A simple procedure for the preparation of protected 2'-O-methyl or 2'-O-ethyl ribonucleoside-3'-O-phosphoramidites

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Received July 1, 1991; Revised September 19; Accepted October 10, 1991

ABSTRACT

Protected 2'-O-methyl and 2'-O-ethyl ribonucleoside-3'-O-phosphoramidites were prepared via alkylation of the ribonucleosides at an early stage in the synthesis. Using a strategy of minimal protection, the alkylation was performed with unprotectected cytidine and adenosine, or with Oβ-protected guanosine and N3,5'-O-protected uridine using methyl or ethyl iodide and sodium hydride. In subsequent steps, the introduction of standard protective groups for oligonucleotide synthesis and the concomitant separation from 3'-O-alkylated isomers was accomplished. A modification of the phosphylation procedure permitted facile isolation of the desired phosphoramidites which show high coupling efficiencies in oligomer assembly.

INTRODUCTION

2'-O-Methyl oligoribonucleotides are oligonucleotide analogs which exhibit high resistance to both DNA- and RNA-specific nucleases and form hybrids of high thermal stability with complementary RNA (1-7). These analogs, as well as the recently described 2'-O-allyl oligoribonucleotides (8,9), have proven to be valuable antisense compounds for studying snRNP-mediated pre-mRNA splicing and processing (10-15). We have demonstrated sequence-specific inhibition of histone pre-mRNA processing in vitro using 2'-O-methyl or 2'-O-ethyl oligoribonucleotide 19mers complementary to the 5'-end of the U7-snRNP-RNA. These compounds inhibited processing at a 300-fold lower concentration than that required using the corresponding DNA oligomer (16).

Considerable effort has been directed toward developing efficient alkylation reactions that yield 2'-O-alkylribonucleoside building blocks suitable for oligonucleotide assembly. Various alkyllating agents such as dimethylsulfate (17), organostannous compounds (18), trimethylsulfoniumhydroxide (19,20) and most commonly diazomethane (21-25) have been used. To synthesize pyrimidine and adenosine 2'-O-methylribonucleotides, Inoue et al. (4) used methyl iodide/silver oxide to alkylate ribonucleoside that were 3'-5'-protected with the Markiewicz disiloxane reagent (26). The 2'-O-methyladenosine building block was synthesized in 7 steps starting from the 6-chloropurine nucleoside. In the case of guanosine, diazomethane was used to monomethylate the unprotected 2',3'-cis-diol. In alternative procedures developed by Sproat et al. (6), efficient 2'-O-alkylation of 3'-5'-disiloxane-protected ribonucleosides was achieved using methyl iodide/BDDDP. By this method the adenosine amidite was synthesized in an 8-step procedure starting from the 6-chloropurine nucleoside, and the guanosine amidite in 12 steps from the 2-amino-6-chloropurine nucleoside. As described recently (9), these procedures were also adapted for the preparation of 2'-O-allyl ribonucleoside monomers utilizing allyl bromide/BDDDP or palladium(O)-catalyzed alkylation.

To prepare 2'-O-methyl building blocks, we initially exploited the preferred alkylation by diazomethane at the 2'-O position to obtain the properly protected amidites in few steps starting from the ribonucleosides (27). Separation from 3'-O-methyl isomers was performed by vacuum-flash-chromatography (VFC, (28)). A major drawback of diazomethane alkylation, besides the inherent risks in the use of a toxic and potentially explosive reagent, is its restriction to the introduction of methyl groups.

In this paper we describe a simple and efficient procedure for the preparation of 2'-O-methylated or 2'-O-ethylated ribonucleoside building blocks for oligomer synthesis. Alkylation of the nucleosides is performed at an early step of the synthesis using methyl or ethyl iodide. The direct methylation of unprotected adenosine has already been described (29). Analogously, we alkylated cytidine without any protective groups, whereas with uridine or guanosine it was crucial to prevent N3- or Oβ-alkylation by protection. The selectivity of this reaction for the 2'-O position of the purine nucleosides is considerably higher than that seen using the diazomethane procedure. The ratio of the 2'-O-isomer to the 3'-O-isomer exceeded 4:1 for all four nucleosides. Because the alkylation reaction is carried out at an early stage of the synthesis, the 3'-O-isomers can easily be separated at subsequent reaction steps. Introduction of 5'- and base-protecting groups and phosphitylation leads to the formation of oligonucleotide building blocks in relatively few synthetic steps. As a result of the simple handling of the alkylling agents,

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the use of cheap and easily accessible reagents, and the few synthetic steps, these procedures are useful for the preparation of 2'-O-alkylated nucleotides.

