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FUNCTIONALIZATION OF NUCLEOSIDE ANALOGS: REGIOSPECIFIC CONVERSION OF 4"-HYDROXYL GROUPS TO THIOESTERS AND DEOXYGENATION OF 3'-HYDROXYL GROUPS

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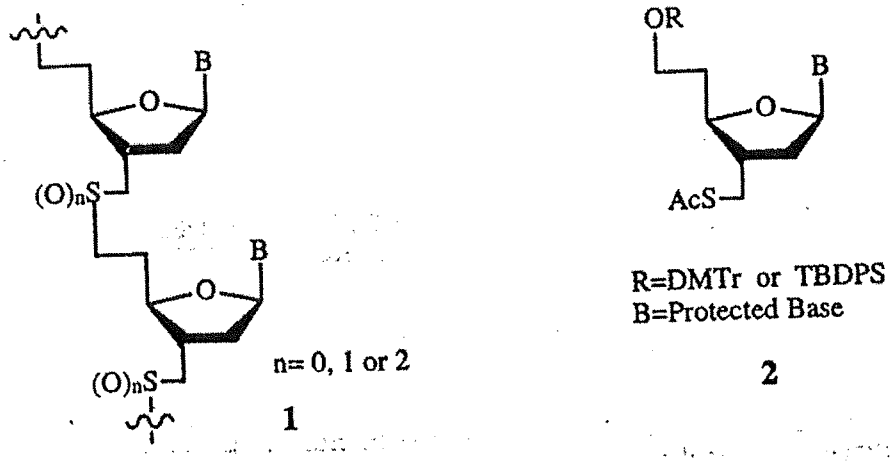
ABSTRACT: Nucleoside analogs **3a-e** were conveniently functionalized by regiospecifically converting 4"-hydroxyl groups to thioesters **4a-e** (>90% yield) and reducing 3'-hydroxyl groups to give building blocks **2a-e** in 85-90% yields.

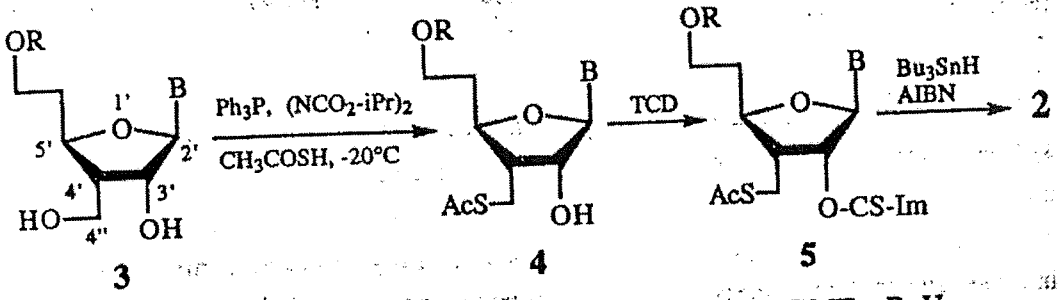
Methods that regioselectively transform functional groups, important throughout organic synthesis, are especially important when preparing analogs of sugars, nucleosides and nucleotides, which frequently place the same class of functional group in different environments. For example, building blocks **2** (in fully protected form) were the key intermediates in the synthesis of sulfide-, sulfoxide- and sulfone-linked nucleotide analogs **1** (Figure),[2,3] which have been interesting probes of nucleic acid structure[3c-f] and protein-nucleic acid interactions.[3g] A variety of routes have generated precursors to these building blocks.[2,3] Each has encountered the synthetic challenge embodied in the need to selectively convert the primary 4"-hydroxyl group of **3** (**Scheme**) to a corresponding thioester **4** in the presence of the adjacent secondary 3'-hydroxyl group, which proved critical to controlling stereochemistry at the 2'-position, but which must be removed to yield the analog of DNA. Many methods for converting alcohols to thioesters and deoxygenation are known,[4,5] but none efficiently addressed this challenge. In particular, methods that convert the 4"-OH group to a thioester via a halide or a tosylate were not selective without further protection and deprotection steps, and even then yielded the thioester only in low yields.

