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# SYNTHESIS OF A *N*-ACYLSULFAMIDE LINKED DINUCLEOSIDE AND ITS INCORPORATION INTO AN OLIGONUCLEOTIDE

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**Abstract**: A *N*-acylsulfamide linked thymidine dinucleoside was synthesized and incorporated into an oligonucleotide (ON). The interest is in a linkage analog that has a higher pKa relative to a phosphodiester and when incorporated into ONs is capable of helix formation with complementary RNA. The hybridization property of the resultant ON with RNA was shown to result in significant destabilization. 

The phosphorothioate internucleotide analog has emerged as the linkage of choice for the development of antisense oligonucleotides (ONs) as therapeutic agents.[1] The phosphorothioate linkage imparts to an antisense ON, nuclease stablity, reasonable affinity to its complementary RNA and the ability to recruit RNase H cleavage of the bound RNA.[2] Despite these virtues, new analogs are still of interest due to the poor cellular permeation properties[1,2] of phosphothioate ONs. Numerous backbone modifications have been made to increase the cellular permeability of ONs but none have resulted in an improvement.[3]

Anionic oligonucleotides such as phosphorothioates have been shown to strongly associate with cells and to be taken up into endosomes.[4] These resulting endosomes acidify.[4] The ONs subsequently remain trapped and do not efficiently cross the lipid bilayer into the cytoplasm. This pathway offers the opportunity to develop oligonucleotide analogs that can exploit the acidification process to effect permeation into the cytoplasm of cells. The acidification trigger for endosomal release is well documented for organic acids,[5] bile acids,[6] and carboxylic porphyrins.[7] We speculated that an internucleotide modification with the appropriate pKa (approximately 5 to 7) could facilitate diffusion from the acidic endosome into the cytoplasm of the cell. In media or blood (pH ~7.2) the linkage would be largely an anion and therefore confer good water solubility and the trafficking into endosomes observed for conventional anionic phosphorothioates. In the endosome (pH ~5.5) this linkage would be largely neutral and more lipophillic. Improved diffusion across the lipid

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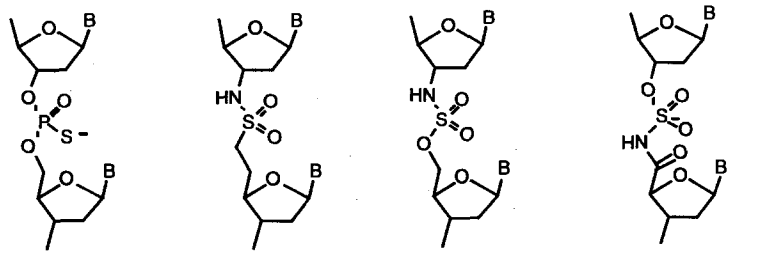
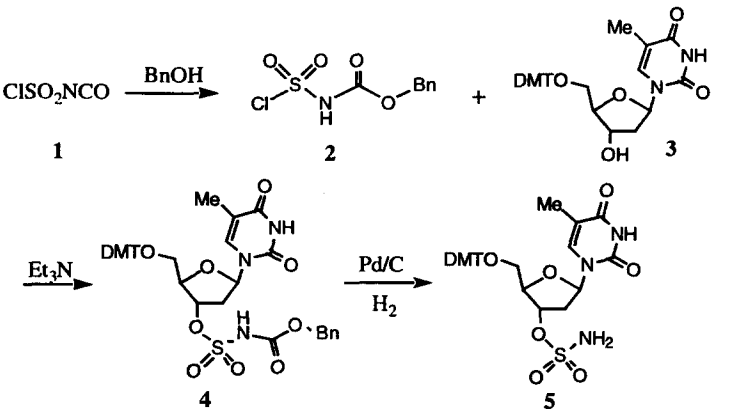
bilayer and permeation into the cytoplasm could be possible. Once in the cytoplasm (pH ~7.2), the linkage would again be largely an anion and may confer RNase H activation when hybridized with RNA molecules. The first requirement of such an ON analog is maintaining the ability to hybridize to complementary RNA.



