Oligoribonucleotide Synthesis. II.[1] Preparation of 2′-*O*-tetrahydropyranyl Derivatives of Adenosine and Cytidine Necessary for Insertion in Stepwise Synthesis

THOMAS NEILSON AND EVA S. WERSTIUK
Department of Biochemistry, McMaster University, Hamilton, Ontario

Received July 30, 1970

The syntheses of N^6-benzoyl-2′-*O*-tetrahydropyranyladenosine (**2**) and N^4-benzoyl-2′-*O*-tetrahydropyranylcytidine (**11**) and their corresponding 5′-*O*-*p*-methoxytrityl derivatives, **3** and **12** respectively, are described. Compounds **2**, **3**, **11**, and **12** are the protected derivatives of adenosine and cytidine required for insertion into a new oligoribonucleotide synthesis. Compound **2** was prepared from the known 2′-*O*-tetrahydropyranyladenosine by perbenzoylation followed by de-*O*-benzoylation. Compound **11** is prepared from cytidine by the following sequence: orthoacetate protection of the 2′- and 3′-hydroxyls; benzoylation of the 5′- and N^4-positions; orthoacetate ring opening provided a mixture of the 2′- and 3′-monoacetates from which the major, 3′-*O*-acetyl-N^4,5′-*O*-dibenzoylcytidine (**7**) was isolated; dihydropyran treatment on the 2′-hydroxyl of **7** and subsequent selective deacylation studies were carried out. Specific de-*O*-acylation gave **11**.

Canadian Journal of Chemistry, 49, 493 (1971)

Synthesis of oligoribonucleotides of defined length and sequence is essential for the investigation of the many remaining problems surrounding the phenomenon of protein synthesis, in particular, the nature of transfer RNA binding sites in aminoacyl synthetases and ribosomes. Although enzymic synthesis can supply a few oligonucleotides of this type, at present only chemical synthesis is potentially capable of supplying all possible combinations. Additional advantages inherent in chemical synthesis stem from its ready extension to the preparation of analogues, for example, changing the bases of any given sequence, say one at a time.

No synthetic material longer than a triribonucleotide has been made by purely chemical means following the preparation of the 64 triplets by Lohrmann *et al.* (2). Their approach was relatively laborious and tended to be inefficient resulting often in yields of a few o.d. units. Two developments however, have encouraged further interest in this general area.

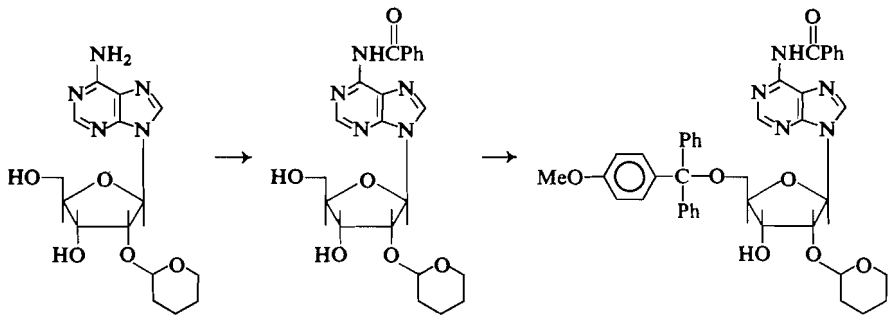
(*a*) Ready solubility of tertiary phosphates in non-polar solvents means that relatively large quantities of intermediates can be handled by silica gel chromatography (3–5). Consequently the slower DEAE–cellulose chromatographic procedures can be by-passed, at least until after the various protecting groups have been removed late in overall synthesis.

(*b*) Removal of the *O*-tetrahydropyranyl grouping can be accomplished using mild mineral acid (p*H* 2) (6) with negligible isomerization of the diphosphate ester linkage. Consequently certain acid-labile protecting groups can also be used to protect the hydroxyl groups of the ribofuranoside ring.

A new stepwise oligoribonucleotide synthesis incorporating these salient features has been developed (1) and its relative convenience illustrated by the synthesis of uridyl[3′–5′]uridyl[3′–5′]uridine (UpUpU) from 2′-*O*-tetrahydropyranyluridine. To show the general nature of this synthetic procedure, 2′-*O*-tetrahydropyranyl derivatives of adenosine, cytidine, and guanosine must be available to allow preparation of oligonucleotides of any sequence. Although the uracil base requires no protection, the amino groups of adenine, cytosine, and guanine must be protected to ensure efficient 3′–5′ diphosphate coupling. *N*-Aroyl groups, in particular *N*-benzoyl and anisoyl, are most often used for this purpose.

In this communication we describe the synthesis of protected intermediates, N^6-benzoyl-2′-*O*-tetrahydropyranyladenosine (**2**) and N^4-benzoyl-2′-*O*-tetrahydropyranylcytidine (**11**) and their corresponding 5′-*O*-*p*-methoxytrityl derivatives, **3** and **12** (the latter pair destined to become the precursors of the 5′-terminal nucleoside unit of an oligoribonucleotide). Subsequent stepwise incorporation of these compounds into a linear sequence by diphosphate coupling will provide synthesis of protected oligoribonucleotides

[1]For part 1 see ref. 1.



1 2 3

containing any predetermined sequence of uridine, adenosine, and cytidine.

2′-*O*-Tetrahydropyranyladenosine (**1**) was prepared by the method of Griffin *et al.* (6). The better crystallizing lower R_F diastereoisomer was used in subsequent conversions to ensure complete and specific blocking of the 2′-position of adenosine. This will guarantee high fidelity in all future 3′–5′ internucleotidic linkages.