RESULTS AND DISCUSSION

The syntheses of the cytidine and adenosine building blocks started with the monomethylation of the unprotected ribonucleosides (Fig. 1).

The methylation of cytosine (1) was performed with methyl iodide (1.1 equiv.) in DMF after deprotonation with sodium hydride (1.6 equiv.). The 2'- and 3'-monomethyl isomers showed almost identical behavior on chromatography and were separated from small amounts of higher methylated side products by VFC. Treatment with benzoyl chloride in pyridine after transient protection (50) of the sugar hydroxyls with TMS chloride, and desilylation with aqueous-methanolic ammonia gave a mixture of the N4-benzoylated O-methyl isomers 2α/5α (81:19 as determined from 1H-NMR and the methoxy signals of the 1H-NMR spectrum). After reaction with DMTrCl in pyridine the 5'-tritylated products could be separated by VFC to give 25% (based on 1) of the 2'-isomer 3α and 5% of the 3'-isomer 6α. In order to characterize the 5'-protected isomers, 3α and 6α were detritylated to give pure 2α and 5α.

The ethylation of 1 was accomplished analogously using sodium hydride (1 equiv.) and ethyl iodide (20 equiv.) in DMF. After benzoylation the 2'-O-ethyl product 2b was obtained in pure form by recrystallization from ethyl acetate in a yield of 24% (based on 1) and was converted to the dimethoxytritylated product 3b.

The direct alkylation of adenosine 7 was performed in a similar fashion as already described for the case of methylation (29). Subsequent treatment of the transiently TMS-protected monoalkylated products with phenoxyacetic anhydride yielded after desilylation a mixture of N6-phenoxyacetyl-protected 2'- and 3'-O alkylated isomers 8 and 11. In the case of methylation a selectivity in the products 8α/11α of 84:16 was obtained; with ethylation a significantly higher selectivity (8b/11b 91:9) was found. Treatment of the isomeric mixtures with DMTrCl in pyridine gave the corresponding tritylated compounds that could be easily separated into the pure isomers by VFC. Thus, tritylated 2'-O-methyl-N6-(phenoxyacetyl)adenosine 9a was obtained in an overall yield of 25% (based on 7). The yield of the corresponding ethylated product 9b was low, mainly as a result of a non-optimized procedures for the introduction of phenoxyacetyl and dimethoxytrityl protective groups; the monoethylated products 8b/11b were obtained in about 50%.

Base-protected 2'-O-alkylguanosines were synthesized by starting from O6-nitrophenylethyl guanosine 13 (Fig. 2), sincealkylation of unprotected, N2-, or N1 and N2-protected guanosine preferentially occurred at the base moiety (4,31). The nitrophenylethyl (NPE) protective group (32) was chosen as it offers also a guanosine O6-protection that is compatible with the oligonucleotide synthesis (33). Methylation of 13 was performed with sodium hydride and methyl iodide at low temperature (-50° to -15°C) in order to prevent the loss of the NPE group by elimination. After purification by VFC a crystalline mixture of the methyl ethers 14a/b was obtained in 48% yield with a ratio of 86:14 according to 1H-NMR analysis. From this mixture the pure 2'-O-methyl isomer 14a was isolated by recrystallization.

For practical reasons both the pure isomer 14a as well as the isomeric mixture 14a/b, obtained from chromatographic purification of the mother liquors of the recrystallization, were converted to the phenoxyacetylated compounds 15a/20a. Further
treatment with DMTrCl allowed the efficient chromatographic separation of the 2'-O-methyl isomer 16a from the 3'-O-methyl isomer 21a. Removal of the NPE group with diazabicyclo[5.4.0]undec-7-en (DBU) in pyridine, neutralization with acetic acid and purification by chromatography gave 18a in high yield.

The ethylation of 13 required a higher reaction temperature (0°C) as compared to the methylation. As a consequence of partial elimination of the NPE group the yield of 2'-O-isomer 14b was significantly lower (13%). Conversion to 15b and further to 16b was accomplished as described for the methylelated analogs.

