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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Received August 31, 2011; Revised October 3, 2011; Accepted October 4, 2011

ABSTRACT
2′-O-Aminooxymethyl ribonucleosides are prepared from their 3′,5′-disilylated 2′-O-phthalimidoxy-

methyl derivatives by treatment with NH₄F in MeOH. The reaction of these novel ribonucleosides with 1-pyrenecarboxaldehyde results in the efficient for-
mation 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 ribo-
nucleosides 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-phthalimidoxyethyl 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 revers-

ible 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 appli-
cations 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 ribo-
nucleosides 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 bind-
ing 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 func-
tional 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-aminoxyethyl 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-aminoxyethyl 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-aminoxyethyl 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-aminoxyethyl thymidine (25, Scheme 3) has also been prepared for the first time and the addition of its pyrenylated conjugates 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-aminoxyethyl 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.

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 Ac2O (15 ml). The solution was stirred at 50°C until completion of the reaction (16 h), which was monitored by TLC (CHCl3:MeOH 95:5 v/v). The solution was transferred to a 21 Erlenmeyer flask to which was added, under vigorous stirring, a solution of K2CO3 (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 CH2Cl2 as the eluent. Fractions containing pure 2a were collected, evaporated to a foam under low pressure, and dissolved in dry C6H6 (~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-diy)-2′-O-(phthalimidooxymethyl)uridine (4a)

To a solution of thoroughly dried 2a (1.1 g, 2.0 mmol) in anhydrous CH$_2$Cl$_2$ (20 ml) was added sulfuric chloride (176 µl, 2.20 mmol); the solution was stirred at $\sim$25°C for 2 h and was then concentrated under reduced pressure to give the 2′-O-chloromethyluridine derivative 3a as an amorphous solid. N-Hydrophthalimide (1.3 g, 8.0 mmol) was placed into a separate reaction vessel to which was added anhydrous CH$_2$Cl$_2$ (10 ml) and DBU (1.04 ml, 7.00 mmol). After 10 min, the red solution was added to the reaction mixture was stirred at $\sim$25°C for 24 h at which point CH$_2$Cl$_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$_2$SO$_4$, 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$_2$Cl$_2$ 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-$d_6$): $\delta$ 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, 1H), 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-$d_6$): $\delta$ 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, 17.1, 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$_3$O$_7$Si$_2$ [M + H]$^+$ 662.2560, found 662.2560.

2′-O-(Aminooxymethyl)uridine (5a)

Purified 4a (300 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 $\sim$25°C for 2 h and ammonium fluoride (185 mg, 5.00 mmol) was added. The solution was stirred at $\sim$25°C for 2 h and was then concentrated under reduced pressure to provide 5a. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 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 (dd, $J = 12.0$, 5.0, 3.1 Hz, 1H), 3.56 (dd, $J = 12.0$, 5.0, 3.1 Hz, 1H), 3.16 (d, $J = 5.0$ Hz, 1H). $^13$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 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$_3$O$_7$ [M + H]$^+$ 290.0983, found 290.0986.

2′-O-(Pyren-1-ylmethanimine-N-oxyxymethyl)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$_4$F-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$_2$Cl$_2$ (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$_2$Cl$_2$ 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$_{25}$H$_{33}$N$_3$O$_7$ [M + H]$^+$ 502.1609, found 502.1609.

5′-O-(4,4′-dimethoxytrityl)-2′-O-(pyren-1-ylmethanimine- N-oxyxymethyl)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 $\sim$25°C and was then evaporated to a gum under reduced pressure. The material was dissolved in CHCl$_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$_2$SO$_4$, 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$_2$Cl$_2$ containing 0.2% Et$_3$N 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-oxyxymethyl)uridine (8a)

To a solution of 7a (250 mg, 311 µmol) in anhydrous CH$_2$Cl$_2$ (3 ml) containing Et$_3$N (167 µl, 1.20 mmol) was added 2-cyanoethyl N,N-diisopropylchlorophosphoramide (140 µl, 622 µmol). The reaction mixture was stirred at $\sim$25°C under argon until complete disappearance of 7a was observed (2 h) by TLC [C$_6$H$_5$Et$_3$N (9:1 v/v)]. The reaction mixture was then poured into water (3 ml) and was extracted with CH$_2$Cl$_2$ (10 ml). The organic layer was dried over anhydrous Na$_2$SO$_4$ 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$_6$H$_5$Et$_3$N (9:1 v/v) as the eluent. Fractions containing the pure product were pooled
N^4-Acetyl-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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 (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). 13C NMR (75 MHz, DMSO-d_6): δ 156.6, 155.3, 140.9, 97.7, 94.0, 88.2, 83.9, 79.3, 68.3, 59.9. +ESI-HRMS: Calcd for C_{16}H_{16}N_4O_6 [M + H]^+ 289.1143, found 289.1145.