Benzoylation of **1** in pyridine followed by careful de-*O*-benzoylation (7) gave N^6-benzoyl-2′-*O*-tetrahydropyranyladenosine (**2**) in 87% yield as a solid foam. Treatment of **2** with 1 equiv of *p*-anisylchlorodiphenylmethane in pyridine gave the expected N^6-benzoyl-5′-*O*-*p*-methoxytrityl-2′-*O*-tetrahydropyranyladenosine (**3**) in 88% yield.

Treatment of 2′,3′-*O*-methoxyethylidenecytidine (**5**) (8) with benzoyl chloride in pyridine gave N^4,5′-*O*-dibenzoyl-2′,3′-*O*-methoxyethylidenecytidine (**6**) in 95% yield over two steps from cytidine (4). Treatment with 5% acetic acid opened the cyclic orthoacetate ring system to give a mixture of the 3′- and 2′-*O*-acetyl derivatives, **7** and **8**. The p.m.r. data indicated an approximate 3:1 mixture of the isomers. Fractional crystallization gave 3′-*O*-acetyl-N^4,5′-*O*-dibenzoylcytidine in 35% yield. The p.m.r. data of the 3′-*O*-acetate, **7**, agreed with the empirical rules established by Fromageot *et al.* (9).

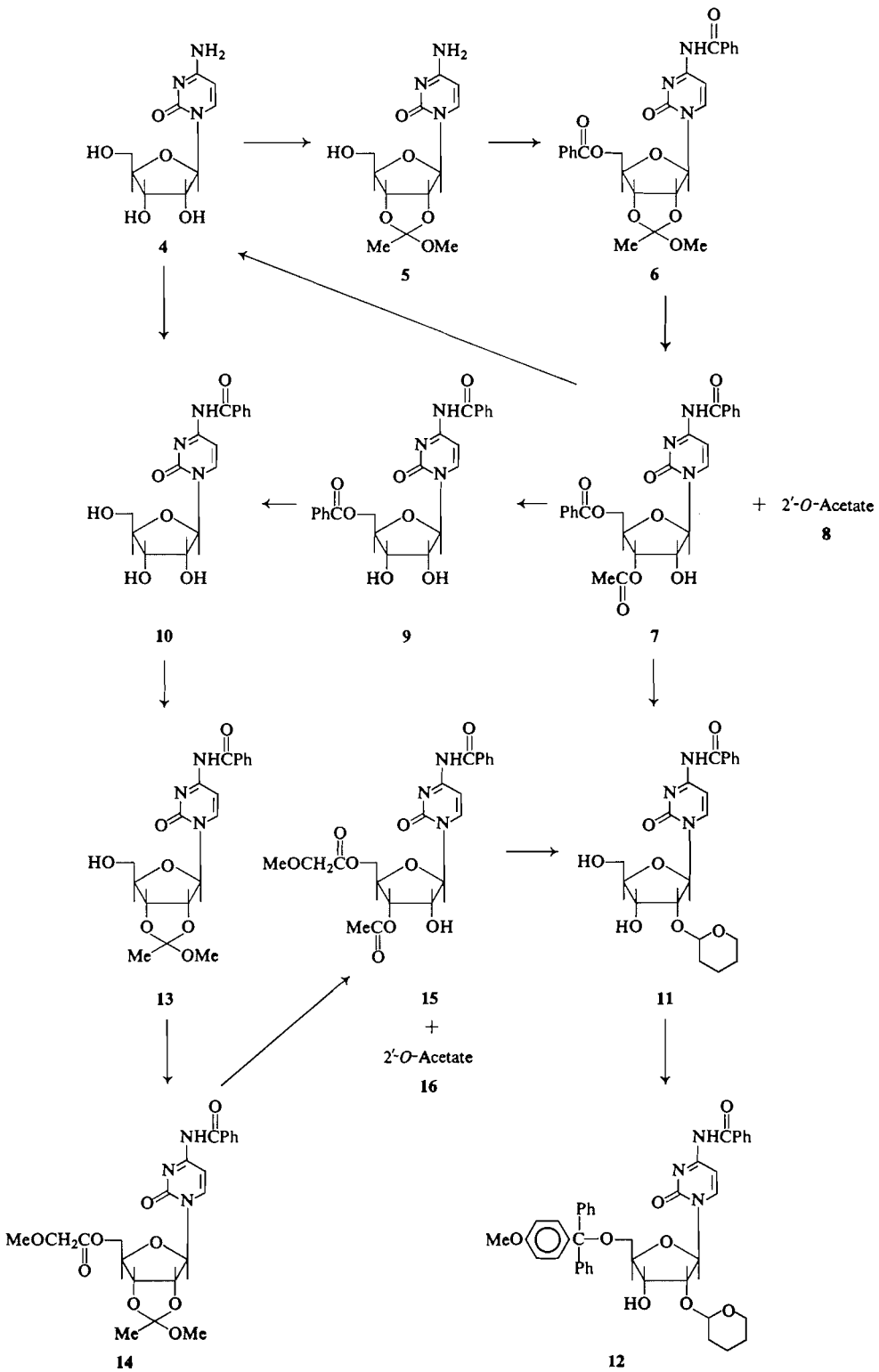
Deacylation studies on **7** showed that it was possible to remove the protecting groups selectively. Half-saturated methanolic ammonia treatment for 2.5 h at ambient temperature gave 85% yield of the N^4,5′-*O*-dibenzoylcytidine (**9**). Sodium hydroxide (0.5 *N*) treatment on **7** in ethanol–pyridine solution gave N^4-benzoylcytidine in 57% yield. Prolonged methanolic ammonia exposure on **7** for 2 days at ambient temperature gave cytidine (**4**) in 69% yield. Such mild conditions for the removal of an *N*-benzamido group should prove useful in an oligoribonucleotide deblocking procedure.

