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# AN EFFICIENT SYNTHESIS OF Y-NUCLEOSIDE (WYOSINE) BY REGIOSPECIFIC METHYLATION OF $N^4$-DESMETHYLWYOSINE USING ORGANOZINC REAGENT

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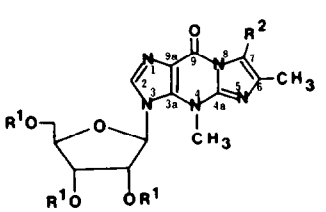
Summary: The reaction of the organozinc reagent, produced by the reaction of $CH_2I_2$ + $Zn(C_2H_5)_2$ + glyme in diethylether at 20 °C, regioselectively methylates the $N^4$-nitrogen of $N^4$-desmethylwyosine-triacetate 3 to give pure wyosine-triacetate 1e in 76% isolated yield; no trace of the isomeric $N^5$ methylated product 1s found in the latter reaction. Synthesis of a new congener of Y-nucleoside 1f is also reported for the first time from compound 5 in a high overall yield using a similar procedure.

The hypermodified, fluorescent Y-nucleosides 1a-1d which occur adjacent to the 3'-end of the anticodon of yeast phenylalanine transfer ribonucleic acid ($tRNA^{phe}$), are perhaps the most chemically interesting group of naturally occurring nucleosides[1,2] because of the challenge it has posed, due to its unstable nature[3] and unique site of methylation at N-4, for an unambiguous synthesis in a high overall yield[4-7]. Two Japanese groups have reported multistep synthesis of Wyosine 1a (nucleoside $Y_t$) with poor overall yields from 5-(methylamino)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-imidazole-4-carboxamide 2[5,6]. On the other hand, Golankiewicz et al.[7] have reported a 3% yield of wyosine-triacetate 1e, by the reaction of a dichloromethane solution of diazomethane with 4-desmethylwyosine-triacetate 3; this reported yield[7] was not however reproducible in our hand. The main product formed in the latter reaction, as reported by Golankiewicz et al.[7], was $N^5$-methylated isomer of wyosine 4. We were however convinced that it should be possible to devise an appropriate methylating condition to convert 4-desmethylwyosine-triacetate 3 to wyosine-triacetate 1e because Y-nucleosides are derived from guanosine biosynthetically[7,8]. We herein report a successful straightforward synthesis of wyosine-triacetate 1e in 76% yield by the reaction of 4-desmethylwyosine-triacetate 3 (1 g. scale) with "the zinc reagent" (Simmons-Smith reaction)[9] produced by the reaction of methylene iodide with diethylzinc. Three different conditions for the synthesis of wyosine-triacetate 1e by Simmons-Smith reaction[9] were attempted: (a) $CH_2I_2$ + $Zn(C_2H_5)_2$ in diethylether at 20 °C (putative $ICH_2ZnI$ as "the zinc reagent"); (b) $CH_2I_2$ + $Zn(C_2H_5)_2$ in diethylether with glyme at 20 °C (putative $ICH_2ZnCH_2I$ as "the zinc reagent" and (c) $CH_2N_2$ in diethylether + dry $ZnI_2$ + glyme at 20 °C (putative $ICH_2ZnCH_2I$ as the "zinc reagent"). Of these three conditions, both procedures (b) and (c) gave clean reactions with 3, in 0.2 mmol scale, to an almost single product 1e on Tlc; however due to the potential hazard of handling of diazomethane solution in a large scale reaction, the following preparative procedure, using condition (b), is recommended:



1a: $R^1 = R^2 = H$

1b: $R^1 = H$, $R^2 = CH_2CH_2CH(NHCO_2Me)CO_2Me$

1c: $R^1 = H$, $R^2 = CH_2CH(O_2H)CH(NHCO_2Me)CO_2Me$

1d: $R^1 = H$, $R^2 = CH_2CH(OH)CH(NHCO_2Me)CO_2Me$

1e: $R^1 = COCH_3$, $R^2 = H$

1f: $R^1 = H$, $R^2 = CH_3$

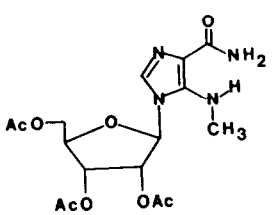
1g: $R^1 = COCH_3$, $R^2 = CH_3$

Preparation of wyosine-triacetate 1e;

