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Permanent or reversible conjugation of 2′-*O*- or 5′-*O*-aminooxymethylated nucleosides with functional groups as a convenient and efficient approach to the modification of RNA and DNA sequences

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ABSTRACT

2′-*O*-Aminooxymethyl ribonucleosides are prepared from their 3′,5′-disilylated 2′-*O*-phthalimidooxymethyl derivatives by treatment with NH_4F in MeOH. The reaction of these novel ribonucleosides with 1-pyrenecarboxaldehyde results in the efficient formation of stable and yet reversible ribonucleoside 2′-conjugates in yields of 69–82%. Indeed, exposure of these conjugates to 0.5 M tetra-*n*-butylammonium fluoride (TBAF) in THF results in the cleavage of their iminoether functions to give the native ribonucleosides along with the innocuous nitrile side product. Conversely, the reaction of 5-cholesten-3-one or dansyl chloride with 2′-*O*-aminooxymethyl uridine provides permanent uridine 2′-conjugates, which are left essentially intact upon treatment with TBAF. Alternatively, 5′-*O*-aminooxymethyl thymidine is prepared by hydrazinolysis of its 3′-*O*-levulinyl-5′-*O*-phthalimidooxymethyl precursor. Pyrenylation of 5′-*O*-aminooxymethyl thymidine and the sensitivity of the 5′-conjugate to TBAF further exemplify the usefulness of this nucleoside for modifying DNA sequences either permanently or reversibly. Although the versatility and uniqueness of 2′-*O*-aminooxymethyl ribonucleosides in the preparation of modified RNA sequences is demonstrated by the single or double incorporation of a reversible pyrenylated uridine 2′-conjugate into an RNA sequence, the conjugation of 2′-*O*-aminooxymethyl ribonucleosides with aldehydes, including those generated from their acetals, provides reversible 2′-*O*-protected ribonucleosides for potential applications in the solid-phase synthesis of native RNA sequences. The synthesis of a chimeric polyuridylic acid is presented as an exemplary model.

INTRODUCTION

Over the past decade, the 2′-hydroxy function of ribonucleosides has been extensively modified for the purpose of identifying the biophysical and biochemical parameters necessary for effective and lasting RNA interference-mediated gene silencing activities (1–4). Actually, 2′-hydroxy modifications are known to impart high binding affinity to RNA sequences, increased lipophilicity, enhanced chemical stability and resistance to nucleases (1,2,5). The 2′-hydroxyl group of ribonucleosides is also an attractive function for conjugation reactions; there are numerous examples of ribonucleoside 2′-conjugates that have been reported in various structural studies (6,7) as well as in therapeutic and diagnostic applications (8,9). Although 2′-*O*-alkylation of ribonucleosides with functional groups has often been employed in the synthesis of ribonucleoside 2′-conjugates (8,9), this method is generally lacking the regioselectivity needed for the production of conjugates free of isomeric impurities. An alternate strategy to the preparation of ribonucleosides 2′-conjugates is the use of the oxyamino-aldehyde coupling reaction (10–12), which incidentally has extensively been applied to the derivatization of oligonucleotides (13–20).

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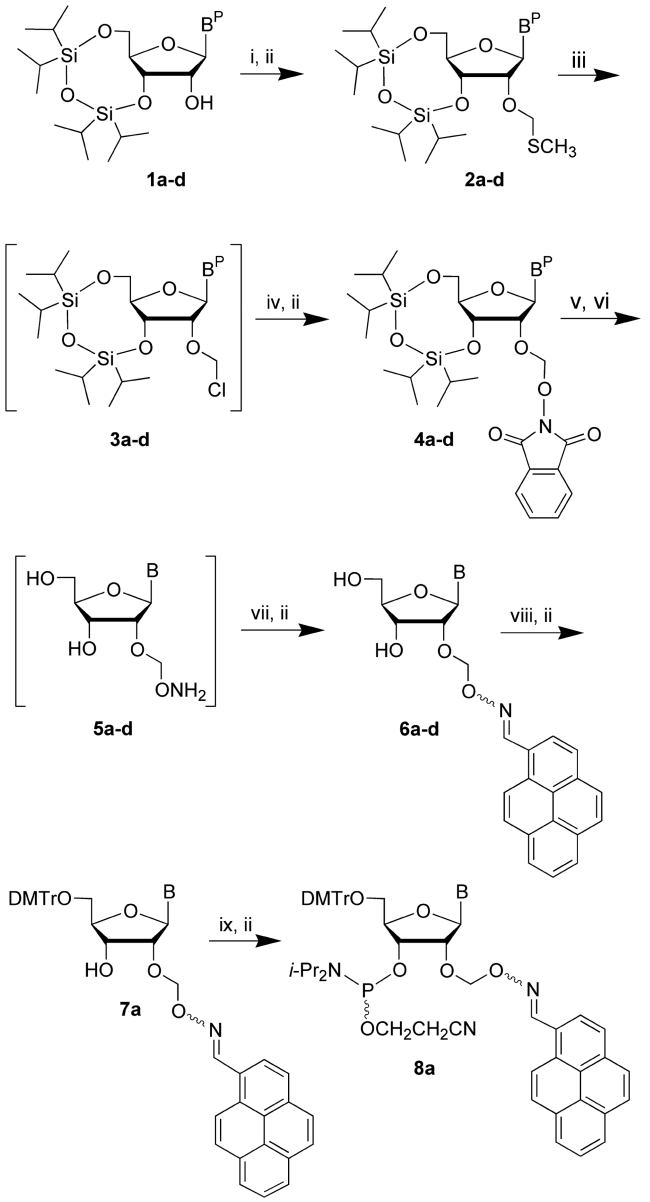
Although the reversibility of the oxyamino-aldehyde coupling reaction has, to the best of our knowledge, never been demonstrated, we rationalized that the conjugation of 2′-*O*-aminooxymethyl ribonucleosides with various functional groups may provide a powerful tool for the preparation and incorporation of permanent or reversible ribonucleoside 2′-conjugates into RNA sequences. Furthermore, reversible ribonucleoside 2′-conjugates may especially be useful in identifying novel ribonucleoside 2′-hydroxyl protecting groups, which have historically been shown to be of critical importance in RNA synthesis (21) and may lead to an improved approach to the solid-phase synthesis of native or modified RNA sequences. Given that the preparation of 2′-*O*-aminooxymethyl ribonucleosides has not been described in the scientific literature, we are now reporting an efficient method for the synthesis of these ribonucleosides (**5a–d**, Scheme 1) and that of several permanent or reversible 2′-conjugates (Figure 1). With the objective of demonstrating the reversibility of 2′-*O*-aminooxymethyl ribonucleoside conjugates, the details of an unprecedented fluoride-mediated conversion of conjugates **6a–d**, **12**, **14**, **16** and **18** to their native ribonucleosides (Scheme 2 and Figure 2) will be discussed. Furthermore, 5′-*O*-aminooxymethyl thymidine (**25**, Scheme 3) has also been prepared for the first time and the addition of its pyrenylated conjugate **26** to the 5′-terminus of a DNA sequence serves as a relevant example for the permanent or reversible functionalization of DNA sequences at their 5′-termini. A single or a double incorporation of the 2′-*O*-pyrenylated ribonucleoside conjugate **6a** into a chemically synthesized oligoribonucleotide (21-mer) is performed to further substantiate the permanent/reversible properties of the modified RNA sequence. Moreover, the phosphoramidite derivative of the reversible uridine 2′-conjugate **29** (Scheme 4) is incorporated into a chimeric polyuridylic sequence (21-mer) in order to provide convincing evidence of the usefulness and versatility of 2′-*O*-aminooxymethyl ribonucleoside conjugates in the design and implementation of novel 2′-hydroxyl protecting groups for potential applications in the synthesis of modified or native RNA sequences.



Scheme 1. Synthesis of 2′-*O*-pyrenylated ribonucleosides derivatives. (i) DMSO, Ac_2O, AcOH, 50°C, 16 h; (ii) silica gel chromatography; (iii) SO_2Cl_2, CH_2Cl_2, 25°C, 2 h; (iv) *N*-hydroxyphthalimide, DBU, CH_2Cl_2, 25°C, 24 h; (v) NH_4F, MeOH, 25°C, 16 h; (vi) concd aq NH_3, 55°C, 1 h; (vii) 1-pyrenecarboxaldehyde, MeOH, 55°C, 1 h; (viii) DMTrCl, pyridine, 25°C, 16 h; (ix) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2, 25°C, 2 h. Abreviations. B^P: **a**, uracil-1-yl; **b**, N^4-acetylcytosin-1-yl; **c**, N^6-isobutyryladenin-9-yl; **d**, N^2-phenoxyacetylguanin-9-yl; B: **a**, uracil-1-yl; **b**, cytosin-1-yl; **c**, adenin-9-yl; **d**, guanin-9-yl; DMTr, 4,4′-dimethoxytrityl.

MATERIALS AND METHODS

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(methylthiomethyl)uridine (2a)

The preparation of **2a** was performed with minor modifications of a published procedure (22,23). To a solution of commercial 5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (**1a**, 7.3 g, 15 mmol) in DMSO (15 ml) was added glacial AcOH (23 ml) and Ac_2O (15 ml). The solution was stirred at 50°C until completion of the reaction (16 h), which was monitored by TLC ($CHCl_3$:MeOH 95:5 v/v). The solution was transferred to a 2 l Erlenmeyer flask to which was added, under vigorous stirring, a solution of K_2CO_3 (31 g) in water (200 ml). The precipitated material was isolated either by filtration or decantation and was redissolved in a minimum volume of THF (15–20 ml). The resulting solution was then poured into water (250 ml) to give the crude product as a gummy material. Most of the water was decanted; the crude product was carefully dried by consecutive coevaporation with pyridine (30 ml), toluene (3 × 30 ml) and dichloromethane (30 ml). The crude ribonucleoside **2a** was purified by chromatography on silica gel using a gradient of MeOH (0 → 3%) in CH_2Cl_2 as the eluent. Fractions containing pure **2a** were collected, evaporated to a foam under low pressure, and dissolved in dry C_6H_6 (~20 ml); the solution was

frozen and then lyophilized under high vacuum affording a white powder (7.00 g, 12.8 mmol, 85%). Characterization data obtained from ^{1}H and ^{13}C NMR analysis of **2a** are in agreement with those reported by Semenyuk *et al.* (23).

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(phthalimidooxymethyl)uridine (4a)

To a solution of thoroughly dried **2a** (1.1 g, 2.0 mmol) in anhydrous CH_2Cl_2 (20 ml) was added sulfuryl chloride (176 µl, 2.20 mmol); the solution was stirred at ~25°C for 2 h and was then concentrated under reduced pressure to give the 2′-*O*-chloromethyluridine derivative **3a** as an amorphous solid. *N*-Hydroxyphthalimide (1.3 g, 8.0 mmol) was placed into a separate reaction vessel to which was added anhydrous CH_2Cl_2 (10 ml) and DBU (1.04 ml, 7.00 mmol). After 10 min, the red solution was added to unpurified **3a**; the reaction mixture was kept stirring at ~25°C for 24 h at which point CH_2Cl_2 (80 ml) was added. The solution was vigorously mixed with aqueous 1 M acetic acid (20 ml); the aqueous layer was discarded and the organic phase was washed twice with a saturated aqueous solution of $NaHCO_3$ (20 ml). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, and evaporated to a foamy solid under reduced pressure. The crude product **4a** was purified by chromatography on silica gel using a gradient of MeOH (0 → 3%) in CH_2Cl_2 as the eluent. Fractions containing **4a** were collected and evaporated under vacuum to give a solid (1.24 g, 1.88 mmol) in a yield of 94% based on the molar amount of starting material (**2a**) that was employed. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.41 (d, J = 2.2 Hz, 1H), 7.88-7.80 (m, 4H), 7.65 (d, J = 8.2 Hz, 1H), 5.62 (dd, J = 8.2, 2.2 Hz, 1H), 5.39 (d, J = 7.2 Hz, 1H), 5.34 (d, J = 7.2 Hz, 1H), 4.87 (d, J = 5.2 Hz, 1H), 4.63 (dd, J = 5.2, 5.2 Hz, 1H), 4.03 (dd, J = 13.0, 3.0 Hz, 1 H), 3.90 (dd, J = 13.0, 3.0 Hz, 1H), 3.79 (dt, J = 9.0, 3.0 Hz, 1H), 1.07–0.95 (m, 28 H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.2, 163.0, 150.0, 142.6, 134.8, 128.4, 123.3, 101.3, 98.0, 90.6, 80.2, 77.9, 69.8, 60.2, 17.2, 17.1, 17.0, 16.81, 16.77, 16.7, 12.5, 12.3, 12.1, 12.0. +ESI-HRMS: Calcd for $C_{30}H_{43}N_3O_{10}Si_2$ $[M+H]^+$ 662.2560, found 662.2560.

2′-*O*-(Aminooxymethyl)uridine (5a)

Purified **4a** (330 mg, 500 µmol) was dissolved in methanol (3 ml), and ammonium fluoride (185 mg, 5.00 mmol) was added. The heterogenous reaction mixture was stirred at ~25°C until desilylation and dephthalimidation were complete (16 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. The reaction product was purified by silica gel chromatography using a gradient of MeOH (0 → 12%) in CH_2Cl_2 as the eluent. Fractions containing the product were collected and evaporated to dryness under low pressure to provide **5a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.4 (br s, 1H), 7.93 (d, J = 8.1 Hz, 1H), 6.21 (br s, 2H), 5.87 (d, J = 4.4 Hz, 1H), 5.64 (d, J = 8.1 Hz, 1H), 5.17 (t, J = 4.9 Hz, 1H), 4.74 (s, 2H), 4.11 (m, 2H), 3.88 (m, 1H), 3.65 (ddd, J = 12.0, 5.0, 3.1 Hz, 1H), 3.56 (ddd, J = 12.0, 5.0, 3.1 Hz, 1H), 3.16 (d, J = 5.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.1, 150.7, 140.4, 101.8, 98.0, 86.7, 84.9, 79.1, 69.0, 60.4. +ESI-HRMS: Calcd for $C_{10}H_{15}N_3O_7$ $[M+H]^+$290.0983, found 290.0986.

