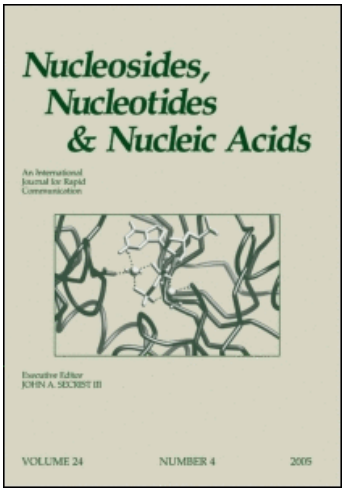

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METHYLATION OF A MESOIONIC TRICYCLIC NUCLEOSIDE RELATED TO WYOSINE

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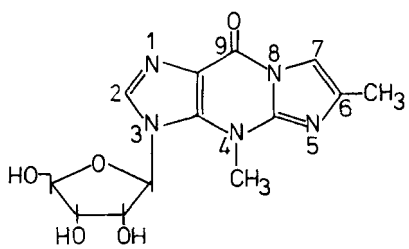
Abstract. 4-Desmethylwyosine /2/, a formal precursor of a hypermodified nucleoside wyosine /1/ was modified by N-1 benzylation. Mesoionic character of the resulting compound 3b, despite the similarities to wyosine in electron density distribution as shown by ^{13}C NMR, does not enforce the change of methylation direction towards the desired N-4 position.

Wyosine /1/ is the simple representative of a family of hypermodified, fluorescent, the so called Y nucleosides occuring in $tRNA^{Phe}$. Its total multistep syntheses have been recently communicated [1,2].



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In search for synthetic approaches, more closely related to the biosynthetical route, we have found[3] that wyosine may be obtained as a minor product of methylation of 4-desmethylwyosine /2/[4], the tricyclic precursor, easily accessible from guanosine. The major products of various methylation procedures were, however, isomers of wyosine, methylated at N-1 or at N-5. In order to change the methylation product patterns in favour of the formation of the naturally occuring N-4 methylated nucleoside we investigated and describe in the present work methylation of the N-1 substituted derivative, followed by N-1 deblocking. The presence of N-1 substituent imposes a mesoionic character upon the base moiety similar to that of 7-methylguanosine.

It has been found[5] that N-1 methylation of 2 leads to distinctive changes in ^{13}C NMR chemical shifts of some carbons, close to those caused by N-4 methylation, whereas N-5 methylation has very small effect.

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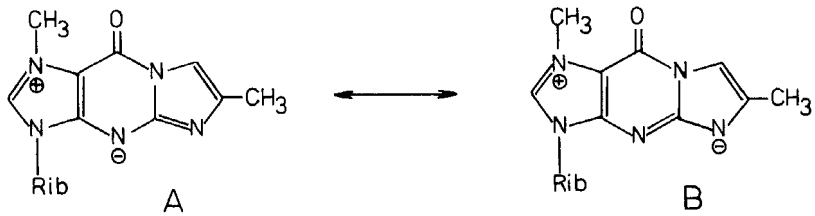
Especially the C-6 signal, which appears at 126.18 ppm for desmethyl compound 2 is shifted as much as to 137.21 ppm for wyosine 1, to 137.93 ppm for the N-1 derivative 3a, and only to 127.81 ppm for the N-5 analogue 7. ^{13}C NMR spectra suggest therefore that in the mesoionic 3a the resonance structure A may be more important than B