N^4-Acetyl-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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 isolated as a white solid (8.5 g, 13.3 mmol, 89%). 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), 7.56 (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). 13C 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, 16.5, 12.5, 12.3, 12.2, 11.9. +ESI-HRMS: Calcd for C_{23}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). 13C NMR (75 MHz, DMSO-d_6): δ 156.6, 155.3, 140.9, 97.7, 94.0, 88.2, 83.9, 79.3, 68.3, 59.9. +ESI-HRMS: Calcd for C_{16}H_{16}N_4O_6 [M + H]^+ 289.1143, found 289.1145.

2'-O-(Pyren-1-ylmethanimine-N-oxyethyl)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_{22}H_{24}N_4O_6 [M + H]^+ 501.1769, found 501.1769.

N^6-Isobutyryl-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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-3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-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), 2.39 (s, 3H), 1.08–0.88 (m, 28H). 13C 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, 16.5, 12.5, 12.3, 12.2, 11.9. +ESI-HRMS: Calcd for C_{33}H_{46}N_4O_{10}Si_2 [M + H]^+ 703.2825, found 703.2825.
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\(_6\)): \(\delta\) 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, 26H).

\(^{13}\)C NMR (75 MHz, DMSO-d\(_6\)): \(\delta\) 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: Calecd for C\(_{28}\)H\(_{49}\)N\(_6\)O\(_5\)Si\(_2\) [M + H]\(^+\) 755.3251, found 755.3250.

N\(^2\)-Phenoxycetacetyl-3',5'-O-(1,1,3,3-tetraisopropylsiloxane-1,3-diy)-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\(^2\)-phenoxycetacetyl-5'-O-(1,1,3,3-tetraisopropylsiloxane-1,3-diy)guanosine (1d, 9.9 g, 15 mmol) in DMSO (22.5 ml) was added glacial AcOH (22.5 ml) and Ac\(_2\)O (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\(_2\)CO\(_3\) (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\(_6\)): \(\delta\) 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\(_6\)): \(\delta\) 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: Calecd for C\(_{33}\)H\(_{49}\)N\(_6\)O\(_8\)Si\(_2\) [M + H]\(^+\) 720.2913, found 720.2918.
pressure providing 5d. $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 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$_6$): $\delta$ 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$_{14}$H$_{25}$N$_3$O$_4$S [M+H]$^+$ 514.1830, found 514.1829.

**2′-O-(Pyrenyl)-1-methanamine-N-oxyxymethyl)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 $\mu$mol) based on the molar amount of starting material (4d) that was utilized. +ESI-HRMS: Calcd for C$_{21}$H$_{21}$N$_6$O$_6$ [M+H]$^+$ 400.1324, found 400.1324.

**N-(2-Oxoethyl)biotinamide**

The acetal 11 (280 mg, 850 $\mu$mol) 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-(Aminoxyxymethyl)uridine (5a) was prepared as described above from silica gel-purified 4a at a scale and under conditions identical to those described for the preparation of 10. After complete NH$_4$F-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 oxidation 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$_2$Cl$_2$ 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$_4$O$_3$S [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$_2$Cl$_2$ (10 ml) was added aminoacetalddehyde dimethyl acetal (130 ml, 1.20 mmol) and Et$_3$N (170 ml, 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$_2$Cl$_2$ as the eluent. Fractions containing the product were collected and evaporated to dryness under low pressure affording 13 as a solid (318 mg, 940 $\mu$mol, 94%). $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 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$_6$): $\delta$ 152.1, 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$_{23}$N$_3$O$_4$S [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 CH2Cl2 (10 ml) and the solution was washed with NaHCO3 (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 NH4F-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 [CHCl3: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 CH2Cl2 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 C25H26N2O4S [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 CH2Cl2 (10 ml) was added 4-aminobutyraldehyde diethyl acetal (237 μl, 1.20 mmol) and Et3N (179 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%). 1H NMR (300 MHz, DMSO-d6): 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). 13C NMR (75 MHz, DMSO-d6): 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 C20H30N2O4S [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 C26H32N2O4S [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 CH2Cl2 (5 ml) was added aminooacetaldheyde dimethyl acetal (130 μl, 1.20 mmol) and Et3N (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 CH2Cl2 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%). 1H NMR (300 MHz, DMSO-d6): 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). 13C NMR (75 MHz, DMSO-d6): 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 C19H24N4O4S [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) I2 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 CH2Cl2 (10 ml) and washed with an aqueous solution of 5% (w/v) sodium bisulfite (5 ml) followed by a saturated aqueous solution of NaHCO3 (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 oxidation reaction (1 h) as indicated by TLC [CHCl₃: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 CH₂Cl₂ as the eluent. Fractions containing the pure product were collected and evaporated to dryness under reduced pressure producing 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 C₂₆H₃₂N₈O₈S [M+H]⁺ 467.2137, found 467.2134. 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 CH₂Cl₂ (1 ml) for 10 min at ~25°C. The reaction product was analyzed by TLC (CHCl₃:MeOH (95:5 v/v)) and was purified by chromatography on silica gel using a gradient of MeOH (0 → 8%) in CH₂Cl₂ 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. 1H NMR (300 MHz, DMSO-d₆): 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). 13C NMR (75 MHz, DMSO-d₆): δ 151.3, 135.5, 129.5, 129.0, 128.9, 128.3, 127.8, 123.5, 119.9, 118.8, 118.5, 115.1, 44.9, 40.9, 25.2, 13.4. +ESI-HRMS: Calcd for C₁₇H₂₄N₂O₇S [M+H]⁺ 401.1377, found 401.1377.