2′-*O*-(Pyren-1-ylmethanimine-*N*-oxymethyl)uridine (6a)

2′-*O*-(Aminooxymethyl)uridine (**5a**) was prepared from **4a** at the scale and under conditions identical to those described above. After complete NH_4F-mediated desilylation and dephthalimidation, 1-pyrenecarboxaldehyde (460 mg, 2.00 mmol) was added to the reaction mixture, which was then heated at 55°C in a 4-ml screw-cap glass vial until completion of the oximation reaction (1 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. The reaction mixture was transferred to a 20-ml screw-cap glass vial to which was added CH_2Cl_2 (7 ml) and a saturated aqueous solution of $NaHCO_3$ (2 ml); after vigorous shaking the organic phase was collected and evaporated to dryness under vacuum. The pyrenylated ribonucleoside **6a** was purified by chromatography on silica gel employing a gradient of MeOH (0 → 8%) in CH_2Cl_2 as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure affording **6a** as a yellowish powder (206 mg, 410 µmol) in a yield of 82% based on the molar amount of starting material (**4a**) that was used. +ESI-HRMS: Calcd for $C_{27}H_{23}N_3O_7$ $[M+H]^+$ 502.1609, found 502.1609.

5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)uridine (7a)

To a solution of dry **6a** (200 mg, 400 µmol) in anhydrous pyridine (1 ml) was added 4,4'-dimethoxytrityl chloride. The solution was allowed to stir for 16 h at ~25°C and was then evaporated to a gum under reduced pressure. The material was dissolved in $CHCl_3$ (10 ml) and was washed with a saturated aqueous solution of $NaHCO_3$ (3 ml). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and evaporated to a solid under low pressure. The crude product **7a** was purified by chromatography on silica gel using a gradient of MeOH (0 → 2%) in CH_2Cl_2 containing 0.2% Et_3N as the eluent. Fractions containing **7a** were collected and evaporated under vacuum to give a solid (293 mg, 364 µmol, 91%).

5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-[(*N,N*-diisopropylamino) (2-cyanoethyl)]phosphinyl-2′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)uridine (8a)

To a solution of **7a** (250 mg, 311 µmol) in anhydrous CH_2Cl_2 (3 ml) containing Et_3N (167 µl, 1.20 mmol) was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (140 µl, 622 µmol). The reaction mixture was stirred at ~25°C under argon until complete disappearance of **7a** was observed (2 h) by TLC [C_6H_6:Et_3N (9:1 v/v)]. The reaction mixture was then poured into water (3 ml) and was extracted with CH_2Cl_2 (10 ml). The organic layer was dried over anhydrous Na_2SO_4 and then filtered. The filtrate was evaporated to dryness under reduced pressure. The crude phosphoramidite product was purified by chromatography on silica gel using C_6H_6:Et_3N (9:1 v/v) as the eluent. Fractions containing the pure product were pooled

together and evaporated to dryness under vacuum. The material was dissolved in dry C_6H_6 (3 ml) and the resulting solution was added to cold (−78°C) stirred hexane (100 ml). The pure ribonucleoside phosphoramidite precipitated immediately as a yellow solid. After careful decantation of hexane, the solid was dissolved in dry C_6H_6 (3 ml); the solution was frozen and then lyophilized under high vacuum. Et_3N-free **8a** was isolated as a yellowish powder (275 mg, 0.27 mmol, 88%). ^{31}P NMR (121 MHz, C_6D_6): δ 151.9, 150.2. +ESI-HRMS: Calcd for $C_{57}H_{58}N_5O_{10}P$ $(M+Na)^+$ 1026.3813, found 1026.3796.

N^4-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(methylthiomethyl)cytidine (2b)

The preparation of **2b** was performed with minor modifications of a published procedure (22,23). To a solution of commercial N^4-acetyl-5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine (**1b**, 7.9 g, 15 mmol) in DMSO (15 ml) was added glacial AcOH (15 ml) and Ac_2O (10 ml). The solution was stirred at 50°C until completion of the reaction (16 h), which was monitored by TLC [$CHCl_3$:MeOH (95:5 v/v)]. The solution was transferred to a 2 l Erlenmeyer flask to which was added, under vigorous stirring, a solution of K_2CO_3 (31.2 g) in water (240 ml). The precipitated material was worked-up, purified and processed under conditions identical to those employed in the preparation of **2a**. The ribonucleoside **2b** was isolated as a white solid (8.30 g, 14.1 mmol, 94%). ^1H NMR (300 MHz, DMSO-d_6): δ 11.08 (s, 1H), 8.19 (d, J = 7.5 Hz, 1H), 7.35 (d, J = 7.5 Hz, 1H), 5.77 (s, 1H), 5.14 (d, J = 11.2 Hz, 1H), 5.04 (d, J = 11.2 Hz, 1H), 4.44 (d, J = 4.4 Hz, 1H), 4.22 (m, 3H), 3.93 (dd, J = 13.6, 1.9 Hz, 1H), 2.16 (s, 3H), 2.14 (s, 3H), 1.08-0.95 (m, 28H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.9, 162.6, 154.1, 143.1, 95.0, 88.9, 81.3, 77.0, 73.0, 67.0, 59.2, 24.2, 17.1, 17.0, 16.9, 16.8, 16.7, 16.5, 12.6, 12.4, 12.3, 12.2, 11.8. +ESI-HRMS: Calcd for $C_{25}H_{45}N_3O_7SSi_2$ $[M+H]^+$ 580.2590, found 580.2597.

N^4-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(phthalimidooxymethyl) cytidine (4b)

The preparation and purification of **4b** were performed at a scale and under conditions identical to those described above for the preparation of **4a**. The ribonucleoside **4b** was obtained as a solid (1.04 g, 1.48 mmol) in a yield of 74% based on the molar amount of starting material (**2b**) that was utilized. ^1H NMR (300 MHz, DMSO-d_6): δ 10.98 (s, 1H), 8.10 (d, J = 7.5 Hz, 1H), 7.87–7.80 (m, 4H), 7.22 (d, J = 7.5 Hz, 1H), 5.76 (s, 1H), 5.52 (d, J = 7.0 Hz, 1H), 5.45 (d, J = 7.0 Hz, 1H), 4.70 (d, J = 4.8 Hz, 1H), 4.41 (dd, J = 4.8, 4.8 Hz, 1H), 4.15 (dd, J = 12.9, 1.2 Hz, 1H), 4.00–3.88 (m, 2H), 2.10 (s, 3H), 1.04–0.89 (m, 28H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.0, 162.9, 162.7, 154.0, 145.2, 134.7, 134.2, 128.5, 123.2, 122.9, 98.1, 95.0, 90.1, 80.8, 79.3, 68.3, 59.7, 24.3, 17.2, 17.12, 17.09, 17.0, 16.8, 16.7, 16.6, 12.5, 12.3, 12.2, 11.9. +ESI-HRMS: Calcd for $C_{32}H_{46}N_4O_{10}Si_2$ $[M+H]^+$ 703.2825, found 703.2825.

2′-*O*-(Aminooxymethyl)cytidine (5b)

Silica gel-purified **4b** (351 mg, 500 μmol) was dissolved in methanol (3 ml), and ammonium fluoride (185 mg, 5.00 mmol) was added. The heterogenous reaction mixture was stirred at ∼25°C until desilylation and dephthalimidation were complete (16 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. A stream of air was used to remove MeOH from the reaction mixture and was followed by the addition of commercial concentrated aqueous NH_3 (3 ml); the resulting solution was kept at 55°C for 1 h in a tightly closed 4-ml screw-cap glass vial. Excess ammonia was removed under a stream of air; the material left was purified by silica gel chromatography using a gradient of MeOH (0 → 25%) in CH_2Cl_2 as the eluent. Fractions containing the product were collected and evaporated to dryness under low pressure to give **5b**. ^1H NMR (300 MHz, DMSO-d_6): δ 7.92 (d, J = 7.4 Hz, 1H), 7.26 (m, 2H), 5.83 (d, J = 3.2 Hz, 1H), 5.74 (d, J = 7.4 Hz, 1H), 5.15 (br s, 1H), 4.77 (q, J = 7.2 Hz, 2H), 4.04 (m, 2H), 3.84 (m, 2H), 3.70 (dd, J = 12.2, 2.2 Hz, 1H), 3.70 (dd, J = 12.2, 2.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.6, 155.3, 140.9, 97.7, 94.0, 88.2, 83.9, 79.3, 68.3, 59.9. +ESI-HRMS: Calcd for $C_{10}H_{16}N_4O_6$ $[M+H]^+$ 289.1143, found 289.1145.

2′-*O*-(Pyren-1-ylmethanimine-*N*-oxymethyl)cytidine (6b)

2′-*O*-(Aminooxymethyl)cytidine (**5b**) was prepared from **4b** at a scale and under conditions identical to those described above. After removal of excess ammonia, the material left was suspended in MeOH (3 ml), reacted with 1-pyrenecarboxaldehyde and processed under conditions identical to those employed for the preparation of **6a**. The pyrenylated ribonucleoside was purified on silica gel using chromatographic conditions identical to those used for the purification of **6a** affording **6b** as a yellow powder (188 mg, 375 μmol) in a yield of 75 % based on the molar amount of starting material (**4b**) that was utilized. +ESI-HRMS: Calcd for $C_{27}H_{24}N_4O_6$ $[M+H]^+$ 501.1769, found 501.1769.

N^6-Isobutyryl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(methylthiomethyl) adenosine (2c)

The preparation of **2c** was performed with minor modifications of a published procedure (22,23). To a solution of commercial N^6-isobutyryl-5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**1c**, 8.7 g, 15 mmol) in DMSO (23 ml) was added glacial AcOH (23 ml) and Ac_2O (15 ml). The solution was stirred at 50°C until completion of the reaction (16 h), which was monitored by TLC [$CHCl_3$:MeOH (95:5 v/v)]. The solution was transferred to a 2 l Erlenmeyer flask to which was added, under vigorous stirring, a solution of K_2CO_3 (46.2 g) in water (230 ml). The precipitated material was worked-up and purified under conditions identical to those employed in the preparation of **2a**. The ribonucleoside **2c** was isolated as a white solid (8.5 g, 13.3 mmol, 89%). ^1H NMR (300 MHz, DMSO-d_6): δ 10.69 (s, 1H), 8.56 (s, 1H), 8.49 (s, 1H), 6.11 (d, J = 1 Hz, 1H), 5.00 (dd, J = 5.3, 5.2 Hz, 1H), 4.97 (d, J = 11.4 Hz, 1H), 4.91 (d, J = 11.4 Hz, 1H),

4.89 (d, $J = 4.8$ Hz, 1H), 4.08 (dd, $J = 12.9$, 2.5 Hz, 1H), 4.02 (dt, $J = 9.0$, 2.5, Hz, 1H), 3.93 (dd, $J = 12.9$, 2.3 Hz, 1H), 2.96 (sept, $J = 6.7$ Hz, 1H), 2.08 (s, 3H), 1.12 (d, $J = 6.7$ Hz, 6H), 1.09-0.97 (m, 28H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.2, 151.4, 150.8, 149.9, 142.6, 124.1, 87.6, 80.8, 76.7, 73.7, 69.3, 59.9, 34.2, 19.1, 17.2, 17.1, 17.0, 16.9, 16.8, 16.7, 12.7, 12.6, 12.3, 12.1, 11.9. +ESI-HRMS: Calcd for $C_{28}H_{49}N_5O_6SSi_2$ $[M+H]^+$ 640.3015, found 640.3016.

N^6-Isobutyryl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(phthalimidooxymethyl) adenosine (4c)

The preparation and purification of **4c** were performed at a scale and under conditions identical to those described above for the preparation of **4a**. The ribonucleoside **4c** was obtained as a solid (1.24 g, 1.64 mmol) in a yield of 82% based on the molar amount of the starting material (**2c**) that was used. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.72 (s, 1H), 8.59 (s, 1H), 8.45 (s, 1H), 7.84-7.74 (m, 4H), 6.06 (d, $J = 1.1$ Hz, 1H), 5.45 (d, $J = 7.5$ Hz, 1H), 5.37-5.30 (m, 3H), 4.04–3.89 (m, 3H), 2.97 (sept, $J = 6.8$ Hz, 1H), 1.15 (d, $J = 6.8$ Hz, 3H), 1.14 (d, $J = 6.8$ Hz, 3H), 1.04–0.96 (m, 28H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 175.2, 163.0, 151.2, 150.9, 149.9, 144.3, 134.8, 134.2, 128.3, 124.3, 123.2, 122.9, 98.4, 87.7, 80.2, 77.8, 70.3, 59.9, 34.3, 19.2, 19.1, 17.1, 17.0, 16.9, 16.8, 16.7, 12.6, 12.3, 12.1. +ESI-HRMS: Calcd for $C_{35}H_{50}N_6O_9Si_2$ $[M+H]^+$ 755.3251, found 755.3250.

2′-*O*-(Aminooxymethyl)adenosine (5c)

The preparation of **5c** from **4c** was performed at a scale and under conditions identical to those used for the preparation of **5b**. After removal of excess ammonia under a stream of air, 2′-*O*-(aminooxymethyl)adenosine was purified by silica gel chromatography employing a gradient of MeOH (0 → 10%) in CH_2Cl_2 as the eluent. Fractions containing the product were collected and evaporated to dryness under low pressure affording **5c**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.38 (s, 1H), 8.13 (s, 1H), 7.37 (br s, 2H), 6.21 (br s, 2H), 6.06 (d, $J = 6.0$ Hz, 1H), 5.43 (m, 2H), 4.68 (q, $J = 7.3$ Hz, 2H), 4.67 (m, 1H), 4.33 (br s, 1H), 3.99 (q, $J = 3.4$ Hz, 1H), 3.68 (m, 1H), 3.57 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.1, 152.4, 148.9, 139.7, 119.2, 98.2, 86.3, 86.1, 79.3, 69.8, 61.4. +ESI-HRMS: Calcd for $C_{11}H_{16}N_6O_5$ $[M+H]^+$ 313.1255, found 313.1256.

2′-*O*-(Pyren-1-ylmethanimine-*N*-oxymethyl)adenosine (6c)

The preparation of **5c** from **4c** was performed at a scale and under conditions identical to those described above. After removal of excess ammonia, the material left was suspended in MeOH (3 ml), reacted with 1-pyrenecarboxaldehyde and processed under conditions identical to those employed for the preparation of **6a**. The pyrenylated ribonucleoside was purified on silica gel using chromatographic conditions identical to those used for the purification of **6a** affording **6c** in a yield of 77% (200 mg, 385 μmol) based on the molar amount of starting material (**4c**) that was employed. +ESI-HRMS: Calcd for $C_{28}H_{24}N_6O_5$ $[M+H]^+$ 525.1881, found 525.1882.