Reaction of **7** with dihydropyran and *p*-toluenesulfonic acid in anhydrous dioxan gave the corresponding 2′-*O*-tetrahydropyranyl derivative which on immediate de-*O*-acylation using methanolic ammonia, gave a diastereoisomeric mixture of N^4-benzoyl-2′-*O*-tetrahydropyranylcytidine (**11**). Silica gel column chromatography separated this mixture into low R_F (high melting) and high R_F (low melting) components in 30 and 34% yields respectively. Treatment of **11** (low R_F) with 1 equiv of *p*-anisylchlorodiphenylmethane in anhydrous pyridine gave the expected N^4-benzoyl-5′-*O*-methoxytrityl-2′-*O*- tetrahydropyranylcytidine (**12**) in 96% yield.

A further check on exclusive 2′-*O*-tetrahydropyranyl substitution of cytidine was provided by preparing **11** from 3′-*O*-acetyl-N^4-benzoyl-5′-*O*-methoxyacetylcytidine (**15**). The isolation of **15** and its 2′-isomer **16** as crystalline solids has been reported (10), thorough p.m.r. and chemical studies on this pair being carried out to ascertain their substitution pattern. Unfortunately no preparative data exist for these compounds and so **15** was prepared in the following manner.

Cytidine (**4**) was converted to its N^4-benzoyl derivative **10** in 59% yield by reaction of benzoic anhydride in methanol. Trimethyl orthoacetate treatment of **10** in anhydrous acidic conditions gave N^4-benzoyl-2′,3′-*O*-methoxyethylidinecytidine (**13**) as a glass in 90% yield. Reaction of **13** with methoxyacetic acid[2] activated by triiso-

[2]This constitutes a convenient general method for esterification as it uses the more stable and more readily available free carboxylic acids rather than their corresponding acid chlorides or anhydrides.



propylbenzenesulfonyl chloride in pyridine gave the corresponding 5′-*O*-methoxyacetate (**14**) as a solid foam. Orthoacetate ring opening of **14** using aqueous 5% acetic acid solution gave the expected mixture of 2′- and 3′-*O*-acetate isomers **15** and **16** in 33% yield over the last two steps. Fractional crystallization of this mixture gave a sample of pure 3′-*O*-acetate (**15**). Condensation of **15** with dihydropyran and subsequent methanolic ammonia treatment gave a diastereoisomeric mixture of N^4-benzoyl-2′-*O*-tetrahydropyranylcytidine (**11**) which on silica gel column chromatography gave identical samples of the components obtained by the route described above.

Thus, suitably protected intermediates are available for insertion into the stepwise synthesis (1). Work describing the triphosphate coupling reactions and subsequent deblocking procedures to give oligoribonucleotides of predetermined sequence and length is in progress.

Experimental

Melting points were taken on a Gallenkamp hot-block melting point apparatus and are uncorrected. The p.m.r. spectra were recorded on a Varian T60 spectrometer in 10–15% (w/v) DMSO-d_6 – D_2O solutions, and line positions are reported in p.p.m. from the reference (TMS). Mass spectra were determined on an Associated Electrical Industries MS-9 double focusing high resolution mass spectrometer. Silica gel (Baker Analyzed Reagent) was used for column chromatography; and Analtech prescored silica gel plates were employed for t.l.c. The plates were developed in 5 or 10% methanol in methylene chloride mixtures.

N^6-Benzoyl-2′-O-tetrahydropyranyladenosine (2)

The lower R_F component of the diastereoisomeric mixture of 2′-*O*-tetrahydropyranyladenosine (**1**) was prepared from adenosine (6). Compound **1** (1 g) was dissolved in anhydrous pyridine (10 ml) and the solution cooled to 0°. Benzoyl chloride (2 ml) was added dropwise, the reaction mixture stirred for 2 h, and then allowed to return to ambient temperature whereupon pyridine hydrochloride separated. The use of t.l.c. in 5% CH_3OH–CH_2Cl_2 showed the reaction to be complete (R_F 0.1 → 0.9). Ice (2–3 g) was added and the quenching allowed to proceed for 20 min. The mixture was poured into water (100 ml) and the solution extracted with methylene chloride (3 × 50 ml). The combined extracts were washed with water (2 × 50 ml) and evaporated *in vacuo* to give an oil. The crude tribenzoate was dissolved in a solution mixture of ethanol (15 ml) and pyridine (8 ml). A sodium hydroxide (2*N*) solution (16 ml) and additional ethanol (16 ml) were added. The de-*O*-benzoylation was allowed to proceed for *only* 5 min, then Dowex-50 resin (pyridinium form) was added to the reaction mixture until the solution p*H* was approximately 7. The t.l.c. in 5% CH_3OH–CH_2Cl_2 showed complete conversion (R_F 0.9 → 0.3). The resin was removed by filtration and the filtrate was reduced to a third of its volume by evaporation *in vacuo* whereupon it was extracted by methylene chloride (4 × 20 ml). The combined extracts were washed with water (2 × 25 ml) and evaporated *in vacuo* to a solid foam, the last traces of pyridine being removed by co-distillation *in vacuo* with toluene (2 × 20 ml). This foam was subjected to silica gel column chromatography and N^6-benzoyl-2′-*O*-tetrahydropyranyladenosine was obtained as a solid foam (1.13 g, 87%) upon elution with 2% CH_3OH–CH_2Cl_2. Crystallization could not be effected but the material was sufficiently pure for further conversions.

