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# Dinucleoside Monophosphate Analogues Containing Disulfide Linkages

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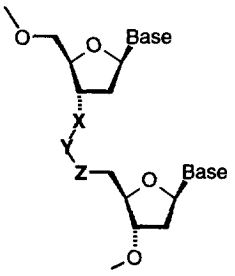
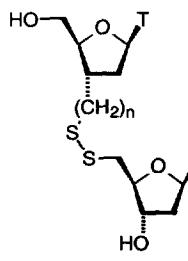
***Abstract:*** The synthesis of two dinucleoside monophosphate analogues is described in which the 3'-O-P-O-5' phosphodiester linkage is replaced by either an isosteric 3'-C-S-S-5' or a shorter 3'-S-S-5' linkage. In both cases the -S-S- bond was formed through a disulfide exchange reaction using an activated *S*-nucleosidyl *S*-aryl disulfide and a suitably protected derivative of 5'-thiothymidine.


Synthetic oligonucleotides are of considerable importance because of their ability to inhibit gene expression through the antisense effect.[1] Although the first generation of modified antisense oligonucleotides retained the phosphorus atom in the internucleoside linkage, as in the phosphorothioate and methylphosphonate analogues, backbone modifications in which the phosphodiester linkage is completely replaced with a non-ionic, hydrolytically stable, non-chiral linkage have attracted increasing attention.[1,2] It is noteworthy that interest in polynucleotides containing these "dephospho" linkages as cell permeable agents with the potential to interfere with mRNA function, was first expressed by Cassidy and Jones[3] over 30 years ago and predates Zamecnik and Stephenson's[4] proposal of the antisense therapeutic principle using synthetic oligonucleotides. More than 50 of these second generation analogues have been prepared and whilst all result in increased nuclease stability, they generally display lower affinity for their RNA targets than the phosphodiester control; exceptions to this include the methylene(methylimino) (MMI)[5] (**1**), thioformacetal[6] (**2**) and amide[7-9] (**3**, **4**) linkages that all exhibit similar or increased RNA binding properties.




| | X | Y | Z |
|---|---|---|---|
| **1** | $CH_2$ | $NCH_3$ | O |
| **2** | S | $CH_2$ | O |
| **3** | $CH_2$ | CO | NH |
| **4** | $CH_2$ | NH | CO |

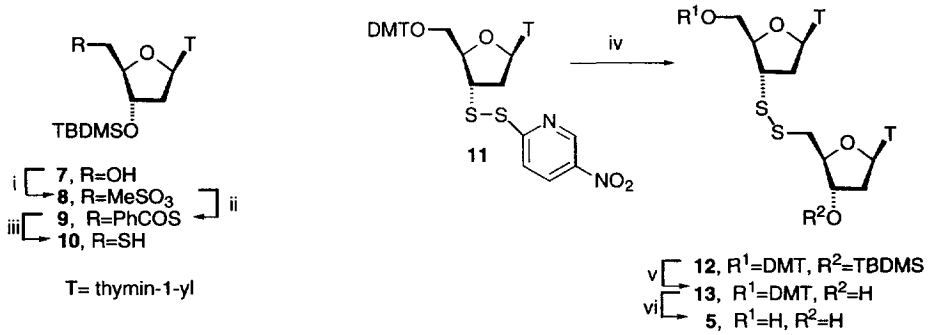



**5** n=0
**6** n=1

To our knowledge there has been no investigation of backbone linkages that contain disulfide groups (such as **5** and **6**) and yet this type of linkage appears to have properties that make it an attractive surrogate for the phosphodiester linkage. In the case of the amide analogues the conformational rigidity

of the amide bond leads to preorganisation of the linkage in a conformation that is favourably disposed to duplex formation. Solution conformation studies have shown that disulfide bonds also exist as rigid systems which are conformationally restricted[10] and their inclusion in internucleoside linkages could potentially yield oligonucleotide analogues that are similarly predisposed. For the isosteric disulfide linkage (**6**) the 3'-oxygen atom is replaced by a $CH_2$ group. This substitution, which favours the 3'-*endo* sugar pucker, is known to have a beneficial influence on duplex stability and is largely responsible for the relatively high affinity that oligomers containing linkages **1**[11] and **3**[12] exhibit for RNA. Indeed modelling studies show that the disulfide linkage **6** has many structural parameters that are similar to the promising MMI linkage (**1**).[13]