N^2-Phenoxyacetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(methylthiomethyl) guanosine (2d)

The preparation of **2d** was performed with minor modifications of a published procedure (22,23). To a solution of commercial N^2-phenoxyacetyl-5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine (**1d**, 9.9 g, 15 mmol) in DMSO (22.5 ml) was added glacial AcOH (22.5 ml) and Ac_2O (15.0 ml). The solution was stirred at 50°C until completion of the reaction (~16 h), which was monitored by TLC [$CHCl_3$:MeOH (95:5 v/v)]. The solution was transferred to a 2 l Erlenmeyer flask to which was added, under vigorous stirring, a solution of K_2CO_3 (51.0 g) in water (270 ml). The precipitated material was worked-up and purified under conditions identical to those employed in the preparation of **2a**. The ribonucleoside **2d** was isolated as a white solid (9.3 g, 13 mmol, 87 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.84 (br s, 1H), 11.83 (br s, 1H), 8.05 (s, 1H), 7.32 (d, $J = 7.6$ Hz, 1H), 7.30 (d, $J = 8.4$ Hz, 1H), 6.98 (m, 3H), 5.91 (d, $J = 1.1$ Hz, 1H), 4.95 (s, 2H), 4.84 (s, 2H), 4.52 (m, 2H), 4.16 (dd, $J = 12.9$, 2.5 Hz, 1H), 4.06 (dt, $J = 8.2$, 2.5 Hz, 1H), 3.95 (dd, $J = 12.9$, 2.5 Hz, 1H), 2.08 (s, 3H), 1.06–0.95 (m, 28H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.7, 157.5, 154.8, 147.6, 147.3, 136.1, 129.4, 121.3, 120.5, 114.5, 86.5, 81.3, 77.8, 73.8, 68.8, 66.2, 60.0, 17.2, 17.16, 17.13, 17.1, 17.05, 17.03, 16.8, 16.74, 16.70, 12.8, 12.7, 12.6, 12.3, 12.2, 11.9. +ESI-HRMS: Calcd for $C_{32}H_{49}N_5O_8SSi_2$ $[M+H]^+$ 720.2913, found 720.2918.

N^2-Phenoxyacetyl-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2'-*O*-(phthalimidooxymethyl)guanosine (4d)

The preparation and purification of **4d** were performed at a scale and under conditions identical to those described above for the preparation of **4a**. The ribonucleoside **4d** was obtained as a solid (1.10 g, 1.32 mmol) in a yield of 66% based on the molar amount of starting material (**2d**) that was employed. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.81 (br s, 1H), 11.55 (br s, 1H), 8.15 (s, 1H), 7.85–7.70 (m, 4H), 7.34–7.28 (m, 3H), 6.99–6.94 (m, 3H), 5.94 (d, $J = 1.3$ Hz, 1H), 5.48 (d, $J = 7.0$ Hz, 1H), 5.39 (d, $J = 7.0$ Hz, 1H), 5.48 (dd, $J = 5.2$, 1.2 Hz, 1H), 4.75 (m, 2H), 4.64 (m, 1H), 4.07–3.89 (m, 3H), 1.07–0.95 (m, 28H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.5, 162.9, 157.5, 154.9, 148.1, 147.2, 137.2, 134.8, 129.5, 128.3, 123.2, 121.3, 114.5, 98.4, 86.0, 80.7, 78.6, 70.2, 66.2, 60.1, 17.2, 17.1, 16.9, 16.8, 12.8, 12.6, 12.3, 12.05, 12.02. +ESI-HRMS: Calcd for $C_{39}H_{50}N_6O_{11}Si_2$ $[M+H]^+$ 835.3149, found 835.3148.

2′-*O*-(Aminooxymethyl)guanosine (5d)

The preparation of **5d** from **4d** was performed under conditions identical to those used for the preparation of **5b**. After removal of excess ammonia under a stream of air, 2′-*O*-(aminooxymethyl)guanosine was purified by silica gel chromatography using a gradient of MeOH (0 → 25%) in CH_2Cl_2 as the eluent. Fractions containing the product were collected and evaporated to dryness under reduced

pressure providing **5d**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.77 (br s, 1H), 7.96 (s, 1H), 6.59 (br s, 2H), 5.85 (d, J = 6.0 Hz, 1H), 5.12 (t, J = 5.2 Hz, 1H), 4.72 (m, 2H), 4.46 (dd, J = 6.0, 5.7 Hz, 1H), 4.26 (dd, J = 4.8, 4.8 Hz, 1H), 3.91 (q, J = 3.8 Hz, 1H), 3.61 (dt, J = 11.8, 4.4 Hz, 1H), 3.52 (dt, J = 11.8, 4.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.6, 153.8, 151.1, 135.3, 116.5, 98.0, 85.6, 84.8, 79.6, 69.6, 61.1. +ESI-HRMS: Calcd for $C_{11}H_{16}N_6O_6$ $[M+H]^+$ 329.1204, found 329.1211.

2′-*O*-(Pyren-1-ylmethanimine-*N*-oxymethyl)guanosine (6d)

The preparation of **5d** from **4d** was performed at a scale and under conditions identical to those described above. After removal of excess ammonia, the material left was suspended in MeOH (3 ml), reacted with 1-pyrenecarboxaldehyde and processed under conditions identical to those employed for the preparation of **6a**. The pyrenylated ribonucleoside was purified on silica gel using chromatographic conditions identical to those used for the purification of **6a** affording **6d** in a yield of 69% (187 mg, 345 μmol) based on the molar amount of starting material (**4d**) that was utilized. +ESI-HRMS: Calcd for $C_{28}H_{24}N_6O_6$ $[M+H]^+$ 541.1830, found 541.1829.

2′-*O*-(5-Cholesten-3-imine-*N*-oxymethyl)uridine (10)

2′-*O*-(Aminooxymethyl)uridine (**5a**) was prepared as described above from silica gel-purified **4a** (132 mg, 200 μmol). After complete NH_4F-mediated desilylation and dephthalimidation of **4a**, 5-cholesten-3-one (**9**, 154 mg, 400 μmol) was added to the reaction mixture, which was then processed under conditions identical to those described in the preparation of **6a**. The reaction product was purified by chromatography on silica gel using a gradient of MeOH (0 → 4%) in CH_2Cl_2 as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure giving **10** as a white powder (90 mg, 0.14 mmol) in a yield of 69% based on the molar amount of starting material (**4a**) that was used. +ESI-HRMS: Calcd for $C_{37}H_{57}N_3O_7$ $[M+H]^+$ 656.4269, found 656.4269. The normal phase HPLC profile of **10** is shown in Supplementary Figure S1.

N-(2,2-Dimethoxyethyl)biotinamide (11)

To a suspension of D-(+)-biotin 2-nitrophenyl ester (365 mg, 1.00 mmol) in MeCN (20 ml) was added aminoacetaldehyde dimethyl acetal (130 μl, 1.20 mmol) and Et_3N (170 μl, 1.20 mmol). The suspension was gently heated until a solution was obtained; the solution was then stirred for 16 h at ∼25°C. The reaction mixture was evaporated to dryness under reduced pressure and the material left was purified by silica gel chromatography using a gradient of MeOH (0 → 10%) in CH_2Cl_2 as the eluent. Fractions containing the product were collected and evaporated to dryness under low pressure affording **11** as a solid (300 mg, 910 μmol, 91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87 (t, J = 5.8 Hz, 1H), 6.43 (s, 1H), 6.37 (s, 1H), 4.32 (t, J = 5.5 Hz, 1H), 4.28 (d, J = 5.5 Hz, 1H), 4.12 (ddd, J = 7.6, 4.4, 1.8 Hz, 1H), 3.25 (s, 6H), 3.13 (t, J = 5.7 Hz, 2H), 2.82 (dd, J = 12.3, 12.3 Hz, 1H), 2.56 (d, J = 12.3 Hz, 1H), 2.07 (t, J = 7.4 Hz, 2H), 1.66-1.22 (m, 7H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 172.2, 162.6, 102.0, 61.0, 59.1, 55.4, 53.1, 40.2, 39.8, 34.9, 28.1, 28.0, 25.2. +ESI-HRMS: Calcd for $C_{14}H_{25}N_3O_4S$ $[M+H]^+$ 332.1639, found 332.1641.

N-(2-Oxoethyl)biotinamide

The acetal **11** (280 mg, 850 μmol) was dissolved in MeOH (2 ml) and commercial concentrated HCl (0.5 ml) was added to the solution, which was allowed to stir for 1 h at ∼25°C. The reaction mixture was evaporated to dryness under reduced pressure to yield the aldehyde, the total amount of which was used without further purification in the preparation of **12**.

Preparation of the biotinylated uridine conjugate 12

2′-*O*-(Aminooxymethyl)uridine (**5a**) was prepared from silica gel-purified **4a** at a scale and under conditions identical to those described for the preparation of **10**. After complete NH_4F-mediated desilylation and dephthalimidation, all of the *N*-(2-oxoethyl)biotinamide produced above was dissolved in MeOH (2 ml) and added to the reaction mixture, which was heated at 55°C in a 4-ml screw-cap glass vial until completion of the oximation reaction (1 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. The reaction mixture was purified by chromatography on silica gel employing a gradient of MeOH (0 → 20%) in CH_2Cl_2 as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure providing **12** as a white powder (74 mg, 0.13 mmol) in a yield of 66% based on the molar amount of starting material (**4a**) that was utilized. +ESI-HRMS: Calcd for $C_{22}H_{32}N_6O_9S$ $[M+H]^+$ 557.2024, found 557.2024. The RP-HPLC profile of **12** is shown in Supplementary Figure S2A.

N-(2,2-Dimethoxyethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (13)

To a solution of dansyl chloride (270 mg, 1.00 mmol) in CH_2Cl_2 (10 ml) was added aminoacetaldehyde dimethyl acetal (130 μl, 1.20 mmol) and Et_3N (170 μl, 1.20 mmol); the solution was allowed to stir for 1 h at ∼25°C. The reaction mixture was then evaporated to dryness under vacuum and the material left was purified by silica gel chromatography using a gradient of MeOH (0 → 1%) in CH_2Cl_2 as the eluent. Fractions containing the product were collected and evaporated to dryness under low pressure affording **13** as a solid (318 mg, 940 μmol, 94%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.45 (dt, J = 8.5, 1.1 Hz, 1H), 8.29 (dt, J = 8.8, 0.9 Hz, 1H), 8.16 (t, J = 5.5 Hz, 1H), 8.10 (dd, J = 7.3, 1.1 Hz, 1H), 7.61 (t, J = 8.5 Hz, 1H), 7.58 (t, J = 8.5 Hz, 1H), 7.25 (dd, J = 7.6, 0.7 Hz, 1H), 4.11 (t, J = 5.5 Hz, 1H), 3.06 (s, 6H), 2.89 (t, J = 5.5 Hz, 2H), 2.81 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.2, 136.2, 129.3, 128.9, 127.9, 127.7, 123.5, 119.1, 115.0, 102.4, 53.3, 44.9, 43.9. +ESI-HRMS: Calcd for $C_{16}H_{22}N_2O_4S$ $[M+H]^+$ 339.1373, found 339.1374.

N-(2-Oxoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide

The acetal **13** (287 mg, 850 μmol) was dissolved in MeOH (1 ml) and concentrated HCl (0.5 ml) was added to the solution, which was stirred for 1 h at ~25°C. The reaction mixture was then evaporated to dryness under reduced pressure; the material left was dissolved in CH_2Cl_2 (10 ml) and the solution was washed with $NaHCO_3$ (2 ml of a saturated aqueous solution). The organic layer was collected and was evaporated under low pressure to give the aldehyde as a pale green foam, the total amount of which was used without further purification in the preparation of **14**.

Preparation of the dansylated uridine conjugate 14

2′-*O*-(Aminooxymethyl)uridine (**5a**) was prepared from silica gel-purified **4a** at a scale and under conditions identical to those described for the preparation of **10**. After complete NH_4F-mediated desilylation and dephthalimidation, all of the *N*-(2-oxoethyl)-5-(dimethylamino) naphthalene-1-sulfonamide generated above was dissolved in MeOH (1 ml) and added to the reaction mixture, which was heated at 55°C in a 4-ml screw-cap glass vial until completion of the oximation reaction (1 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. The reaction mixture was then worked-up and processed exactly as described in the preparation of **10**. The product was purified by chromatography on silica gel employing a gradient of MeOH (0 → 6%) in CH_2Cl_2 as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure to give **14** as a pale green powder (82 mg, 0.14 mmol) in a yield of 70 % based on the molar amount of starting material (**4a**) that was employed. +ESI-HRMS: Calcd for $C_{24}H_{29}N_5O_9S$ $[M+H]^+$ 564.1759, found 564.1759. The RP-HPLC profile of **14** is shown in Supplementary Figure S3A.

N-(4,4-Diethoxybutyl)-5-(dimethylamino)naphthalene-1-sulfonamide (15)

To a solution of dansyl chloride (270 mg, 1.00 mmol) in CH_2Cl_2 (10 ml) was added 4-aminobutyraldehyde diethyl acetal (237 μl, 1.20 mmol) and Et_3N (170 μl, 1.20 mmol). The solution was stirred for 1 h at ~25°C and was then evaporated to dryness under low pressure. The material left was processed and purified under conditions identical to those described for the processing and purification of **13**. Fractions containing the product were collected and evaporated to dryness under low pressure affording **15** as a solid (366 mg, 930 μmol, 93%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.45 (dt, J = 8.5, 1.1 Hz, 1H), 8.30 (dt, J = 8.8, 0.9 Hz, 1H), 8.09 (dd, J = 7.3, 1.2 Hz, 1H), 7.89 (t, J = 5.5 Hz, 1H), 7.61 (t, J = 8.5 Hz, 1H), 7.58 (t, J = 8.5 Hz, 1H), 7.25 (dd, J = 7.6, 0.7 Hz, 1H), 4.19 (t, J = 5.3 Hz, 1H), 3.36 (m, 2H), 3.23 (m, 2H), 2.81 (s, 6H), 2.77 (t, J = 5.5 Hz, 2H), 1.31 (m, 4H), 0.99 (t, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.2, 136.1, 129.2, 128.9, 128.1, 127.7, 123.5, 119.1, 114.9, 101.6, 60.2, 44.9, 42.2, 30.1, 24.4, 15.1. +ESI-HRMS: Calcd for $C_{20}H_{30}N_2O_4S$ $[M+H]^+$ 395.1999, found 395.2000.