A solution of freshly distilled diiodomethane (3.6 ml, 44.8 mmol) in dry diethylether (12 ml) was introduced in a 250 ml 3-necked flask fitted with a reflux condenser, a guard tube (silica gel) and a rubber septum, under a gentle stream of dry argon. Then a solution of diethylzinc (3 ml, 22.5 mmol) in dry diethylether (15 ml) was added with a syringe at 20 °C over a period of 20 min at a rate such that the reaction was under a smooth reflux. After 30 min, the exothermic reaction was complete and dry dimethoxyethane (2.3 ml, 22.5 mmol) was added. After a few minutes, a white precipitate of $ZnI_2$-dimethoxyethane complex separated. "The zinc reagent" thus synthesized[9] was cooled at 10 °C, and a solution of 4-desmethylwyosine-triacetate 3 (1.1 g, 2.46 mmol) in dry dichloromethane (20 ml) was added quickly. A Tlc examination (5% methanol-$CHCl_3$) upon an aqueous work-up after 1 min, showed a complete consumption of starting material and a single higher $R_f$ fluorescent spot. After 4 min, ice-cold 1 M aqueous ammonium bicarbonate solution (50 ml) was added to the reaction mixture resulting in a strong effervescence of ethane. When the hydrolysis was complete, chloroform (50 ml) was added and the two-phase system, thus produced, was filtered through a celite bed and washed with an excess of chloroform. Combined organic phase was washed with 0.1 M aqueous sodium thiosulfate (75 ml) and water (75 ml). The organic phase was evaporated in a rotavapor in vacuo to dryness leaving a foam (1.2 g) which was dissolved in ethylacetate (free of acid and freshly distilled) and loaded onto a short column of silica gel (3.5 mm x 30 mm) packed with ethylacetate of latter quality. The column was eluted with ethylacetate (50 ml), 1% methanol-ethylacetate mixture (150 ml) and finally with 2% methanol-ethylacetate mixture. Evaporation of appropriate fractions gave a fluorescent white foam of 1e, 860 mg (75%)[10]. Further elution of column with 5% methanol-chloroform gave a powder (46 mg, 8%) which after recrystallization from water gave 20 mg of $N^1$-methylwyebase[11]. Wyosine-triacetate 1e was finally deprotected to give wyosine[12] 1a by the action of methanolic ammonia at 20 °C in 88% yield.

Recently McCloskey et al.[13] have reported the isolation of a new derivative of the Y-nucleoside 1f from archaebacterium tRNA. This has prompted us to devise the synthesis of the new isomer 1f from its 4-desmethyl-triacetate precursor 5 using a similar general strategy as described above for wyosine-triacete 1e. We have thus synthesized 4-desmethyl-triacetate 5 by a reaction of 2-bromobutanone[17] and guanosine in DMSO in presence of NaH, followed by an acetylation step, using procedures almost identical to the preparation of compound 3[7,14]. Compound 5[18] (1 mmol) was then selectively methylated according to the procedure (a)[9] (same as procedure (b) but dimethoxyethane was omitted) to give the new congener of Y-nucleoside 1g[19] in 65% yield which was subsequently deprotected to give 1f[20] in 70% yield as crystals (water).

Although Simmons-Smith reaction[9] has been proved to be a versatile and convenient method for the stereospecific synthesis of cyclopropanes from olefins, but "the zinc reagent" has been reported to be quite resistant to methylene transfer to the $>C=N-$ except one example[15]. We found that the $N^4$-methylation, under above condition, does not proceed at all if a proton on $N^5$ is not available as in $N^5$-methylated or acetylated compounds 4 and 6, respectively. It is not clear to us why the above reaction undergoes such a regiospecific $N^4$-methylation to give 1e without any trace of $N^5$-methylated product 4. It is possible that "the zinc reagent" undergoes a nucleophilic displacement reaction[16] at the methylenic-carbon upon abstraction of the $N^5$-proton by iodide as shown in the complex 7 which then undergoes cleavage of the carbon-metal bond to give compounds 1e or 1g. An alternative mechanism involving cyclopropanation[9] across $>C^{4a}=N^4-$, as shown in 9, does not explain the latter regiospecific addition over two other double bonds in compound 3; furthermore it is difficult to explain the driving force, other than the resonance stabilization of the final product 1e, for the breaking of the aziridine carbon-carbon bond in the intermediate 9 under the above experimental condition. It is also not clear, that if 9 were an intermediate in the above reaction, why an adduct like 9 could not be isolated by the reaction of "the zinc reagent" with $N^5$-methylated or acetylated compounds 4 and 6, respectively.