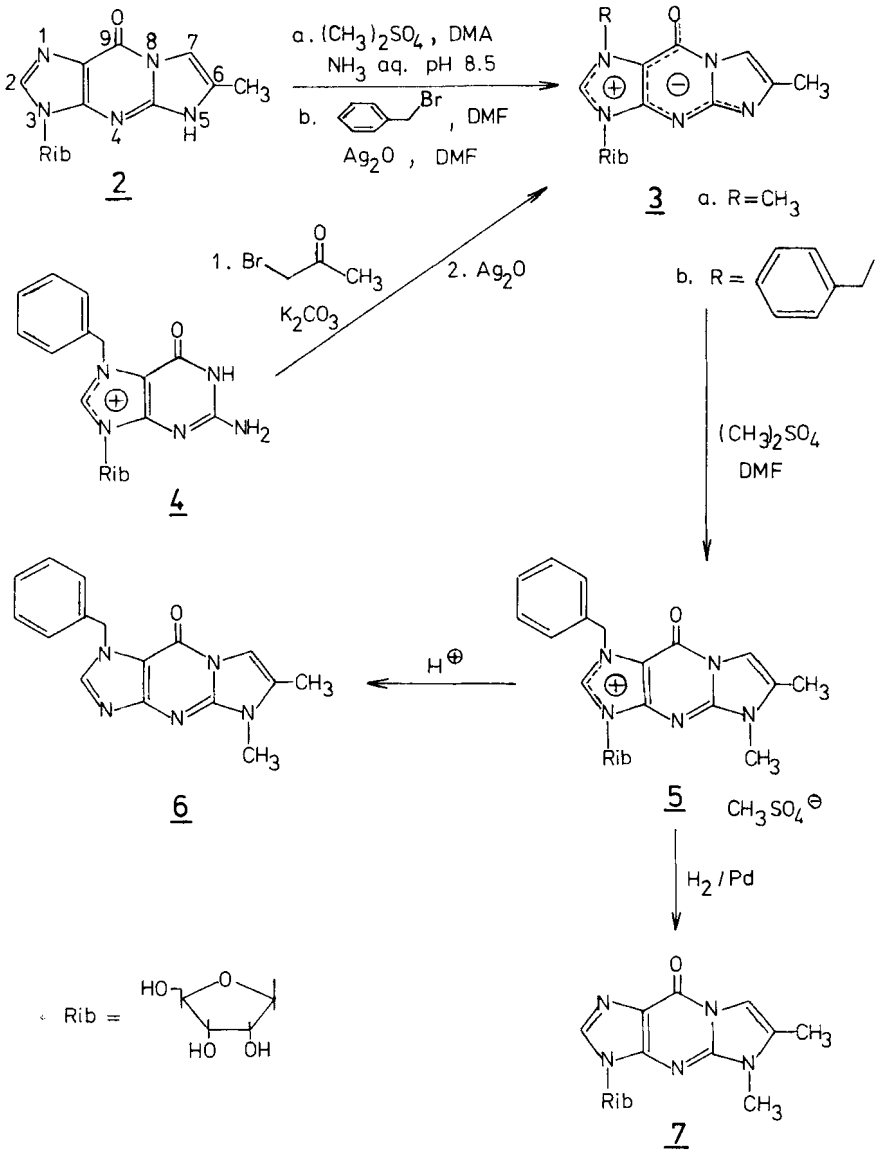
and that the changed, in comparison to 2, negative charge distribution may result in higher percentage of the desired N-4 methylation.

Introductory experiments on N-1 deblocking showed that demethylation with lithium 2-methylpropane-2thiolate,which according to [6] had quantitatively transformed 7-methylguanosine into guanosine, in the case of 3a could not be achieved. We employed, therefore, benzyl group as a quaternizing substituent, that could be easily removed by catalytic hydrogenolysis.

The action of benzyl bromide on 4-desmethylwyosine /2/ in dimethylformamide gave smoothly 1-benzyl-4-desmethylwyosine hydrobromide in 64% yield. Transformation of this salt into mesoionic nucleoside 3b could not be accomplished under mild alkaline conditions, working in the case of mesoionic compounds with methyl groups as quaternizing substituents[3,7]. Hydrolytic opening of the imidazolium ring occurred already at pH 8. Finally, mesoionic nucleoside 3b was obtained by treatment of the hydrobromide with silver oxide in anhydrous dimethylformamide /84%/. This new reagent seems to be promising for preparation of other mesoionic nucleosides.

Another, even more convenient way towards 3b was the modification of the procedure involving the formation of the third ring onto 7-substituted guanosine[3]. Starting with 7-benzylguanosine hydrobromide 4,1-benzyl-4-desmethylwyosine was obtained in 65% yield. The latter compound exhibited in ^{13}C NMR characteristic chemical shifts,similar to its 1-metyl congener /eg C-6 appeared at 135.99 ppm/.

Methylation of the mesoionic compound 3b with dimethylsulfate in dimethylformamide took strictly one direction. Thin-layer chromatography in 3 solvent systems did not show other products even in traces.