N-(4-Oxobutyl)-5-(dimethylamino)naphthalene-1-sulfonamide

This aldehyde was prepared from acetal **15** at a scale and under conditions identical to those employed for the preparation *N*-(2-oxoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide from acetal **13**. *N*-(4-Oxobutyl)-5-(dimethylamino)naphthalene-1-sulfonamide was obtained as a pale green foam, the total amount of which was used without further purification in the preparation of **16**.

Preparation of the dansylated uridine conjugate 16

This conjugate was prepared and purified exactly as reported for the preparation and purification of the dansylated uridine conjugate **14**. The dansylated uridine conjugate **16** was isolated as a light green powder (96 mg, 0.16 mmol) in a yield of 81 % based on the molar amount of starting material (**4a**) that was used. +ESI-HRMS: Calcd for $C_{26}H_{33}N_5O_9S$ $[M+H]^+$ 592.2072, found 592.2071. The RP-HPLC profile of **16** is shown in Supplementary Figure S4A.

N-(2,2-Dimethoxyethyl)-4-(dimethylamino)azobenzene-4′-sulfonamide (17)

To a solution of 4-(dimethylamino)azobenzene-4′-sulfonyl chloride (324 mg, 1.00 mmol) in CH_2Cl_2 (5 ml) was added aminoacetaldehyde dimethyl acetal (130 μl, 1.20 mmol) and Et_3N (179 μl, 1.20 mmol). The solution was allowed to stir for 16 h at ~25°C. The reaction mixture was evaporated to dryness under reduced pressure and the material left was purified by silica gel chromatography using a gradient of MeOH (0 → 2%) in CH_2Cl_2 as the eluent. Fractions containing the product were collected and evaporated to dryness under reduced pressure affording **17** as a solid (373 mg, 950 μmol, 95%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.82 (d, J = 9.3 Hz, 2H), 7.19 (m, 4H), 6.85 (d, J = 9.3 Hz, 2H), 4.29 (t, J = 5.4 Hz, 1H), 3.19 (s, 6H), 3.08 (s, 6H), 2.89 (t, J = 5.6 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.4, 153.0, 142.5, 140.5, 127.7, 125.3, 122.1, 111.5, 102.3, 53.3, 44.1, 39.8. +ESI-HRMS: Calcd for $C_{18}H_{24}N_4O_4S$ $[M+H]^+$ 393·1591, found 393.1596.

N-(2-Oxoethyl)-4-(dimethylamino)azobenzene-4′-sulfonamide

The acetal **17** (287 mg, 850 μmol) was dissolved in a solution of 10% (w/v) I_2 in acetone (10 ml) (24). The resulting solution was stirred at ~25°C for 16 h and was then evaporated to dryness under reduced pressure. The material left was dissolved in CH_2Cl_2 (10 ml) and washed with an aqueous solution of 5% (w/v) sodium bisulfite (5 ml) followed by a saturated aqueous solution of $NaHCO_3$ (5 ml). The organic layer was collected and was evaporated to dryness under vacuum. The total amount of the orange product was used in the preparation of **18**.

The dabsylated cytidine conjugate 18

2′-*O*-(Aminooxymethyl)cytidine (**5b**) was prepared from silica gel-purified **4b** (140 mg, 0.2 mmol) as described above. After removal of excess ammonia, the material left was suspended in MeOH (3 ml) and all of the *N*-(2-oxoethyl)-4-(dimethylamino)azobenzene-4′-sulfonamide produced above was suspended in MeOH (1 ml) and added to the reaction mixture, which was heated at 55°C in a 4-ml screw-cap glass vial until completion of the oximation reaction (1 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. The reaction mixture was then worked-up and processed exactly as described in the preparation of **10**. The product was purified by chromatography on silica gel employing a gradient of MeOH (0 → 8%) in CH_2Cl_2 as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure providing **18** as an orange powder (74 mg, 0.12 mmol) in a yield of 61% based on the molar amount of starting material (**4b**) that was employed. +ESI-HRMS: Calcd for $C_{26}H_{32}N_8O_8S$ $[M+H]^+$ 617.2137, found 617.2134. The RP-HPLC profile of **18** is shown in Supplementary Figure S5A.

N-(4-Cyanobut-1-yl)-5-(dimethylamino)naphthalene-1-sulfonamide (20)

4-Aminobutyronitrile was prepared from the reaction of 4-chlorobutyronitrile (207 mg, 2.00 mmol) with potassium phthalimide (407 mg, 2.20 mmol) under the conditions described by McKay *et al*. (25) with the following modification: the crude 4-aminobutyronitrile, instead of purified 4-aminobutyronitrile hydrochloride, was reacted with a stirred solution of dansyl chloride (135 mg, 500 µmol) in CH_2Cl_2 (1 ml) for 10 min at ~25°C. The reaction product was analyzed by TLC [$CHCl_3$:MeOH (95:5 v/v)] and was purified by chromatography on silica gel using a gradient of MeOH (0 → 3%) in CH_2Cl_2 as the eluent. Fractions containing **20** were collected and evaporated under vacuum affording the pure product (123 mg, 390 µmol) in a yield of 78% based on the molar amount of dansyl chloride used in the reaction. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.47 (dt, J = 8.5, 1.1 Hz, 1H), 8.28 (dt, J = 8.5, 1.1 Hz, 1H), 8.11 (dd, J = 7.2, 1.2 Hz, 1H), 8.02 (t, J = 5.8 Hz, 1H), 7.63 (t, J = 7.5 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.26 (dd, J = 7.5, 0.7 Hz, 1H), 2.85 (m, 2H), 2.82 (s, 6H), 2.39 (t, J = 7.0 Hz, 2H), 1.60 (quint, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.3, 135.5, 129.5, 129.0, 128.9, 128.3, 127.8, 123.5, 119.9, 118.8, 115.1, 44.9, 40.9, 25.2, 13.4. +ESI-HRMS: Calcd for $C_{16}H_{19}N_3O_2S$ $[M+H]^+$ 318.1271, found 318.1271.

2′-*O*-[5-(Dimethylamino)naphthalene-1-sulfonamidyl-*N*-oxymethyl]uridine (22)

Silica gel-purified 2′-*O*-(aminooxymethyl)uridine (**5a**, 87 mg, 0.30 mmol) was dissolved in pyridine (2 ml) and dansyl chloride (135 mg, 500 µmol) was added. The solution was stirred at ~25°C for 2 h and was then evaporated to dryness under vacuum. The crude product **22** was purified by silica gel chromatography using a gradient of MeOH (0 → 8%) in CH_2Cl_2 as the eluent. Fractions containing **22** were collected and evaporated under vacuum giving a yellow solid (130 mg, 250 µmol, 83%). +ESI-HRMS: Calcd for $C_{22}H_{26}N_4O_9S$ $[M+H]^+$ 523.1493, found 523.1493. The RP-HPLC profile of **22** is shown in Supplementary Figure S6A.

3′-*O*-(Levulinyl)-5′-*O*-(methylthiomethyl)-2′-deoxythymidine (23)

To a solution of commercial 3′-*O*-(levulinyl)-2′-deoxythymidine (3.0 g, 8.8 mmol) in DMSO (9 ml) was added glacial AcOH (13 ml) and Ac_2O (9 ml). The solution was stirred at ~50°C until completion of the reaction (16 h), which was monitored by TLC [$CHCl_3$:MeOH (95:5 v/v)]. AcOH and Ac_2O were evaporated under vacuum and the remaining material was mixed with 15 g of silica gel. Residual DMSO was allowed to evaporate from the silica gel over a period of 16 h at room temperature. The silica gel mix was layered on the top of a glass column packed with silica gel. The product was eluted using a gradient of MeOH (0 → 3%) in CH_2Cl_2 as the eluent. Fractions containing pure **23** were collected, evaporated to a foam under low pressure, and dissolved in dry C_6H_6 (~20 ml); the solution was frozen and then lyophilized under high vacuum affording a white powder (2.8 g, 7.0 mmol, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ. 11.33 (s, 1H), 7.55 (s, 1H), 6.19 (t, J = 6.2 Hz, 1H), 5.18 (d, J = 5.6 Hz, 1H), 4.74 (s, 2H), 4.11 (s, 1H), 3.69 (s, 2H), 3.31 (s, 1H), 2.74 (t, J = 6.2 Hz, 2H), 2.50 (t, J = 6.2 Hz, 2H), 2.27 (m, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 206.7, 171.9, 163.5, 150.3, 135.4, 109.8, 83.7, 82.2, 74.8, 74.7, 67.6, 37.3, 35.9, 29.4, 27.6, 13.4, 12.6. +ESI-HRMS: Calcd for $C_{17}H_{24}N_2O_7S$ $[M+H]^+$ 401.1377, found 401.1379.

3′-*O*-(Levulinyl)-5′-*O*-(phthalimidooxymethyl)-2′-deoxythymidine (24)

To a solution of thoroughly dried **23** (2.8 g, 7.0 mmol) in anhydrous CH_2Cl_2 (70 ml) was added neat sulfuryl chloride (626 µl, 7.73 mmol); the solution was stirred at ~25°C for 2 h and was then concentrated under reduced pressure to give 5′-*O*-chloromethyl-3′-*O*-levulinyl-2′-*O*-deoxythymidine as an amorphous solid. *N*-Hydroxyphthalimide (4.58 g, 28.1 mmol) was placed into a separate reaction vessel to which was added anhydrous CH_2Cl_2 (35 ml) and DBU (3.60 ml, 24.6 mmol). After 10 min, the red solution was added to unpurified 5′-*O*-chloromethyl-3′-*O*-levulinyl-2′-*O*-deoxythymidine; the reaction mixture was kept stirring at ~25°C for 24 h at which point CH_2Cl_2 (150 ml) was added. The solution was extracted twice with aqueous 1 M acetic acid (30 ml); the organic phase was collected, washed with a saturated aqueous solution of $NaHCO_3$ (3 × 100 ml), dried over anhydrous Na_2SO_4, filtered, and evaporated to a foamy solid under reduced pressure. The crude product was purified by chromatography on silica gel using a gradient of MeOH (0 →3%) in CH_2Cl_2 as the eluent. Fractions containing **24** were collected and evaporated under vacuum to give a solid (2.1 g, 4.1 mmol, 58%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.31 (s, 1H), 7.87 (s, 4H),

7.48 (s, 1H), 6.17 (dd, J = 6.0, 2.5 Hz, 1H), 5.24 (m, 3H), 4.13 (m, 3H), 2.75 (t, J = 6.2 Hz, 2H), 2.50 (m, 2H), 2.25 (m, 2H), 2.12 (s, 3H), 1.64 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 206.7, 171.8, 163.4, 163.1, 150.3, 135.6, 134.8, 128.4, 123.2, 109.6, 100.0, 83.7, 81.8, 74.3, 69.3, 37.3, 35.6, 29.4, 27.6, 11.9. +ESI-HRMS: Calcd for $C_{24}H_{25}N_3O_{10}$ $[M+H]^+$ 516.1613, found 516.1618.

5′-*O*-(Aminooxymethyl)-2′-deoxythymidine (25)

Under an inert atmosphere, 1 M hydrazine hydrate in pyridine:acetic acid (3:2 v/v, 7.3 ml) was added to a solution of **24** (1.2 g, 2.3 mmol) in anhydrous pyridine (11 ml). The reaction mixture was stirred for 1 h at 25°C and was then concentrated under vacuum to a volume of ~3 ml. The crude product was purified by chromatography on silica gel using a gradient of MeOH (0 →3%) in CH_2Cl_2 as the eluent. Fractions containing **25** were collected and evaporated under vacuum to give a solid (318 mg, 1.11 mmol, 48%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.27 (br s, 1H), 7.61 (s, 1H), 6.19 (dd, J = 6.5, 2.5 Hz, 1H), 5.30 (br s, 2H), 4.70 (s, 2H), 4.26 (br s, 1H), 3.90 (br s, 1H), 3.71 (m, 3H), 2.12 (m, 2H), 1.78 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.6, 150.3, 135.9, 109.4, 98.5, 85.2, 83.8, 70.6, 67.7, 39.0, 12.1. +ESI-HRMS: Calcd for $C_{11}H_{17}N_3O_6$ $[M+H]^+$ 288.1190, found 288.1196.

5′-*O*-(Pyren-1-ylmethanimine-*N*-oxymethyl)-2′-deoxythymidine (26)

A solution of **25** (300 mg, 1.05 mmol) and 1-pyrenecarboxaldehyde (1.1 g, 5.0 mmol) in MeOH (2 ml) was heated at 55°C in a 4-ml screw-cap glass vial until completion of the oximation reaction (1 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. The reaction mixture was transferred to a 20-ml screw-cap glass vial to which was added CH_2Cl_2 (7 ml) and a saturated aqueous solution of $NaHCO_3$ (2 ml); after vigorous shaking the organic phase was collected and evaporated to dryness under vacuum. The pyrenylated product was purified by chromatography on silica gel employing a gradient of MeOH (0 → 5%) in CH_2Cl_2 as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure affording **26** as a yellowish powder (400 mg, 800 µmol, 80%). +ESI-HRMS: Calcd for $C_{28}H_{25}N_3O_6$ $[M+Na]^+$ 522.1636, found 522.1642.

