Supplementary Data

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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Materials and Methods. Common chemicals and solvents in addition to DMSO, glacial acetic acid, acetic anhydride, potassium carbonate, elemental iodine, sodium bisulfite, pyridine, triethylamine, Et₃N•3HF, DEPC-treated H₂O, 30% hydrogen peroxide, sulfuryl chloride, N-hydroxyphthalimide, 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU), hydrazine hydrate, ammonium fluoride, tetra-n-butylammonium fluoride, 1-pyrenecarboxaldehyde, pyrene-1-carbonitrile, 5-cholesten-3-one, D-(+)-biotin 2-nitrophenyl ester, 5-dimethylamino-1-naphthalenesulfonfyl chloride (dansyl chloride), 4-(dimethylamino)azobenzene-4’-sulfonyl chloride (dabsyl chloride), acetaldehyde, aminoacetaldehyde dimethyl acetal, 4-aminobutyraldehyde diethyl acetal, (methylthio)acetaldehyde dimethyl acetal, 4-chlorobutyronitrile, potassium phthalimide, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, and anhydrous solvents (MeCN, CH₂Cl₂, C₆H₆, pyridine, THF) and DMSO-d₆ were all purchased from commercial sources and used without further purification.

N⁴-acetyl-5’-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine, N⁶-isobutyryl-5’-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine, 5’-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine, N²-phenoxyacetyl-5’-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine, 3’-O-(levulinyl)-2’-deoxythymidine, 2’-O-(tert-butyldimethysilyl) A¹Bu, G¹Pac, C¹Ac and U phosphoramidite monomers, dA¹Bu, dG¹Pac, dC¹Ac and dT phosphoramidite monomers, PD-10 (Sephadex G-25M) columns, 0.45 M 1H-tetrazole in MeCN, 0.25 M 5-benzylthio-1H-tetrazole and all ancillary reagents commonly used in solid-phase DNA/RNA synthesis including succinyl long chain alkylamine controlled-pore glass (CPG) support functionalized with 2’-deoxythymidine as the leader nucleoside were obtained from reputable sources and used as received.

Flash chromatography purifications were performed on glass columns (6.0 cm or 2.5 cm I.D.) packed with silica gel 60 (230-400 mesh), whereas analytical thin-layer chromatography (TLC) analyses were conducted on 2.5 cm × 7.5 cm glass plates coated with a 0.25 mm thick layer of silica gel 60 F₂₅₄. Analytical RP-HPLC analyses were performed using 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 is pumped at a flow rate of 1 mL/min for 40 min or, as indicated, 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. In all RP-HPLC chromatograms, peak heights are normalized to the highest peak, which is set to 1 arbitrary unit. 2 M Triethylammonium acetate buffer was purchased from Applied Biosystem and diluted to 0.1 M with HPLC grade water prior to use.

All NMR experiments were performed using a spectrometer at the field of 300.13, 75.47 and 121.5 MHz for one-dimensional ¹H, ¹H-decoupled ¹³C and ¹H-decoupled ³¹P, respectively. Samples were maintained at a temperature of 298 K. All spectra were recorded in deuterated solvents or as indicated and chemical shifts δ were reported in parts per million (ppm) relative to appropriate internal references. High resolution mass spectra used to confirm the elemental composition of new compounds were obtained on a Bruker Daltonics ApexQ FT-ICR mass spectrometer equipped with a 12 T magnet. Electrospray ionization in positive ion mode was used to generate [M+H]⁺ and [M+Na]⁺ ions out of test samples (0.01 mg dissolved in 1 mL of 10 mM ammonium acetate in MeCN:H₂O (1:1 v/v)). Spectra were externally calibrated using 0.5 mg/mL solution of CsI in water, which yielded a series of peaks in the mass range used for analysis (200-2000 m/z).
Figure 1. Normal phase HPLC analysis of silica gel purified 10.

