Tetrahedron Letters,Vol.31,No.3,pp 335-338,1990 0040-4039/90 $3.00 + .00
Printed in Great Britain Pergamon Press plc

BUILDING BLOCKS FOR OLIGONUCLEOTIDE ANALOGS WITH DIMETHYLENE-SULFIDE, -SULFOXIDE, AND -SULFONE GROUPS REPLACING PHOSPHODIESTER LINKAGES

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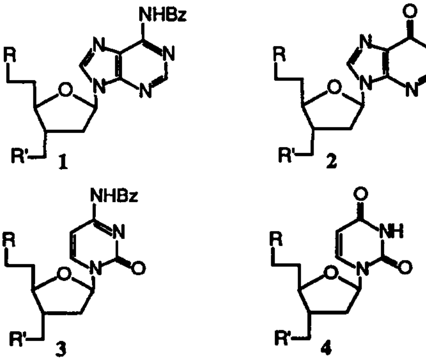
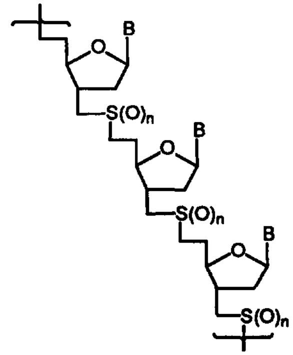
Summary
Routes are presented for the synthesis of 3',5'-bishomo-deoxyribonucleosides, building blocks needed to synthesize oligonucleotide analogs where the -O-PO_2-O- groups are replaced by -CH_2-SO_2-CH_2-,-CH_2-S-CH_2–, and -CH_2-SO-CH_2- units. As isosteric, diastereomerically pure (in the first two cases), non-ionic analogs of natural oligonucleotides, such molecules have potential application as "anti-sense" oligonucleotide analogs.

Non-ionic oligonucleotide analogs are important synthetic targets, as they are likely to be stable to biological degradation, cross biological barriers, bind to natural oligonucleotides with a complementary sequence, and disrupt their biological function.[1] It is well-recognized that if such compounds could be found and applied *in vivo*, they could be used to treat virtually every disease involving the unwanted expression of genetic information.[2] As all viral diseases, many cancers, most bacterial and parasitic maladies, and many other diseases are of this type, the excitement has been understandable.

Some time ago we reported that sulfones (such as dimethylsulfone and sulfolane) as cosolvents can assist the penetration of natural oligonucleotides into cells.[3] This observation suggested to us that incorporating the sulfone unit directly into an oligonucleotide by replacing the phosphodiester (-O-PO_2-O-) groups by sulfone (-CH_2-SO_2-CH_2-) groups might yield an especially attractive oligonucleotide analog capable of penetrating cell membranes without co-solvents. Further, sulfones are non-ionic, achiral, isosteric analogs of phosphate diesters, and are stable to both chemical and biochemical degradation, making them ideal analogs for phosphate esters on other grounds as well.

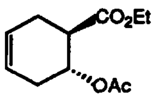


a: R = SH, R' = OH
b: R = SAc, R' = OBz

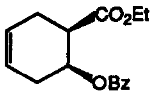


5 (n=0,1,2; B=A,C,G,U)

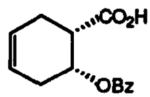
We report here a route for the synthesis of deoxyribonucleoside analogs **1-4**, analogs bearing functionalization appropriate for their use as building blocks in the synthesis of oligonucleotide analogs having the phosphodiester groups replaced by dimethylene-sulfide, -sulfoxide, or -sulfone units (**5**). The building blocks are prepared in their protected forms (**1b-4b**), as these derivatives are more suitable for long term storage, and are readily converted to their unprotected forms immediately prior to coupling by standard procedures.[4]