5′-*O*-(Pyren-1-ylmethanimine-*N*-oxymethyl)-3'-*O*-[(*N,N*-diisopropylamino) (2-cyanoethyl)]phosphinyl-2′-deoxythymidine (27)

To a solution of 5′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)-2′-deoxythymidine (**26**, 350 mg, 700 µmol) in anhydrous CH_2Cl_2 (5 ml), containing Et_3N (0.39 ml, 2.8 mmol), was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.31 ml, 1.4 mmol). The reaction mixture was stirred at ~25°C under argon until complete disappearance of **26** (2 h) was confirmed by TLC [C_6H_6:Et_3N (9:1 v/v)]. The reaction mixture was then poured into water (5 ml) and was extracted with CH_2Cl_2 (15 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The crude phosphoramidite product was purified by chromatography on silica gel using C_6H_6:Et_3N (9:1 v/v) as the eluent. Fractions containing the pure product were pooled together and evaporated to dryness under vacuum. The material was dissolved in dry C_6H_6 (4 ml) and the resulting solution was added to cold (−78°C) stirred hexane (100 ml). The pure deoxyribonucleoside phosphoramidite precipitated immediately as a yellowish solid. After careful decantation of hexane, the solid was dissolved in dry C_6H_6 (4 ml); the solution was frozen and then lyophilized under high vacuum. Et_3N-free **27** was isolated as a yellowish powder (451 mg, 640 µmol, 92%). ^{31}P NMR (121 MHz, C_6D_6): δ 148.1, 147.7. +ESI-HRMS: Calcd for $C_{37}H_{42}N_5O_7P$ $(M+H)^+$ 700.2895, found 700.2904.

2′-*O*-[2-(Methylthio)ethanimine-*N*-oxymethyl]uridine (28)

Commercial 2-methylthioacetaldehyde dimethylacetal was converted *in situ* to methylthiomethylacetaldehyde under conditions identical to those employed for the preparation of *N*-(2-oxoethyl)biotinamide with the exception of the reaction scale, which was 10-fold larger. The acidic solution of methylthioacetaldehyde in aqueous methanol was used without workup in the preparation of **28**. 2′-*O* (Aminooxymethyl)uridine (**5a**) was prepared from silica gel-purified **4a** at a scale of 2 mmol under conditions identical to those described for the preparation of **6a**. After complete NH_4F-mediated desilylation and dephthalimidation, all of the acidic methylthioacetaldehyde solution prepared above was added to the reaction mixture, which was heated at 55°C in a 4-ml screw-cap glass vial until completion of the oximation reaction (1 h) as indicated by TLC [$CHCl_3$:MeOH (9:1 v/v)]. The reaction mixture was purified by chromatography on silica gel employing a gradient of MeOH (0 → 5%) in CH_2Cl_2 as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure providing **28** as a white powder (557 mg, 1.54 mmol) in a yield of 77% based on the molar amount of starting material (**4a**) that was utilized. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.31 (br s, 1H), 7.87 (d, J = 8.1 Hz, 0.25H), 7.85 (d, J = 8.1 Hz, 0.75H), 7.34 (t, J = 6.7 Hz, 0.75H), 6.89 (t, J = 6.0 Hz, 0.25H), 5.90 (d, J = 5.9 Hz, 0.75H), 5.88 (d, J = 5.9 Hz, 0.25H)), 5.66 (d, J = 8.1 Hz, 0.75H), 5.65 (d, J = 8.1 Hz, 0.25H), 5.22-5.11 (m, 3H), 5.05 (d, J = 7.8 Hz, 1H), 4.25 (dt J = 5.5, 5.3 Hz, 1H), 4.13 (m, 1H), 3.87 (dt, J = 3.3, 3.1 Hz, 1H), 3.65-3.51 (m, 2H), 3.28 (ddd, J = 6.4, 5.9, 5.7 Hz, 1H), 3.11 (ddd, J = 6.9, 6.6, 6.5 Hz, 1H), 2.01 (s, 0.75H), 1.96 (s, 2.25H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.9, 150.4, 149.5, 149.0, 140.5, 101.9, 101.8, 95.7, 95.6, 85.9, 85.7, 85.3, 85.2, 78.7, 78.5, 68.7, 68.6, 60.8, 60.7, 30.9, 26.4, 14.6, 13.8.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[2-(methylsulfinyl)ethanimine-*N*-oxymethyl]uridine (29)

To a solution of **28** (500 mg, 1.38 mmol) in methanol (20 ml) was added 30% H_2O_2 (5 ml). The solution was allowed to stir at ~25°C until completion of the reaction (2 h) as monitored by TLC [($CHCl_3$:MeOH (9:1 v/v)]. The reaction mixture was evaporated to dryness under reduced

pressure and the residue was purified by chromatography on silica gel using a gradient of CH_3OH (0→7%) in CH_2Cl_2. Fractions containing the pure product were collected and evaporated to a foam under low pressure. The oxidized material was dried by co-evaporation with anhydrous pyridine (3 × 5 ml) under reduced pressure. Dry pyridine (10 ml) was added and was followed by 4,4′-dimethoxytrityl chloride (474 mg, 1.40 mmol). TLC analysis [($CHCl_3$:MeOH (95:5 v/v)] of the reaction showed a complete reaction within 16 h at ~25°C. The reaction mixture was then poured into a saturated solution of $NaHCO_3$ (200 ml) and was extracted with CH_2Cl_2 (3 × 150 ml). The combined organic layers were dried over anhydrous Na_2SO_4. Following filtration, the filtrate was evaporated under reduced pressure and the material left was purified by chromatography on silica gel using a gradient of CH_3OH (0→2%) in CH_2Cl_2 containing 0.2% Et_3N as the eluent. Fractions containing pure **29** were collected and evaporated to a foam under low pressure. The purified product was dissolved in dry C_6H_6 (10 ml); the resulting solution was frozen and was then lyophilized under high vacuum to provide **29** as a powder (830 mg, 1.22 mmol, 89%). The stereochemical complexity of **29** precluded its facile characterization by NMR spectroscopy. The ribonucleoside **29** was characterized as its 3′-phosphoramidite derivative **30**.

5′-*O*-(4,4'-Dimethoxytrityl)-3′-*O*-[(*N,N*-diisopropylamino) (2-cyanoethyl)]phosphinyl-2′-*O*-[2-(methylsulfinyl)ethanimine-*N*-oxymethyl]uridine (30)

This compound was prepared from **29**, purified and processed under conditions similar to those described for the preparation of the phosphoramidite **8a**. The phosphoramidite **30** was isolated as a white powder (801 mg, 0.91 mmol, 83%). ^{31}P NMR (121 MHz, C_6D_6): δ 151.7, 151.6, 150.5, 150.1, 150.0. +ESI-HRMS: Calcd for $C_{43}H_{54}N_5O_{11}PS$ $(M + Na)^+$ 902.3170, found 902.3187.

General procedure for the removal of functional groups from the ribonucleoside conjugates 6a–d, 12, 14, 16 and 18

Purified **6a** (5.0 mg, 10 μmol) was placed in a 4-ml screw-cap glass vial and 0.5 M TBAF in THF (100 μl) was added. The tightly closed vial was heated at 55°C; progress of the reaction was monitored by RP-HPLC. Excess solvent was removed under a stream of air; the material left was dissolved in HPLC buffer A (0.1 M triethylammonium acetate, pH 7.0, 500 μl). An aliquot (2 μl) was analyzed by RP-HPLC according to the following conditions: starting from 0.1 M triethylammonium acetate pH 7.0, a linear gradient of 1% MeCN/min was pumped at a flow rate of 1 ml/min for 40 min; the gradient was then increased to 6% MeCN/min for 10 min at the same flow rate and was kept isocratic for an additional 15 min. Peak heights were normalized to the highest peak, which was set to 1 AU.

Solid-phase synthesis of modified chimeric RNA sequences

The solid phase syntheses of 5′-r(U*AUCCGUAGCUAA CGUCAUG)dT (**31**) and 5′-r(U*AUCCGUAGCU*AAC GUCAUG)dT (**32**) [U* and dT correspond to 2′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)uridine and 2′-deoxythymidine residues, respectively] and 5′-r(UAUCCG UAGCUAACGUCAUG)dT (**33**) were conducted on a scale of 0.2 μmole in the 'trityl-off' mode using a succinyl long chain alkylamine controlled-pore glass (CPG) support functionalized with 2′-deoxythymidine as the leader nucleoside. The syntheses were carried out using a DNA/RNA synthesizer and commercial 2′-*O*-(*tert*-butyldimethylsilyl) A^{Pac}, G^{Pac}, C^{Ac} and U phosphoramidite monomers (Pac and Ac correspond to phenoxyacetyl and acetyl, respectively), which were dissolved in dry MeCN to give 0.15 M solutions. The pyrenylated ribonucleoside phosphoramidite **8a** was also used as a 0.15 M solution in dry MeCN. 5-Benzylthio-1*H*-tetrazole (0.25 M in MeCN) and all other ancillary reagents necessary for oligonucleotide synthesis were obtained from commercial sources. The reaction time for each phosphoramidite coupling step was set to 5 min. The dedimethoxytritylation, capping and oxidation steps of any synthesis cycle were each performed over a period of 60 s.

Solid-phase synthesis of modified DNA sequences

The solid-phase syntheses of 5′-d(T*ATCCGTAGCTAA CGTCATGT) [T* corresponds to 5′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)-2′-deoxythymidine] (**34**) and 5′-d(TATCCGTAGCTAACGTCATGT) (**35**) were carried out using the 5′-pyrenylated deoxyribonucleoside phosphoramidite **27** and commercial dA^{Pac}, dG^{Pac}, dC^{Ac} and dT phosphoramidite monomers, as 0.1 M solutions in dry MeCN, under conditions identical to those employed in the syntheses of **31–33** with the following exceptions: (i) 1 *H*-tetrazole (0.45 M in MeCN) was used for phosphoramidite activation; (ii) the reaction time for each phosphoramidite coupling step was set to 3 min and; (iii) the dedimethoxytritylation, capping and oxidation steps of any synthesis cycle were each performed over a period of 60 s, 30 s and 30 s, respectively.

Solid-phase synthesis of r(U_{20})dT (37) from the phosphoramidite derivative of a reversible uridine 2′-conjugate

The solid phase syntheses of r(U^+_{20})dT (**36**) [U^+ and dT correspond to 2′-*O*-[2-(methylsulfinyl)ethanimine-*N*-oxymethyl]uridine and 2′-deoxythymidine residues, respectively] and the corresponding control sequence r(U_{20})dT (**37**) were conducted using the 2′-*O*-[2-(methylsulfinyl)ethanimine-*N*-oxymethyl]uridine phosphoramidite **30** and commercial 2′-*O*-(*tert*-butyldimethylsilyl)uridine phosphoramidite monomers, respectively, as 0.2 M solutions in dry MeCN, under conditions identical to those employed in the syntheses of **31–33** with the exception of the coupling reaction time for phosphoramidite **30**, which was set to 3 min.

Deprotection and characterization of the chimeric RNA sequences

The solid-phase-linked 5′-dedimethoxytritylated RNA oligonucleotide (**31**, **32** or **33**) was placed into a 4 ml

screw-capped glass vial to which was added concentrated aqueous NH_3 (1 ml). The suspension was shaken occasionally over a period of 30 min at ~25°C. The ammoniacal solution was then transferred to another 4 ml glass screw-capped and was left standing at ~25°C for 16 h. A sample of the ammoniacal solution (5 OD_{260}) was evaporated to dryness using a stream of air. The oligonucleotide was then dissolved in DMSO (50 µl) and $Et_3N{\bullet}3HF$ (65 µl) was added to the solution, which was heated to for 2.5 h at 65 °C. The solution was then concentrated under a stream of air, diluted in 0.1 M triethylammonium acetate pH 7.0 and purified by RP-HPLC using a 5 µm Supelcosil LC-18S column (25 cm × 4.6 mm) under the following conditions: starting from 0.1 M triethylammonium acetate pH 7.0, a linear gradient of 1% MeCN/min was pumped at a flow rate of 1 ml/min for 40 min and was then held, isocratically, for 20 min. Fractions containing the pyrenylated (**31** or **32**) or unmodified oligonucleotide (**33**) were pooled together, concentrated to a volume of ~250 µl and loaded onto a PD-10 (Sephadex G-25M) column. The oligonucleotide was eluted from the column using DEPC-treated H_2O as the eluent. Fractions of 1 ml were collected and those containing the RNA oligomer (A_{260}) were pooled together for analysis by RP-HPLC and characterization by mass spectrometry. –MALDI-TOF MS (**31**): Calcd for $C_{217}H_{239}N_{75}O_{147}P_{20}$ $[M-H]^+$ 6868, found 6868. –MALDI-TOF MS (**32**): Calcd for $C_{235}H_{250}N_{76}O_{148}P_{20}$ $[M-H]^+$ 7125, found 7126. –MALDI-TOF MS (**33**): Calcd for $C_{199}H_{228}N_{74}O_{146}P_{20}$ $[M-H]^+$ 6611, found 6611. Samples (1 OD_{260}) of the purified and desalted oligoribonucleotides **31** and **32** were evaporated to dryness using a stream of air and were treated with 0.5 M TBAF in DMSO (0.1 ml) for 2 h at 55°C. Each solution was diluted using 0.1 M triethylammonium acetate pH 7.0 (1 ml) and desalted as described above through a PD-10 column prior to RP-HPLC analysis and characterization by mass spectrometry. –MALDI-TOF MS (**31**→**33**): Calcd for $C_{199}H_{228}N_{74}O_{146}P_{20}$ $[M-H]^+$ 6611, found 6613. –MALDI-TOF MS (**32**→**33**): Calcd for $C_{199}H_{228}N_{74}O_{146}P_{20}$ $[M-H]^+$ 6611, found 6611.

Deprotection and characterization of DNA sequences

The solid-phase-linked 5′-dedimethoxytritylated 5′-pyrenylated oligodeoxyribonucleotide **34** or DNA oligonucleotide **35** was placed into a 4 ml screw-capped glass vial to which was added concentrated aqueous NH_3 (1 ml). The suspension was shaken occasionally over a period of 30 min at ~25°C. The ammoniacal solution was then transferred to another 4 ml glass screw-capped and was left standing at ~25°C for 16 h. A sample of the ammoniacal solution (1 OD_{260}) was evaporated to dryness using a stream of air, redissolved in 0.1 M triethylammonium acetate pH 7.0 and purified by RP-HPLC under conditions identical to those used for the purification of **31**–**33**. The purified DNA sequences were desalted under conditions identical to those used for the RNA sequences and were analyzed by RP-HPLC (Supplementary Figure S7) and characterized by mass spectrometry. –MALDI-TOF MS (**34**): Calcd for $C_{223}H_{251}N_{75}O_{127}P_{20}$ $[M-H]^+$ 6632, found 6633. –MALDI-TOF MS (**35**): Calcd for $C_{205}H_{240}N_{74}O_{126}P_{20}$ $[M-H]^+$ 6375, found 6372. A sample (1 OD_{260}) of the purified and desalted oligodeoxyribonucleotide **34** was evaporated to dryness using a stream of air and was treated with 0.5 M TBAF in DMSO (0.1 ml) for 1 h at 55°C. After diluting the solution with 0.1 M triethylammonium acetate pH 7.0 (1 ml), the solution was desalted as described above through a PD-10 column prior to RP-HPLC analysis (Supplementary Figure S7) and characterization by mass spectrometry. –MALDI-TOF MS (**34**→**35**): Calcd for $C_{205}H_{240}N_{74}O_{126}P_{20}$ $[M-H]^+$ 6375, found 6378.