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 Ac₂O (9 ml). The solution was stirred at ~50°C until completion of the reaction (16 h), which was monitored by TLC (CHCl₃:MeOH (95:5 v/v)). AcOH and Ac₂O 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 CH₂Cl₂ as the eluent. Fractions containing pure 23 were collected, evaporated to a foam under low pressure, and dissolved in dry C₆H₆ (~20 ml); the solution was frozen and then lyophilized under high vacuum affording a white powder (2.8 g, 7.0 mmol, 80%). 1H NMR (300 MHz, DMSO-d₆): 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). 13C NMR (75 MHz, DMSO-d₆): 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 C₁₇H₁₉N₂O₃S [M+H]⁺ 401.1377, found 401.1377.

3′-O-(Levulinyl)-5′-O-(phthalimidooxymethyl)-2′-deoxythymidine (24) To a solution of thoroughly dried 23 (2.8 g, 7.0 mmol) in anhydrous CH₂Cl₂ (70 ml) was added neat sulfuryl chloride (626 μl, 7.33 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′-deoxythymidine as an amorphous solid. N-Hydroxypthalimide (4.58 g, 28.1 mmol) was placed into a separate reaction vessel to which was added anhydrous CH₂Cl₂ (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′-deoxythymidine; the reaction mixture was kept stirring at ~25°C for 24 h at which point CH₂Cl₂ (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 NaHCO₃ (3 × 100 ml), dried over anhydrous Na₂SO₄, 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 → 8%) in CH₂Cl₂ as the eluent. Fractions containing 24 were collected and evaporated under vacuum to give a solid (2.1 g, 4.1 mmol, 58%). 1H NMR (300 MHz, DMSO-d₆): 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). $^1$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$_{32}$H$_{25}$N$_3$O$_6$ [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) 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% to 3%) in CH$_2$Cl$_2$ 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), 3.17 (m, 3H), 2.12 (s, 2H), 1.78 (s, 3H). $^1$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$_3$O$_6$ [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-pyrene-carboxaldehyde (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$_2$Cl$_2$ (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 using a gradient of MeOH (0 to 3%) in CH$_2$Cl$_2$ as the eluent. Fractions containing 26 were collected and evaporated under vacuum to give a solid (400 mg, 0.80 mmol, 80%). +ESI-HRMS: Calcd for C$_{28}$H$_{25}$N$_3$O$_6$ [M + Na]$^+$ 522.1636, found 522.1642. 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$_4$F-mediated desilylation and deprotection, 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 oxidation 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 to 5%) in CH$_2$Cl$_2$ 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 (t, J = 6.7 Hz, 0.75H), 7.34 (J, 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)ethyl]aminooxymethyl]uridine (29)