To synthesize 5'-O-tritylated 2'-O-alkyl-uridines 25a and 25b (Fig. 3), it was necessary to protect the N2-lactam group from alkylation, similar to the guanosine synthesis. Therefore the cyanoethyl group, which has been used for the protection of thymidine (33), was introduced at N3 by treatment of 5'-O-tritylated uridine 22 with sodium hydride/acrylonitrile in DMF. Purification by VFC gave MMTr-protected 23a or DMTr-protected 23b in a yield of 45% or 55%, respectively. To prevent elimination of the cyanoethyl group during the methylation procedure, deprotonation of 23a was performed with limiting amounts of sodium hydride (0.9 equiv.) at low temperature (−40°C), and upon addition of methyl iodide (1.9 equiv.) the reaction was allowed to warm up slowly to room temperature. The resulting 2'-O- and 3'-O-methyl isomers 24a and 27a could be easily separated by chromatography. The ratio of isomers 24a/27a, as determined by yield, was about 81:19. As a side product dimethylated uridine 28a was isolated. Deprotection of 24a with potassium tert.-butylate gave 5'-O-MMTr-2'-O-methyluridine 25a. The compound was further characterized by detritylation to 2'-O-methyl uridine 29a. The ethylation of 23b afforded 24b/27b in an isomeric ratio of 83:17. Deprotection of 24b from the cyanoethyl group gave 5'-O-DMTr-2'-O-ethyl uridine 25b.

Phosphitylation of the protected alkylated nucleosides was accomplished as described (35) with a modified workup procedure (Table 1). The carefully dried precursor nucleoside was treated with (2-cyanoethoxy)-N,N-dimethylaminomethylphosphine and diisopropylethylamine in THF. To avoid chromatographic purification, which in the case of the guanosine compound resulted in partial cleavage of the phenoxyacetamide protecting group, the reaction was quenched with an lipophilic secondary alcohol (iso-propanol or sec-butanol). Thus the excess of phosphitylating agent is converted to a highly soluble amidite compound.

Figure 3. Synthesis of uridine building block. a, R=methyl, T=MMTr; b, R=ethyl, T=DMTr.

Table 1. Phosphitylation of protected 2'-O-methyl and 2'-O-ethylribonucleosides. TLC solvents: A, acetone/DCM 3:7; E, ethylacetate; D, DCM/ethylacetate 1:1. To avoid decomposition, TLC was developed after delay (less than 30 sec) after spotting the sample onto the plate.
Amidite in petroleum ether. A small amount of N,N-diisopropylamino-O-cya noethyl-H-phosphonate 31, a hydrolysis product of the phosphorylating agent, coprecipitated with the amidite but did not cause any detectable decrease in subsequent coupling efficiency. The protected 2'-O-methyl- and 2'-O-ethylribonucleoside-3'-O-phosphoramidites have been successfully used for oligomer assembly (16). Employing standard methodology with an increased coupling time of 5 minutes, average coupling efficiencies of greater than 98% were obtained.

**EXPERIMENTAL SECTION**

**Abbreviations:** BDDDP, 2-tetradecyl-methylene; BDDDD TP, 2-tert.-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin; DCM, dichloromethane; DMF, dimethylformamide; DMTr, dimethoxytrityl; DMT, dimethoxytrityl chloride; LC, column chromatography; MMTr, monomethoxytrityl; NPE, 2-(4-nitrophenyl)ethyl; DMTrCl, dimethoxytrityl chloride; PhOAc, Phenoxyacetyle; RT, room temperature; TLC, thin layer chromatography; THF, tetrahydrofuran; TMS, trimethylsilyl; VFC, vacuum flash chromatography;

Ribonucleosides were obtained from Fluka (Switzerland) and dried (80°C/10^-2 min / 20 hours) before use. Preparative liquid chromatography [LC, VFC (28)] and thin layer chromatography (TLC) were performed on a JEOL FX90Q, a Bruker AC-200, and a Bruker AC-250 spectrometer with variable temperature unit and QNP probehead; characteristic 1H and 13C signals of described compounds are shown in Table 2.