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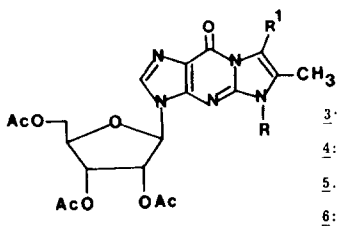
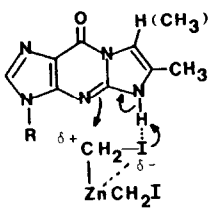



3: R = $R^1$ = H

4: R = $CH_3$, $R^1$ = H

5: R = H, $R^1$ = $CH_3$

6: R = $COCH_3$, $R^1$ =



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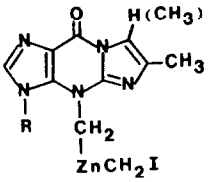

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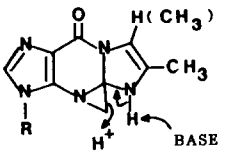

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10. UV (MeOH): $\lambda_{max}$ = 235 nm (25.300), 291 nm (6.480), (pH 7); 234 nm (26.200), 291 nm (6.340), (pH 12); 227 (23.300), 273 nm (9.500), (pH 2). MS ($FAB^+$): calc. for $(M + H)^+$ 462.1625, found 462.1623. $[\alpha]_D^{20}$ = -15 ° (c = 0.25, MeOH). $^1$H-NMR ($CDCl_3$): δ = 7.73 (s, 1H) H-2; 7.38 (q, $^4J_{H7-CH_3}$ = 1 Hz, 1H) H-7; 6.27 (d, $J_{1',2'}$ = 5.8 Hz, 1H) H-1'; 5.87 (dd, $J_{2',3'}$ = 5.5 Hz, 1H) H-2'; 5.49 (dd, $J_{3',4'}$ = 4.0 Hz, 1H) H-3'; 4.51 (m, 1H) H-4'; 4.31 (d, 2H) H-5',5''; 4.19 (s, 3H) N-$CH_3$; 2.31 (d, 3H) 6-$CH_3$; 2.18, 2.16, 2.09 (3s, 3 x 3H) 3 x OAc. $^{13}$C-NMR ($CDCl_3$): δ= 151.8 (C-9); 142.3 (C-4a); 139.7 (C-3a); 138.1 (C-6); 133.6 (d, $J_{CH}$ = 217 Hz) C-2; 116.6 (C-9a); 106.4 (d, $J_{CH}$ = 195 Hz) C-7; 86.2 (d, $J_{CH}$ = 168 Hz) C-1'; 80.9 (d, $J_{CH}$ = 152 Hz) C-4'; 72.9 (d, $J_{CH}$ = 155 Hz) C-2'; 70.6 (d, $J_{CH}$ = 157 Hz) C-3'; 62.8 (t, $J_{CH}$ = 150 Hz) C-5'; 33.9 (N-$CH_3$); 14.25 (6-$CH_3$).
11. mp = 209-10 °C ($H_2O$), MS ($FAB^+$): calc. for $(M + H)^+$ 218.1042, found 218.1074. UV (water): $\lambda_{max}$ = 231 nm (24.900), 262 nm (4.700), 310 nm (5.300), (pH 7 and 13); 222 nm (sh), 231 nm (29.400), 285 nm (6.900) (pH 2): $^1$H-NMR ($CDCl_3$): δ = 7.65 (s, 1H) H-2; 7.38 (q, 1H) H-7; 4.10 (d, 3H) 1-$CH_3$; 3.96 (s, 3H) 4-$CH_3$; 2.36 (d, 3H) 6-$CH_3$.