Because of its bulk, it was conjectured that the reactive intermediate in the Mitsunobu reaction involving triphenylphosphine[6] might react more rapidly with a primary hydroxyl group than a secondary hydroxyl group. If the steps preceding the breakdown of the phosphonium ion to give product are not in rapid equilibrium, this might be synthetically



1 2

Figure



3 4 5 2

3a: R=DMTr, B=U
3b: =TBDPS, =T
3c: =TBDPS, =C^{Bz}
3d: =TBDPS, =A^{Bz}
3e: =TBDPS, =G^{iBu}

4a: R=DMTr, B=U
4b: =TBDPS, =T
4c: =TBDPS, =C^{Bz}
4d: =TBDPS, =A^{Bz}
4e: =TBDPS, =G^{iBu}

5a: R=DMTr, B=U
5b: =TBDPS, =T
5c: =TBDPS, =C^{Bz}
5d: =TBDPS, =A^{Bz}
5e: =TBDPS, =G^{iBu}

Scheme

useful as a way of converting a primary hydroxyl group to a thioester more rapidly than a secondary hydroxyl group. The increased size of the thioester group (relative to the alcohol group) might then be expected to obstruct further reaction of an adjacent secondary hydroxyl group with the bulky reactive Mitsunobu intermediate.

To test this conjecture, diol precursors carrying a complete set of nucleobases (**3a-e**) were prepared and subjected to optimized Mitsunobu reaction conditions (Experimental). The diols were found to be converted exclusively to the desired monothioesters **4** in high yields (>90%). Model studies showed (perhaps surprisingly) that the thioester was stable

during the subsequent 3'-deoxygenation step following the Barton reduction procedure,[7] as was the 4,4'-dimethoxytrityl (DMTr) group, the *tert*-butyldiphenylsilyl (TBDPS) group, and the protecting groups on the nucleobases. Thus, **4a-e** could be directly transformed to the intermediates **5a-e**, without any manipulation of functionality, by treatment with 1,1'-thiocarbonyl diimidazole (TCD) in high yield (>85%). These were transformed directly to deoxyribose analogs **2a-e** by reduction of **5** by tributyltin hydride (Bu_3SnH) in the presence of azo-bis-*iso*butyronitrile (AIBN) in refluxing toluene (85-90% yield, **Scheme**).

With the growing interest in nucleoside and oligonucleotide analogs with modified backbones, these synthetic transformations should be useful.

EXPERIMENTAL

N^4-Benzoyl-1-[(2R,3R,4R,5R)-4-acetylthiomethyl-5-(2-*t*-butyldiphenylsilyloxyethyl)-3-hydroxytetrahydrofuran-2-yl]-cytosine (4c)

Di*iso*propyl azodicarboxylate (0.81 mL, 90-95%, 1.5 eq.) and triphenylphosphine (0.97 g, 1.5 eq.) were dissolved in dry THF (18 mL), and the mixture was stirred at -20°C (ice-salt bath) under Ar. After a yellow precipitate appeared, **3c** (1.512 g, 2.47 mmol, in dry THF, 5 mL) and thioacetic acid (263 µL, 1.5 eq.) in THF (1.7 mL) were injected separately and simultaneously. The temperature was allowed to rise to RT, and the reaction mixture was stirred at RT for 1 hr (TLC, 7.5% MeOH/CH_2Cl_2, **3c**, R_f=0.42). The solvent was removed under reduced pressure, and the residue purified by chromatography on silica gel (from 2% to 3% MeOH/CH_2Cl_2) to give **4c** (1.561 g, 92%) and obtained as a white foam.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.08 (s, 9H, 3xCH_3), 1.84 (m, 1H, H-4'), 2.03 (s, 3H, CH_3COS), 2.05-2.15 (m, 2H, CH_2CH_2O-DPTBS), 3.91 (t, J=6.6, 2H, CH_2O-DPTBS), 4.14 (dd, J=6.1, 11.4, 1H, 4'-CH_3COSCH_2), 4.32-4.52 (m, 3H, H-3', H-5', 4'-CH_3COSCH_2), 4.90 (br., 1H HO), 5.71 (s, 1H, H-2'), 7.48-7.55 (m, 8H, ar.-H, H-5), 7.62 (m, 2H, ar.-H), 7.70 (m, 4H, ar.-H), 7.80 (d, J=7.5, 1H, H-6), 7.96 (m, 2H, ar.-H), 9.18 (br., 1H, NH);

^{13}C-NMR ($CDCl_3$) δ: 19.2 ($SiCCH_3$), 20.8 (CH_3COS), 26.9 (3xCH_3), 37.5 ($HOCH_2CH_2$), 45.8 ($C_{4'}$), 60.4 ($HOCH_2$), 60.9 ($C_{4''}$), 76.7 ($C_{5'}$), 80.4 ($C_{3'}$), 94.5 ($C_{2'}$), 96.3 (C_5), 127.6 (Si-0-ar.-CH), 129.1 (Si-p-ar.-CH), 129.7, 129.4 (CO-Ph-o-CH, CO-Ph-m-CH), 132.9 (Si-ar.-C), 133.5 (CO-Ph-p-CH), 133.3 (CO-Ph-C), 135.7 (Si-m-ar.-CH), 143.4 (C_6), 155.8 (C_4), 162.6 (C_2), 166.3 (Ph-CO), 170.8 (COS);

