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SYNTHESIS OF OLIGORIBONUCLEOTIDES CONTAINING 4-THIOURIDINE USING THE CONVERTIBLE NUCLEOSIDE APPROACH AND THE 1-(2-FLUOROPHENYL)-4-METHOXYPIPERIDIN-4-YL GROUP.

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ABSTRACT

Oligoribonucleotides containing 4-thiouridine were prepared using the Fpmp group for protection of the 2'-OH. Two uridine derivatives with the 1,2,4-triazolyl and the 2-nitrophenyl groups at position 4 were used to obtain 4-thiouridine by postsynthetic substitution with sodium hydrogen sulfide. Both uridine derivatives allow the preparation of the desired oligonucleotides in good yields.

Keywords: 4-thiouridine, oligonucleotides, RNA,

INTRODUCTION

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Photochemical cross-linking can be used to characterize RNA-protein interactions by trapping transient complexes, which can be difficult to isolate.¹⁻³ One of the most common strategies used in photochemical cross-linking is to incorporate photoreactive nucleotide analogs at defined positions. These photoreactive nucleotides can be incorporated into the RNA chains by *in vitro* transcription and/or RNA ligation.¹⁻³ Recent advances in chemical synthesis of RNA have opened up the possibility of obtaining short RNA molecules containing some of these photoreactive nucleosides by chemical synthesis. Specifically the synthesis of oligoribonucleotides containing the photoreactive nucleosides 5-bromo-, 5-iodo- and 4-thiouridine has been described.⁴⁻⁸ For the preparation of 5-thiouridine oligoribonucleotides, the S-cyanoethyl-protected 4-thiouridine phosphoramidite derivative^{4,6,7} has been used.

Another possibility is to prepare an uridine derivative carrying a leaving group in position 4. After the assembly of the desired sequence this derivative can be converted to thiouridine by nucleophilic displacement with a thiol. This strategy, known as the convertible nucleoside approach⁹ or the postsynthetic modification approach¹⁰, has been used to prepare oligoribonucleotides carrying N-alkyl-cytosine derivatives¹¹ and 4-thiouridine^{5,8}. In these studies, 2'-OH were protected as a *tert*-butyldimethylsilyl (TBDMS) ethers.

We are interested in the preparation of oligoribonucleotides carrying photoreactive nucleosides such as 4-thiouridine. In this paper, we describe the preparation and use of two uridine derivatives for the synthesis of 4-thiouridine oligoribonucleotides. The 2'-OH of the phosphoramidite derivatives is protected by the 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp) group¹²⁻¹⁴. In this way, Fpmp-protected RNA intermediates can be isolated, facilitating the characterization of the products.

RESULTS AND DISCUSSION

Preparation of Oligonucleotides Carrying 4-Thiouridine Using the 1,2,4-Triazolyl Derivative of Uridine.

Nucleoside derivatives carrying good leaving groups at specific positions can be used to obtain oligonucleotides carrying modified bases^{4,5,8-11}. The 1,2,4-triazolyl group^{5,15,16} was first selected to activate the position 4 of the uracil base in order to obtain 4-thiouridine. To

study the displacement conditions we prepared the 1,2,4-triazolyl derivative of 2',3',5'-tri-*O*-acetyluridine. 2',3',5'-Tri-*O*-acetyluridine¹⁷ (**1**, Figure 1) was reacted with a solution of phosphoryl *tristriazolide*^{5,16} prepared by mixing POCl₃, 1,2,4-triazol and triethylamine in dry CH₃CN. Compound **2** was then treated with a 0.2 M solution of thiolacetic acid in CH₃CN and with a 0.2 M solution of NaSH in dimethylformamide (DMF). Both gave the desired 2',3',5'-tri-*O*-acetyl-4-thiouridine (**3**, Figure 1) but the reaction with NaSH was faster finishing after 3 hr at room temperature.

FIGURE 1

Next, the commercially available uridine phosphoramidite carrying the Fpmp group for the protection of the 2'-OH was reacted with the phosphoryl *tristriazolide*⁵ solution giving compound **4** (Figure 1) in 85% yield after purification on silica gel.