1-Benzyl-5-methyl derivative in form of methyl sulfate /5/ was isolated from the reaction mixture in 82% yield. The position of methylation was deduced from spectroscopic data and confirmed by chemical transformation and comparison with known compounds. In ^{1}H NMR the new methyl group appeared at 3.67 ppm/δ/, the chemical shift characteristic for N-5 methyl group of tricyclic nucleosides[3,8] whereas N-4 methyl signal was found around 4 [1,2,3]. In ^{13}C NMR, the newly introduced methyl group gave signal at 28.92 ppm, the values for known N-5 and N-4 methyl substituted nucleosides being 28,40 and 30.81 ppm, respectively [3,5]. Acidic hydrolysis of 5 provided a non-fluorescent base 6 different also in other respects from 1-benzyl wye base described in literature [9]. Removal of 1-benzyl grcup from 5 by catalytic hydrogenolysis over palladium resulted in N-5 methyl isomer of wyosine. This product was identical in all respects with 5-methyl-4-desmethylwyosine [3,8].

Compound 3b reacted exclusively at N-5 also with methyl iodide. Diazomethane in methanol could not be used as a methylating agent because of the opening of the imidazolium ring under the reaction conditions.

The reasons possibly responsible for the exlusive N-5 methylation are presently under investigation.

EXPERIMENTAL

General methods were as given previously[3]. Thin-layer chromatography /TLC/ was conducted on Merck precoated silica gel F_{254} Type 60 plates using the following solvent systems /measured by volume/ : A, isopropanol-concd.ammonia-water /7:1:2/; B, n-butanol- glacial acetic acid-water /5:3:2/; C,chloform-methanol /4:1/. All evaporations were carried out in vacuo below 40°C.

Materials.-4-Desmethylwyosine /2/ was obtained from guanosine using the modified [3]procedure of Kasai et al[8]. 7-Benzylguanosine hydrobromide /4/ was synthesized according to Brookes et al [10]. 1-Methyl-4-desmethylwyosine /3a/and 5-methyl-4-desmethylwyosine /7/ were prepared earlier [3] in our laboratory.

Attempted demethylation of 1-methyl-4-desmethylwyosine /3a/

An anhydrous suspension of 3a /33.5 mg, 0.1 mmol/ in hexamethylphosphoramide /HMPA, 0.1 mL/ was treated with 2.5 mmol of freshly prepared lithium 2-methylpropane-2-thiolate in HMPA /0.5 mL/. The

reaction mixture was then heated at 50° under atmosphere of argon for 48 h, After this time the obtained solution was evaporated to an oil, which was dissolved in ethanol-water 9:1 /2 mL/ and chromatographed on a silica gel column /H_{254} Merck,1.3x8 cm/ in an ethanol-water gradient, 2-mL fractions. Fractions 3-10 contained 1-methyl-4-desmethylwye base /69% yield, as determined by quantitative UV-measurment/, identical in all respects with the base obtained by hydrolysis of 3a in 1N HCL: $\lambda_{max}^{H_2O}$230nm/ε 34,600/, 259/6,600/ and 303 /8,800/; R_F 0.60/B/,0.58/C/. Fractions 11-14 contained the imidazolium ring-opened product / 12% of yield / and fractions 17-26- the unreacted substrate /14%/.

1-Benzyl-4-desmethylwyosine /3b/

Method A. To a suspension of 4-desmethylwyosine /2, 643 mg.2.0mmol/ in dry dimethylformamide /10 mL/ a portion of benzyl bromide /403 mg, 2.4 mmol/ was added.The reaction mixture was strirred at room temperature for 20 h, then evaporated to dryness. The residual yellow oil was dissolved in ethanol /10 mL/ and evaporated in the presence of silica gel /100-200 mesh, ca 10 mL/. The gel was dried under vacuum and applied on the top of a silica gel short column /4,5 x 7 cm,HF_{254} Macherey-Nagel/. Products were eluted with solvent C, 15-mL fractions. Fractions 11-34 containing the chromatogarphically pure main product were pooled and evaporated to yield 622 mg /64%/ of the hydrobromide of 1-benzyl-4-desmethylwyosine as a pale-yellow solid foam.

TLC : R_F 0.53/B/, 0.16/C/.