Deprotection of r(U^+_{20})dT (36) and characterization of its conversion to r(U_{20})dT (37)

The solid-phase-linked 5′-dedimethoxytritylated RNA oligonucleotide **36** or **37** was placed into a 4 ml screw-capped glass vial to which was added concentrated aqueous NH_3 (1 ml). The suspension was shaken occasionally over a period of 30 min at ~25°C. The ammoniacal solution was then transferred to another 4 ml glass screw-capped and was evaporated to dryness using a stream of air. The oligonucleotide was then dissolved with 0.5 M TBAF in DMSO (0.1 ml) and was heated for 16 h at 55°C. After diluting the solution with 0.1 M triethylammonium acetate pH 7.0 (1 ml), the solution was desalted, as described above, through a PD-10 column prior to RP-HPLC analysis (Figure 6) and characterization by mass spectrometry. –MALDI-TOF MS (**36**→**37**): Calcd for $C_{190}H_{214}N_{42}O_{165}P_{20}$ $[M-H]^+$ 6344, found 6349. –MALDI-TOF MS (**37**): Calcd for $C_{190}H_{214}N_{42}O_{165}P_{20}$ $[M-H]^+$ 6344, found 6350.

RESULTS AND DISCUSSION

Synthesis and oximation of 2′-*O*-aminooxymethyl ribonucleosides

The preparation of the pyrenylated ribonucleosides **6a–d** from commercially available ribonucleosides (**1a–d**) is outlined in Scheme 1. The reaction of **1a–d** with DMSO, acetic anhydride and acetic acid produced the ribonucleoside 2′-thioacetals **2a–d** in yields of 85–94%; these acetals were efficiently converted to their chloromethyl ether derivatives (**3a–d**) by treatment with sulfuryl chloride (26,27) in CH_2Cl_2 and were isolated, without purification, as amorphous materials. The addition of a pre-mixed solution of *N*-hydroxyphthalimide and a limiting amount of DBU (~0.9 molar equiv) in CH_2Cl_2 to **3a-d** gave the 2'-*O*-phthalimidooxymethyl ribonucleosides **4a–d** in yields of 66–94% relative to the molar amounts of **2a–d** that were used as starting materials. Desilylation and, unexpectedly, dephthalimidation of **4a–d** occurred when treated with a suspension of NH_4F in MeOH (23), thereby affording the novel 2′-*O*-aminooxymethyl ribonucleosides **5a–d** after *N*-deacylation of the nucleobases upon exposure to concentrated aqueous NH_3. For characterization purposes, analytical samples of **5a–d** were purified by silica gel chromatography and were analyzed by ^1H and ^{13}C NMR spectroscopies and high resolution mass spectrometry (HRMS). Otherwise, unpurified **5a–d** was

reacted with 1-pyrenecarboxaldehyde in MeOH and afforded the pyrenylated ribonucleoside conjugates **6a–d** in post-purification yields of 69–82%; the identities of **6a–d** were confirmed by HRMS analysis. It should be noted that partial deacylation of the nucleobases occurred during the desilylation and dephthalimidation of **4b–d**. However, if required, *N*-acylation of the nucleobases can be easily achieved by transient protection of the hydroxyl functions of **6b–d** by treatment with chlorotrimethylsilane in dry pyridine followed by reaction with the desired acylating reagent as described by Ti *et al*. (28). It is also worth noting that when the desilylation of **4a** was effected by treatment with 0.5 M TBAF in THF, uridine was the only nucleosidic product detected by RP-HPLC analysis of the deprotection reaction. Similarly, when **4a** was successively treated with hydrazine hydrate to release the aminooxymethyl function and with NH_4F in methanol to desilylate the 5′-and 3′-hydroxy groups, RP-HPLC analysis of the reaction revealed only uridine as the nucleosidic product. Further investigations are necessary to fully assess the mechanistic implications of these findings, which convincingly underscore the uniqueness of the concomitant desilylation and dephtalimidation of **4a–d** by NH_4F in MeOH.

With the intent of further substantiating the versatility of **4a–d** in the preparation of ribonucleoside 2′-conjugates, the ribonucleoside **4a** was converted to 2′-*O*-aminooxymethyl uridine (**5a**), as described above, by treatment with NH_4F/MeOH, and was reacted with either cholesten-3-one (**9**) or with aldehydes derived from *N*-(2,2-dimethoxyethyl)biotinamide (**11**), *N*-(2,2-dimethoxyethyl)-5-(dimethylamino)-naphthalene-1-sulfonamide (**13**) and *N*-(4,4-diethoxybutyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**15**) to give the uridine 2′-conjugates **10**, **12**, **14** and **16**, respectively (Figure 1). The reaction of 2′-*O*-aminooxymethyl cytidine (**5b**) with the aldehyde derived from *N*-(2,2-dimethoxyethyl)-4-(dimethylamino)-azobenzene-4′-sulfonamide (**17**) afforded the cytidine 2′-conjugate **18** (Figure 1). The acetals **11**, **13**, **15** and **17** were conveniently prepared from the reaction of aminoacetaldehyde dimethyl acetal or 4-aminobutyraldehyde diethyl acetal with D-(+)-biotin 2-nitrophenyl ester, dansyl chloride and dabsyl chloride in the presence of triethylamine. These acetals were isolated in yields of 91–95%. The equilibrium between acetals **11**, **13**, **15** and their corresponding aldehydes upon exposure to concentrated HCl in MeOH led to efficient conjugation with **5a** to provide the conjugates **12**, **14** and **16** in isolated yields of 66–81%. The acetal **17** did not significantly convert to the aldehyde under acidic conditions presumably because protonation of the acetal's azo function might have considerably decreased its solubility in MeOH. This shortcoming was avoided by the reaction of **17** with a solution of 10% iodine in acetone as described in the literature (24); under these conditions, the crude *N*-(2-oxoethyl)-4-(dimethylamino)azobenzene-4′-sulfonamide was obtained and, without further purification, was reacted with the 2′-*O*-aminooxymethyl ribonucleoside **5b**. The cytidine 2′-conjugate **18** was obtained in a yield exceeding 60%.

Reversibility of ribonucleoside 2′-conjugates to their ribonucleoside precursors

Ribonucleoside 2′-conjugates **6a–d**, **12**, **14**, **16** and **18** are stable conjugates, which can be conveniently and efficiently converted to their native ribonucleoside precursors upon treatment with 0.5 M TBAF in THF. A proposed mechanism for these transformations is shown in Scheme 2 and is supported by representative RP-HPLC profiles illustrating the conversion of **6a**, **12** and **14** to uridine (Figure 2, Supplementary Figures S2 and S3, respectively). The RP-HPLC chromatograms demonstrate the formation of pyrene-1-carbonitrile (**19**) and *N*-(4-cyanobut-1-yl)-5-(dimethylamino)naphthalene-1-sulfonamide (**20**, Figure 3) as side products from the fluoride-assisted cleavage of the 2′-iminooxymethyl ether function from **6a** and **16**, respectively (Figure 2 and Supplementary Figure S4). The identities of **19** and **20** were confirmed by their RP-HPLC retention times (t_R = 61.5 and 48.2 min, respectively), which were found identical to those of authentic commercial (**19**) or chemically synthesized (**20**) samples (see Figures 2 and 3, respectively). Although it had been reported that the oxime phosphate ester **21** (Figure 3) underwent base-catalyzed decomposition to produce diphenyl phosphate and 4-nitrobenzonitrile (29), the fluoride-mediated cleavage of ribonucleoside 2′-*O*-aryl or -alkyliminooxymethyl ethers has not, to the best of our knowledge, been reported in the literature.

It should be noted that the synthesis of 2′-*O*-phthalimidooxy*ethyl* ribonucleosides had been reported earlier by Kawasaki *et al*. (30) with the purpose of preparing 2′-*O*-aminooxyethyl-modified antisense oligonucleotides for potential therapeutic applications. Although 2′-*O*-aminooxyethyl ribonucleosides can be amenable to conjugation with various functional groups, these permanent conjugates cannot be easily converted back to unmodified ribonucleosides.

In this regard, the reaction of the 2′-*O*-aminooxy*methyl* ribonucleoside **5a** with cholesten-3-one (**9**) and dansyl chloride also gave, as expected, the permanent uridine 2′-conjugates **10** (Figure 1) and **22** (Figure 3), respectively. These conjugates were both found stable to TBAF/THF under the conditions used for the conversion of **6a-d**, **12**, **14**, **16** and **18** to their corresponding ribonucleosides. Indeed, treatment of **22** with 0.5 M TBAF in THF for 72 h at 55°C produced uridine to the extent of <1%, as determined by RP-HPLC analysis of the reaction products (data shown in Supplementary Figure S6).

The conjugates **6a–d**, **12**, **14**, **16** and **18** exist as a mixture of *E*- and *Z*-geometrical isomers; one of these isomers appears to undergo fluoride-assisted cleavage of the 2′-iminooxymethyl ether function at a faster rate than the other geometrical isomer, as judged by RP-HPLC analysis of the cleavage reactions. Our findings are consistent with those reported earlier by others (31) indicating that both *syn*- and *anti*-piperonaldoxime acetates produced a nitrile via β-elimination under basic conditions; the *trans*-β-elimination from the *anti*-acetate proceeding faster than the *cis*-β-elimination. It is also worth noting that the proximity of an electron-donating group to

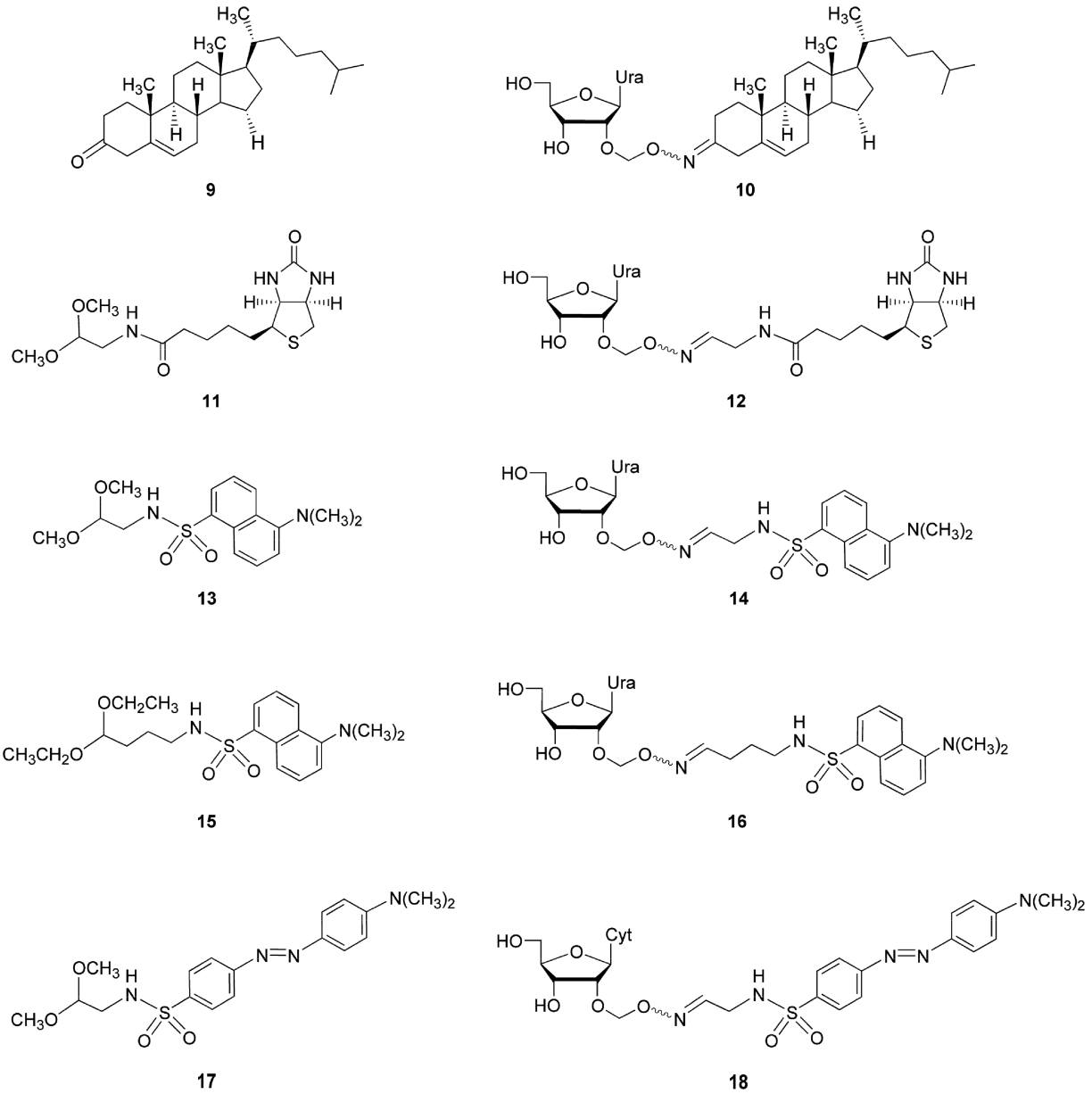
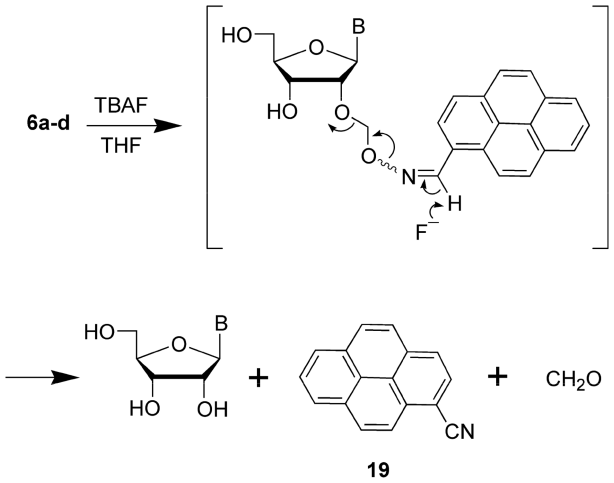


