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Selective Protection and Deprotection Procedures for Thiol and Hydroxyl Groups

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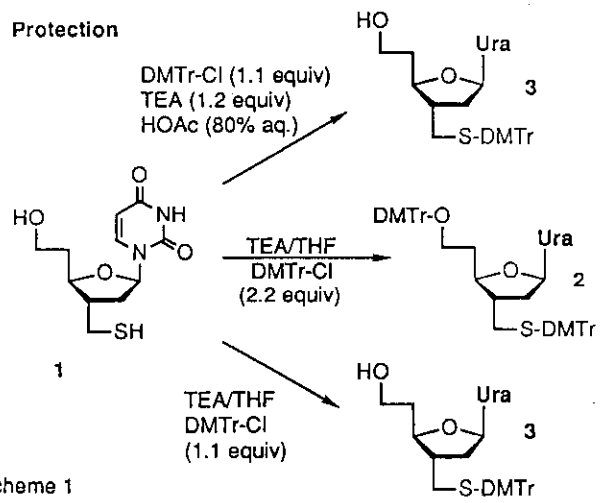
Abstract: A protecting group strategy has been developed that permits the convergent synthesis of oligonucleotide analogs containing dimethylene sulfone groups replacing of phosphate diester groups. The strategy is based on experimental conditions that allow selective removal of dimethoxytrityl groups from oxygen and sulfur.

The preparation of oligonucleotide analogs having dimethylene sulfide, sulfoxide, and sulfone linking groups replacing the phosphodiester groups[1] found in natural DNA involves the joining of building blocks in either a stepwise or convergent fashion. The simplest approach involves an S_N2 reaction between a thiolate anion (as the nucleophile) on one coupling unit and a mesylate (as the electrophile) on a second (Scheme 1). While simple hydroxyl groups do not compete with thiol groups in such S_N2 reactions, heterocyclic amino groups and other thiol groups do, and a protecting group strategy is needed to obtain oligomeric products efficiently.

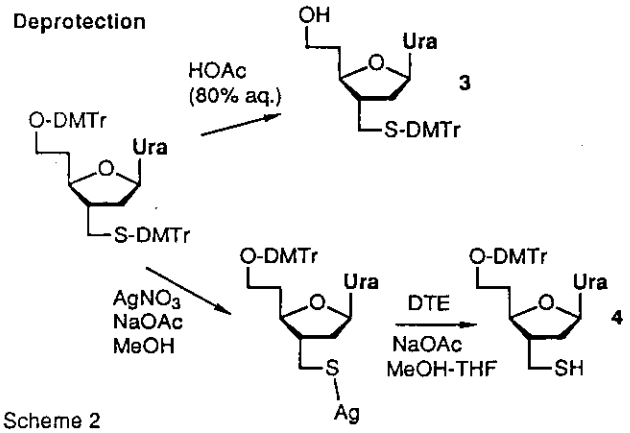
In one strategy, reported earlier,[1] the thiol group of the terminal building block was irreversibly blocked by alkylation in the first step of the synthesis. Once blocked, oligomers could be prepared only by repetitive addition of single nucleoside analogs. Obviously, for the synthesis of longer oligonucleotide analogs, a convergent strategy that allows the joining of oligonucleotide analogs would be more efficient. To effect such a strategy, a set of conditions is needed for protecting and deprotecting a thiol group in the presence of other functionality commonly found in oligonucleotide analogs. These cannot involve either strong base, which would disturb standard protecting groups on the nucleoside bases, or strong acid, which would lead to depurination. Preliminary studies showed that acylation, disulfide bond formation, and other simple strategies were inadequate. Therefore, we developed a protecting group strategy involving dimethoxytrityl groups. As this strategy might find use in the synthesis of other sulfur-containing oligonucleotide analogs[2] or other non-repetitive polymers joined by thioether linking groups, it is appropriate to present it at this time.