To a solution of 28 (500 mg, 1.38 mmol) in methanol (20 ml) was added 30% H$_2$O$_2$ (5 ml). The solution was allowed to stir at ~25°C until complete disappearance of 28 (2 h) was confirmed by TLC [[CHCl$_3$:MeOH (9:1 v/v)]. The reaction mixture was evaporated to dryness under reduced pressure. The crude phosphoramidite product was purified by chromatography on silica gel using C$_6$H$_6$:Et$_3$N (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$_6$H$_6$ (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$_6$H$_6$ (4 ml); the solution was frozen and then lyophilized under high vacuum. Et$_3$N-free 27 was isolated as a yellowish powder (451 mg, 640 µmol, 92%). $^{31}$P NMR (121 MHz, C$_6$D$_6$): δ 148.1, 147.7. +ESI-HRMS: Calcd for C$_{37}$H$_{42}$N$_5$O$_7$P [M + H]$^+$ 700.2895, found 700.2904.
pressure and the residue was purified by chromatography on silica gel using a gradient of CH$_3$OH (0→7%) in CH$_2$Cl$_2$. Fractons 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$_2$Cl$_2$ (3×150 ml). The combined organic layers were dried over anhydrous Na$_2$SO$_4$. Following filtration, the filtrate (801 mg, 0.91 mmol, 83%).

31P NMR (121 MHz, C$_6$D$_6$): The phosphoramidite 5 was characterized as its (2-cyanoethyl)phosphinyl-2-(pyren-1-ylmethanimine-N-oxymethyl)uridine (30) phosphoramidite derivative 50.

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%). 31P NMR (121 MHz, C$_6$D$_6$): δ 151.7, 151.6, 150.5, 150.1, 150.0. +ESI-HRMS: Calcd for C$_{43}$H$_{56}$N$_3$O$_{11}$PS (M + Na)$^+$ 902.3170, found 902.3187.

**General procedure for the removal of functionalities 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*AUCCGUAGCUAAGUCUA)GCUAUG)dT (32) and 5'-r(U*AUCCGUAGCUAAGUCUA)GCUAUG)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'-deoxyxymethylinidine 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 phenoxyacetoyl 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-1H-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*ATCCGTAAGCTAGCCCTAGCTATG) [T* corresponds to 5'-O-(pyren-1-ylmethanimine-N-oxymethyl)-2'-deoxyxymethylinidine] (34) and 5'-d(TATCCGTAAGCTAGCTCTTAGCTATG) (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'-deoxyxymethylinidine 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
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 OD260) was evaporated to dryness using a stream of air. The oligonucleotide was then dissolved in DMSO (50 μl) and Et3N•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 H2O as the eluant. Fractions of 1 ml were collected and those containing the RNA oligomer (A260) were pooled together for analysis by RP-HPLC and characterization by mass spectrometry. –MALDI-TOF MS (31): Caled for C219H250N75O127P20 [M – H] + 6611, found 6618. –MALDI-TOF MS (32): Caled for C217H239N75O147P20 [M – H] + 6611, found 6611. –MALDI-TOF MS (33): Caled for C199H228N74O146P20 [M – H] + 6611, found 6611. Samples (1 OD260) 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 loaded onto a PD-10 column prior to RP-HPLC analysis (Figure 6) and characterization by mass spectrometry. –MALDI-TOF MS (31–33): Caled for C190H214N42O165P20 [M – H] + 6375, found 6372. –MALDI-TOF MS (32–33): Caled for C190H214N42O165P20 [M – H] + 6375, found 6378. Deprotection of r(U20)dT (36) and characterization of its conversion to r(U20)dT (37) The solid-phase-linked 5′-dimethoxytritylated RNA oligonucleotide 36 or 37 was placed into a 4 ml glass screw-capped vial to which was added concentrated aqueous NH3 (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 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): Caled for C205H240N74O126P20 [M – H] + 6375, found 6378. 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 CH2Cl2 and were isolated, without purification, as amorphous materials. The addition of a pre-mixed solution of N-hydroxylamine and a limiting amount of DBU (~0.9 molar equiv) in CH2Cl2 to 3a-d gave the 2'-O-phthalimidoxy methyl 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 NH4F in MeOH (23), thereby affording the novel 2'-O-aminooxymethyl ribonucleosides 5a–d after N-deacylation of the nucleobases upon exposure to concentrated aqueous NH3. For characterization purposes, analytical samples of 5a–d were purified by silica gel chromatography and were analyzed by 1H and 13C NMR spectroscopies and high resolution mass spectrometry (HRMS). Otherwise, unpurified 5a–d was...
reacted with 1-pyrene carboxaldehyde 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-acetylation 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 acetylating 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 NH₄F 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 dephthalimidation of 4a–d by NH₄F 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 NH₄F/MeOH, and was reacted with either cholest-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 dansyl 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 (<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
the 2'-iminoxyethyl 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 (pKₐ ~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 (pKₐ ~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
The 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).