**N4-Benzoyl-5'-O-demethoxyltrityl-2'-O-methylcytidine 3a**

A solution containing 40 g (0.164 mol) of cytidine in 650 ml of dry DMF was treated with 4.33 g (0.18 mol) sodium hydride of dry DMF was treated with 4.33 g (0.18 mol) sodium hydride and 2.33 g (0.018 mol) of NPE. The resulting solution was stirred for 45 min at 0°C. During a period of 4 hours 36.9 g (0.26 mol) of 4.33 g (0.18 mol) sodium hydride was added, and the reaction mixture was then allowed to warm up to RT. As the fraction of dimethylated products started to increase (after ca. 6 h, according to TLC analysis; DMF of...
a TLC sample was removed in high vacuum at RT) the reaction mixture was filtered from precipitated sodium iodide and evaporated in vacuo. LC of the residue (chloroform/methanol 5:1) gave 22.2 g of a crude mixture of 2' and 3'-O-methylcytidine (still containing some sodium iodide; TLC: chloroform/methanol 3:2, R_f = 0.3 for both isomers). After coevaporation with benzene/pyridine 20.8 g of the product mixture were dissolved in 250 ml of dry pyridine and treated with 34.9 g (0.32 mol) of trimethylchlorosilane for 1 hour at RT. After addition of 13.5 g (0.096 mol) of benzoyl chloride the reaction was kept at RT overnight. The mixture was poured onto 300 ml water, diluted with 300 ml of methanol and then 250 ml of 25% aqueous ammonia was added. After 20 min the solution was extracted with DCM several times. The combined organic phases after drying with sodium sulfate, filtration and evaporation gave 32 g of a mixture containing 2a/5a (ratio 81:19; TLC: DCM/methanol 10/1, R_f = 0.2, for both isomers). This mixture, after coevaporation with benzene/pyridine was dissolved in dry pyridine and treated with 33 g (0.0976 mol) of dimethoxymethyl chloride for 2 hours at RT. The mixture was concentrated in vacuo and partitioned between DCM and aqueous sodium bicarbonate. The aqueous phase was extracted with DCM several times; the combined DCM extracts were dried with sodium sulfate and evaporated. LC of the residue (still containing small amounts of pyridine; 1200 g silica gel, eluent: DCM, then DCM/acetonitrile 8:1) yielded 25.3 g (25%) 3a and 5.1 g (5%) 6a as slightly yellow solids (TLC: DCM/acetonitrile 1:3, R_f = 0.24 for 3a, R_f = 0.28 for 6a).

N^-Benzoyl-2'-O-methylcytidine 2a: was obtained in pure form by detritylation of 3a [2% trichloroacetic acid in DCM, 5 min/RT] followed by extraction with water, evaporation of aqueous phase and purification of the residue by LC (DCM/acetonitrile first 4:1, then 3:2), 94% yield. 2a: white foam; TLC: DCM/acetonitrile 1:1, R_f = 0.32.

N^-Benzoyl-3'-O-methylcytidine 5a was characterized as the product obtained by detritylation of 6a [2% hydrochloric acid in DCM/ether (50:1); purification by LC (DCM/acetonitrile 2:1), precipitation from ethylacetate/methanol; 90% yield]. 5a: white foam; TLC: DCM/acetonitrile 1:1, R_f = 0.43.

N^-Benzoyl-2'-O-ethylcytidine 2b Compound 2b was obtained as described for 3a except that 45 g (0.185 mol) of cytidine was treated with 0.185 mol of sodium hydride and 40.52 g (3.825 mol) of ethyl iodide. LC purification gave 25.5 g of isomeric mixture containing monomethylated products as colorless foam. Subsequent reaction with 32.8 g (0.301 mol) of trimethylchlorosilane and 19.8 g (0.140 mol) of benzoyl chloride gave a crude product which after crystallization from ethylacetate and drying yielded 16.6 g (24%) pure 2b with a melting point of 193–194°C. TLC: DCM/isopropanol 9/1, R_f = 0.34.

N^-Benzoyl-5'-O-dimethoxymethyl-2'-O-ethylcytidine 3b A solution containing 7.30 g (19.4 mmol) of 2b in dry pyridine was treated with 9.88 g (29.2 mmol) of dimethoxymethyl chloride and worked up according to the tritylation procedure for 3a. Purification by LC (DCM/Isopropanol 30:1) gave 7.43 g (56.6%) of pure 3b as a pale yellow solid. TLC: DCM/isopropanol 9/1, R_f = 0.55.