Both the oxygen and sulfur of nucleoside analog **1** can be protected simultaneously by reacting with dimethoxytrityl chloride (DMTr-Cl, 2.2 eq) in THF containing triethylamine (TEA, 10 eq), affording **2** in 97% yield. Use of only 1.1 eq of DMTr-Cl under the same conditions yields **3**, with only the thiol protected, as the major product. In acetic acid (80%) containing triethylamine (TEA, 1.2 eq), treatment of **1** with dimethoxytrityl chloride (DMTr-Cl, 1.1 eq) protected the thiol group yielding **3** as the sole product (91%).



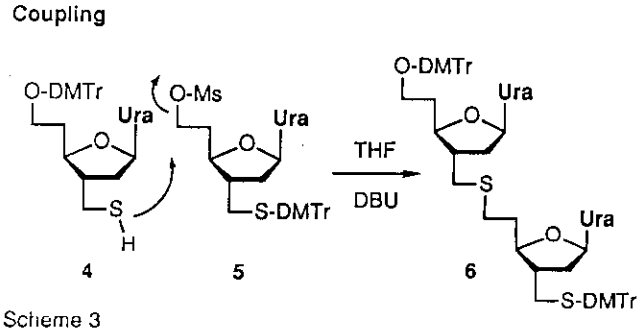
Scheme 1

The DMT group can be removed from oxygen essentially quantitatively by treating with acetic acid (80%, RT, 10 min). The DMT protection remains on the thiol group under these conditions. When treated with methanolic $AgNO_3$ (RT, 1 min) in the presence of NaOAc as buffer, the DMT is essentially quantitatively removed from sulfur without deprotection of oxygen. In the absence of buffer, the DMT group migrates from oxygen to sulfur. The product bearing a free thiol group forms a precipitate as a silver salt, which can be recovered by digestion with a solution of dithioerythritol (DTE).



Scheme 2

Mesylation of **3** yields a synthetic intermediate **5** that can enter an S_N2 reaction as an electrophile. Coupling of **4** with the mesylate **5** in THF using DBU as base yields the dinucleotide analog **6** protected at both ends (96%) contaminated with a small amount of the disulfide of **4**. Removal of the DMT group from sulfur yields a synthetic intermediate that can enter a further coupling reactions as the nucleophile; removal of the DMT from oxygen followed by mesylation yields a synthetic intermediate that can enter a further coupling reactions as the electrophile. Dimers, tetramers, hexamers and octamers have now been constructed by repeating these procedures.



Scheme 3

Experimental

1-{(2R,4R,5R)-5-[2-(4,4′-dimethoxytriphenylmethoxy)ethyl]-4-(4,4′-dimethoxytriphenylmethylthiomethyl)-tetrahydrofuran-2-yl}uracil (**2**)

Thiol and hydroxyl groups were protected simultaneously using the following procedure. Triethylamine (1.4 ml, 10 eq.) and THF (20 ml) were injected simultaneously into a solid mixture of **1** (280 mg, 1.03 mmol) and DMT-Cl (767 mg, 2.06 mmol, 2.2 eq) under Ar with stirring. The solution (0.05 M) was stirred at RT for 2 hr (TLC 5% MeOH/CH_2Cl_2, R_f=0.50). After the reaction was completed, MeOH (2 ml) was added to consume the excess reagent. The mixture was stirred (5 min), the solvents were removed by rotary evaporation, the residue resuspended in EtOAc, the insoluble salts removed by filtration, and the solvents removed under reduced pressure. The residue was chromatographed on silica gel (EtOAc:Hexane1:1, R_f=0.43) to give product (874 mg, 97% yield) as an oil.