Phosphoramidite **4** was reacted with thymidine linked to a controlled pore glass (CPG) support to obtain after regular phosphite oxidation the dinucleotide sequence A: 5' ^{tri}UT 3' (where ^{tri}U is 4-(1,2,4-triazol-1-yl)-2-pyrimidon-1-yl-β-D-ribofuranoside). The coupling reaction of phosphoramidite **4** proceeded in 95% yield, according to the absorbance of the DMT group released after the coupling reaction. The resulting support was then treated with a 0.2 M NaSH solution in DMF at room temperature for 3 hours, and, afterwards, with conc. NH₃ for 1 hr at room temperature to release the dinucleotide from the support and remove the cyanoethyl group. Only one peak was observed, which was collected and found to have the expected molecular weight.

Oligoribonucleotide sequences B: 5' ^{tri}UG 3' and C: 5'CUAGU(^{tri}U)CGAU 3' were assembled on a DNA synthesizer using Fpmp-protected nucleoside 2-cyanoethyl phosphoramidites and solid supports. The resulting supports were treated with a 0.2 M NaSH solution in DMF, and with conc. NH₃. In these oligonucleotides ammonia treatment must be longer than one hour in order to remove the protecting groups of the bases. The effect of the ammonia treatment was studied on the dinucleotide B. Overnight treatment at 50°C removed the thiol group of 4-thiouridine yielding the dinucleotide carrying cytidine or uridine. The side product is more polar than the desired oligonucleotide and it is easily separated by HPLC (see Figure 2b). In this way, the side product was isolated and

characterized by mass spectrometry (MS, electrospray, found 1002.4 expected for $C_{Fpmp}G_{Fpmp}$ 1002.8, expected for $U_{Fpmp}G_{Fpmp}$ 1003.8). The formation of this side product is reduced by lowering the temperature (24 hr, at room temperature) or the time of the ammonia treatment (4 h, 50°C, Figure 2a). Using a 4 h treatment at 50°C decamer C was obtained as the major product, and had the expected molecular weight. Dinucleotide 5' ^{45}UG 3' was used to initiate the transcription by T7 RNA polymerase. In this way, longer RNA transcripts with 4-thiouridine at the 5'-end were obtained and used to study proteins involved in RNA splicing, as described previously¹⁸.

FIGURE 2

We also found that samples with the (1,2,4-triazolyl) derivative should be processed rapidly. After three months, both the phosphoramidite **4** and oligonucleotide supports carrying the (1,2,4-triazolyl) derivative had lost the capacity to produce 4-thiouridine. It is believed that the (1,2,4-triazolyl) derivative is hydrolyzed by humidity to yield uridine even if the samples are maintained frozen. On the other hand, the 1,2,4-triazolyl derivative of thymidine is much more stable. The presence of the methyl group in thymidine may prevent hydrolysis by steric and electronic effects. For this reason, a new uridine derivative was prepared.

Preparation of Oligonucleotides Carrying 4-Thiouridine Using the 2-Nitrophenyl Derivative of Uridine.

The synthesis of the phosphoramidite derivative of 4-(2-nitrophenyl)uridine is shown in Figure 3. 3',5'-*O*-Tetraisopropylidisiloxane-1,3-dilyl)-4-*O*-(2-nitrophenyl)uridine (**5**) was prepared as described¹⁹. Compound **5** was reacted with 1-(2-fluorophenyl)-4-methoxy-1,2,5,6 *tetrahydropyridine*¹² to give the 2'-Fpmp derivative **6** in 62% yield. Removal of the silyl group with tetrabutylammonium fluoride produced compound **7** in 86% yield. 2'-Fpmp derivative **7** was reacted with dimethoxytrityl chloride and the resulting compound **8** was reacted with 2-cyanoethoxy-*N,N*-diisopropylamino-chlorophosphine to obtain the desired phosphoramidite **9** in 91% yield.

FIGURE 3

Phosphoramidite **9** was reacted with thymidine linked to a controlled pore glass (CPG) support to obtain the dinucleotide sequence A: 5' ^{np}UT 3' (being ^{np}U: 4-(2-nitrophenyl)-uridine. The coupling reaction of phosphoramidite **9** proceeded in 94% yield, similar to the Fpmp phosphoramidites of the natural nucleosides. The resulting support was then treated with a 0.2 M NaSH solution, and with conc. NH₃ at room temperature. Only one peak was observed, which was collected and found to have the expected molecular weight. Oligoribonucleotide sequence E: 5' UAC(^{np}U)GU 3' was prepared using the appropriate Fpmp phosphoramidites. The support was treated with a 0.2 M NaSH solution and the resulting support was then treated with conc. ammonia as described above, producing the desired thiouridine oligonucleotide in good yields. This phosphoramidite maintained its properties for at least two years when frozen.