We now report the synthesis of two dithymidine monophosphate analogues that contain disulfide linkages in which the 3'-O-P-O-5' phosphodiester linkage is replaced by either an isosteric 3'-C-S-S-5' (**6**) or a shorter 3'-S-S-5' (**5**) linkage. For both thymidine dimers it was anticipated that the -S-S- bond would be formed through a disulfide exchange reaction using an activated *S*-nucleosidyl *S*-aryl disulfide and a suitably protected derivative of 5'-thiothymidine. The latter compound was readily accessible from 3'-*O*-*t*-butyldimethylsilylthymidine (**7**)[14] in three steps. Reaction of **7** with methanesulfonyl chloride in pyridine gave the mesylate (**8**, **Scheme 1**) in 95%. Conversion to the thiobenzoate ester (**9**) was achieved in 84% by displacement with sodium thiobenzoate in DMF. Subsequent hydrolysis of the thiobenzoate ester with NaOH in aqueous ethanol under an inert nitrogen atmosphere, gave 3'-*O*-*t*-butyldimethylsilyl-5'-thiothymidine (**10**) in 75% yield. With the lower 5'-thiothymidine portion of both dimers in hand, attention was focused on the upper 3'-section.

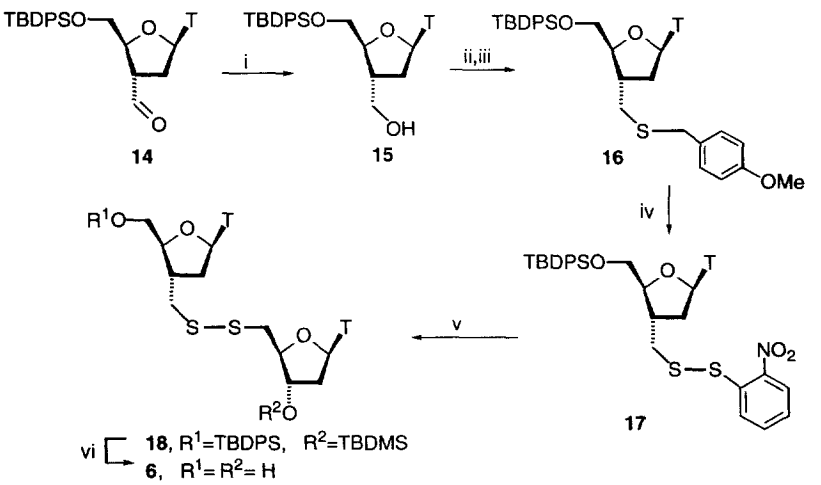



**Scheme 1** *Reagents and conditions:* i) $MeSO_2Cl$, pyridine, 0°C, 15 h, 95%; ii) NaSBz, DMF, 90°C, 2 h, 84%; iii) 2 M NaOH in $EtOH/H_2O$ (9:1), $N_2$, 0°C, 1 h then AcOH, 75%; iv) **10**, $CH_2Cl_2$, rt, 2 h, 69%; v) $Et_3N.3HF$, THF, rt, 15 h, 84%; vi) 80% AcOH, rt, 0.5 h, 84%.

The synthesis of **5** required 3'-deoxy-5'-*O*-dimethoxytrityl-3'-*S*-(5-nitropyridyl-2-disulfanyl)thymidine (**11**) as the substrate for the disulfide exchange reaction. The pyridyl disulfide **11** was prepared by a similar procedure to that previously reported for the corresponding monomethoxytrityl derivative.[15] The key disulfide exchange reaction between **11** and **10** (1.5 equiv) was performed in $CH_2Cl_2$ and progression of the reaction was accompanied by the generation of a deep red colour produced on release of 2-mercapto-5-nitropyridine. Following chromatography the fully protected dimer (**12**) was isolated in 69% yield. The TBDMS group was readily removed with

$Et_3N.3HF$ to yield the DMT-protected dimer (**13**) (84%) which is the immediate precursor to the dinucleotide phosphoramidite required for oligonucleotide synthesis. Complete deprotection to yield **5** was achieved by an additional treatment with 80% aqueous acetic acid (70%).

The 400 MHz $^1H$ NMR spectrum of **5** showed a distinctive doublet of doublets (6.16 ppm, $^3J$=4.7, 6.7 Hz) and a triplet (6.24 ppm, $^3J$=7.1 Hz) for H1' of the 3'-thiothymidine and 5'-thiothymidine moieties, respectively. The multiplet for the H3' proton of the 5'-thiothymidine moiety, which has a neighbouring oxygen atom, was observed at 4.34 ppm, considerably downfield from the H3' proton of the 3'-thiothymidine portion (3.68 ppm) which is next to sulfur.




**Scheme 2** *Reagents and Conditions:* i) $NaBH_4$, MeOH, 0°C, 0.5 h, 95%; ii) $MeSO_2Cl$, pyridine, 0°C, 15 h, 84%; iii) 4-methoxy-α-toluenethiol, NaH, DMA, 100°C, 0.5 h, 55%; iv) 2-nitrophenylsulfenyl chloride, AcOH-DCM (1:9 v/v), 0°C, 1 h, 75%; v) **10**, $CH_2Cl_2$, 1.5 h, rt, 91%; vi) $Et_3N.3HF$, THF, 15 h, rt, 22%.