The p.m.r. spectrum showed signals (δ TMS) at 8.82 and 8.78 (2H, 2 singlets; 2 and 8 protons of adenine); 7.95 (2H, multiplet; *o*-benzoate protons); 7.55 (3H, multiplet; other benzoate protons); 6.26 (1H, doublet $J \sim 6$ Hz; 1′-anomeric proton); 4.88 (1H, triplet $J \sim 5$ Hz; 3′-sugar proton); 4.73 (1H, singlet; THP acetal proton); 4.42 and 4.11 (1H each, multiplets; 2′- and 4′-sugar protons); 3.72 (4H, multiplet; *O*-methylene of THP and 5′-sugar protons); and 1.60 p.p.m. (6H, multiplet; *C*-methylene THP protons).

Anal. Calcd. for $C_{22}H_{25}N_5O_6$ (mol. wt. 455.46): N, 15.3. Found: N, 14.7. The mass spectrum of N^6-benzoyl-2′-*O*-tetrahydropyranyladenosine (recorded at 250–260°, probe 1) showed a strong M^+ peak at 455 *m/e*; and further peaks at 426 (M-29, loss of CHO); 351 (M-104, loss of C_6H_5CO—); 266 (M-189, loss of C_6H_5CO— and THP); 136, 135 (M-319, and M-320 respectively, loss of C_6H_5CO—, THP and ribose).

N^6-Benzoyl-5′-O-p-methoxytrityl-2′-O-tetrahydropyranyladenosine (3)

N^6-Benzoyl-2′-*O*-tetrahydropyranyladenosine (280 mg) was dissolved in anhydrous pyridine (2 ml) and methylene chloride (10 ml). *p*-Anisylchlorodiphenylmethane (280 mg, 1.1 equiv) was added and the reaction mixture was sealed and left overnight in the dark at ambient temperature. The t.l.c. in 5% CH_3OH–CH_2Cl_2 showed the reaction was complete (R_F 0.3 → 0.9). The reaction was quenched with ethanol (2 ml) and poured into water (25 ml). The mixture was extracted with methylene chloride (3 × 25 ml). The combined extracts were washed with water (3 × 25 ml) and evaporated *in vacuo* to a gum, the last traces of pyridine being removed by co-distillation *in vacuo* with toluene (2 × 20 ml). Column chromatography on silica gel was applied. Elution with 1% CH_3OH–CH_2Cl_2 gave the desired 5′-*O*-monotrityl material as a solid foam (390 mg, 88%) which was sufficiently pure for oligonucleotide coupling reactions. The p.m.r. spectrum showed signals (δ TMS) at 8.20 and 8.12 (2H, 2 singlets; 2 and 8 protons of adenine); 7.65 (2H, multiplet; *o*-benzoate protons); 7.15 (3H, multiplet; other benzoate protons); 6.90 (14H, multiplet; trityl aromatic protons); 5.95 (1H, doublet $J \sim 5$ Hz; 1′-anomeric proton); 4.80 (1H, triplet $J \sim 4$ Hz; 3′-sugar proton); 4.57 (1H, singlet; THP acetal proton); 4.41 and 4.11 (1H each, multiplets; 2′- and 4′-sugar protons); 3.62 (4H, multiplet; *O*-methylene of THP and 5′-sugar protons); 3.41 (3H, singlet; MeO of *p*-methoxytrityl); and 1.6 p.p.m. (6H, broad multiplet; *C*-methylenes of THP).

2′,3′-O-Methoxyethylidenecytidine (5)

This compound was prepared in 95% yield as described

(8) and was used in the next reaction without further purification.

*N^4-5′-O-Dibenzoyl-2′,3′-O-methoxyethylidenecytidine (**6**)*

Benzoyl chloride (2.5 ml, 0.021 mol) was added dropwise, with stirring to a cooled (ice) solution of 2′,3′-*O*-methoxyethylidenecytidine (**5**) (2.95 g, 0.01 mol) in anhydrous pyridine (30 ml). When the addition was completed, the reaction mixture was allowed to warm up to room temperature and stirred for a further 1 h. The t.l.c. indicated complete conversion into a product of R_F: 0.85 in 10% CH_3OH–CH_2Cl_2. Water was then added (1 ml) and stirring continued for a further 1 h. The reaction mixture was then concentrated *in vacuo* to *ca.* 10 ml, dilute triethylammonium bicarbonate buffer (p*H* 7.5) was added (25 ml), and the product extracted repeatedly with chloroform (3 × 25 ml). The combined organic extracts were washed, dried (Na_2SO_4), and evaporated to dryness *in vacuo*. The pale yellow glass (4.34 g, 86%, R_F: 0.85 in 10% CH_3OH–CH_2Cl_2) was used in the next reaction without further purification.

