SYNTHESIS OF N,O-PROTECTED DERIVATIVES OF 2'-O- METHYLCYTIDINE AND OF 2'-O-METHYL- AND N_1-METHYLGUANOSINES

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Using selective 3',5'-O-tetraisopropyldisiloxane protection, N-acyl-2'-O-methyl derivatives of cytidine and guanosine and of 2'-O-tetrahydropyranyl-N_1-methylguanosine have been synthesized. It has been shown that the spatial screening of the amino groups by the neighboring methyl substituents in N_1-methylguanosine leads to its inactivation in acylation, tetrahydropyranylation, and tritylation reactions.

In nucleoside section 27-43, the anticodon loop of $tRNA^{Phe}$ contains the following modified methylated nucleosides: 2'-O-methylcytidine (32), 2'-O-methylguanosine (34), the guanosine derivative wybutosine (37), and 5-methylcytidine (40) [1, 2]. A nucleoside of simpler structure — N_1-methylguanosine is frequently found in position 37 of the tRNAs of prokaryotes and eukaryotes.

We have studied the methylation and the introduction of protective groups in the carbohydrate and heterocyclic moieties of the cytidine and guanosine molecules. In addition, protected methylnucleosides are of interest as the initial compounds for obtaining 3'-phospho derivatives and then for the solid-phase oligoribonucleotide synthesis of a somewhat simplified anticodon section 27-43 of yeast $tRNA^{Phe}$.

The methylation of unprotected nucleosides is a process taking place ambiguously, according to the choice of methylating agent and the conditions of the reaction, which is completed by the formation of a complex mixture of N,O-methyl derivatives [3]. In spite of the fact that 2'-O-methyl- and 3'-O-methyl-substituted cytidines and adenosines can be obtained by treating the nucleosides with diazomethane in 1,2-dimethoxyethane [4, 5], this method is not very suitable since, while the yields of 2'-O-methylnucleosides are low, the separation of the 3'-isomers remains fairly laborious.

The directed 2'-O-methylation of nucleosides becomes feasible after the selective blocking of the most reactive centers, which are the O^6-atom and the N^2- and N^4-exoamino functions of guanine and cytosine, and the 3'- and 5'-hydroxyls of ribofuranose. For the 2'-O-methylation of nucleosides protective groups were selected in the light of their correspondence to this aim and also in the subsequent reactions of obtaining the 3'-phospho derivatives and oligonucleotides.

N^4-Benzoylcytidine (Ia) was synthesized under the conditions of the combined reactions of blocking the hydroxyls of the ribofuranose by silylation and acylation of the amino group [6].

Among the protective agents for the O^6-position of a guanine residue [7-9], the little-studied diphenylcarbamoyl (DPC) protective group [9] possesses such advantages as accessibility and ease of introduction and elimination. In spite of the lability of carbamoyl derivatives in an alkaline medium, O^6-diphenylcarbamoyl-N^2-isobutyryl guanosine (Ib) was obtained in good yield on the O-deacylation of the O^6-diphenylcarbamoyl-N^2,2',3',5'-tetraisobutyryl derivative.

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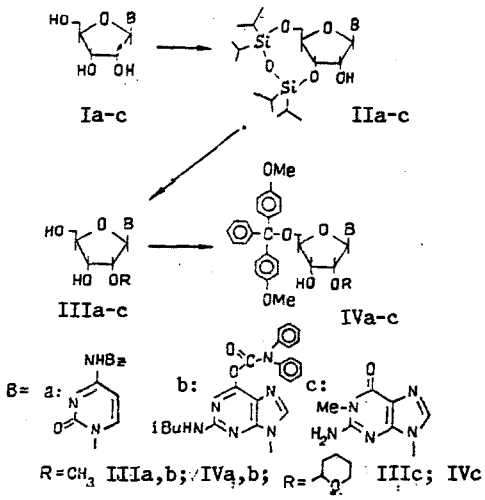