Synthesis of the top half of dimer **6** started from the previously reported aldehyde[16] (**14**, **Scheme 2**). Almost quantitative reduction to the hydroxymethyl nucleoside (**15**) was achieved using excess sodium borohydride in MeOH at 0°C. This procedure is analogous to that which we have recently reported for the reduction of the aldehyde function in 2'-deoxy-2'-α-*C*-(2-oxoethyl)-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxyl)uridine.[17] Subsequent treatment of the hydroxymethyl nucleoside with methanesulfonyl chloride in pyridine gave the mesylate (84%) which, on reaction with the sodium salt of 4-methoxy-α-toluenethiol in *N,N*-dimethylacetamide (DMA) at 100°C, gave the benzyl sulfide **16** (55%). Conversion to the activated disulfide **17** was achieved under mild conditions according to the general procedure recently published by Reese and co-workers.[18] Thus, treatment of **16** with 2-nitrobenzenesulfenyl chloride in a mixture of $CH_2Cl_2$/AcOH at 0°C yielded the activated disulfide **17** in 75%. Reaction of the activated disulfide **17** with 3'-*O*-*t*-butyldimethylsilyl-5'-thiothymidine, as described above, gave the fully protected dinucleoside disulfide (**18**) in 91% yield. Removal of the silyl protecting groups could be achieved using $Et_3N.3HF$. The reaction appeared to proceed relatively

cleanly, as determined by TLC analysis, but it proved difficult to separate the dinucleoside disulfide (**6**) from the unwanted triethylammonium salts and consequently the purified yield of **6** was low (22%).

The $^1$H NMR spectrum of **6** showed a distinctive pair of triplets at 6.10 ($^3J$=6.1 Hz) and 6.25 ppm ($^3J$=6.7 Hz), for H1' of the 3'-deoxy-3'-mercaptomethylthymidine and 5'-thiothymidine moieties, respectively. Preliminary studies show that the disulfide linkage of both dimers is stable to the conditions used in the oxidation, detritylation and ammonia deprotection steps of oligonucleotide synthesis and it should therefore be possible to prepare oligomers containing these linkages. We believe that thermal melting studies on oligonucleotide duplexes containing these disulfide linkages will be useful in determining the structural features that are necessary for the design of effective antisense agents.

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**References**

1. De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995,** *28*, 366-374.
2. Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993,** *36*, 1923-1937.
3. Cassidy, F.; Jones, A. S. *Eur. Polym. J.* **1966,** *2*, 319.
4. Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1978,** *75*, 280-284.
5. Vasseur, J.-J.; Debart, F.; Sanghvi, Y. S.; Cook, P. D. *J. Am. Chem. Soc.* **1992,** *114*, 4006.
6. Jones, R. J.; Lin, K.-Y.; Milligan, J. F.; Wadwani, S.; Matteucci, M. D. *J. Org. Chem.* **1993,** *58*, 2983.
7. Lebreton, J.; Mesmaeker, A. D.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Frier, S. M. *Tetrahedron Lett.* **1993,** *34*, 6383.
8. Lebreton, J.; Waldner, A.; Lesueur, C.; Mesmaeker, A. D. *Synlett* **1994,** 137.
9. De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Synlett* **1993,** 733.
10. Pappalardo, C. C.; Ronsisvalle, G. *Tetrahedron* **1972,** *28*, 4147.
11. Mohan, V.; Griffey, R. H.; Davies, D. H. *Tetrahedron* **1995,** *51*, 8655.
12. De Mesmaeker, A.; Lesueur, C.; Bevierre, M.-O.; Waldner, A.; Fritsch, V.; Wolf, R. M. *Angew. Chem. Int. Ed. Engl.* **1996,** *35*, 2790-2794.
13. Sanghvi, Y. S.; Singh, U. C. .
14. Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L. *Can. J. Chem.* **1978,** *56*, 2768.
15. Higson, A. P.; Scott, G. K.; Earnshaw, D. J.; Baxter, A. D.; Taylor, R. A.; Cosstick, R. *Tetrahedron* **1996,** *52*, 1027.
16. Sanghvi, Y. S.; Bharadwaj, R.; Debart, F.; Mesmaeker, A. D. *Synthesis* **1994,** 1163.
17. Lawrence, A. J.; Pavey, J. B. J.; Cosstick, R.; O'Neil, I. A. *J. Org. Chem.* **1996,** *61*, 9213.
18. Johnson, R.; Joshi, B. V.; Reese, C. B. *J. Chem. Soc., Chem. Commun.* **1994,** 133.

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