FAB-MS (m/e, relative intensities): 656 ([M-31]$^+$, 24), 598 (6), 460 (3), 399 (4), 351 (2), 307 (30), 289 (15), 279 (32), 216 (86), 199 (17), 183 (5), 154 (100), 131 (81), 105 (48), 91 (20), 77 (30).

N^4-Benzoyl-1-[(2R, 3R, 4R, 5R)-4-acetylthiomethyl-5-(2-*t*-butyldiphenylsilyloxyethyl)-3-(1-imidazothiocarbonyloxy)tetrahydrofuran-2-yl]cytosine (5c).

A mixture of 4c (755 mg) and 1,1'-thiocarbonyl diimidazole (295 mg, 1.5 eq.) in dry DMF (11 mL) was stirred at RT for 1.5 hr (TLC, 7.5% MeOH/CH_2Cl_2, R_f=0.51). The DMF was then removed under vacuum (0.5 torr, 55°C). The residue was purified by chromatography on silica gel (gradient from EtOAc/hexane =8:2 to 9:1) to give 5c (764 mg, 87%) as a white foam.

^{1}H-NMR ($CDCl_3$) δ: 1.08 (s, 9H, 3xCH_3), 1.80 (m, 2H, CH_2CH_2O-DPTBS), 2.18 (s, 3H, CH_3COS), 2.79 (m, 1H, H-4'), 2.94 (m, 2H, 4'-CH_3COSCH_2), 3.88 (t, J=4.7, 2H, CH_2O-DPTBS), 4.30 (m, 2H, H-3', H-5'), 5.73 (s, 1H, H-2'), 7.48-7.55 (m, 9H, ar.-H, H-5, H^{Im}-5), 7.62 (m, 2H, ar.-H), 7.70 (m, 6H, ar.-H, H^{Im}-2,4), 7.74 (d, J=7.5, 1H, H-6), 7.96 (m, 2H, ar.-H), 9.48 (br., 1H, NH);

^{13}C-NMR ($CDCl_3$) δ: 19.2 (SiCCH$_3$), 23.8 ($C_{4''}$), 26.7 (3xCH_3), 30.2 (CH_3COS), 36.8 (HOCH$_2$CH_2), 47.2 ($C_{4'}$), 60.7 (HOCH_2), 76.3 ($C_{5'}$), 80.9 ($C_{3'}$), 94.4 ($C_{2'}$), 96.7 (C_5), 127.8, 128.6 (Si-o-ar.-CH, $^{Im}C_5$), 129.3 (Si-p-ar.-CH), 129.6, 129.9 (CO-Ph-o-CH, CO-Ph-m-CH), 131.2, 132.9, 133.4 (Si-ar.-C, $^{Im}C_4$, $^{Im}C_2$), 134.9 (CO-Ph-p-CH), 135.7 (Si-m-ar.-CH), 143.7 (C_6), 155.6 (C_4), 162.4 (C_2), 166.3 (Ph-CO), 170.8 (COS), 196.0 (OCS-Im);

FAB-MS (m/e, relative intensities): 672 ([M-127]$^+$, 16), 628 (4), 614 (6), 557 (6), 469 (2), 414 (3), 379 (2), 321 (3), 279 (98), 216 (100), 199 (32), 183 (15), 154 (22), 13 (55), 105 (68), 91 (16), 77 (26).

N^4-Benzoyl-1-[(2R, 4R, 5R)-4-acetylthiomethyl-5-(2-*t*-butyldiphenylsilyloxyethyl)-tetrahydrofuran-2-yl]cytosine (2c).

5c (124 mg, 0.155 mmol), Bu_3SnH (164 μL, 4 eq.) and AIBN (10 mg, 0.4 eq., 10%) were dissolved in toluene (1.5 mL). The mixture was slowly injected into refluxing toluene (dry , 1.5 mL) under Ar and stirred for 2 min (TLC, 5% MeOH/CH_2Cl_2, R_f=0.32). The toluene was then evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (gradient from 1 to 4% MeOH/CH_2Cl_2) to give 2c (89 mg, 86% yield) as a white foam.

