°(c=0.03, MeOH), IR (nujol) 3189, 2361, 1748, 1692, 1303, 1216, 1136, 1101, 1054, 1016, 779, 725 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.81, 9.39(1H.2s, br, N^3H), 7.05(1H, s, br, H6), 7.10-6.86(1H, m, *J*=7.5 Hz, H5'), 5.57(1H, m, *J*=9.0 Hz, H4'), 4.65(1H, m, H2'), 3.78, 3.77(3H, 2s, $NCOOCH_3$), 3.68, 3.67, 3.64, 3.63(3H, 4s, $COOCH_3$), 3.6-2.7(1H, m, H3'), 2.45(1H, m, H3'), 2.18(3H, s, CH_3CO), 1.98, 1.96(3H, 2s, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.3($NCOOCH_3$, carbonyl), 173.2($NCOOCH_3$, carbonyl), 170.0($COOCH_3$, carbonyl), 169.9($COOCH_3$, carbonyl), 163.9(C4), 153.1(CH_3CO, carbonyl), 151.8(C2), 135.5(C6), 134.9(C6), 110.3(C5), 109.4(C5), 70.9(C5'), 70.5(C5'), 70.0(C5'), 69.9(C5'), 66.8(C4'), 66.0(C4'), 64.9(C4'), 60.4(C4'), 59.1(C2'), 59.0(C2'), 58.8(C2'), 53.6(CH_3O), 53.0(CH_3O), 52.8(CH_3O), 35.5(C3'), 34.3(C3'), 21.0(CH_3CO), 13.0(CH_3), 12.8(CH_3); MS (CI) *m/z* 370(M+H)$^+$; UV λ_{max}= 264 nm (ε= 11818); HRMS (EI) calcd. for $C_{15}H_{19}N_3O_8$ $(M)^+$ 369.1172 found 369.1182.

1-[(2*S*, 4*R*, 5*R*)*N*-Methyloxycarbonyl-4-hydroxy-2-pyrrolidine-5-ylcarboxylic acid methyl ester]-5-methyluracil (17).

To 1-[(2*S*, 4*R*, 5*R*)-*N*-methyloxycarbonyl-4-acetoxy-2-pyrrolidin-5-ylcarboxylic acid methyl ester]-5-methyluracil (**12**) (0.12 g, 0.57 mmol) in dry THF (3 mL) at 0°C was added $NaBH_4$ (0.02 g, 0.57 mmol) with stirring. The mixture was stirred at ambient temperature for 10 hours, after which the mixture was carefully acidified with concd.HCl until a neutral solution was obtained. The solution was then diluted with chloroform, washed with brine and dried over magnesium sulfate. Concentration of the filtrate gave a white foam which was subjected to column chromatography (90:10 dichloromethane-ethanol) to give the title

compound as a white foam (yield= 0.12 g, 66%). $[\alpha]_D^{25}$= +65.5(c=0.08, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.75, 10.65(1H, 2s, br, N^3H), 8.34, 8.26(1H, 2s, H6), 5.9(1H, m, H5'), 5.5(1H, m, H4'), 4.7(1H, br, OH), 4.45(1H, m, H2'), 3.84(3H, s, CH_3O), 3.76, 3, 72(3H, 2s, CH_3O), 3.4(1H, m, H3'), 2.4(1H, m, H3'), 1.95, 1.93(3H, 2s, CH_3); ^{13}C NMR ($CDCl_3$) δ173.4($NCOOCH_3$, carbonyl), 164.9($COOCH_3$, carbonyl), 155.0(C4), 151.6(C2), 136.2(C6), 111.8(C5), 111.2(C5), 79.5(C5'), 78.7(C5'), 74.5(C4'), 73.6(C4'), 59.8(C2'), 59.0(C2'), 53.9(CH_3O), 53.0(CH_3O), 52.7(CH_3O), 52.3(CH_3O), 35.0(C3'), 34.0(C3'), 12.5(CH_3), 12.3(CH_3); MS (CI) *m/z* 328(M+H)$^+$; UV λ_{max}= 263 nm (ε= 8035); HRMS (EI) calcd. for $C_{13}H_{17}N_3O_7$ $(M)^+$ 327.1066 found $(M)^+$ 327.1064.

1-[(2*S*, 4*R*, 5*R*)-*N*-Methyloxycarbonyl-2-hydroxymethyl-4-hydroxypyrrolidin-5-yl]-5-methyluracil (18). To 1-[2*S*, 4*R*, 5*R*)-*N*-methyloxycarbonyl-4-hydroxy-2-pyrrolidin-5-ylcarboxylic acid methyl ester]-5-methyluracil **(17)** (0.1 g, 0.31 mmol) in ethanol (2 mL), was added $NaBH_4$ (0.03 g, 0.8 mmol). The solution was then stirred for 10 hours at ambient temperature, after which the solution was acidified with concd.HCl until a neutral solution was obtained. The solution was then filtered through a celite pad and concentrated under vacuum to give a white foam. Column chromatography (85:15 dichloromethane-ethanol) afforded the title compound as a white foam (0.02 g, 22%). ^{1}H NMR (300 MHz, d_6-DMSO) δ 11.26(1H, s, br, N^3H), 8.15(1H, s, H6), 5.70(1H, m, H5'), 5.57(1H, d, *J*=6.0 Hz, H4'), 5.32(1H, m, H2'), 4.11(1H, br, OH), 3.93(1H, m, CH_2OH), 3.68-3.54(3H, m, $NCOOCH_3$), 3.51(1H, m, CH_2OH), 2.09(1H, m, H3'), 1.80(1H, m, H3'), 1.71(3H, s, CH_3), 1.8-1.6(2H, br, OH); ^{13}C NMR (d_6-DMSO) δ 163.9(C4), 155.5($NCOOCH_3$, carbonyl), 150.6(C2), 136.6(C6), 108.1(C5), 72.9(C5'), 59.8(CH_3O), 52.8(CH_3O), 20.8(C2'), 12.3(CH_3); MS (CI) *m/z* 300(M+H)$^+$; UV λ_{max}= 265 nm (ε= 10299); HRMS (EI) calcd. for $C_{12}H_{17}N_3O_6$ $(M)^+$ 299.1117 found $(M)^+$ 299.1116.

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