S'-O-Dimethoxymethyl-2'-O-methyl-N^-6-(phenoxyacetyl) adenosine 9a A solution containing 50 g (0.187 mol) of adenosine in 700 ml of dry DMF was cooled to 0°C and treated with 5.34 g (0.224 mol) of sodium hydride for 45 min at 0°C. During a period of 4 hours 42.2 g (0.30 mol) of methyl iodide as a 20% (w/v) solution in DMF, was added in several portions the reaction mixture was then allowed to warm up to RT. As the fraction of dimethoxymethylated products started to increase (according to TLC analysis; DMF of a TLC sample was removed in high vacuum at RT) the reaction mixture was filtered from precipitated sodium iodide and evaporated in vacuo. VFC of the residue (chloroform/methanol gradient 19:1 to 5:1) gave 42 g of a crude mixture of 2'- and 3'-O-methyl-adenosine (still containing some sodium iodide; TLC: chloroform/ethanol 3:1, R_f = 0.3 for both isomers). After coevaporation with benzene/pyridine the product mixture was dissolved in 500 ml of dry pyridine and treated with 64.8 g (0.60 mol) of trimethylchlorosilane for 2 hours at RT. After addition of 51.2 g (0.179 mol) of phenoxyacetic anhydride the reaction was kept at RT overnight. The mixture was poured into 1000 ml of water and diluted with 400 ml of methanol. After 40 min about 25 g of solid sodium bicarbonate was added to the resulting clear solution, the mixture was stirred for a further 10 min and was then extracted with DCM several times. The combined organic phases were dried with sodium sulfate, filtered and evaporated in vacuo. After coevaporation with benzene/pyridine the crude product containing 8a/11a (45 g, ratio 86:14) was dissolved in dry pyridine and treated with 53.2 g (0.157 mol) of dimethoxymethyl chloride for 2 hours at RT. The mixture was concentrated in vacuo and partitioned between DCM and aqueous sodium bicarbonate. The aqueous phase was extracted with DCM several times; the combined DCM extracts were dried with sodium sulfate and evaporated in vacuo. VFC of the residue (DCM then DCM/acetonitrile 8:1) yielded 32.8 g (25%) of 9a and 3.3 g (2.5%) of 12a as colorless solids (TLC: DCM/acetonitrile 7:3, 9a: R_f = 0.52, 12a: R_f = 0.48).

2'-O-Methyl-N^-6-(phenoxyacetyl)adenosine 8a: The crude product containing 8a/11a, obtained according to the procedure given above, was purified by LC (400 g silica gel; DCM/acetonitrile 4:1) to give 5.5 g (7.05% based on 7) of 8a, with given analytical data, and 14.1 g (18.1% based on 7) of mixture of 8a/11a (TLC: DCM/methanol = 9:1, 8a: R_f = 0.50; 11a: R_f = 0.48).

3'-O-Methyl-N^-6-(phenoxyacetyl)adenosine 11a was characterized as the product obtained by detritylation of 12a [2% hydrochloric acid in DCM/ether (50:1); -5°C for 1 min; purification by VFC (DCM/acetonitrile 3.5:1), 92% yield]. 11a: white foam; TLC: DCM/methanol 9:1, R_f = 0.48.

5'-O-Dimethoxymethyl-2'-O-ethyl-N^-6-(phenoxyacetyl) adenosine 9b Compound 9b was synthesized according to the procedure used for preparation of 9a, except that 50 g (187 mmol) of adenosine was treated with 5.40 g (225 mmol) sodium hydride and 81.3 g (525 mmol) of ethyl iodide for 4 hours at 0°C. The following LC purification gave 30 g of isomeric mixture containing monomethylated products as colorless foam. Subsequent reaction with 4.1 g (406 mmol) of trimethylchlorosilane and 58.4 g (203 mmol) of phenoxyacetic anhydride gave a crude product which upon partial purification by VFC (DCM/THF 1:1) and drying in vacuo gave 19.7 g (25%) of a mixture containing 8b/11b (ratio
10:1) as colorless foam. Tritylation of 19.6 g of 8b/11b in 100 ml of dry pyridine with 23.2 g (69 mmol) of dimethoxytrityl chloride, as described for 9a, and VPC purification (DCM/THF gradient from 12:1 to 6:1) gave 4.8 g of 9b (3.5% overall yield from adenosine) as white foam. 9b: TLC: DCM/isopropanol 16:1, Rf = 0.42. (3'-regioisomer 12b, not isolated, Rf = 0.46).