*3′-O-Acetyl-N^4-5′-O-dibenzoylcytidine (**7**)*

A mixture of N^4-5′-*O*-dibenzoyl-2′,3′-*O*-methoxyethylidenecytidine (**6**) (4.34 g, 0.0086 mol) and 5% acetic acid (50 ml) was stirred at room temperature. After 30 min t.l.c. indicated the presence of products of R_F: 0.89, 0.80, and 0.66 in 10% CH_3OH–CH_2Cl_2. The reaction mixture was then repeatedly extracted with methylene chloride (4 × 25 ml), the combined organic extracts were washed, dried (Na_2SO_4), and evaporated *in vacuo*. The colorless glass (4.08 g) was purified by chromatography on silica gel (60 g) in methylene chloride. Elution with 2% methanol – methylene chloride (400 ml) and 2.5% methanol – methylene chloride mixtures (400 ml) gave a mixture of **7** and **8** (1.85 g, 44% yield, R_F: 0.66 in 10% CH_3OH–CH_2Cl_2).

Pure 3′-*O*-acetyl-N^4-5′-*O*-dibenzoylcytidine (**7**), 1.48 g, 35% yield of m.p. 192–194°, was obtained by crystallization from absolute ethanol (50 ml). The p.m.r. spectrum showed signals (δ TMS) at 8.28–7.28 (12H, multiplet, two C_6H_5CO—, and 6-, 5-cytidine protons); 5.90 (1H, doublet, $J \sim 5$ Hz; 1′-anomeric proton); 5.12 (1H, triplet; 2′-sugar proton) and at 2.1 p.p.m. (3H, sharp singlet; —$COCH_3$ protons).

Anal. Calcd. for $C_{25}H_{23}N_3O_8$ (mol. wt. 492.46): C, 60.85; H, 4.70; N, 8.52. Found: C, 60.36; H, 4.71; N, 8.26.

Concentration of the mother liquor to a small volume yielded a *ca.* 1:1 mixture of N^4-benzoyl-2′(3′)-*O*-acetates **8** and **7** respectively of m.p. 177–185°. The p.m.r. spectrum showed signals (δ TMS) at 5.99 (1H, two doublets of the 1′-anomeric protons of **7** and **8** superimposed); 5.48 and 5.23 (1H, two triplets of 2′-proton of **7** and 3′-proton of **8** superimposed); and at 2.18 and 2.15 p.p.m. (3H, two sharp singlets; of 3′-$COCH_3$ protons of **7** and 2′-$COCH_3$ protons of **8** respectively).

*N^4-Benzoyl-2′-O-tetrahydropyranylcytidine (**11**)*

(*a*) A solution of *p*-toluenesulfonic acid monohydrate (0.86 g, 4.3 mmol) in dry dioxan (15 ml) containing molecular sieves (0.25 g) was cooled in ice until solidified. Dihydropyran (5.24 g, 6.0 ml; 0.062 mol) was then added dropwise with stirring, followed by a suspension of 3′-*O*-acetyl-N^4-5′-*O*-dibenzoylcytidine (3.0 g, m.p. 190–192°; 0.06 mol, R_F: 0.62 in 10% CH_3OH–CH_2Cl_2) in dry dioxan (15.0 ml). The reaction mixture was then allowed to warm up to room temperature (*ca.* 10 min) and kept at this temperature with continued stirring. After 2 h t.l.c. indicated complete conversion into products of R_F: 0.84 and 0.73 in 10% CH_3OH–CH_2Cl_2. The colorless solution was then cooled in ice and neutralized with concentrated ammonia. The precipitated ammonium tosylate was filtered off, washed well with methylene chloride, and the combined filtrate and washings evaporated *in vacuo*. The yellow oil (3.2 g) obtained was suspended in a mixture of ethanol (30 ml) and pyridine (15 ml), and 50% ethanolic sodium hydroxide (containing 15 ml 2 *N* NaOH and 15 ml absolute ethanol) was added with stirring, and the reaction mixture set aside at room temperature. After 5 min t.l.c. indicated two products of R_F: 0.69 and 0.61 in 10% CH_3OH–CH_2Cl_2 in about 1:1 proportion. The p*H* of the solution was then adjusted to 7.0 with Dowex 50W-X8 resin (pyridinium form). The resin was then filtered off, washed well with methylene chloride – ethanol, and the combined filtrate and washings evaporated *in vacuo*. The resulting mixture of diastereoisomers was separated on silica gel (40 g) in methylene chloride. Elution with 3% methanol – methylene chloride mixture (400 ml) yielded 0.88 g (34.1%) of the low m.p. diastereoisomer of R_F: 0.61 in 10% CH_3OH–CH_2Cl_2. Continued elution with 4% methanol – methylene chloride mixture (200 ml) gave 0.46 g (17.8%) of a mixture of the two diastereoisomers R_F: 0.61 and 0.54 in 10% CH_3OH–CH_2Cl_2. Finally, the high m.p. isomer of R_F: 0.54 (0.41 g, 15.5%) was obtained on elution with 4.0–4.5% methanol – methylene chloride mixtures (200 + 400 ml). The mixture of diastereoisomers (0.46 g; R_F: 0.61 and 0.54 in 10% CH_3OH–CH_2Cl_2) was rechromatographed on silica gel (15 g) to yield further 0.38 g (14.5%) of the high m.p. diastereoisomer of R_F: 0.54. An analytically pure sample of m.p. 162–164°, R_F: 0.61 was prepared by crystallization from methylene chloride – hexane. Its p.m.r. spectrum showed signals (δ TMS) at 8.05–7.25 (6H, multiplet, C_6H_5CO—, and 5-cytidine proton); 5.85 (1H, singlet, 1′-anomeric proton); 5.02 (1H, singlet, acetal proton of THP); 3.78 (4H, —O—CH_2— protons of THP and 5′-sugar protons); and a broad peak at 1.6 p.p.m. (6H, multiplet, THP protons).