FIGURE 4

CONCLUSION

We have shown that oligoribonucleotides containing 4-thiouridine can be prepared from two different precursors: first, the 4-(1,2,4-triazolyl) derivative which can be obtained from the readily available uridine phosphoramidite carrying the Fpmp group at position 2' but which has a limited shelf-life; second, the 4-(2-nitrophenyl) derivative, which requires a longer synthetic scheme but, once prepared, presents a longer shelf-life. These derivatives may be also used for N-15 labelling and preparation of oligonucleotides carrying N-alkyl-C derivatives similarly as described with the 4-chlorophenyl uridine derivative.¹¹

EXPERIMENTAL SECTION

Abbreviations: A₂₆₀: absorbance at 260 nm, controlled pore glass (CPG), DMF: *N,N*-dimethylformamide, DMT: dimethoxytrityl, Fpmp: 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl, OD: optical density units at 260 nm, THF: tetrahydrofuran, ^{np}U: 4-*O*-(2-

nitrophenyl)uridine, ^{4S}U: 4-thiouridine; ^{tri}U: 4-(1,2,4-triazol-1-yl)-2-pyrimidon-1-yl-β-D-ribofuranoside.

General Procedures. Solvents, including those of HPLC grade, were from SDS and E. Merck. Reagents were from Aldrich and Fluka and were used without further purification. Analytical TLC was run on aluminium sheets coated with silica gel 60 F₂₅₄ from Merck. Silica gel column chromatography was performed with Chromatogel 60 A C.C. (40-60 microns, 230-400 mesh, SDS). Fpmp-protected phosphoramidites and 1-(2-fluorophenyl)piperidin-4-one were obtained from Cruachem Ltd (Scotland). 1-(2-Fluorophenyl)-4-methoxy-1,2,5,6 *tetrahydropyridine* was prepared from 1-(2-fluorophenyl)piperidin-4-one as described¹². 2',3',5'-tri-*O*-acetyluridine (**1**) was prepared by the reaction of uridine with acetic anhydride in pyridine¹⁷. 3',5'-*O*-Tetraisopropylidisiloxane-1,3-dilyl)-4-*O*-(2-nitrophenyl)uridine (**5**) was prepared as described with minor modifications¹⁹. ¹H-NMR (250 MHz), ¹³C-NMR (63 MHz) and ³¹P-NMR (75 MHz) spectra were recorded on a Bruker AM-250. HPLC chromatography was performed on an HPLC Waters equipped with an UV detector at 260 nm.

Standard Work-Up. All reactions were carried out in oven-dried glassware, under a nitrogen or argon atmosphere, unless specified otherwise. Before use, starting materials were dried by evaporation with the dry solvent that will be used for the reaction. Once the reaction was completed, solutions were concentrated to dryness and the residues were dissolved in CH₂Cl₂ or CHCl₃ and washed with 5% aqueous NaHCO₃ and saturated aqueous NaCl. The organic phase was dried (Na₂SO₄) and the solvent was removed.

2',3',5'-*O*-Triacetyl-4-(1,2,4-triazol-1-yl)-2-pyrimidon-1-yl-β-D-ribofuranoside (**2**).

To an ice-cooled stirred suspension of 1,2,4-triazole (1.5 g, 21.7 mmol) in dry CH₃CN (30 ml) 0.42 ml (4.2 mmol) of POCl₃ were added, followed by 3.27 ml (23.4 mmol) of dry Et₃N. After 30 min a solution of 2',3',5'-tri-*O*-acetyluridine¹⁷ (**1**) (2 mmol) in dry CH₃CN was added over a period of 15 min and stirred for 2 h. The reaction was stopped by addition of 25 ml of a saturated NaHCO₃ aqueous solution and, 50 ml of ethyl acetate were added. The organic phase was washed with brine, dried over Na₂SO₄ and concentrated to dryness.