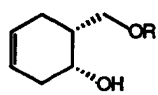
(±)**6**



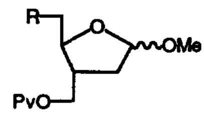
(±)**7**



(-)**8**

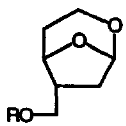


9: R=H
10: R=Pv

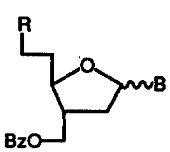


11: R=CHO
12: R=CH_2OH

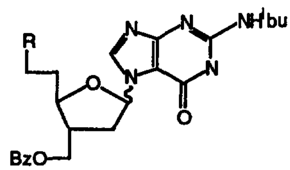
The synthesis started from **6**,[5] which was deacetylated with NaOEt in EtOH, and then converted to cis-benzoate **7** by treatment with PPh_3, diisopropylazodicarboxylate (DIAD) and benzoic acid in THF (72 % overall). Benzoate **7** was resolved into its antipodes by enantioselective hydrolysis with pig liver esterase[6] in H_2O/t-BuOH 9:1 at pH 7 (maintained by automatic titration with dilute NaOH) to give, after addition of 0.45 mol eq. of hydroxide and extractive workup, acid (–)**8** ($[\alpha]_D$=-89.5°, c=6.35, acetone) with >99 % ee (determined, after conversion to the corresponding methylester with diazomethane, by NMR in the presence of $Eu(hfc)_3$). The absolute configuration of (–)**8** was assigned by direct correlation to glucose.[7] (–)**8** was reduced with $LiAlH_4$ in THF to diol **9** (mp: 65-66°C), which was selectively protected with pivaloyl chloride (PvCl) in pyridine at -15°C to furnish **10** in an 85% overall yield. **10** was converted to **12** in 63 % yield by the following one pot procedure: (1) ozonization in methanol at -78°C, (2) reductive work-up with dimethylsulfide, (3) selective acetalization to the cyclic acetal **11** by stirring the reaction mixture in methanol for 14 days, and (4) reduction of **11** with excess $NaBH_4$. Presumably, traces of acid which formed during ozonolysis catalyse the selective acetalization in step 3. The 6-hydroxyl group of **12** was internally protected by acid catalysed cyclization (Dowex® H+) in refluxing toluene (85%) to give **13**. Since the pivaloate group in **13** is too stable to be removed in the presence of amide protected bases, **13** was converted to benzoate **14** by hydrolysis with 1 M NaOH (3 h, 25°C), followed by benzylation (BzCl, pyridine, 83% overall).



13: R=Pv
14: R=Bz



15: R=OH, B=A^{Bz}
16: R=OH, B=$G^{i\text{-}Bu}$
17: R=SAc, B=$G^{i\text{-}Bu}$
18: R=OH, B=C^{Bz}
19: R=SAc, B=C^{Bz}
20: R=OH, B=U
21: R=SAc, B=U



22: R=OH
23: R=SAc

The appropriately protected bases were (after bis-silylation with trimethylsilyl chloride/hexamethyldisilazane (TMSCl/HMDS) or, preferentially, *in situ* using N-methyl-N-TMS-trifluoroacetamide, MSTFA) introduced into **14** via Lewis-acid catalysis using TMSOTf in acetonitrile.[8] The thioacetate moiety was introduced in all cases by a Mitsunobu reaction involving the slow addition of a mixture of the nucleoside analog and AcSH (2 eq.) to a pre-formed complex of PPh_3 (2 eq.) and DIAD in THF at 0°C.[9] Thus, **14** was reacted with bis-TMS-benzoyladenine in the presence of TMS-triflate (TMSOTf, 0.5 eq.) at 40°C for 30 min. to furnish **15** as a 1:1 mixture of anomers in 64% yield. The anomers were separated by HPLC (silica gel, CH_2Cl_2/THF 65:35) and the β-anomer was then converted to thioacetate **1b** in 69% yield. Reaction of **14** with bisTMS-^{i}butyrylguanine in the presence of TMSOTf (0.5 eq.) at 40°C for 1h gave the guanosine analog **16** as a 2:1 mixture of N^9- and N^7-isomers, each as a 1:1 mixture of anomers. The regio-isomers were separated by silica gel chromatography (CH_2Cl_2/MeOH 9:1) to furnish the N^9-isomer **16** in 56% yield and the N^7-isomer **22** (30%). After conversion of the N^9-isomer (**16**) to thioacetate **17** (73%) the anomers were separated by HPLC (silica gel, $CHCl_3$/EtOH 40:1, H_2O-saturated), to give β-anomer **2b**. The benzoylcytosine derivative **18** was obtained as a 1:1 mixture of anomers in 64% yield by treatment of **14** with bis-TMS-benzoylcytosine at 40°C for 1h, employing TMSOTf (1.8 eq.). The anomers were separated, after conversion of **18** to the thioacetate **19** (87%), by HPLC (silica gel, EtOAc/hexane/H_2O 7:3:0.07) to furnish pure β-anomer **3b**. Uracil derivative **20** (as a 1:1 mixture of anomers) was synthesized by reaction of **14** with bis-TMS-Uracil and TMSOTf (0.3 eq.) for 15 min. at room temperature (75%). Conversion of **20** to thioacetate **21** (78%), followed by HPLC-resolution of the anomers (silica gel, EtOAc/hexane/H_2O 7:3:0.07) afforded pure β-anomer **4b**.[10]