Anal. Calcd. for $C_{21}H_{25}N_3O_7$ (mol. wt. 431.43): C, 58.46; H, 5.84; N, 9.74. Found: C, 58.33; H, 5.61; N, 9.27.

Crystallization from methylene chloride – hexane provided an analytical sample of m.p. 194–196° of the other isomer of R_F: 0.54 (Reese reported (11) m.p. 195–197°). Its p.m.r. spectrum showed signals (δ TMS) at 8.45 (1H, doublet, $J \sim 8$ Hz; 6-cytidine proton); 8.08–7.2 (6H, multiplet, C_6H_5CO—, and 5-cytidine proton); 6.03 (1H, doublet, $J \sim 4$ Hz; 1′-anomeric proton); 4.80 (1H, singlet, acetal proton of THP); and a broad peak at 1.58 p.p.m. (6H, multiplet, THP protons).

Anal. Calcd. for $C_{21}H_{25}N_3O_7$ (mol. wt. 431.43): C, 58.46; H, 5.84; N, 9.74. Found: C, 58.55; H, 5.82; N, 8.94.

(*b*) A solution of *p*-toluenesulfonic acid monohydrate (142 mg, 0.75 mmol) in dry dioxan (5 ml) containing molecular sieves (0.05 g) was cooled in ice till solidified.

Dihydropyran (0.92 g, 1 ml, 1.1 mmol) was then added dropwise with stirring, followed by a suspension of 3′-*O*-acetyl-N^4-benzoyl-5′-*O*-methoxyacetylcytidine (**15**) (0.461 g, 1.0 mmol; m.p. 95–97° R_F: 0.69 in 10% CH_3OH–CH_2Cl_2) in dry dioxan (2.5 ml). The reaction mixture was then allowed to warm up to ambient temperature (*ca.* 10 min) and maintained with continued stirring. After 3.5 h, t.l.c. indicated complete conversion into products of R_F: 0.93 and 0.86 in 10% CH_3OH–CH_2Cl_2. The colorless solution was then cooled in ice, neutralized with concentrated ammonia, the precipitated ammonium tosylate filtered off, and washed well with methylene chloride and the combined filtrate and washings evaporated *in vacuo*. The yellow oil obtained was suspended in a mixture of ethanol(5.0 ml) – pyridine(2.5 ml), 50% ethanolic sodium hydroxide (containing 2.5 ml 2 N NaOH and 2.5 ml absolute ethanol) was added with stirring, and the reaction mixture set aside at room temperature. After 5 min, t.l.c. indicated two products of R_F: 0.69 and 0.61 in 10% CH_3OH–CH_2Cl_2. The p*H* of the solution was then adjusted to 7.0 with Dowex 50W-X8 resin (pyridinium form). The resin was then filtered off, washed well with methylene chloride – ethanol, and the combined filtrate and washings evaporated *in vacuo*. The resulting colorless glass (0.4 g) was purified by chromatography on silica gel (15 g) in methylene chloride. Elution with 3% methanol – methylene chloride mixture (200 ml) first yielded pure N^4-benzoyl-2′-*O*-tetrahydropyranylcytidine (62 mg, 14%; R_F: 0.61); then some mixture (0.044 g, R_F: 0.69 and 0.61). The second diastereoisomer (0.161 g, 37%; R_F: 0.61) was obtained on further elution with 4% methanol – methylene chloride mixture (200 ml). An analytical sample of m.p. 193–195°, R_F: 0.61 was obtained by crystallization from methylene chloride – hexane. The p.m.r. spectrum was identical with that of a sample obtained from the N^4-5′-*O*-dibenzoyl-3′-*O*-acetylcytidine (**7**) prepared previously (*a*).

*N^4-Benzoyl-5′-O-p-methoxytrityl-2′-O-tetrahydropyranylcytidine (**12**)*

N^4-Benzoyl-2′-*O*-tetrahydropyranylcytidine (**11**) (107 mg, 0.25 mmol; m.p. 193–195°; R_F: 0.54 in 10% CH_3OH–CH_2Cl_2) was evaporated from anhydrous pyridine (2 × 10 ml) and finally its volume reduced to *ca.* 3 ml. *p*-Anisylchlorodiphenylmethane (90 mg, 0.27 mmol; 1.1 equiv) was added and the reaction mixture set aside at room temperature in the dark. After 16 h t.l.c. indicated the presence of a product of R_F: 0.73 and some starting material of R_F: 0.51 in 10% CH_3OH–CH_2Cl_2. Further amount of the reagent (45 mg, 0.13 mmol) was then added, and the reaction allowed to continue till completed (*ca.* 2–3 days). Absolute ethanol (5 ml) was then added, and the mixture evaporated *in vacuo*. The resulting oil was dissolved in methylene chloride, washed with water, and evaporated to dryness *in vacuo*. The last traces of pyridine were removed by co-distillation with toluene (2 × 5 ml). The colorless glass (280 mg) obtained was purified by chromatography on silica gel (12 g) in methylene chloride. Elution with 1–2% methanol – methylene chloride mixtures (200 + 200 ml) yielded N^4-benzoyl-5′-*O*-*p*-methoxytrityl-2′-*O*-tetrahydropyranylcytidine (**12**) (170 mg, 96%) (R_F: 0.73 in 10% CH_3OH–CH_2Cl_2). The p.m.r. spectrum showed signals (δ TMS) at 8.35–6.85 (21H, multiplet, C_6H_5CO—, 6-, and 5-cytidine protons; and *p*-methoxytrityl protons); 6.10 (1H, doublet $J \sim 2$ Hz; 1′-anomeric proton); 4.98 (1H, broad singlet, acetal proton of THP); 3.68 (3H, sharp singlet; OCH_3); and 1.62 p.p.m. (6H, broad multiplet; THP protons).