^{1}H-NMR ($CDCl_3$) δ: 1.08 (s, 9H, 3xCH_3), 1.80-2.0 (m, 2H, CH_2CH_2O-DPTBS), 2.05 (s, 3H, CH_3COS), 2.18-2.50 (m, 3H, H-3', H-4'), 3.88 (dd, J=6.8, 13.8, 2H, CH_2O-DPTBS), 4.10 (m, 3H, 4'-CH_3COSCH_2, H-5'), 5.98 (dd, J=2.8, 6.9, 1H, H-2'), 7.38-7.55 (m, 8H, ar.-H, H-5), 7.62 (m, 2H, ar.-H), 7.70 (m, 4H, ar.-H), 7.74 (d, J=7.5, 1H, H-6), 7.96 (m, 2 H, ar.-H), 8.75 (br., 1H, NH);

^{13}C-NMR ($CDCl_3$) δ: 19.19 (SiCCH$_3$), 20.74 (CH_3COS), 26.88 (3xCH_3), 37.04 ($C_{3'}$), 37.55 (HOCH$_2$CH_2), 41.43 ($C_{4'}$),, 60.79 (HOCH_2), 63.77 ($C_{4''}$), 77.22 ($C_{5'}$), 80.93

($C_{2'}$), 87.40 (C_5), 128.44 (Si-o-ar.-CH), 129.10 (Si-p-ar.-CH), 129.4, 129.9 (CO-Ph-o-CH, CO-Ph-m-CH), 133.22 (Si-ar.-C), 133.58 (CO-Ph-p-CH), 135.57 (Si-m-ar.-CH), 143.7 (C_6), 155.9 (C_4), 162.2 (C_2), 170.5 (COS).

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REFERENCES

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2. (a) Schneider, K. C.; Benner, S. A. *Tetrahedron Lett.* **1990**, *31*,, 335-338; (b) Huang, Z.; Schneider, K. C.; Benner, S. A. *J. Org. Chem.* **1991**, *56*, 3869-3882; (c) Huang, Z.; Benner, S. A. *Synlett.* **1993**, 83-84.
3. (a) Huang, Z., *Synthesis of Sulfide and Sulfone 2'-Deoxyribonucleotide analogs.* ETH Diss. No. 10429, **1993**, 10429; (b) Richert, C. *Dimethylensulfon-verbrückte Oligoribonucleotide.* ETH Diss.No. 10895 **1994**, 10895; (c) Roughton, A. L.; Portmann, S.; Benner, A. S.; Egli, M. *J. Am. Chem. Soc.* **1995**, *117*, 7249-7250; (d) Richert, C.; Roughton, A. L.; Benner, S. A. *J. Am. Chem. Soc.* **1996**, *118*, 4518-4531; (e) Hyrup, B.; Richert, C.; Schulte-Herbrüggen, T.; Benner, S. A.; Egli, M. *Nucl. Acids Res.* **1995**, *23*, 2427-2433; (f) Bäschlin, D. K.; Hyrup, B.; Benner, S. A.; Richert, C. *J. Org. Chem.* **1996**, *61*, 7620-7626; (g) Blättler, M. *Dimethylensulfon-modifizierte Oligodesoxyribonukleotid-analoga.* ETH Diss. 12109 (1997).
4. (a) Volante, R. P. *Tetrahedron Lett.* **1981**, *22*, 3119-3122; (b) Mukaiyama, T.; Matsueda, R.; Suzuki, M. *Tetrahedron Lett.* **1970**, *11*, 901-904; (c) Nishio, T.; *J. Chem. Soc. Chem. Commun.* **1989**, 205-206; (d) Gingras, M.; Harpp, D. N. *Tetrahedron Lett.* **1990**, *31*, 1397-1400.
5. (a) Marriott, J. H.; Mottahedeh, M.; Reese, C. B. *Tetrahedron Lett.* **1990**, *31*, 7485-7488; (b) Beretta, E.; Cinquini, M.; Colonna, S.; Fornasier, R. *Synthesis* **1974**, *6*, 425-426.
6. (a) Mitsunobu, O.; Eguchi, M. *Bull. Chem. Soc. Jap.* **1971**, *44*, 3427-3429; (b) Mitsunobu, O. *Synthesis* **1981**, *13*, 1-28.
7. (a) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc. Perkin I* **1975**, 1574-1585; (b) Barton, D. H. R.; Subramanian, R. *J. Chem. Soc. Perkin I* **1977**, 1718-1723.