Structure assignments: The N^7- and N^9-isomers of the purine nucleoside analogs were distinguished by NMR spectroscopy. Uniformly in N-alkylated purines, the H-8 and H-1 proton signals and the C-4, C-8 and C-1' carbon signals of the N^7-isomers are shifted downfield relative to the corresponding resonances of the N^9-isomers, while the signal for C-5 of the N^7-isomer is more shielded relative to the signal of the N^9-isomer.[11] In the case of the analogs of guanosine, the N^7-isomers **22** and the N^9-isomers **17** were isolated. The α- and β-anomers of **22** were separated by crystallization from CH_2Cl_2/pentane, and the β-anomer converted to thioacetate **β-23**. The H-8 and H-1' proton signals and the C-4, C-8 and C-1' carbon signals of the chromatographically more mobile compound, assigned as the N^7-isomer **23**, are downfield, and the C-5 resonance upfield relative to the corresponding resonances of its isomer **2b**, assigned as the the N^9-isomer.

For the adenine derivative **1b**, the ^{13}C NMR spectrum of **1b** was compared to the spectrum of N^6-benzoyl-2'-deoxyadenosine and other benzoylated adenosines.[12] The chemical shifts of the purine ^{13}C signals of **2b** and N^6-benzoyl-2'-deoxyadenosine are similar within a range of 1.5 ppm, but differ, in the manner outlined above, from the chemical shifts reported for N^7-ribofuranosyl-adenine.[13]

The anomeric configurations of compounds and **1b**-**4b** were assigned by NOE-difference spectroscopy. In all cases irradiation at H-1' gave a significant enhancement of the H-4' and H-2'α protons for the compound assigned the β-anomeric configuration, whereas the isomers assigned the α-anomeric configuration showed significant enhancement upon irradiation of H-1' only at H-2'β. The anomeric configuration of the N^7-isomer **23** was tentatively assigned as β from the chemical shift of the H-4' signal. It was found that for all nucleoside analogs synthesized here, the H-4' signal of the α-anomer is shifted downfield relative to the signal of the β-anomer, presumably because of the anisotropic effect of the bases in the α-position. In the case of **3b** and **4b**, upon irradiation of H-1' NOE-enhancement is also found at H-6, which confirms that the N^1-isomers were obtained.

In conclusion, building blocks **1b**-**4b** have been synthesized as the necessary first step for the synthesis of analogs of oligonucleotides having $-CH_2-S-CH_2-$, $-CH_2-SO-CH_2-$, and $-CH_2-SO_2-CH_2-$ units replacing the -O-PO_2-O- groups in natural oligonucleotides. Preliminary work on the coupling of these building blocks has produced tetramers in satisfactory yield. Progress in this area will be reported separately.