N_1-Methylguanosine (Ic) was obtrained by treating the unprotected nucleoside with methyl-iodide in dry dimethyl sulfoxide [10], and the structure of the methyl derivative of guanosine obtained was checked by PMR spectroscopy. To protect the amino group in (Ic) we used the widely practised method of obtaining N-acylnucleosides that consists in the complete acylation of the nucleoside and its subsequent O-deblocking, and also the variant of N-acylation described in [6]. However, under the reaction conditions given no N^2-isobutyryl-(Ic) was obtained. The use of acylating agents with less voluminous substituents (Ac_2O or AcCl) was also unsuccessful. The difficulty of acylating the amino group in (Ic) is probably connected with its spatial screening by neighbouring N_1-methyl substituents. In view of the low reactivity of the amino group of (Ic), it was decided to perform all the subsequent synthesis of 2',5'-protected derivatives of (Ic) with the nucleoside unprotected at the amino group.

In the preparation of 2'-O-methyl-(Ia and b) and of 2'-O-tetrahydropyranyl-(Ic) derivatives, for blocking the 3',5'-hydroxyls of the ribofuranose we used selective 1,1,3,3-tetraisopropyldisiloxane (TPDS) protection (see scheme). The methylation of the 3',5'-TPDS derivatives of N^4-benzoylcytidine (IIa) and of O^6-DPC-N^2-isobutyrylguanosine (IIb) was carried out with methyl iodide in the presence of silver oxide. 2'-O-methyluridine has been obtained previously [11] by a similar method. The methylated (IIa and b), without additional purification, were subjected to the elimination of the TPDC protection; the N^4-benzoyl-2'-O-methylcytidine (IIIa) and O^6-DPC-N^2-isobutyryl-2'-O-methylguanosine (IIIb) were purified by column chromatography. In the 2'-O-methylation of (IIb), the formation of a considerable amount of by-products was observed. According to the results of TLC, these were polar compounds with low mobilities in various solvent systems. It may be assumed that the O^6-DPC-group (IIb) has little effect on the electronic properties of the N_7-atom of the heterocycle and this, therefore, as the most nucleophilic center, underwent methylation. The N_7-methyl-(IIb) formed as an intermediate, like all the guanosine derivatives of this series [12], apparently underwent decomposition when the pH of the medium was changed at the stage of separating the 2'-O-methyl derivative (IIb). This also led to the formation of a set of polar substances detected in the TLC of the reaction mixture from the 2'-O-methylation of (IIb).

In the preparation of 2'-O-tetrahydropyranyl-(IIc) and -(IIIc) formation of the N^2-tetrahydropyranyl derivative was not excluded. An analogous reaction of dihydropyran with the unprotected amino group of guanosine is used to obtain alkali-stable N^2-protected derivatives [13]. However, in the case of the 2'-O-tetrahydropyranylation of (IIc) no formation of the N^2-derivative was observed, which is apparently connected with the spatial screening of the amino group of (IIc) by the neighboring N_1-methyl substituent or is a consequence of its protonation by the strong trifluoroacetic acid used as the catalyst for this process.

The treatment of (IIIa-c) with 4,4'-dimethoxytrityl chloride in pyridine gave the 5'-O-dimethoxytrityl derivatives (IVa-c). Under the conditions of this reaction the tritylation of the unprotected amino group of (IIIc) is possible. The introduction of mono- or dimethoxytrityl protection into the amino functions of adenosine and guanosine is a well-studied reaction [13, 14]. However, the formation of N^2,5'-O-bis-dimethoxytrityl-(IIIc) was not recorded either by the TLC method or by the results of elementary analysis and of PMR

spectroscopy. In the PMR spectrum of (IVc), because of the accumulation of the signals of the protons of the amino group and of the protons of the phenyl residues of the dimethoxytrityl group (6.5-7.0 ppm) the integral values in the calculation of the intensities of the signals were evaluated in relation to the intensity of the signal of the proton at the C_8-atom of the heterocycle (7.6 ppm). The signals of the protons of the amino groups in (Ic) and (IIc) appeared at 7.03 and 6.62 ppm, depending on the properties of the solvents [$(CD_3)_2SO$ and $CDCl_3$, respectively].