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*AUCGUAGCUAAGCUCAUG)dT (31), 5'-r(U*AUCGUAGCUAAGCUAUG)dT (32) [U* and dT correspond to 2'-O-(pyren-1-ylmethanimine-N-oxymethyl)uridine and 2'-deoxythymidylic residues, respectively] and 5'-r(UAUCGUAGCUAAGCUCAUG)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-butyldimethylsiloxymethyl)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 NH3 at ambient temperature 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 Et3N•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 Et3N•3HF under the conditions used for complete cleavage of the 2'-O-TBDMS groups. The RP-HPLC retention times of the purified...
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-aminoxythymine deoxyribonucleosides are similar to 2'-O-aminoxythymine ribonucleosides in their abilities to produce conjugates for the functionalization of DNA sequences at their 5'-termini; 5'-O-aminoxythymyl thymidine (25) served as an appropriate model for this purpose. The synthesis of 25 (Scheme 3) began with the thioacetalization of commercial 3'-O-levalyl 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-hydroxypthalimide in the presence of DBU to provide 24. Hydrazinolysis of 24 resulted in the cleavage of the pthalimido and levulinyl groups affording 5'-O-aminoxythymyl thymidine (25). Oximation of 25 with pyrenezcarboxaldehyde in MeOH at 55°C led to the 5'-pyrenylated thymidine derivative 26, which was purified and phosphorylated using 2-cyanoethyl N,N-disopropylchlorophosphoramide and triethylamine under anhydrous conditions to give the 5'-pyrenylated thymidine phosphoramide 27. The DNA sequences 5'-d(T*ATCCGTAGCTAACGTACATGT) (34) [T* corresponds to 5'-O-(pyren-1-ylmethanimine-N-oxymethyl)-2'-deoxythymidine] and 5'-d(TATCCGTAGCTAACGTACATGT) (35) were synthesized, using the phosphoramidite 27 and commercial dAPac, dGPac, dCAc and dT phosphoramidite monomers under the conditions described in the experimental section. Post-synthesis oligonucleotide deprotection was carried out by treatment with concentrated aqueous NH₃ 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 (tR = 33.4 min) considerably larger than that of the control DNA sequence 35 (tR = 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 and 35 were completely converted to 33, as shown by RP-HPLC (Figures 4 and 5) and mass spectrometry analyses of the fully deprotected oligonucleotides.
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 methylthioacetaldehyde, 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 oxime 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 3. Preparation of the pyrenylated deoxyribonucleoside phosphoramidite 27. (i) DMSO, Ac$_2$O, AcOH, 50°C, 16 h; (ii) silica gel chromatography; (iii) SO$_2$Cl$_2$, CH$_2$Cl$_2$, 25°C, 2 h; (iv) N-hydroxyphthalimide, DBU, CH$_2$Cl$_2$, 25°C, 24 h; (v) 1 M hydrazine hydrate in pyridine:AcOH (3:2 v/v), 25°C, 1 h; (vi) 1- pyrene-carboxaldehyde, MeOH, 55°C, 1 h; (vii) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, Et$_3$N, CH$_2$Cl$_2$, 25°C, 2 h. Lev, levulinyl; Thy, thymin-1-yl.

Scheme 4. Preparation of the ribonucleoside phosphoramidite 30. (i) NH$_4$F, MeOH, 25°C, 16 h; (ii) methylthioacetaldehyde, MeOH, 55°C, 1 h; (iii) silica gel chromatography; (iv) 30% H$_2$O$_2$, MeOH, 25°C, 2 h; (v) DMTrCl, pyridine, 25°C, 16 h; (vi) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, Et$_3$N, CH$_2$Cl$_2$, 25°C, 2 h. Ura, uracil-1-yl.
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\textsuperscript{20})dT bearing the 2’-(methylsulfonyl)ethanimine-N-oxymethyl group for 2’-hydroxyl protection. The iterative coupling efficiency of 30 exceeded 99% and the yield of r(U\textsuperscript{20})dT was 80 ± 5% as determined by the colorimetric trityl assay. Upon phosphate deprotection and release of r(U\textsuperscript{20})dT from the CPG support effected by aqueous NH\textsubscript{3}, the fluoride-mediated cleavage of the 2’-O-[2-(methylsulfonyl)ethanimine-N-oxymethyl] group was subsequently performed under anhydrous conditions with 0.5 M TBAF in DMSO over a period of 16 h at 55°C. Unpurified r(U\textsuperscript{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\textsuperscript{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-aminoxyoxymethyl 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-aminoxyoxymethyl 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-phthalimidoxyoxymethyl 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-pyreneboroxine-aldehyde 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 cholesterolene 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-aminoxyoxymethyl 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’-aminoxyoxymethyl 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.
FUNDING

Funding for open access charge: Intramural research funding.

Conflict of interest statement.

No declared.

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