Figure 1. Ribonucleoside 2′-conjugates produced from the reaction of **5a** or **5b** with cholesten-3-one (**9**) or with aldehydes derived from various acetals (**11**, **13**, **15** and **17**).

the 2′-iminooxymethyl ether function clearly affects the rates of the fluoride-assisted cleavage reaction. As shown in Figure 2, the fluoride-mediated conversion of **6a** to uridine was complete within 4 h at 55°C while the conversion of **12** to uridine took 6 h under identical conditions (Supplementary Figure S2). One might argue that in the presence of fluoride ion, which is a strong base in aprotic solvent, the amide function of **12** (pK_a ~25) may become negatively charged to some extent and decrease the acidity of the nearby oximic proton as a consequence of the electron-donating properties of the partially ionized amide function. The reduced acidity of the oximic proton would then result in a slower fluoride-mediated β-elimination reaction. This argument is further supported by the considerably slower fluoride-assisted conversion of **14** to uridine, which was only 15% complete after 24 h at 55°C (Supplementary Figure S3). The relatively acidic sulfonamide function of **14** (pK_a ~10) is presumably ionized to a larger extent than that of an amide function by the strongly basic fluoride ion, thereby decreasing further the acidity of the oximic proton, which led to slower β-elimination rates relative to those of **6a–d** and **12** under identical conditions. Also consistent with this argument is that when the sulfonamide function is increasingly distal to the oximic proton, the electron-donating properties of the ionized sulfonamide have a lesser effect on the acidity of the oximic proton and result in relatively faster β-elimination rates. Typically, the fluoride-assisted



Scheme 2. Fluoride-assisted conversion of 2′-*O*-pyrenylated ribonucleosides (**6a–d**) to native ribonucleosides. TBAF, tetra-*n*-butylammonium fluoride; B, uracil-1-yl, cytosin-1-yl, adenin-9-yl, or guanin-9-yl.

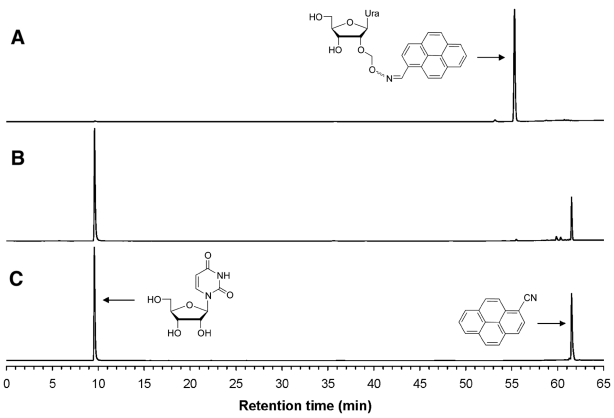


Figure 2. RP-HPLC analysis of the fluoride-assisted conversion of silica gel-purified 2′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)uridine (**6a**) to uridine. (**A**) Chromatogram of the silica gel purified **6a**. (**B**) Chromatogram of the conversion of **6a** to uridine by treatment with 0.5 M tetra-*n*-butylammonium fluoride in THF for 3 h at 55°C. (**C**) Chromatogram of mixed uridine and pyrene-1-carbonitrile commercial samples. Conditions: RP-HPLC analysis was performed using UV detection (254 nm) and a 5 µm Supelcosil LC-18S column (25 cm × 4.6 mm) according to the following conditions: starting from 0.1 M triethylammonium acetate pH 7.0, a linear gradient of 1% MeCN/min was pumped at a flow rate of 1 ml/min for 40 min; the gradient was then increased to 6% MeCN/min for 10 min at the same flow rate and kept isocratic for an additional 15 min. Peak heights are normalized to the highest peak, which is set to 1 AU.

conversion of **16** to uridine was complete within 48 h at 55°C (Supplementary Figure S4); this β-elimination rate is comparatively faster than that of **14** but still significantly slower than those of **6a–d** and **12**. Given the structural similarity of **18** and **14** in terms of proximity of the sulfonamide function to the oximic proton, the conversion of **18** to cytidine by treatment with 0.5 M TBAF in THF was comparable to that of **14** to uridine, as it was only 25% complete after 24 h at 55°C (Supplementary Figure S5).

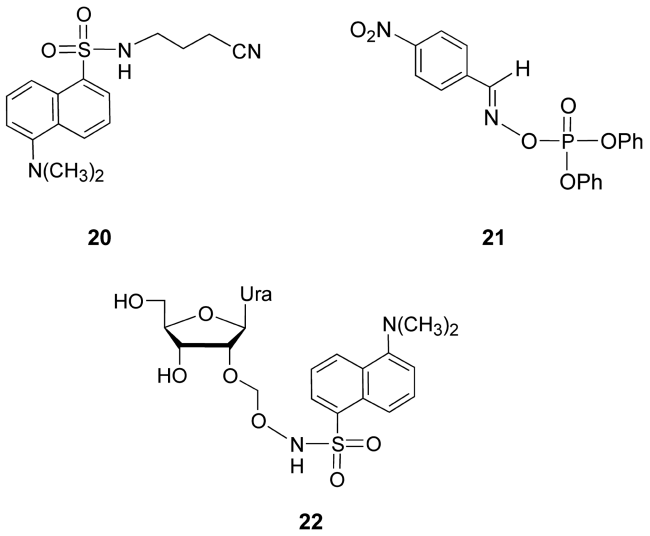


Figure 3. Structures of compounds **20**, **21** and **22**.

Single or double incorporation of a reversible ribonucleoside 2'-conjugate into chimeric RNA sequences: synthesis, deprotection and characterization

With the objective of demonstrating the ability of 2′-*O*-aminooxymethyl ribonucleoside conjugates to modify RNA sequences, the pyrenylated ribonucleoside conjugate **6a** was reacted with 4,4′-dimethoxytrityl chloride in pyridine to provide **7a**, which after purification, was reacted with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite and triethylamine to give the pyrenylated ribonucleoside phosphoramidite **8a** (Scheme 1). The RNA sequences 5′-r(U*AUCCGUAGCUAACGUCAUG)dT (**31**), 5′-r(U*AUCCGUAGCU*AACGUCAUG)dT (**32**) [U* and dT correspond to 2′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)uridine and 2'-deoxythymidine residues, respectively] and 5'-r(UAUCCGUAGCUAACGUCAUG)dT (**33**) were prepared using solid-phase techniques (32) to show that the single or double incorporation of pyrenylated ribonucleoside phosphoramidite **8a** into RNA sequences constructed from commercial 2′-*O*-(*tert*-butyldimethylsilyl) ribonucleoside phosphoramidites (Supplementary 'Materials and Methods' section) proceeded without compromising the overall yields of the RNA sequences on the basis of the stepwise colorimetric determination of the dimethoxytrityl cation concentration after the first and last coupling steps of each oligonucleotide assembly. Treatment of the solid-phase-linked oligonucleotides with concentrated aqueous NH_3 at ambient temperature (~25°C) resulted in the cleavage of all nucleobase and phosphate protecting groups with the release of the 2′-*O*-protected oligonucleotides from the CPG support. Exposure of 2′-*O*-protected oligonucleotides to $Et_3N{\bullet}3HF$ (33,34) in DMSO at 65°C resulted in the exclusive cleavage of the 2′-*O*-TBDMS protecting groups. Although the 2′-*O*-pyrenylated ribonucleoside conjugates **6a–d** are cleaved by TBAF, these conjugates are totally stable to $Et_3N{\bullet}3HF$ under the conditions used for complete cleavage of the 2′-*O*-TBDMS groups. The RP-HPLC retention times of the purified

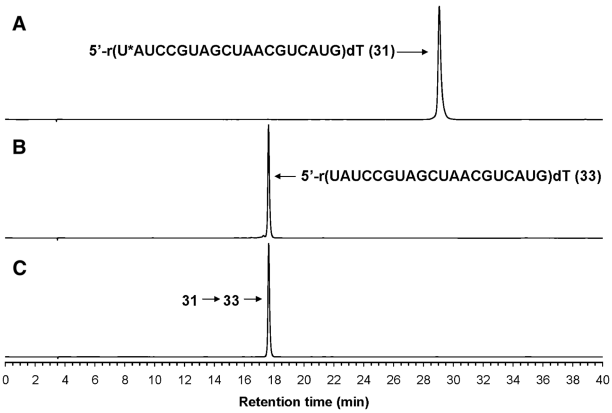


Figure 4. RP-HPLC analysis of purified and desalted 5′-r(U*AUCCGUAGCUAACGUCAUG)dT (**31**) [U* and dT represent 2′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)uridine and 2′-deoxythymidine residues, respectively] and its conversion to 5′-r(UAUCCGUAGCUAACGUCAUG)dT (**33**). (**A**) Chromatogram of **31** that was prepared from the 2′-*O*-pyrenylated ribonucleoside phosphoramidite **8a** and commercial 2′-*O*-(*tert*-butyldimethylsilyl) A^{Pac}, G^{Pac}, C^{Ac} and U phosphoramidite monomers, deprotected, RP-HPLC purified and desalted as delineated in the 'Materials and Methods' section. (**B**) Chromatogram of **33** that was prepared from commercial 2′-*O*-(*tert*-butyldimethylsilyl) A^{Pac}, G^{Pac}, C^{Ac} and U phosphoramidite monomers, and processed as described in (A). (**C**) Chromatogram of RP-HPLC purified and desalted **31** that was treated with 0.5 M TBAF in DMSO for 2 h at 55°C and then desalted. RP-HPLC analysis was performed using UV detection (254 nm) and a 5 μm Supelcosil LC-18S column (25 cm × 4.6 mm) according to the following conditions: starting from 0.1 M triethylammonium acetate pH 7.0, a linear gradient of 1% MeCN/min was pumped at a flow rate of 1 ml/min for 40 min and was then held, isocratically, for 20 min. Peak heights are normalized to the highest peak, which is set to 1 AU.

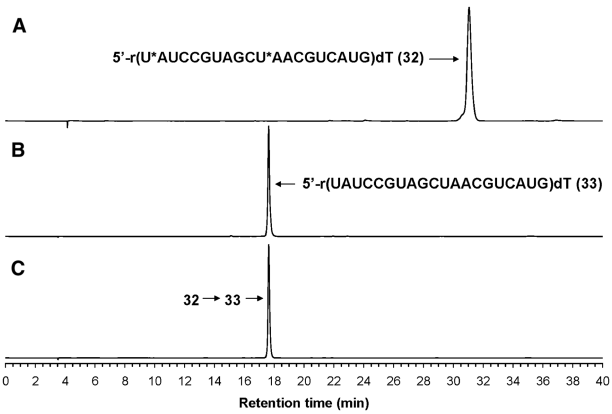
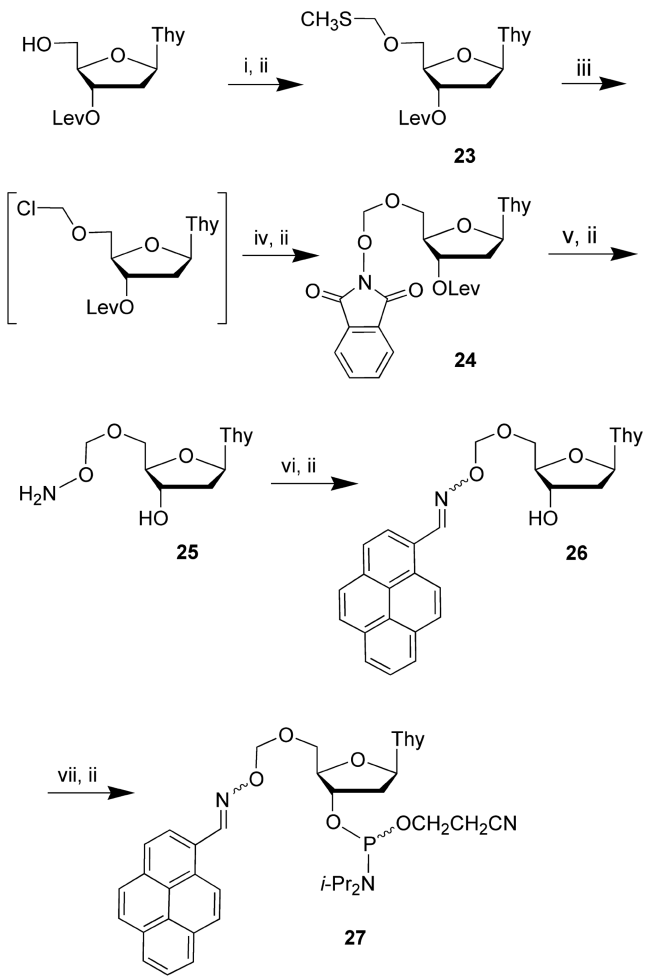


Figure 5. RP-HPLC analysis of purified and desalted 5′-r(U*AUCCGUAGCU*AACGUCAUG)dT (**32**) [U* and dT represent 2′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)uridine and 2′-deoxythymidine residues, respectively] and its conversion to 5′-r(UAUCCGUAGCUAACGUCAUG)dT (**33**). (**A**) Chromatogram of **32** that was prepared from the 2′-*O*-pyrenylated ribonucleoside phosphoramidite **8a** and commercial 2′-*O*-(*tert*-butyldimethylsilyl) A^{Pac}, G^{Pac}, C^{Ac} and U phosphoramidite monomers, deprotected, RP-HPLC purified and desalted as delineated in the 'Materials and Methods' section. (**B**) Chromatogram of **33** that was prepared from commercial 2′-*O*-(*tert*-butyldimethylsilyl) A^{Pac}, G^{Pac}, C^{Ac} and U phosphoramidite monomers, and processed as described in (A). (**C**) Chromatogram of RP-HPLC purified and desalted **32** that was treated with 0.5 M TBAF in DMSO for 2 h at 55°C and then desalted. RP-HPLC analysis was performed as described in the caption of Figure 4.

oligonucleotides **31**, **32** and **33** (t_R = 29.0, 31.0 and 17.6 min, respectively) were compared and found consistent with the composition of each oligonucleotide under identical chromatographic conditions (Figures 4 and 5). MALDI-TOF analyses of **31**, **32** and **33** corroborated their expected molecular weights. The reversibility of the 2′-*O*-pyrenylated oligonucleotides **31** and **32** to **33** was demonstrated by the reaction of **31** and **32** with 0.5 M TBAF in DMSO at 55°C. Under these conditions, **31** and **32** were completely converted to **33**, as shown by RP-HPLC (Figures 4 and 5) and mass spectrometry analyses of the fully deprotected oligonucleotides.