Notes and References

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10. All compounds were fully characterized by elemental analysis and/or UV-, mass-, IR-, and NMR-spectroscopy: **1b**: ^{1}H-NMR ($CDCl_3$) δ 1.98-2.21 (m, 2H), 2.32 (s, 3H), 2.57 (ddd, J=7,9,13.5 Hz, 1H), 2.78-2.99 (m, 3H, C3'-H), 3.18 (ddd, J=5, 5, 13.5 Hz, 1H), 4.16 (td, J=3.5,8.5 Hz, 1H), 4.47 (m, 2H), 6.37 (dd, J = 3,7 Hz, 1H), 7.45-7.65 (m, 6H), 8.04 (m, 4H), 8.29 (s, 1H), 8.81 (s, 1H), 9.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.9, 30.6, 35.3, 35.9, 42.2, 64.4, 82.7, 85.2, 123.9, 127.9, 128.6, 128.8, 129.6, 132.7, 133.4, 133.7, 141.5, 149.6, 151.2, 152.5, 164.8, 166.3, 195.5. **2b**: ^{1}H NMR ($CDCl_3$) δ 1.27, 1.28 (2d, J=8.5 Hz, 6H), 1.97, 2.12 (2m, 2H), 2.29 (s, 3 H), 2.40 (ddd, J=13.5,8.5,7.5 Hz, 1H), 2.72 (m, 2H), 2.95 (m, 2H, C3'-H), 3.11 (ddd, J=5,5,13.5, 1H), 4.05 (ddd=td, J=8,3.5 Hz, 1H), 4.39 (mc, 2H), 6.05 (dd, J=7.5,3.5 Hz, 1H), 7.49 (mc, 2 H), 7.60 (mc, 1 H), 7.80 (s, 1H), 8.87 (s, 1H), 12.08 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 19.0, 19.1, 25.6, 30.6, 34.7, 35.6, 36.2, 41.8, 64.9, 82.5, 84.7, 121.8, 128.5, 129.5, 133.3, 137.6, 147.7, 147.8, 155.8, 166.3, 179.5, 196.0. **3b**: ^{1}H NMR ($CDCl_3$) δ 1.97-2.24 (2m, 2 H), 2.30-2.50 (s, m, 5H), 2.59 (13.5,8,6.5 Hz, 1H), 2.94 (ddd, J = 13.5,8.5,7.5, 1H), 3.29 (ddd, J=13.5,9.5,7 Hz, 1H), 4.10 (td, J=10,7,3 Hz, 1H), 4.40 (m, 2H), 6.11 (dd, J=6.5,3 Hz, 1H), 7.44 (m, 7H), 7.91 (d, J=8 Hz, 2H), 8.02 (m, 2H), 8.20 (d, J=7.5 Hz, 1H). **4b**: ^{1}H NMR ($CDCl_3$) δ 1.91-2.17(2m, 2H), 2.28 (m, 1H), 2.33 (s, 3H), 2.42 (m, 2H), 2.89, 3.18 (2mc, 2H), 3.99 (ddd=td, J=4.5,4.5,1.5 Hz, 1H), 4.38 (mc, 2H), 5.81 (d, J=8 Hz), 6.09 (dd, J=6.5,4 Hz, 1H), 7.26 (m, 2H), 7.39 (m, 1H), 7.56 (d, J=8 Hz), 8.02 (m, 2H), 9.04 (s, 1H). **23**: ^{1}H NMR ($CDCl_3$) δ 1.22 (2d=t, J=7 Hz, 6 H), 1.99-2.17 (m, 2H), 2.32 (s, 3H), 2.50-2.64 (m, 3H), 3.00 (m, 2H), 3.21 (dddd=sept., J=5,8.5,14 Hz,1H), 4.12 (dt, 3,9 Hz, 1H), 4.37, 4.45 (2dd, J=5,11 Hz, 2H), 6.53 (t, J=5 Hz, 1H), 7.46 (m, 3H), 7.59 (m, 2H), 8.18 (s, 1H), 10.52 (s, 1H), 12.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.1, 19.2, 26.1, 30.6, 35.2, 35.9, 38.3, 41.6, 64.2, 83.0, 128.6, 129.6, 133.4, 140.7, 148.1, 153.1, 158.0 (C-4), 166.3, 180.1, 195.5.
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(Received in Germany 16 October 1989)