Anal. Calcd.for $C_{20}H_{21}N_5O_5$. HBr.0.5 H_2O /501.34/:C, 47.91; H, 4.62; N, 13.76; Br, 15.93. Found: 47.96; H, 4.84; N, 13.15; Br, 16.23.

UV :$\lambda_{max}^{H_2O}$ 232 nm /ε 25,200/, 279 /8,800/, 306 /7,300/

^{1}H NMR:/$(CD_3)_2SO$ /δ: 2.23/d,3,CH_3/, 3.72/m,3,4'and 5'H/, 4.18/t, 1,3'H/, 4.54/t,1,2'H/, 5.32/m,2,3' and 5'OH/, 5.83/s and d,3,CH_2Ph and 2'OH/, 6.04/d,1,1'H/,7.24-7.58/m,5,C_6H_5/, 7.66/d,1,7-H/,10.04/s,1,2-H/. In the presence of D_2O signals of hydroxyl groups and 2-H disappear.

A solution of the hydrobromide /210 mg, 0.427 mmol/ in anhydrous DMF /5 mL/ was added to a suspension of Ag_2O in DMF /5 mL,prepared from 0.7 mmol of $AgNO_3$ and 7 mL of 0.1N NaOH, then washed with water, acetone and finally with dry DMF/. The reaction mixture was stirred at room temperature for 30 min, then filtered through Celite 535. The filtrate was concentrated to a volume of ca 2 mL and diluted with chloroform /2 mL/. This solution was applied on a silica gel

layer /3 cm, HF_{254} Merck, on a suction funnel, ø 7 cm/ and eluted with chloroform, followed by chloroform-methanol 4:1, then 1:1, 100-mL fractions. Fractions 5-8 were evaporated to yield 147.3 mg /84%/ of 3b as a white solid.

TLC: R_F 0.73/A/, 0.53/B/, 0.06/C/.

Anal. Calcd. for $C_{20}H_{21}N_5O_5 \cdot H_2O$ /429.43/: C, 55.94; H, 5.40; N, 16.31. Found: C, 56.27; H, 5.04; N, 16.27.

UV: $\lambda_{max}^{H_2O}$ 232 nm /ε 23,200/, 279 / 8,000/, 306 / 5,700/.

^{13}C NMR: /$(CD_3)_2SO$/Assignments by comparison with the spectra of 3a and related tricyclic nucleosides [3,5].

δ: 14.63/6-CH_3/, 51.20/CH_2Ph/, 60.74/C-5'/, 69.62/C-3'/, 73.30/C-2'/ 36.15/C-4'/,89.83/C-1'/, 101.86/C-7/,103.49/C-9a/, 128.14,128.40,128.73 / C_6H_5/, 135.13/C-2/, 135.99/C-6/, 146.29/C-3a/, 148.90/C-4a/,149.97 /C-9/.

Method B. To a suspension of 7-benzylguanosine hydrobromide / 4, 440 mg, 0.986 mmol/ in dry DMF /10 mL/ were added: well powdered potassium carbonate /147.2 mg, 1.065 mmol/ and bromoacetone / 145.8 mg, 1.065 mmol/. The obtained suspension was stirred at room temperature till TLC in solvent C showed a completed conversion to 3b hydrobromide /2h/. The reaction mixture was then filtered through Celite and evaporated to a volume of ca 2 mL. This was added to a suspension of Ag_2O /1,5 mmol, prepared like in method A/. The reaction was carried out and the product was purified as in method A, yielding 258 mg of 3b /total yield 4→3b 65%/. The product was identical in all respects with that obtained in method A.

Alkaline hydrolysis of 1-benzyl-4-desmethylwyosine /3b/

An ultraviolet spectrum of 3b /1.2 A_{232}/ in 0.1M Tris-HCL, pH 10.0, 23°C, was recorded at 5-min time intervals. The increase of absorbance at 236 nm corresponded to the imidazole ring-opened product, $\lambda_{max}^{H_2O}$ 236,278 nm, R_F 0.36 in solvent C, half-time of hydrolysis $t_{1/2}$ 20 min.