^{1}H-NMR ($CDCl_3$) d :1.78-1.90 (m, 2H, CH_2CH_2ODMTr), 2.04-2.23 (m, 4H, 2H-3', H-4', H-4"), 2.32 (dd, J=5.4, 12.3 Hz, 1H, H-4"), 3.18 (t, J=6.7 Hz, 2H, CH_2ODMTr), 3.50 (td, J=3.2, 8.9 Hz, 1H, H-5'), 3.77 (d, 12H, 4 x CH_3), 5.63 (d, J=8.1 Hz, 1H, H-5), 5.85 (t, J=5.0 Hz, 1H, H-2'), 6.75-6.85 (m, 8H, H-ar.), 7.15 (d, J=8.2 Hz, 1H, H-6), 7.24-7.3 (m, 14H, H-ar.), 7.36-7.42 (m, 4H, H-ar.), 8.3 (br, 1H, NH)

^{13}C-NMR ($CDCl_3$) d: 33.33 ppm (DMTrOCH$_2$CH$_2$), 34.61 (C_3'), 38.99 (C_4"), 42.12 (C_4'), 55.22, 55.26 (4 x CH_3O), 60.40 (DMTrOCH_2), 66.14 (ar$_3$CS), 82.60 (C_5'), 85.16 (C_2'), 86.28 (ar$_3$C-O),101.81 (C_5), 113.1 (d, CH-C-OCH$_3$ in ar.),126.7 (d, CHCHCH-C- in Ph), 127.8 (d, CH-C- in Ph),128.1 (d, CHCH-C- in Ph),129.3, 129.9 (CHCH-C-OCH$_3$ in ar),136.2, 136.8 (CCHCH-C-OCH$_3$ in ar.),139.3 (C_6), 145.0 (d, C in Ph group),149.8 (C_2), 158.3 (d, CH_3O-C), 162.8 (C_4).

1-[(2R,4R,5R)-4-(4,4'-dimethoxytriphenylmethylthiomethyl)-5-(2-hydroxyethyl)-tetrahydrofuran-2-yl] uracil (3)

Thiol groups were protected selectively in the presence of hydroxyl groups using the following procedure. To **1** (97 mg, 0.364 mmol) and DMTr-Cl (136 mg, 0.4 mmol, 1.1 eq.) in a round bottom flask (10 ml) was added simultaneously triethylamine (61 ml, 1.2 eq.) and 80% aq HOAc (3.6 ml) under Ar. The initial red color faded upon stirring at RT for 1 hr (TLC 10% MeOH in CH_2Cl_2, R_f=0.46). The solvents were removed under high vacuum and the residue chromatographed on silica gel (2% to 4% MeOH/CH_2Cl_2 gradient) to give product (190 mg, 91%) as a white foam.

^{1}H-NMR ($CDCl_3$) d: 1.62-1.80 (m, 2H, CH_2CH_2ODMTr), 1.95-2.12 (m, 4H, 2H-3', H-4'), 2.20-2.40 (m, 2H, 2H-4"), 3.65 (td, J=3.3, 8.9 Hz, 1H, H-5'), 3.72 (m, 2H, CH_2ODMTr), 3.83 (s, 6H, 2 x CH_3), 5.74 (dd, J=3.3, 8.0 Hz, 1H, H-5), 5.97 (dd, J=4.1, 8.3 Hz, 1H, H-2'), 6.75-6.88 (m, 4H, H-ar.), 7.20-7.45 (m, 10H, H-6, 9H-ar.), 8.9 (br, 1H, NH)

^{13}C-NMR ($CDCl_3$) d: 33.16 ppm (DMTrOCH$_2$CH$_2$), 36.38 (C_3'), 38.74 (C_4"), 42.40 (C_4'), 55.28 (2 x CH_3O), 60.40 (DMTrOCH_2), 66.24 (ar$_3$CS), 83.81 (C_5'), 85.18 (C_2'), 102.24 (C_5), 113.26 (CH-C-OCH$_3$ in ar),126.75 (CHCHCH-C- in Ph), 127.98 (CH-C- in Ph),129.33 (CHCH-C- in Ph), 130.63 (CHCH-C-OCH$_3$ in ar), 136.77 (CCHCH-C-OCH$_3$ in ar),139.34 (C_6), 144.99 (C in Ph group),150.16 (C_2), 158.19 (CH_3O-C), 163.25 (C_4).