EXPERIMENTAL

Cytidine and guanosine produced by the Biolar NPO [Scientific Production Combine] were used. The diphenylcarbamoyl chloride and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane were obtained by known methods [15, 16]. Column chromatography was performed on silica gel L 40/100, and TLC on Silufol UV_{254} plates (Chemapol). The eluents were mixtures of chloroform and ethanol – system 1) (9:1, by volume); 2) (12:1); and 3) (14:1); and 4) NH_4OH—DMFA—isopropanol (10:25:65). UV spectra were recorded in ethanol on a Spectord UV-VIS spectrometer. PMR spectra were recorded in $CDCl_3$ (0 – TMS) on a Bruker WP 100SY instrument.

N^4-Benzoylcytidine (Ia) was obtained by the procedure of [6] with a yield of 94%; $C_{12}H_{17}N_3O_6$, mp 206°C (from water), Rf 0.32 (system 1).

UV spectrum (λ_{max}, nm): 260, 306 (log ε 4.35, 3.99).

O^6-Diphenylcarbamoyl-N^2,2',3',5'-O-tetraisobutyrylguanosine was obtained by a similar procedure to that of [9] with a yield of 94%; light-yellow oil, Rf 0.47 (system 1).

O^6-Diphenylcarbamoyl-N^2-isobutyrylguanosine (Ib). At 0°C, 20 ml of 2 M NaOH was added to a solution of 3.95 mmole of O^6-diphenylcarbamoyl-N^2,2',3',5'-O-tetraisobutyrylguanosine in a mixture of 20 ml of ethanol and 8 ml of pyridine. The mixture was allowed to stand for 7 min (with monitoring by TLC), and then 4 ml glacial acetic acid was added, the solvent was evaporated off in vacuum, and the residue was dissolved in 50 ml of chloroform. The organic layer was washed with water (2 × 20 ml) and was dried with Na_2SO_4, the solvent was evaporated off, the residue was dissolved in a mixture of ethanol and toluene (2:3; 2 × 30 ml) and this solution was evaporated. The product was purified by CC (system 1). Yield of (Ib) 76%; $C_{27}H_{28}N_6O_7 \cdot H_2O$, mp 164-168°C (from aqueous ethanol), Rf 0.38 (system 1).

UV spectrum (λ_{max}, nm): 230, 280 (log ε, 4.55, 4.14).

N_1-Methylguanosine (Ic) was obtained as in [10] with a yield of 42%; $C_{11}H_{15}N_5O_5$, mp 208-210°C (from methanol), according to the literature [10]: mp 225-227°C, Rf 0.46 (system 4).

UV spectrum: λ_{max} 257 nm (log ε 4.04).

PMR spectrum (100 MHz, D_2O): 3.79 (3H, s, N_1—CH_3), 6.25 (1H, d, J = 6.2 Hz, 1'-H), 8.32 (1H, s, C_8—H); $(CD_3)_2SO$: 3.40 (3H, s, N_1—CH_3), 5.72 (1H, d, J = 6 Hz, 1'-H), 7.03 (2H, s, NH_2), 7.94 (1H, s, C_8—H).

3',5'-O-(1,1,3,3-Tetraisopropyldisiloxan-1,3-diyl) Derivatives of N^4-Benzoylcytidine (IIa), of O^6-Diphenylcarbamoyl-N^2-isobutyrylguanosine (IIb), and of N_1-Methylguanosine (IIc). A mixture of 10 mmole of one of compounds (Ia-c) and 11 mmole of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in 100 ml of dry pyridine was stirred for 2 h, the solvent was evaporated off, the residue was dissolved in 150 ml of chloroform, and the solution was washed with water (2 × 10 ml). The organic layer was dried with Na_2SO_4 and evaporated to dryness, and the residue was chromatographed. For (IIa) and (IIb) the eluent was system 3, and for (IIc) it was chloroform.

Yield of (IIa) 73%; $C_{28}H_{43}N_3O_7Si_2$, mp 134-135°C (from acetonitrile), Rf 0.76 (system 1).

Yield of (IIb) 92%; $C_{39}H_{54}N_6O_8Si_2$, solid foam, Rf 0.63 (system 3).

Yield of (IIc) 85%; $C_{23}H_{41}N_6O_5Si_2$, mp 135-137°C (from ethyl acetate), Rf 0.29 (system 1).