The resulting product (0.85 g unpurified with Et₃N) was used in the next step without further purification. TLC (10% MeOH in CH₂Cl₂) one single fluorescent spot of R_f = 0.7. UV (max, MeOH) 243, 302 nm.

2',3',5'-Tri-O-acetyl-4-thiouridine (**3**).

1) Reaction with thiolacetic acid. Compound **2** (0.24 mmol) was treated with 0.142 ml (2 mmol) of thiolacetic acid dissolved in 10 ml of CH₃CN. After one day of magnetic stirring at room temperature conversion to 2',3',5'-triacetyl thiouridine was judged to be around 50% by TLC (10% CH₃OH in CH₂Cl₂, starting material R_f= 0.7 fluorescent, final product R_f= 0.8 non fluorescent). After 2 days the reaction was practically finished. The reaction mixture was concentrated to dryness and treated using the standard work-up described above. The product was purified by column chromatography. Yield: 58% (53 mg, 0.14 mmol). Physical and spectroscopic data agreed with literature values²⁰.

2) Reaction with sodium hydrogen sulfide. 2',3',5'-O-Triacetyl-4-(1,2,4-triazolyl)uridine (0.24 mmol) was treated with 148.2 mg (2 mmol) of sodium hydrosulfide in 10 ml DMF. After 3 hr of magnetic stirring the conversion was judged to be completed by TLC (see above). The reaction mixture was concentrated to dryness and treated using the standard work-up described above. The product was purified by column chromatography. Yield: 62 % (60 mg, 0.15 mmol. Physical and spectroscopic data agreed with literature values²⁰.

5'-O-Dimethoxytrityl-2'-O-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-4-(1,2,4-triazolyl)uridine 3'-O-[2-cyanoethyl-*N,N*-(diisopropylamino)] phosphoramidite (**4**).

The protocol described by Shah *et al.*⁵ was used for the preparation of this phosphoramidite. To an ice-cooled suspension of 1,2,4-Triazole (1.04 g, 15.5 mmol) in dry CH₃CN (20 ml) was added 0.3 ml of POCl₃ was added, followed by 2.25 ml of dry Et₃N. After 30 min a solution of the DMT-U(Fpmp) phosphoramidite (188 mg, 0.25 mmol) in dry CH₃CN was added over a period of 15 min and stirring was continued for 2 hr. After standard work-up, the product was purified by column chromatography (ethyl acetate/ CH₂Cl₂ 2:3 + 1% triethylamine). Yield 170 mg, 85%. ³¹P-NMR (CDCl₃): 151.3, 150.2 (two diastereoisomers).

TABLE 1

3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-2'-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-4-*O*-(2-nitrophenyl)uridine (**6**).

3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-4-*O*-(2-nitrophenyl)uridine¹⁸ (2.43g, 4 mmol) and 1-(2-fluorophenyl)-4-methoxy 1,2,5,6-tetrahydropyridine (4.95 g, 24 mmol) were dissolved in dry THF (40 ml). Mesitylenesulfonic acid dihydrate (0.25 g, 1.05 mmol) was dried by coevaporation of dry CH₃CN (2 x 20 ml) under reduced pressure. The dried mesitylenesulfonic acid was dissolved in dry CH₃CN (10 ml) and added to the THF solution. The reaction mixture was stirred overnight under anhydrous conditions, whereupon TLC showed an almost complete reaction. Triethylamine was added and after 5 minutes, THF was evaporated and the residue was dissolved in CH₂Cl₂. The solution was treated with the standard work-up described above. The residue was purified on silica gel using a 0-4% CH₃OH gradient in CH₂Cl₂. Yield: 2.04 g (2.5 mmol) 62%. Anal. Calcd for C₃₉H₅₅FN₄O₁₀Si₂: C 57.47, H 6.80, N 6.87. Found C 57.05, H 6.67, N 6.61%. MS (FAB+) *m/z*: calculated for C₃₉H₅₅FN₄O₁₀Si₂, 815; found 814.8 [M⁺],.

2'-*O*-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-4-*O*-(2-nitrophenyl)uridine (**7**).