Phosphorothioate Sulfonamide Sulfamide *N*-Acylsulfamide

**Figure 1**

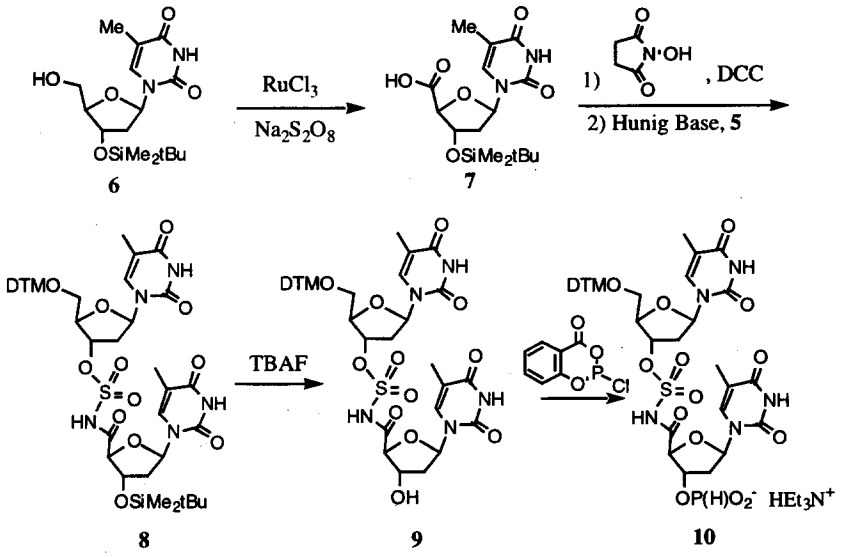
These considerations prompted us to select the *N*-acylsulfamide linkage as a possible candidate for an analog possessing the properties discussed previously. Sulfamides[8] and sulfonamides[9] have been reported as analogs of phosphodiesters (Figure 1) and no biological data have been reported. These linkages would be expected to have pKas of approximately ~10,[10] far to high to confer the permeation properties described above. Electron-withdrawing groups within sulfonamides can confer dramatically lower pKas.[11] Herein we report the synthesis of an ON containing an electron-deficient *N*-acylsulfamide linkage and its hybridization property with a complementary RNA molecule as a first attempt to create a binding competent ON capable of acidification triggered endosomal release.



**Scheme 1**

The synthesis of the dinucleotide linked with the *N*-acylsulfamide started with the readily available chlorosulfonylisocyanate (Scheme 1). Chlorosulfonylisocyanate was reacted with one equivalent of benzyl alcohol in $CH_2Cl_2$ to give **2**. The resulting *N*-carbamoylsulfonyl chloride was then reacted with 5-DMT-thymidine **3** to give **4** in the presence of triethylamine in 90% yield. Upon debenzylation with Pd/C under $H_2$, **4** led to sulfamide **5** in 95% yield.

For the preparation of the 3' nucleoside synthon (Scheme 2), the readily available 3'-O-silylthymidine **6** was oxidized with sodium persulfate and $RuCl_3$ in 1 N NaOH solution.[12] The desired carboxylic acid **7** was obtained in 50% yield. The coupling of the two monomers was performed by first activating carboxylic acid **2** with *N*-hydroxysuccinimide and dicyclohexylcarbodiimide. Subsequently, sulfamide **5** and diisopropylethylamine were added to the reaction mixture to give dimer **8** in 65% yield. Desilylation of the dimer afforded **9** in 95% yield. Compound **9** was derivatized to give the desired dinucleoside H-phosphonate **10** (67% yield). **10** was then incorporated into two positions of ON **11**, 5'-TCTCTCTCTCTCT*TT*TT where * represents the *N*-acylsulfamide linkage and all other linkages are phosphodiester.[13] Maldi mass spectroscopy analysis of **11** confirmed the expected molecular weight.[14] Hybridization of ON **12** with the complementary single stranded RNA showed a Tm of 57 °C, which was a substantial destabilization relative to the hybridization of the control ON consisting completely of phosphodiester linkages (61 °C).[15]




**Scheme 2**

This analog linkage is isostructual with a phosphodiester with the exception of the replacement of a carbonyl group for the 5' methylene and a NH for the 5' oxygen. A and B form helix formation is known to require a guache conformation between the 5' and 4' oxygens in native nucleosides.[16] These substitutions of

the 5' methylene with a carbonyl and the 5' oxygen with a NH may make this conformation energetically unfavorable and result in the decreased binding affinity. This significant reduction in binding affinity suggests ONs completely substituted with this analog linkage would not bind complementary sequences and are of no interest in biological systems. However, future phosphodiester analogs that fulfill the design criterion of a pKa approaching 7 and that are capable of helix formation are of interest for addressing the permeation limitation of conventional phosphorothioates.

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14. Maldi mass spectroscopic analysis of the ON **12**: calcd for $M+H^+$: 4521.84; found: 4521.78.
15. Sequence of the RNA was 5'-AAAAAGAGAGAGAGA. Tm data were measured in a buffer solution of 140 mM KCl/5 mM $Na_2HPO_4$/1 mM $MgCl_2$ at pH 7.2, and the concentration of all ONs was about 2 μM. Error in Tm measurements was 0.5° C.
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