PMR spectrum of (IIc): 1.08—1.12 (28H, m, Si—$CH(CH_3)_2$), 3.50 (3H, s, N_1—CH_3), 5.90 (1H, d, J=2.2 Hz, 1'-H), 6.62 (2H, s, NH_2), 7.78 (1H, s, C_8-H).

2'-O-Methyl Derivatives of N^4-Benzoylcytidine (IIIa) and of O^6-Diphenylcarbamoyl-N^2-isobutyrylguanosine (IIIb). A reaction mixture consisting of 2 mmole of (IIa) or (IIb), 20 mmole of methyl iodide, and 16 mmole of dry freshly precipitated silver oxide in 20 ml of acetone was stirred at 20°C for 20 h. The solid matter was filtered off, the filtrate was

evaporated, the residue was dried in vacuum and dissolved in 20 ml of dry THF, and the solution was treated with 4 ml of a 1 M solution of tetrabutylammonium fluoride in THF. After 10 min, the solution was evaporated and the residue was chromatographed (eluents: for (IIIa), system 1; for (IIIb), system 3).

The yield of (IIIa) was 56%; $C_{17}H_{19}N_3O_6$, mp 174-175°C (from ethanol), Rf 0.25 (system 1).

UV spectrum (λ_{max}, nm): 262, 307 (log ε 4.40, 4.04).

PMR spectrum: 3.62 (3 H, s, O—CH_3); 5.70 (1 H, d, J = 1.5 Hz, 1'-H); 6.82, 7.81-7.88 (7 H, m, H^5 and H^6 of cytosine, and phenyl protons).

Yield of (IIIb), 42%; $C_{28}H_{30}N_6O_7$, mp 138-142°C (from aqueous ethanol), Rf 0.30 (system 3).

UV spectrum (λ_{max}, nm): 230, 280 (log ε 4.55, 4.13).

PMR spectrum: 1.18 and 1.28 (6H, s, s, C—$(CH_3)_2$); 2.60-2.77 (1 H, m, CH— of isobutyryl); 3.39 (3 H, s, O—CH_3); 5.90 (1 H, d, J = 6.3 Hz, 1'-H); 7.27-7.42 (10 H, m, phenyl protons); 8.08 (s, C_8—H); 8.16 (1 H, s, NH).

N_1-Methyl-2'-O-(tetrahydropyran-2-yl)guanosine (IIIc). A mixture of 86 mmole of 3,4-dihydro-2H-pyran and 6.5 mmole of trifluoroacetic acid was added to a suspension of 4.3 mmole of (IIc) in 45 ml of dry chloroform. The reaction mixture was stirred at 20°C for 36 h, and then 10 ml of pyridine and 20 ml of water was added and the chloroform layer was evaporated off. The aqueous layer was extracted with chloroform (2 × 25 ml), and the organic extracts were combined, dried with Na_2SO_4, and evaporated in vacuum. The residue was dissolved in 50 ml of a 2:3 mixture of ethanol and toluene and the solution was evaporated; this operation was repeated, the resulting foam was dissolved in 45 ml of dry THF, and this solution was treated with 18 ml of a 1 M solution of tetrabutylammonium fluoride in THF and the mixture was kept for 3 h. The solvent was evaporated off and the residue was chromatographed (with system 1 as eluent), and the product was reprecipitated in hexane.

The yield of (IIIc) was 61%; $C_{16}H_{23}N_5O_6$, amorphous powder, Rf of the isomers 0.18 and 0.28 (system 1).

UV spectrum: λ_{max} 258 nm (log ε 4.20).

PMR spectra: 1.36-1.82 (8 H, m, protons of the pyran ring); 3.42 (3 H, s, N_1—CH_3); 5.86 and 5.98 (1 H × 2, d, d, J = 6 Hz and J = 7 Hz, 1'-H of the two tetrahydropyranyl isomers); 7.82 (1 H, s, C_8—H).