12. mp = 233 °C (water) decomp. $[\alpha]_D^{20}$ = -57 ° (c = 0.08, $H_2O$). MS ($FAB^+$): calc. for $(M + H)^+$ 336.1308, found 336.1309. UV: $\lambda_{max}$ = 235 nm (32.300), 295 nm (7.400), (0.1 M phosphate buffer, pH 7); $\lambda_{max}$ = 235 nm (33.800), 295 nm (7.600) (pH 13); 227 nm (36.300), 230 nm (36.300), 278 nm (10.700), (pH 2). $^1$H-NMR (DMSO-$d_6$): δ = 8.27 (s, 1H) H-2; 7.37 (d, $^4J_{H7\text{-}CH_3}$ = 1 Hz, 1H) H-7; 6.16 (d, $J_{1',2'}$ = 4.6 Hz, 1H) H-1'; 4.48 (dd, $J_{2',3'}$ = 4.6 Hz, 1H) H-2'; 4.19 (dd, 1H) H-3'; 4.11 (s, 3H) N-$CH_3$; 4.05 (m, 1H) H-4'; 3.67 (m, 2H) H-5',5''; 2.23 (d, 3H) 6-$CH_3$. $^{13}$C-NMR (DMSO-$d_6$): 151.6 (C-9); 142.4 (C-4a); 137.4 (C-6); 135.5 (d, $J_{CH}$ = 219 Hz) C-2; 115.7 (C-9a); 105.7 (d, $J_{CH}$ = 195 Hz) C-7; 88.8 (d, $J_{CH}$ = 168 Hz) C-1'; 85.4 (d, $J_{CH}$ = 150 Hz) C-4'; 74.8 (d, $J_{CH}$ = 149 Hz) C-2'; 69.6 (d, $J_{CH}$ = 151 Hz) C-3'; 60.4 (t, $J_{CH}$ = 141 Hz) C-5'; 33.9 (N-$CH_3$); 14.1 (6-$CH_3$).

    Above data and acid lability (performed by H. Lönnberg et al. of University of Turku, Finland) are identical to the reported values (ref. 6 & 7).