1-Benzyl-5-methyl-4-desmethylwyosine methyl sulfate /5/

A sample of the mesoionic compound 3b /116 mg, 0.283 mmol/ was treated with dimethyl sulfate /46.5 mg, 0.368 mmol/ in anhydrous DMF /5 mL/. The reaction flask was maintained at room temperature at darkness. After 18 h a next portion /0.368 mmol/ of methylating agent was added and reaction was continued for the next day. Then the reaction

solution was evaporated to a brown oil,which was chromatographed on a silica gel short column /HF_{254} Merck ,2.5 x 10 cm/ in methylene chloride-ethanol 4:1,15-mL fractions. Fractions containing the product /8-21/ were pooled and evaporated to a colourless solid. This was dissolved in ethanol /1 mL/ and precipitated in ethyl ether /50 mL/, then filtered off, washed with ether and n-hexane, and dried under diminished pressure over P_2O_5 to give, 124.8 mg /82%/ of 5 as a white powder.

TLC:R_F 0.72/A/, 0.49/B/, 0.24/C/.

Anal.Calcd.for $C_{21}H_{24}N_5O_5 \cdot CH_3SO_4 \cdot 0.5\ H_2O$ /546.56/: C, 48.35; H, 5.16; N, 12.81; S, 5.87. Found: C, 48.16; H, 5.21; N, 12.49; S,6.02.

UV:$\lambda_{max}^{H_2O}$ 232 nm /ε 26,500/, 281 /9,400/, 308 /7,600/.

^{13}C NMR: /$(CD_3)_2SO$/. Assignments by comparison with the spectra of related tricyclic nucleosides[3,5] and SFORD technique. δ: 9.40/6-CH_3/, 23.92 /N-CH_3/, 51.83 /CH_2Ph/, 52.74 /CH_3SO_4/, 60.25 /C-5'/,69.13 /C-3'/ 73.70 /C-2'/, 85.84 /C-4'/, 90.02 /C-1'/, 103.53 /C-7/, 105.88 /C-9a/, 128.14, 128.60, 128.73 /C_6H_5/, 134.21 /C-6/, 138.13 /C-2/, 145.31 /C-3a/, 146.68 /C-4a/, 147.27 /C-9/.

^{1}H NMR: / $(CD_3)_2SO$/δ : 2.37/d,3,6-CH_3/, 3.67/s,6,N-CH_3,CH_3SO_4/, 3.92-4.36/m,4,3',4' and 5'H/, 4.64/t,1,2'H/, 5.13/t,1,5'OH/, 5.34/t,1, 3'OH/, 5.79/s,2,CH_2Ph/, 5.85/d,1,2'OH/, 6.04/d,1,1'H/, 7.33-7.53/m,5, C_6H_5/, 7.67/d,1,7-H/, 9.83/s,1,2-H/.

Acidic hydrolysis of 5. 1-Benzyl-5-methyl-4-desmethylwye /6/

A sample of 5 /24.4 mg, 0.044 mmol / was dissolved in methanol /0.2 mL/ and treated with 0.1N HCL /1.0 mL/ at 70° for 20 h. Then, the reaction mixture was cooled down to room temperature, neutralized with 0.01N NaOH, diluted with water /10 mL/, and extracted with ethyl acetate /two portions, 10 mL each/. The organic layer was dried over Na_2SO_4, and evaporated to an oil. The crude product was crystallized from ethanol, yielding 11.2 mg /87%/ of 1-benzyl-5-methyl-desmethylwye base /6/ as colourless crystals.

TLC: R_F0.64/B/, 0.74/C/.

UV:$\lambda_{max}^{H_2O}$ 235 nm /ε 35.200/, 261 /7.200/, 308 8.900/.

^{1}H NMR: / $CDCL_3$/δ : 2.27/d,3,6-CH_3/, 3.59/s,3,N-CH_3/,5.54/s,2,CH_2Ph/ 7.19-7.35/m,6,C_6H_5 and 7-H/, 7.76/s,1,2-H/.

Catalytic hydrogenolysis of 5. 5-Methyl-4-desmethylwyosine /7/

The methylated product 5 /54 mg. 0.1 mmol / was dissolved in 3 mL of 80% aq.ethanol and hydrogenated under atmospheric pressure over palladium oxide /10 mg/ for 18h. After evaporation of the solvent the debenzylated nucleoside 7 was crystallized from methanol to yield 31.2 mg /93%/ of colourless crystals. The product was identical in all respects with a sample of 5-methyl-4-desmethylwyosine obtained by direct methylation of 2[3]. /TLC in three solvent systems, UV, ^{1}H NMR./

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