5'-O-Dimethoxytrityl Derivatives of N^4-Benzoyl-2'-O-methylcytidine (IVa), of O^6-Diphenylcarbamoyl-N^2-isobutyryl-2'-O-methylguanosine (IVb), and of N_1-Methyl-2'-O-(tetradropyran-2-yl)guanosine (IVc). One of the compounds (IIIa-c) (1.5 mmole) was dried by evaporation with dry pyridine and was dissolved in 15 ml of pyridine, this solution was treated with 2.25 mmole of 4,4'-dimethoxytrityl chloride, and the reaction mixture was stirred for 12 h (with monitoring by TLC). Then it was treated with 3 ml of ethanol, 50 ml of chloroform, and 30 ml of water, and the organic layer was separated off, and dried with Na_2SO_4 and evaporated, and the residue was chromatographed (with the eluents chloroform followed by system 3), and the product (IVa, b, or c) was precipitated in hexane.

The yield of (IVa) was 73%; $C_{38}H_{37}N_3O_8$, Rf 0.62 (system 1).

PMR spectra: 3.76 (3H, s, 2'-O—CH_3); 3.83 (6H, s, Ph—OCH_3); 6.03 (1H, d, J = 0.6 Hz, 1'-H); 6.84-7.38 (18H, m, protons of Ph and $(CH_3O)_2Tr$); 7.52 (1H, d, J = 6.4 Hz, H^5); 7.90 (1H, d, J = 6.4 Hz, H^6); 8.1 (1H, s, N^4—H).

The yield of (IVb) was 87%; $C_{49}H_{48}N_6O_9$, Rf 0.60 (system 1).

PMR spectra: 1.19 and 1.28 (6H, s, s, C—$(CH_3)_2$); 2.62-2.84 (1H, m, CH of isobutyryl); 3.2 (3H, s, 2'-O—CH_3); 3.75 (6H, s, Ph—OCH_3); 6.12 (1H, d, J = 2.4 Hz, 1'-H); 6.74-7.42 (23H, m, protons of Ph and $(CH_3O)_2Tr$); 7.87 (1H, s, C_8—H); 8.17 (1H, s, N^2—H).

The yield of (IVc) was 70%; $C_{37}H_{41}N_5O_8$, Rf of the isomers 0.32 and 0.38 (system 1).

PMR spectra: 1.40-1.85 (8H, m, protons of the pyran ring); 3.46 (3H, s, N_1—CH_3); 3.77 (6H, s, Ph—OCH_3); 5.92 and 6.04 (1H × 2, d, d, J = 5.6 Hz and 6.2 Hz, 1'-H of the two tetrahydropyranyl isomers); 6.80-7.40 (15H, m, protons of $(CH_3O)_2Tr$ and NH_2); 7.66 (1H, s, C_8—H).

SUMMARY

1. The 2'-O-methylation of selectively protected cytidine and guanosine has been

studied. It has been shown that spatial screening by the neighboring methyl substituent of the amino group in N_1-methylguanosine leads to its complete deactivation in acylation, tetrahydropyranylation, and tritylation reactions.

2. N,O-Protected 2'-O-methyl and 2'-O-tetrahydropyranyl derivatives of cytidine, guanosine, and N_1-methylguanosine have been synthesized.

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INSECT PHEROMONES AND THEIR ANALOGUES.

XXII. METHYL-BRANCHED PHEROMONES BASED ON 4-METHYLTETRAHYDROPYRAN.

SYNTHESIS OF RACEMIC 2-ACETOXY-3,7-DIMETHYLPENTADECANE (DIPRIONYL ACETATE)

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A synthesis is proposed of racemic 2-acetoxy-3,7-dimethylpentadecane – the sex attractant (diprionyl acetate) of plane sawflies of the genera Diprion and Neodiprion on the basis of the regioselective transformation of bifunctional products of the cleavage of 4-methyltetrahydropyran.

Syntheses are given in the literature both of racemates [1-7] and of optically active forms [8-12] of 2-acetoxy-3,7-dimethylpentadecane (I) – the sex pheromone of plane sawflies of the genera Diprion and Neodiprion. Since racemic (I), known under the name of diprionyl acetate, exhibits biological activity [1] it is economically desirable to use just this as the attractant.

We have developed a practical synthesis of the racemic acetate (I) based on the regioselective transformations of the methyl-branched bromohydrin (III) obtained by the cleavage of the readily available 4-methyltetrahydropyran (II) [13]. The coupling of compound (III)

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