1-{(2R,4R,5R)-5-[2-(4,4'-dimethoxytriphenylmethoxy)ethyl] -4-mercaptomethyltetrahydrofuran-2-yl}uracil (4)

The dimethyoxytrityl group was removed from sulfur selectively by the following procedure. To a solution of **2** (660 mg, 0.753 mmol) in THF/MeOH (3:1, 16 ml) was added an aqueous solution of NaOAc (1.5 ml, 3 M, 6 eq.). Separately, a solution of $AgNO_3$ (256 mg 2 eq.) in water (1 ml) was diluted with MeOH (5 ml), and the solution added to **2**.

The mixture was stirred for 2 min, the solids were recovered by centrifugation, and the pellet was washed (3 x) with MeOH to remove dimethoxytrityl-containing byproducts. To the pellet was added THF/MeOH (1:1, 40 ml), aqueous NaOAc (1.5 ml, 3 M), and dithioerythritol (DTE, 464 mg, 2 eq. to Ag+)., and the mixture was stirred for 5 min. The yellow solid (the silver complex of DTE) was removed by filtration through a short silica column, which was washed with THF/EtOH (1:1). The eluate was evaporated under reduced pressure, the residue was washed with water, and the solids recovered by filtration through sand.. The product was eluted from the sand by washing with THF. Solvents were removed by rotary evaporation to yield **4** (426 mg, 98.6%, TLC, 5% MeOH/CH_2Cl_2, R_f=0.37), which was dried under high vacuum.

^{1}H-NMR ($CDCl_3$) d: 1.85-2.05 (m, 2H, CH_2CH_2ODMTr), 2.12 (m, 2H, 2H-3'), 2.19 (dd, J=3.8, 7.8 Hz, 1H, H-4"), 2.27 (m, 1H, H-4'), 2.48 (m, 1H, H-4"), 2.67 (m, 1H, H-4"), 3.28 (m, 2H, CH_2ODMTr), 3.79 (s, 6H, 2 x CH_3), 3.93 (m,1H, H-5'), 5.68 (d, J=8.1 Hz, 1H, H-5), 5.97 (dd, J=3.9, 7.1 Hz, 1H, H-2'), 6.81-6.88 (m, 4H, H-ar), 7.22 (d, J=8.1 Hz, 1H, H-6), 7.27-7.45 (m, 7H, H-ar.), 7.43 (m,2H, H-ar.), 8.62 (br, 1H, NH)

^{13}C-NMR ($CDCl_3$) d: 25.95 ppm (DMTrOCH$_2$CH$_2$), 34.72 (C_3'), 38.26 (C_4"), 45.52 (C_4'), 55.23 (2 x CH_3O), 60.16 (DMTrOCH_2), 81.94 (C_5'), 85.15 (C_2'), 86.37 (ar$_3$CO), 102.10 (C_5), 113.13 (CH-C-OCH$_3$ in ar), 126.82 (CHCHCH-C- in Ph), 127.85 (CH-C- in Ph),128.07 (CHCH-C- in Ph), 129.96 (CHCH-C-OCH$_3$ in ar.), 136.14 (CCHCH-C-OCH$_3$ in ar), 139.28 (C_6), 144.93 (C in Ph group),150.18 (C_2), 158.50 (CH_3O-C), 163.23 (C_4).

1-[(2R,4R,5R)-4-(4,4'-dimethoxytriphenylmethylthiomethyl)-5-(2-hydroxyethyl)tetrahydrofuran-2-yl]uracil (3)

Selective removal of DMT from oxygen was effected with the following procedure. HOAc (80% aq., 10.4 ml) was added to **2** (456 mg, 0.521 mmol), and the mixture was stirred for 20 min (TLC, 7.5% MeOH/CH_2Cl_2, R_f=0.36). Na_2CO_3 (sat. aq.) was then added to adjust the pH of the solution to pH 6-7. The solution was extracted with EtOAc, the extracts dried (Na_2SO_4), and the mixture chromatographed on silica gel column (2-4% MeOH/CH_2Cl_2) to give **3** (284 mg, 95% yield) as a foam.

References and Notes

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