Compound **6** (2.04g, 2.5 mmol) was dissolved in dry THF (50 ml) and 1.1 M tetrabutylammonium fluoride in THF (5.5 ml) was added with stirring. Silica gel TLC in ethanol/ CH₂Cl₂ (5: 95, v/v) showed a complete reaction after 5 minutes with a spot of R_f.0.32. The reaction mixture was quenched with 16 ml of pyridine/methanol/water (3:1:1), and the solution was poured into stirred Dowex 50Wx4 (200 mesh, pyridinium form) resin suspended in 100 ml of pyridine/methanol/water (3:1:1). The mixture was stirred for 20 min, the resin filtered off and washed with the same mixture (3 x 50 ml). The combined filtrates and washings were evaporated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a gradient from 0 to 15% ethanol in CH₂Cl₂. Yield: 1.24 g (2.16 mmol) 86%. Anal. Calcd for C₂₇H₃₀FN₄O₉: C 56.54, H 5.27, N 9.77. Found C 56.28, H 5.01, N 9.54%. MS (FAB+) *m/z*: calculated for C₂₇H₃₀FN₄O₉, 573.5 ; found 573.9 [M⁺].

5'-*O*-Dimethoxytrityl-2'-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-4-*O*-(2-nitrophenyl)uridine (**8**).

Compound **7** (1.24 g, 2.16 mmol) was dried by evaporation of pyridine (3 x 1.5 ml) in vacuo. Anhydrous pyridine (30 ml) and 4,4'-dimethoxytrityl chloride (0.9 g, 2.65 mmol) were added with stirring and exclusion of moisture. Silica gel TLC in triethylamine / ethanol / CH₂Cl₂ (1:10:89, v/v/v) showed a complete reaction after 1.5 h with a new spot of R_f 0.65. The reaction was quenched by addition of methanol (5 ml) and the solvent was removed in vacuo. The residual oil was dissolved in ethyl acetate (50 ml) and the solution was washed as described above in the standard work-up. Residual pyridine was removed by evaporation of toluene *in vacuo*. The crude product was purified by column chromatography. The column was packed using a 1% triethylamine solution in CH₂Cl₂ and the product was eluted with a 0-10% methanol gradient in CH₂Cl₂. Compound **8** was obtained as a white foam. Yield: 1.69 g (1.93 mmol) 89%. Anal. Calcd for C₄₈H₄₈FN₄O_{11.2/3} CH₂Cl₂: C 62.76, H 5.34, N 6.02. Found C 62.40, H 5.21, N 6.04%. MS (FAB+) *m/z*: calculated for C₄₈H₄₈FN₄O₁₁, 875.9; found 874.9 [M⁺], 896.7 [M+Na⁺].

5'-*O*-Dimethoxytrityl-2'-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-4-*O*-(2-nitrophenyl)uridine 3'-*O*-[2-cyanoethyl-*N,N*-(diisopropylamino)] phosphoramidite (**9**).

Compound **8** (1 g, 1.14 mmol) was dried by evaporation of dry CH₃CN (3 x 10 ml) in vacuo. The residual foam was dissolved in dry 1,2-dichloroethane (30 ml) containing *N,N*-diisopropylethylamine (0.6 ml, 3.42 mmol) and the solution (under argon) was cooled in an ice bath. 2-Cyanoethoxy-*N,N*-diisopropylamino-chlorophosphine (0.4 ml, 1.71 mmol) was added drop wise with stirring during approx. 1 min. The mixture was kept 10 min at 0°C and was then stirred for 1 h at room temperature. TLC in triethylamine/ CH₂Cl₂ showed a complete reaction with a product spot of R_f 0.55. Dichloromethane (50 ml) was added and the solution was washed as described above in the standard work-up. The product was purified by column chromatography on silica gel using CH₂Cl₂/ *n*-hexane (5:2) containing 2% triethylamine as eluant. Yield: 1.12 g (1.04 mmol) 91%. ³¹P-NMR (Cl₃CD) δ_P: 150.8 and 149.7 ppm. Anal. Calcd for C₅₇H₆₅FN₆O₁₂P: C 63.62, H 6.09, N 7.81. Found C 63.25, H 6.13, N 7.69%. MS (FAB+) *m/z*: calculated for C₅₇H₆₅FN₆O₁₂P, 1076.1; found 1097.1 [M+Na⁺].