*Selective De-O-acylation of 3′-O-Acetyl-N^4-5′-O-dibenzoylcytidine (**7**)*

*(a) N^4-Benzoylcytidine (**10**)*

To a stirred suspension of 3′-*O*-acetyl-N^4-5′-*O*-dibenzoylcytidine (**7**) (200 mg, 0.4 mmol, m.p. 188–190°, R_F: 0.62 in 10% CH_3OH–CH_2Cl_2) in ethanol (1.0 ml) and pyridine (0.5 ml), 50% ethanolic sodium hydroxide (containing 0.5 ml of 2 *N* NaOH and 0.5 ml of absolute ethanol) was added at room temperature. The clear solution obtained was stirred for 5 min, when t.l.c. indicated complete conversion into a product of R_F: 0.16 in 10% CH_3OH–CH_2Cl_2. The p*H* of the solution was then adjusted to 7.0 with Dowex 50W-X8 resin (pyridinium form), the resin was filtered off, washed well with methanol – methylene chloride, and the combined filtrate and washings evaporated *in vacuo*. The white crystals of N^4-benzoylcytidine (**10**) (82 mg, 57%) had m.p. 220–222° (dec.) R_F: 0.16 in 10% CH_3OH–CH_2Cl_2; lit. m.p. 219–220° (12).

The p.m.r. spectrum showed signals (δ TMS) at 8.55 (1H, doublet, $J \sim 8$ Hz; 6-cytidine proton); 7.35 (1H, doublet, $J \sim 8$ Hz; 5-cytidine proton); 8.15–7.40 (5H, multiplet, C_6H_5CO—); and 5.90 p.p.m. (1H, doublet, $J \sim 3$ Hz; 1′-anomeric proton). N^4-Benzoylcytidine (**10**) was also prepared from cytidine by selective benzoylation.

A mixture of cytidine (5.0 g, 0.02 mol) in methanol (250 ml) and benzoic anhydride (1.0 g, 4.4 mmol) was heated under reflux. Further portions of benzoic anhydride (5 × 1 g, 0.022 mol) were added at *ca.* 1 h intervals. After the addition was completed (total added 6.0 g, 0.026 mol) the reaction mixture was cooled, and the precipitated N^4-benzoylcytidine (1.77 g, R_F: 0.16 in 10% CH_3OH–CH_2Cl_2) was filtered off. The t.l.c. indicated still some unreacted starting material (R_F: at origin in 10% CH_3OH–CH_2Cl_2), so the filtrate was concentrated *in vacuo* to *ca.* 150 ml, and was again heated under reflux with further portions of benzoic anhydride (3 × 1 g, 1.3 mmol). On cooling further 2.33 g of product (R_F: 0.16 in 10% CH_3OH–CH_2Cl_2) was filtered off. Total obtained: 4.1 g, m.p. 220–222° (dec.) 59%. The sample of N^4-benzoylcytidine (**10**) was identical with that obtained above (*a*) from (**7**) as confirmed by its p.m.r. spectrum.

*(b) N^4-5′-O-dibenzoylcytidine (**9**)*

3′-*O*-Acetyl-N^4-5′-*O*-dibenzoylcytidine (**7**) (200 mg, 0.4 mmol; m.p. 188–190°, R_F: 0.62 in 10% CH_3OH–CH_2Cl_2) was suspended in dilute methanolic ammonia (4 ml, prepared by diluting saturated (at 20°) methanolic ammonia with an equal volume of methanol) and stirred at room temperature. After 2.5 h t.l.c. indicated complete conversion into a product of R_F: 0.46 in 10% CH_3OH–CH_2Cl_2. The reaction mixture was then evaporated *in vacuo*, and the product crystallized from ethanol. N^4-5′-*O*-Dibenzoylcytidine was obtained (150 mg, 85%; m.p. 193–195°, R_F: 0.46 in 10% CH_3OH–CH_2Cl_2; lit. m.p. 186–190° (8)). Its p.m.r. spectrum showed signals (δ

TMS) at 8.3–7.25 (12H, multiplet, 6-, and 5-cytidine protons, and *two* C_6H_5CO— protons); 5.95 (1H, doublet, $J \sim 2$ Hz; 1′-anomeric proton); and 4.85 and 4.25 p.p.m. (5H, 2 multiplets, sugar protons).

(c) Cytidine (4)

3′-*O*-Acetyl-N^4-5′-*O*-dibenzoylcytidine (**7**) (100 mg, 0.2 mmol; m.p. 188–190°, R_F: 0.62 in 10% CH_3OH–CH_2Cl_2) was suspended in dilute methanolic ammonia (4 ml, prepared by diluting saturated (at 20°) methanolic ammonia with an equal volume of methanol) and stirred at room temperature. After 40 h t.l.c. indicated complete conversion into a product of R_F: at the origin in 10% CH_3OH–CH_2Cl_2. The reaction mixture was then evaporated to dryness *in vacuo* and triturated with ether (3 × 5 ml), and the residue crystallized from ethanol. The cytidine (**4**) obtained (33 mg, 69%; m.p. 210–212°, R_F, at origin in 10% CH_3OH–CH_2Cl_2) was identical with that of an authentic sample.