2'-O-Ethyl-(phenoxyacetyl)adenosine 8b: TLC: DCM/isopropanol 9/1, Rf = 0.34.

2'-O-Methyl-0'-[2-(4-nitrophenyl)ethyl]guanosine 14a
After coevaporation with DMF/benzene 8.1 g of 13 (32) was dissolved in 130 ml of dry DMF and treated with 1.6 equiv. of sodium hydride for 45 min at -50°C. During a 5 h period 8 equiv. of methyl iodide was added and the reaction mixture allowed to warm up to -15°C. Ammonium chloride was then added and the mixture was evaporated in vacuo. The resulting residue was purified by VLC (chloroform/methanol 80:1) to yield 4.0 g (48%) of a mixture containing 14a/19a (86:14). 1.8 g (22%) of colorless crystals (Fp. = 109–111°C, NMR) of pure 14a (less than 1% 19a) was obtained by recrystallization from benzene/chloroform. The mother liquor was evaporated and purified by LC (DCM/isopropanol 30:1) to give 1.7 g (20.4%) crystals containing a mixture of 14a/19a (82:18). TLC: DCM/isopropanol 5:1, Rf = 0.56 (14a), Rf = 0.61 (19a).

2'-O-Ethyl-0'-[2-(4-nitrophenyl)ethyl]guanosine 14b
A solution containing 3.15 g of dry 13 (32) in 30 ml DMF was treated with 0.9 equiv. of sodium hydride at -15°C for 1 h. Then 8 equiv. of ethyl iodide was added during a period of 2 h at -15°C and the solution was subsequently stirred at 0°C for 3 h. The workup was performed as described for 14a, with 0.44 g (13%) of white foam 14b being obtained by LC (DCM/isopropanol 16:1). TLC: DCM/isopropanol 9:1, Rf = 0.37 (14b), Rf = 0.46 (3'-regioisomer 19b, not isolated).

2'-O-Methyl-0'-[2-(4-nitrophenyl)ethyl]-N2-(phenoxyacetyl)guanosine 15a
After drying by coevaporation with pyridine/benzene, 1.5 g of 14a was dissolved in 150 ml of pyridine and 5 equiv. of trimethylchlorsilane added at RT over a duration of 30 min. After 2 h 1.4 equiv. of phenoxyacetic anhydride was added at RT and the reaction mixture stirred over night. The reaction was then quenched by addition of 250 ml of aqueous methanol (50%) and 10 ml of conc. aqueous ammonia, after 10 min the product was extracted with DCM. Following drying (sodium sulfate) of the combined DCM phases and treatment in vacuo, 1.85 g of colorless foam 15a was obtained (NMR). Analogously 1.7 g of a mixture of 14a/19a (82:18) was phenoxyacetylated to give 1.8 g (81%) of a mixture of the two isomers 15a/20a. TLC: Chloroform/methanol 15:1, Rf = 0.36 (15a), Rf = 0.38 (20a).

2'-O-Ethyl-0'-[2-(4-nitrophenyl)ethyl]-N2-(phenoxyacetyl)guanosine 15b
Phenoxyacetylation of 0.44 g of 14b was accomplished as described for 15a. The crude product was purified by LC (DCM/isopropanol 10:1) to yield 0.47 g (82%) of 15b as colorless foam. TLC: DCM/isopropanol 9:1, Rf = 0.45.

5'-O-Dimethoxytrityl-2'-O-methyl-0'-[2-(4-nitrophenyl)ethyl]-N2-(phenoxyacetyl)guanosine 16a
Compound 15a (3.0 g of a mixture containing 0.3 g of regioisomer 20a) was dried by coevaporation with benzene/pyridine and dissolved in 350 ml of pyridine. After addition of 1.2 equiv. of dimethoxytrityl chloride the solution was stirred for 2 h at RT and then evaporated in vacuo. The residue was taken up in aqueous bicarbonate and extracted with DCM. The combined organic phases were dried (sodium sulfate), filtered and evaporated in vacuo. The residue was separated by LC (toluene/isopropanol 30:1) to give 4.1 g (81%) of 16a and 0.3 g (6%) of 21a as pale yellow foams. TLC: toluene/isopropanol 9:1, Rf = 0.24 (16a), Rf = 0.28 (21a).