Functionalization of the 5′-terminus of a DNA sequence with a reversible conjugate: synthesis, deprotection and characterization

It was also our intent to show that 5′-*O*-aminooxymethyl deoxyribonucleosides are similar to 2′-*O*-aminooxymethyl ribonucleosides in their abilities to produce conjugates for the functionalization of DNA sequences at their 5′-termini; 5′-*O*-aminooxymethyl thymidine (**25**) served as an appropriate model for this purpose. The synthesis of **25** (Scheme 3) began with the thioacetalization of commercial 3′-*O*-levulinyl thymidine under conditions similar to those used for the 2′-*O*-thioacetalization of **1a–d**. The 5′-*O*-thioacetal **23** was then treated with sulfuryl chloride to yield the 5′-chloromethylated deoxyribonucleoside intermediate, which was reacted with *N*-hydroxyphthalimide in the presence of DBU to provide **24**. Hydrazinolysis of **24** resulted in the cleavage of the phthalimido and levulinyl groups affording 5′-*O*-aminooxylmethyl thymidine (**25**). Oximation of **25** with pyrenecarboxaldehyde in MeOH at 55°C led to the 5′-pyrenylated thymidine derivative **26**, which was purified and phosphitylated using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and triethylamine under anhydrous conditions to give the 5′-pyrenylated thymidine phosphoramidite **27**. The DNA sequences 5′-d(T*ATCCGTAGCTAACGTCATGT) (**34**) [T* corresponds to 5′-*O*-(pyren-1-ylmethanimine-*N*-oxymethyl)-2′-deoxythymidine] and 5′-d(TATCCGTAGCTAACGT CATGT) (**35**) were synthesized, using the phosphoramidite **27** and commercial dA^{Pac}, dG^{Pac}, dC^{Ac} and dT phosphoramidite monomers under the conditions described in the experimental section. Post-synthesis oligonucleotide deprotection was carried out by treatment with concentrated aqueous NH_3 at ~25°C. The coupling efficiency of **27** was found comparable to that of commercial deoxyribonucleoside phosphoramidites given the similar recovery of both **34** and **35**, as judged by UV spectroscopy at 260 nm. The 5′-pyrenylated DNA sequence **34** exhibited, as expected, a retention time (t_R = 33.4 min) considerably larger than that of the control DNA sequence **35** (t_R = 18.2 min) as a consequence of the notorious hydrophobicity of the pyrenyl function. (Supplementary Figure S7). The identity of both **34** and **35** was confirmed by mass spectrometry. The reversibility of the 5′-pyrenylated DNA sequence **34** was also verified by its reaction with 0.5 M TBAF in DMSO at 55°C. Under these conditions **34** was completely converted to **35** on the basis of

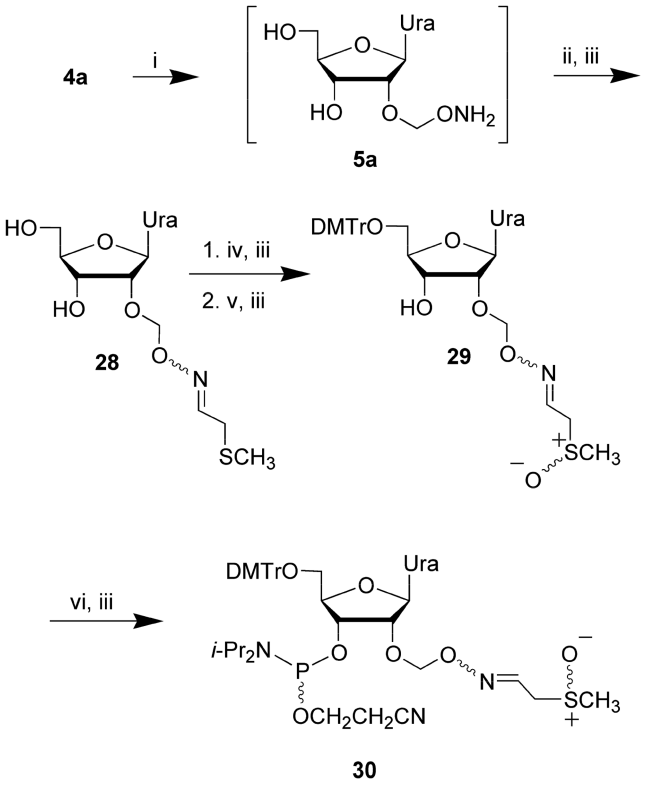


Scheme 3. Preparation of the pyrenylated deoxyribonucleoside phosphoramidite **27**. (i) DMSO, Ac_2O, AcOH, 50°C, 16 h; (ii) silica gel chromatography; (iii) SO_2Cl_2, CH_2Cl_2, 25°C, 2 h; (iv) *N*-hydroxyphthalimide, DBU, CH_2Cl_2, 25°C, 24 h; (v) 1 M hydrazine hydrate in pyridine:AcOH (3:2 v/v), 25°C, 1 h; (vi) 1-pyrenecarboxaldehyde, MeOH, 55°C, 1 h; (vii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2, 25°C, 2 h. Lev, levulinyl; Thy, thymin-1-yl.

RP-HPLC (Supplementary Figure S7) and mass spectrometry analyses.

Reversible ribonucleoside 2′-conjugate in the synthesis of native RNA sequences: synthesis and characterization of the chimeric polyuridylic acid model r(U_{20})dT

Given the pressing need for RNA sequences in RNA interference research (21), the versatility of reversible 2′-*O*-aminooxymethyl ribonucleoside conjugates in the design of novel 2′-hydroxyl protecting groups for the synthesis of native or modified RNA sequences is clearly an asset. The reaction of aldehydes or aldehydes derived from acetals with 2′-*O*-aminooxymethyl ribonucleosides was performed with the purpose of assessing the stability of the resulting 2′-conjugates to the conditions prevailing during the synthesis and deprotection of RNA sequences, and evaluating the reversibility kinetics of these conjugates when exposed to TBAF in DMSO. In preliminary experiments, the 2′-*O*-aminooxymethyl ribonucleoside intermediate **5a** was reacted with either acetaldehyde or methythioacetaldehyde, the latter of which was produced *in situ* from its commercial dimethyl acetal under acidic conditions, to generate the expected oxime conjugates (Scheme 4). Both ribonucleoside 2′-conjugates were found stable to the reagents and conditions used for routine solid-phase RNA synthesis and to the mild basic conditions that are required for *N*-deacylation of the nucleobases, cleavage of the 2-cyanoethyl phosphate protecting groups and release of the 2′-*O*-protected RNA sequence from the CPG support. The conversion of 2′-*O*-[2-(methylthio)ethanimine-*N*-oxymethyl]uridine (**28**) to uridine upon exposure to 0.5 M TBAF in DMSO was complete within 30 min at 55°C and was the fastest of the two 2′-*O*-aminooxymethyl ribonucleoside conjugates investigated. In order to further improve the fluoride-assisted conversion of **28** to uridine, we rationalized that by increasing the acidity of the oximic proton, the reversibility of **28** to uridine should be enhanced. Indeed, reaction of the sulfoxide derivative of **28** with 0.5 M TBAF in DMSO led to its complete transformation to uridine within 5 min at 25°C. These encouraging results prompted us to prepare the ribonucleoside phosphoramidite **30**, which was obtained from the



Scheme 4. Preparation of the ribonucleoside phosphoramidite **30**. (i) NH_4F, MeOH, 25°C, 16 h; (ii) methylthioacetaldehyde, MeOH, 55°C, 1 h; (iii) silica gel chromatography; (iv) 30% H_2O_2, MeOH, 25°C, 2 h; (v) DMTrCl, pyridine, 25°C, 16 h; (vi) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2, 25°C, 2 h. Ura, uracil-1-yl.

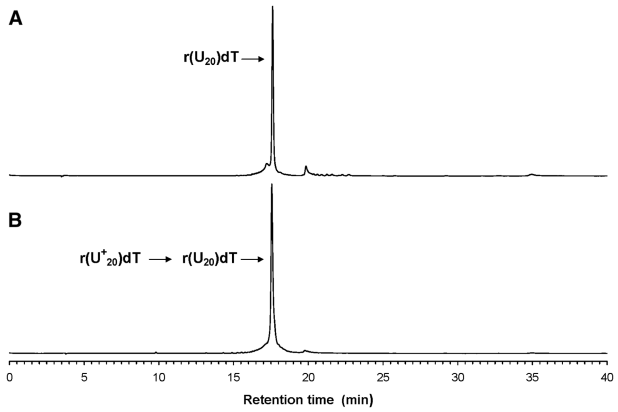


Figure 6. RP-HPLC analysis of the conversion of unpurified and desalted 5′-r(U^{+}_{20})dT) [U^{+} represents 2′-*O*-[2-(methylsulfinyl)ethanimine-*N*-oxymethyl]uridine] (**36**) to 5′-r(U_{20})dT (**37**). (**A**) Chromatogram of r(U_{20})dT **37** that was prepared from commercial 2′-*O*-(*tert*-butyldimethylsilyl)uridine phosphoramidite, deprotected, and desalted as delineated in the 'Materials and Methods' section. (**B**) Chromatogram of **36** that was prepared from the ribonucleoside phosphoramidite monomer **30** and processed as described in (A). RP-HPLC analysis was performed as described in the caption of Figure 4.

5′-dimethoxytritylation of the sulfoxide derivative of **28** to **29** and its subsequent 3′-phosphitylation by 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite under the conditions described in the caption of Scheme 4. The ribonucleoside phosphoramidite **30** was successfully used in the solid-phase synthesis of r(U^{+}_{20})dT bearing the 2-(methylsulfinyl)ethanimine-*N*-oxymethyl group for 2′-hydroxyl protection. The iterative coupling efficiency of **30** exceeded 99% and the yield of r(U^{+}_{20})dT was 80 ± 5% as determined by the colorimetric trityl assay. Upon phosphate deprotection and release of r(U^{+}_{20})dT from the CPG support effected by aqueous NH_3, the fluoride-mediated cleavage of the 2′-*O*-[2-(methylsulfinyl)ethanimine-*N*-oxymethyl] groups was subsequently performed under anhydrous conditions by treatment with 0.5 M TBAF in DMSO over a period of 16 h at 55°C. Unpurified r(U_{20})dT (**37**) was desalted, analyzed by RP-HPLC and successfully characterized by MALDI-TOF mass spectrometry. The RP-HPLC profile of the chimeric polyuridylic acid was compared with the profile of r(U_{20})dT that was prepared from commercial 2′-*O*-(*tert*-butyldimethylsilyl)uridine phosphoramidite monomers and deprotected under standard conditions (32). Figure 6 shows the high similarity of these chromatographic profiles and strongly suggests that reversible 2′-*O*-aminooxymethyl ribonucleoside conjugates may lead to the development of novel 2′-hydroxyl protecting groups for the optimal preparation of native or modified RNA sequences.

CONCLUSIONS

The synthesis of novel 2′-*O*-aminooxymethyl ribonucleosides (**5a–d**) provides facile access to reversible or permanent ribonucleoside 2′-conjugates through an efficient and chemoselective oximation reaction with aldehydes or ketones. The synthetic process, whereby 2′-*O*-phthalimidooxymethyl ribonucleoside derivatives (**4a–d**) have generally been prepared from commercial ribonucleoside precursors (**1a–d**) in relatively high yields is remarkable in terms of simplicity; a unique feature of this process is the one-step removal of the silyl and phthalimido groups from **4a–d** by treatment with methanolic ammonium fluoride. The reaction of **5a–d** with 1-pyrenecarboxaldehyde or **5a** with aldehydes, generated from acetals (**11**, **13** and **15**) under acidic conditions, gave stable but reversible ribonucleoside 2′-conjugates, whereas the reaction of **5a** with the cholestenone **9** or dansyl chloride afforded permanent ribonucleoside conjugates. The conjugation of cholesterol derivatives to antisense oligonucleotides has been reported to enhance the cellular uptake and antisense efficacy of these biomolecules in cell-based systems (35). In this context, it has become apparent that 2′-*O*-aminooxymethyl ribonucleosides (**5a–d**) are capable of forming conjugates with a variety of functional groups. Conversion of these ribonucleoside conjugates, after 5′-*O*- and nucleobase protection, to 3′-phosphoramidite derivatives should permit their incorporation into oligonucleotides. These oligonucleotide conjugates may particularly be useful in the development of innovative approaches to improving the cellular uptake of nucleic acid-based drugs through specific pathways. Cellular uptake and localization of therapeutic oligonucleotides are still the most challenging problems to overcome in order to better control gene expression. In addition, one of the important findings of this work is the facile reversibility of ribonucleoside 2′-conjugates to native ribonucleosides upon treatment with 0.5 M TBAF in DMSO. As discussed above, TBAF induced the β-elimination of 2′-iminooxymethyl ether functions through the formation of innocuous nitriles and thus provides new opportunities for the discovery and implementation of novel 2′-hydroxy protecting groups, which are of crucial importance in the synthesis of native and/or modified RNA sequences for RNA interference applications (21). Furthermore, the reversibility of DNA/RNA oligonucleotides conjugated to specific ligands is particularly useful in the affinity purification of synthetic DNA/RNA from which intact DNA/RNA oligonucleotides can be recovered from the ligand-affinity binding system (36). This approach may find application in the large-scale synthesis and purification of therapeutic DNA/RNA sequences. The reversibility of DNA/RNA oligonucleotides conjugated to affinity ligands may have a much broader appeal when the reversible DNA/RNA conjugate serves as an aptamer for capturing proteins from cell lysates. This strategy may permit the identification and characterization of biomedically relevant DNA/RNA protein complex(es) upon release from the ligand-affinity binding system.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Materials and Methods, and Supplementary Figures S1–S7.

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