N^4-Benzoyl-2′,3′-O-methoxyethylidenecytidine (13)

A mixture of N^4-benzoylcytidine (5.2 g, 0.015 mol), *p*-toluenesulfonic acid monohydrate (0.70 g, 3.7 mmol; 0.25 equiv) and trimethyl orthoacetate (12.5 ml) was stirred at room temperature. After 2.5 h t.l.c. indicated complete conversion into a product of R_F: 0.72 in 10% CH_3OH–CH_2Cl_2. The reaction mixture was then cooled, and neutralized with concentrated ammonia solution, then chloroform was added (50 ml), and the precipitated ammonium tosylate filtered off. The precipitate was washed well with chloroform (3 × 25 ml), and the combined organic solutions evaporated *in vacuo*. The yellow glass obtained (5.0 g, 90%) had R_F: 0.70 in 10% and 0.48 in 5% CH_3OH–CH_2Cl_2. This material was used for the next reaction without any further purification.

3′-O-Acetyl-N^4-benzoyl-5′-O-methoxyacetylcytidine (15)

Methoxyacetic acid (1.15 g, 12.5 mmol) was evaporated from anhydrous pyridine (2 × 25 ml), and finally its volume reduced to *ca.* 10 ml. The solution was sealed under nitrogen, tri-isopropylbenzenesulfonyl chloride (3.5 g, 12.5 mmol) added, and the reaction mixture allowed to stand at room temperature for 1 h. N^4-Benzoyl-2′,3′-*O*-methoxyethylidenecytidine (**13**) (5.0 g, 12.5 mmol) was evaporated from anhydrous pyridine (2 × 25 ml), and finally its volume reduced to *ca.* 10 ml. This solution was added to the methoxyacetic acid – TPS complex (prepared above) and the reaction mixture set aside at room temperature. After 4 h t.l.c. indicated *ca.* 90% conversion into a product of R_F: 0.84 in 5% CH_3OH—CH_2Cl_2. A small piece of ice was then added to the mixture, and stirring continued for a further 1 h. The reaction mixture was then poured into ice-water (50 ml) and repeatedly extracted with methylene chloride (6 × 30 ml). The combined organic extracts were washed and evaporated *in vacuo* to give N^4-benzoyl-5′-*O*-methoxyacetyl-2′,3′-*O*-methoxyethylidenecytidine (**14**) as a cream colored glass (8.0 g). Crude (**14**) (8.0 g) was treated with 5% acetic acid (100 ml), and the mixture stirred at room temperature. After 16 h t.l.c. indicated complete conversion into products R_F: 0.59, and 0.48 in 5% CH_3OH—CH_2Cl_2. The reaction mixture was then repeatedly extracted with methylene chloride (6 × 40 ml). The combined organic extracts were washed, dried (Na_2SO_4), and evaporated *in vacuo*. The colorless glass obtained (8.5 g) was purified by chromatography on silica gel (100 g) in methylene chloride. Elution with 2% methanol – methylene chloride mixture (300 ml) yielded a mixture, (0.6 g, R_F: 0.59, and 0.48 in 5% CH_3OH–CH_2Cl_2). The required 2′(3′)-*O*-acetyl-N^4-benzoyl-5′-*O*-methoxyacetylcytidine (1.9 g, 33.0%, R_F: 0.48 in 5% CH_3OH–CH_2Cl_2) was obtained on elution with 2.5% methanol – methylene chloride mixture (600 ml). Further material (0.58 g, R_F: 0.25 in 5% CH_3OH–CH_2Cl_2) tentatively identified as N^4-benzoyl-2′(3′)-*O*-acetylcytidine by its p.m.r. spectrum, was obtained by washing the column with 3% methanol – methylene chloride (300 ml). The pure 3′-*O*-acetyl-N^4-benzoyl-5′-*O*-methoxyacetylcytidine (**15**) was obtained by repeated crystallization from absolute ethanol – small amount of methylene chloride. The pure 3′-acetate had m.p. 95–97° (lit. m.p. 99–100° (10)). The p.m.r. spectrum showed signals (δ TMS) at 8.2–7.25 (7H, multiplet; 6-, and 5-cytidine protons; and C_6H_5CO— protons); 5.90 (1H, doublet; $J \sim 4$ Hz, 1′-anomeric proton); 5.30 and 4.28 (3H, two multiplets, sugar protons); 3.25 (3H, sharp singlet, CH_3O— protons); and 2.08 p.p.m. (3H, sharp singlet, —$COCH_3$ protons).

Pure 2′-*O*-acetyl-N^4-benzoyl-5′-*O*-methoxyacetylcytidine of m.p. 146–148° (lit. m.p. 155–157° (10)) was also obtained by repeated crystallization from absolute ethanol – trace of methylene chloride.

Attempts to record the mass spectra of cytidine derivatives **10**, **7**, **15**, and the two diastereoisomers of **11** were unsuccessful. The high temperatures (above 280°, probe 3.5) required for volatilization of these compounds gave only decomposition products.

Financial assistance from the Medical Research Council of Canada is gratefully acknowledged.

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