5'-O-Dimethoxytrityl-2'-O-ethyl-0'-[2-(4-nitrophenyl)ethyl]-N2-(phenoxyacetyl)guanosine 16b
Compound 16b was prepared from 0.47 g of 15b according to the procedure given for 16a. LC purification (DCM/acetone 15:1) of the crude product gave 483 mg (68%) of pure 16b as a yellow powder. TLC: DCM/isopropanol 9:1, Rf = 0.60.

5'-O-Dimethoxytrityl-2'-O-methyl-N2-(phenoxyacetyl)guanosine 18a
A solution containing 150 mg of 16a in 5 ml of a 0.5 M 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) in pyridine was stirred for 2 h at RT. Then 1.5 ml of 1 M aqueous acetic acid was added slowly, the mixture diluted with water and the aqueous phase extracted with DCM. The combined organic phases were dried (sodium sulfate) and evaporated in vacuo. VFC of the residue (DCM/acetone/triethylamine 100:10:2) gave 95 mg (76%) of 18a as a colorless oil. TLC: Chloroform/Methanol = 4.8/0.3, Rf = 0.38.

N3-(2-Cyanoethyl)-5'-O-monomethoxytrityluridine 23a
After coevaporation with DMF/benzene 7 g of 22a (prepared according to published procedures(34)) was dissolved in 100 ml of dry DMF, cooled to 0°C and treated with 1 equiv. of sodium hydride for 1 h. During 12 h 20 equiv. of acrylonitrile were added and the reaction mixture heated to 80°C. The reaction was then quenched by pouring onto water/DCM. The crude product obtained by evaporation of the DCM extract was purified by VFC (DCM/acetone 30:1) to yield 3.5 g (45%) of colorless foam 23a. TLC: DCM/acetone 33:2, Rf = 0.42.

N3-(2-Cyanoethyl)-5'-O-dimethoxytrityluridine 23b
Compound 23b (24.0 g) was obtained analogously to 23a from 40 g of 22b (prepared according to published procedures(34)) in 55% yield as colorless solid. 23b: TLC: DCM/isopropanol 9:1, Rf = 0.51.

N3-(2-Cyanoethyl)-2'-O-methyl-5'-O-monomethoxytrityluridine 24a
A solution containing 600 mg of 23a in 10 ml dry DMF was cooled to -40°C and treated with 0.9 equiv. of NaH for 45 min. Then 1.9 equiv. of methyl iodide was added and during a period of 3 h the temperature raised to RT. The reaction was quenched by addition of ammonium chloride and evaporated. The residue was dissolved in water and extracted with DCM. After drying (sodium sulfate) and evaporation of the combined organic phases in vacuo, the reaction products were separated by LC (ether) to give 200 mg (33%) of 24a, 48 mg (8%) of 3'-O-methyl product 27a and 80 mg (13%) of dimethyalted product 28a as colorless foams. TLC: DCM/acetone 3.2:1.8, Rf = 0.80 (24a), Rf = 0.74 (27a), Rf = 0.87 (28a).
2′-O-Methyl-5′-O-monomethoxytrityluridine 25a

A solution containing 100 mg of 24a in DCM was treated with 2 equiv. of KOT-Bu for 30 min and then poured onto aqueous sodium bicarbonate. After extraction with DCM the organic phase was dried (sodium sulfate) evaporated in vacuo and the crude product purified by VFC (DCM/acetone 9:1) to yield 80 mg (85%) of 25a as a colorless solid. TLC: DCM/acetone = 3:2, Rf = 0.41.

5′-O-Dimethoxytrityl-2′-O-ethyluridine 25b

A DMF solution containing 32.4 g of 25b was stirred at -20°C, dried with sodium sulfate and evaporated. The solution was stored at -20°C over 3 i molecular sieve and could be kept for several months without detectable decomposition.

ACKNOWLEDGEMENTS

We appreciate the critical reading of this manuscript by Lisa Ballou and Stephen Phillips and we acknowledge the skilful preparation of figures by Hannes Tkadletz.

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