Oligonucleotide Synthesis and Purification.

Sequences A: 5' ^{tri}UT 3', B: 5' ^{tri}UG 3', C: 5'CUAGU(^{tri}U)CGAU 3', D: 5' ^{np}UT 3', and E: 5' UAC(^{np}U)GU 3' were assembled using 35 mg (1 μ mol) of the appropriate supports and Fpmp-protected nucleoside 2-cyanoethyl phosphoramidites on a DNA synthesizer (Applied Biosystems Mod. 394). Standard 1 μ mol scale RNA synthesis cycles were used. Coupling efficiencies were higher than 96%. The last DMT group was removed before deprotection (DMT off) except in sequences C and E, in which it was not removed (DMT on) so as to facilitate HPLC purification.

After the assembly of the sequences, supports were treated with a 0.2 M NaSH solution in DMF (2 ml) at room temperature for 3 hours. The supports were washed with DMF and CH₃CN and dried. The resulting supports were treated with concentrated ammonia at 50 °C for 4 hours. The resulting Fpmp oligonucleotides were purified by reverse-phase HPLC. HPLC conditions were as follows: Column : Nucleosil 120C18 (200 x 4 mm), flow rate 1 ml / min, a 30 min linear gradient from 10 to 55% CH₃CN over 20 mM aqueous triethylammonium acetate. Overall (synthesis and purification) yields were: sequence A (1 μ mol scale): 5 OD, sequence B (4 μ mol scale): 41 OD, sequence C (1 μ mol scale): 19 OD, sequence D (1 μ mol scale): 4 OD and D: (1 μ mol scale): 11 OD. All oligonucleotides have an UV maximum near 320 nm in addition to the maximum near 260 nm. Oligonucleotides were analyzed by mass spectrometry (electrospray). 5' ⁴⁵U_{Fpmp}T 3': found 771.4 (M) calculated for C₃₁H₃₉FN₅O₁₃PS 771.5; 5' ⁴⁵U_{Fpmp}G_{Fpmp} 3': found 1019.0 (M) calculated for C₄₃H₅₂F₂N₉O₁₄PS 1019.8; 5' DMT-C_{Fpmp}U_{Fpmp}A_{Fpmp} G_{Fpmp}U_{Fpmp}⁴⁵U_{Fpmp} C_{Fpmp}G_{Fpmp}A_{Fpmp}U_{Fpmp} 3': found 5511.1 (M) calculated for C₂₂₇H₂₇₅F₁₀N₄₄O₈₃P₉S 5448.1; 5' DMT-U_{Fpmp}A_{Fpmp}C_{Fpmp}⁴⁵U_{Fpmp}G_{Fpmp}U_{Fpmp} 3': found 3399.3 (M) calculated for C₁₄₉H₁₇₃F₆N₂₅O₄₉P₅S 3398.3. The Fpmp and DMT groups were removed by treatment of the samples with 0.5 ml of 10 mM aqueous HCl (pH 2.5-3) at room temperature overnight^{13,14}. The samples were neutralized by adding 25 μ l of sterile 2M Tris acetate pH 7 followed by ethanol precipitation of RNA with 3 volumes of ethanol and 1/10 of volume of sterile 3M sodium acetate. Mass spectrometry (MALDI-TOF). 5' ⁴⁵UT 3': found 586.4 (M+Na-H)⁻

calculated for $C_{19}H_{25}N_4O_{12}PS$ 564.3; 5' ^{45}UG 3': found 627.5 ($M+Na-H$)⁻ calculated for $C_{19}H_{24}N_7O_{12}PS$ 605.3.

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FIGURE LEGENDS:

Figure 1: Uridine derivatives **1-4**

Figure 2: HPLC purification of dinucleotide 5' ^{45}S U_{Fmp}G_{Fmp} 3'. Oligonucleotide support B: 5' ^{32}P UG 3' was treated with a 0.2 M NaSH solution in DMF at room temperature for 3 hours and the resulting supports were treated with concentrated ammonia a) at 50 °C for 4 hours and b) at 50 °C overnight.

Figure 3: Uridine derivatives **5-9** carrying the 2-nitrophenyl group

Figure 4: HPLC purification of oligonucleotide sequence E.

Figure 1

Figure 2

Figure 3.

Figure 4.