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18. UV (MeOH): λmax 239 nm (11.600), 290 nm (2.800), 314 nm (2.600), (pH 12); 230 nm (12.100), 286 nm (6.800), (pH 7); 230 nm (8.700), 284 nm (4.500), (pH 2). $[\alpha]_D^{20}$ = -14.2 ° (c = 0.5, MeOH). MS ($FAB^+$): calc. for $(M + H)^+$ 462.1625, found 462.1631.
    $^1$H-NMR ($CDCl_3$): δ = 7.89 (s, 1H) H-2; 6.06 (m, 2H) H-1' and -2'; 5.71 (m, 1H) H-3'; 4.37 (m, 3H) H-4' and 5', 5"; 2.69 (s, 3H) 7-$CH_3$; 2.25 (s, 3H) 6-$CH_3$; 2.12, 2.09 and 2.02 (3s, 3 x 3H) 3 x OAc. $^{13}$C-NMR ($CDCl_3$): δ = 154.9 (C-9); 149.2 (C-3a); 146.3 (C-4a); 137.11 (d, $J_{CH}$ = 207.6 Hz) C-2; 121.3 (C-6); 117.2 (C-9a); 116.3 (C-7); 86.9 (d, $J_{CH}$ = 168 Hz) C-1'; 76.8 (d, $J_{CH}$ = 153 Hz) C-4'; 72.5 (d, $J_{CH}$ = 161 Hz) C-2'; 70.8 (d, $J_{CH}$ = 161 Hz) C-3'; 63.3 (t, $J_{CH}$ = 148 Hz) C-5'; 20.6 (Ac); 20.5 (Ac); 11.0, 9.10 (7- & 6-$CH_3$).
19. UV (MeOH): λmax 238 nm (19.500), 297 nm (3.900), (pH 12); 239 nm (18.100), 297 nm (3.900), (pH 7); 230 nm (17.100), 275 nm (7.400), (pH 2). $[\alpha]_D^{20}$ = -19.5 ° (c = 0.52, MeOH). MS ($FAB^+$): calc. for $(M + H)^+$ 476.1782, found 476.1796.
    $^1$H-NMR ($CDCl_3$): δ = 7.74 (s, 1H) H-2; 6.24 (d, $J_{1',2'}$ = 6 Hz, 1H) H-1'; 5.82 (dd, $J_{2',3'}$ = 5.6 Hz, 1H) H-2'; 5.48 (dd, $J_{3',4'}$ = 3.4 Hz, 1H) H-3'; 4.5 (m, 1H) H-4'; 4.32 (m, 2H) H-5', 5"; 4.11 (s, 3H) N-$CH_3$; 2.62 (d, J = 0.7 Hz) 7-$CH_3$; 2.18 (s, 6H) 6-$CH_3$ and one acetate; 2.14 and 2.12 (2s, 2 x 3H) 2 x OAc. $^{13}$C-NMR ($CDCl_3$): δ = 154.2 (C-9); 141.8 (C-4a); 139.2 (C-3a); 133.5 (d, $J_{CH}$ = 220 Hz) C-2; 133.2 (m) C-6; 118.9 (m) C-7; 116.4 (C-9a); 86.1 (d, $J_{CH}$ = 168 Hz) C-1'; 80.9 (d, $J_{CH}$ = 151 Hz) C-4'; 73.1 (d, $J_{CH}$ = 154 Hz) C-2'; 70.8 (d, $J_{CH}$ = 159 Hz) C-3'; 62.9 (t, $J_{CH}$ = 149 Hz) C-5'; 33.9 (N-$CH_3$); 12.3 and 11.0 (6- and 7-$CH_3$).
20. mp = 307 °C (decomp.). UV (MeOH): λmax 239 nm (24.100), 298 nm (4.700), (pH 12); 238 nm (24.100), 298 nm (4.800), (pH 7); 235 nm (28.600), 285 nm (6.100), (pH 2). $[\alpha]_D^{20}$ = -45 ° (c = 0.041, water). MS ($FAB^+$): calc. for $(M + H)^+$ 350.1465, found 350.1452.
    $^1$H-NMR (DMSO-$d_6$): δ = 8.17 (s, 1H) H-2; 6.09 (d, $J_{1',2'}$ = 4.9 Hz, 1H) H-1'; 5.74 (d, 6.1 Hz, 1H) HO-2'; 5.33 (d, 5.4 Hz, 1H) HO-3'; 5.15 (t, 5.1 Hz, 1H) HO-5'; 4.46 (dd, $J_{1',2'}$ = 5.1 Hz, 1H) H-2'; 4.1 (m, 2H) H-2' & -3'; 4.01 (s, 3H) N-$CH_3$; 3.65 (m, 2H) H-5', 5"; 2.55 (s, 3H) 7-$CH_3$; 2.11 (s, 3H) 6-$CH_3$. $^{13}$C-NMR (DMSO-$d_6$): δ = 154.2 (C-9); 142.1 (C-4a); 139.6 (C-3a); 135.1 (d, $J_{CH}$ = 218 Hz) C-2; 132.6 (C-6); 117.4 (m) C-7; 115.8 (C-9a); 88.4 (d, $J_{CH}$ = 170 Hz) C-1'; 85.4 (d, $J_{CH}$ = 146 Hz) C-4'; 74.5 (d, $J_{CH}$ = 138 Hz) C-2'; 69.6 (d, $J_{CH}$ = 138 Hz) C-3'; 60.4 (t, $J_{CH}$ = 140 Hz) C-5'; 33.6 (N-$CH_3$), 12.1, 10.8 (7- & 6-$CH_3$).

    The acid lability of this 7-methyl derivative is very similar to that of Y-nucleoside (H. Lönnberg, unpublished result). This synthetic sample has also been compared with that of the naturally-occurring compound (ref. 13) by UV, Hplc and mass spectroscopy by Professor J.A. McCloskey (University of Utah, U.S.A, personal communication) and have been found to be identical.

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