Chromatogram of silica gel-purified 2’-O-(5-cholesten-3-imine-N-oxyethyl)uridine (10). Normal phase HPLC analysis was performed using UV detection (254 nm) and a 5 μm Agilent Prep-SIL Scalar column (25 cm × 4.6 mm) according to the following conditions: starting from CHCl₃, a linear gradient of 1% MeOH/min was pumped at a flow rate of 1 mL/min for 30 min and from 30% MeOH to CHCl₃ in 10 min. Peak heights are normalized to the highest peak, which is set to 1 arbitrary unit.
Figure 2. RP-HPLC analysis of the fluoride-assisted conversion of the silica gel-purified biotinylated uridine conjugate 12 to uridine

A: Chromatogram of the silica gel purified biotin-uridine conjugate 12. B: Chromatogram of the conversion of 12 to uridine by treatment with 0.5 M tetra-\(n\)-butylammonium fluoride in THF for 6 h at 55 °C. C: Chromatogram of a commercial sample of uridine. RP-HPLC analyses were 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 is pumped at a flow rate of 1 mL/min for 40 min.
Figure 3. RP-HPLC analysis of the fluoride-assisted conversion of the silica gel-purified dansylated uridine conjugate 14 to uridine

A: Chromatogram of the silica gel purified 14. B: Chromatogram of the conversion of 14 to uridine by treatment with 0.5 M tetra-n-butylammonium fluoride in THF for 24 h at 55 °C. C: Chromatogram of a commercial sample of uridine. RP-HPLC analysis was performed under conditions identical to those described for the fluoride-assisted conversion of 6a to uridine in Figure 2 of the original manuscript.
Figure 4. RP-HPLC analysis of the fluoride-assisted conversion of the silica gel-purified dansylated uridine conjugate 16 to uridine

A: Chromatogram of the silica gel purified 16. B: Chromatogram of the conversion of 16 to uridine by treatment with 0.5 M tetra-n-butylammonium fluoride in THF for 48 h at 55 °C. C: Chromatogram of a commercial sample of uridine co-mixed with an analytical sample of N-(4-cyanobut-1-yl)-5-(dimethylamino)naphthalene-1-sulfonamide (20). Conditions: RP-HPLC analysis was performed under conditions identical to those described for the fluoride-assisted conversion of 6a to uridine in Figure 2 of the original manuscript.
Figure 5. RP-HPLC analysis of the fluoride-assisted conversion of the silica gel-purified dabsylated cytidine conjugate 18 to cytidine

A: Chromatogram of the silica gel-purified 18.

B: Chromatogram of the conversion of 18 to cytidine by treatment with 0.5 M tetra-n-butylammonium fluoride in THF for 24 h at 55 °C. C: Chromatogram of a commercial sample of cytidine. Conditions: RP-HPLC analysis was performed under conditions identical to those described for the fluoride-assisted conversion of 6a to uridine in Figure 2 of the original manuscript.
Figure 6. RP-HPLC analysis of the fluoride-assisted conversion of the silica gel-purified dansylated uridine conjugate 22 to uridine

**A:** Chromatogram of the silica gel purified 22. **B:** Chromatogram of the conversion of 22 to uridine by treatment with 0.5 M tetra-n-butylammonium fluoride in THF for 72 h at 55 °C. Conditions: RP-HPLC analysis was performed under conditions identical to those described for the fluoride-assisted conversion of 6a to uridine in Figure 2 of the original manuscript.
Figure 7. RP-HPLC analysis of purified and desalted 5’-d(T*ATCCGTA GCTAACGTCATGT) [T* corresponds to 5’-O-(pyren-1-ylmethanimine-N-oxymethyl)-2’-deoxythymidine] (34) and 5’-d(TATCCGTA GCTAACGTCATGT) (35).

A: Chromatogram of 34 that was prepared from the 5’-O-pyrenylated deoxyribonucleoside phosphoramidite 8a and commercial 2’-O-(tert-butylidimethylsilyl) A<sub>ibu</sub>, G<sub>Pac</sub>, C<sub>Ac</sub> and U phosphoramidite monomers, deprotected, RP-HPLC purified and desalted as delineated in the Experimental section. B: Chromatogram of 35 that was prepared from commercial 2’-O-(tert-butylidimethylsilyl) A<sub>ibu</sub>, G<sub>Pac</sub>, C<sub>Ac</sub> and U phosphoramidite monomers, and processed as described in A. C: Chromatogram of RP-HPLC purified and desalted 34 that was treated with 0.5 M TBAF in DMSO for 1 h at 55 ºC and then desalted. RP-HPLC analyses were 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. Peak heights are normalized to the highest peak, which is set to 1 arbitrary unit.