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SYNTHESIS OF NUCLEOSIDE SULFONATES AND SULFONES

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Abstract: Sulfonate analogues of adenine, cytidine and guanine monophosphates have been synthesized for the first time. The synthesis of ribose 3-sulfonate, a synthesis of sulfonyl disaccharides and dinucleotides and an improved synthesis of a ribose 3-sulfone is also reported.

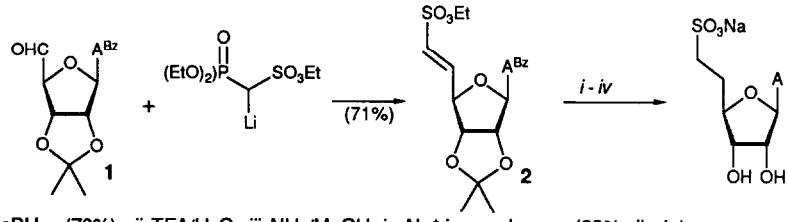
Oligonucleotides known as "antisense" oligonucleotides can inhibit the expression of genetic information.[1] By forming double helices with complementary pieces of messenger RNA (mRNA) even relatively short segments (< 20 nucleotides) of synthetic or naturally occurring oligonucleotides can interfere with the normal process required for the expression of an individual gene. Since the formation of double helices is highly sequence dependent, short, synthetic oligonucleotides can be targeted toward individual mRNAs with great specificity.[2] This powerful technique holds great promise for the development of new therapeutic antiviral and antitumor agents and the amelioration of many disorders caused by the expression of harmful genetic information.

To be effective as an antisense agent, a synthetic oligonucleotide should be resistant to chemical and biochemical degradation, must penetrate through cell membranes, and be capable of forming highly stable double helices with RNA. Naturally occurring RNA and DNA do not meet these criteria because they are susceptible to degradation by cellular nucleases, and form highly stable double helices only under high salt conditions.[3] Synthesis of an oligodeoxynucleotide with a methylphosphonate,[4] or phosphate triester backbone,[5] gives a neutral, nuclease resistant DNA isostere[6] capable of penetrating cell membranes[7] and forming stable double helices *in-vivo*. This methodology has two distinct drawbacks. First, the synthetic oligonucleotides thus produced are highly heterogeneous because of the introduction of uncontrolled chirality at phosphorus. Second, RNA containing phosphate triesters or methylphosphonates is likely to be chemically unstable. Since RNA-RNA helices are more stable than DNA-RNA helices,[8,9] and the predominant *in-vivo* target of antisense oligonucleotides is RNA, it is especially desirable to be able to synthesize stable modified antisense RNA.

A strategy which addresses the concerns raised above is the replacement of phosphorus with sulfur. Some candidates for this replacement that are isosteric to phosphorus diesters are 3', or 5'-sulfonate esters, or the corresponding sulfonamides, or the 3',5'-dialkyl sulfone group. A synthesis of sulfone-linked DNA has recently been reported.[10] This communication reveals a facile method for the synthesis of 3', or 5'-sulfonate containing nucleosides, as well as an improved method for the synthesis of 3-sulfone and sulfonate carbohydrates. The sulfonate analogues of nucleoside monophosphates[11] may prove to have interesting biological properties, and their synthesis should facilitate the synthesis of sulfonyl-containing RNA.

The sulfonate analogue of uridine 5'-phosphate may be easily synthesized by reaction of a protected ribose 5-iodide with an α-sulfonyl anion.[12] Unfortunately, this simple reaction fails with the more complex nucleoside iodides. We next examined the reaction of sulfonyl-stabilized α-phosphonate anions[13] with a protected adenosine aldehyde (**1**)[14] (eq 1). This reaction gave the α,β-unsaturated sulfonate ester (**2**). Reduction of the double bond with $NaBH_4$, followed by hydrolysis of the acetonide and ammonolysis of the sulfonate ester and N-benzoyl protecting groups gave adenosine 5'-sulfonate (41% overall yield based on **1**).

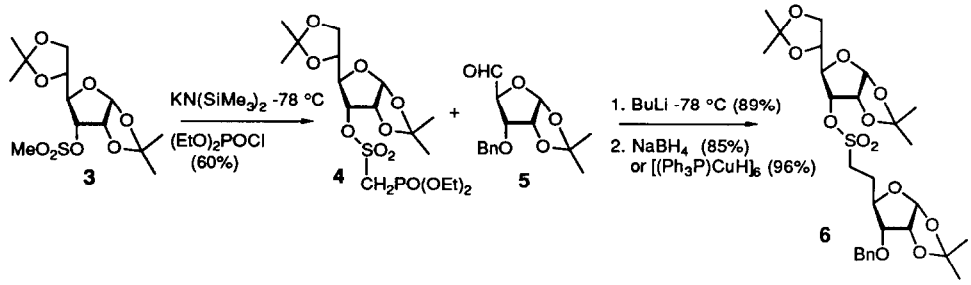
Eq. 1



i, $NaBH_4$, (70%); *ii*, TFA/H_2O; *iii*, NH_3/MeOH; *iv*, Na^+ ion exchange, (83% *ii* - *iv*).

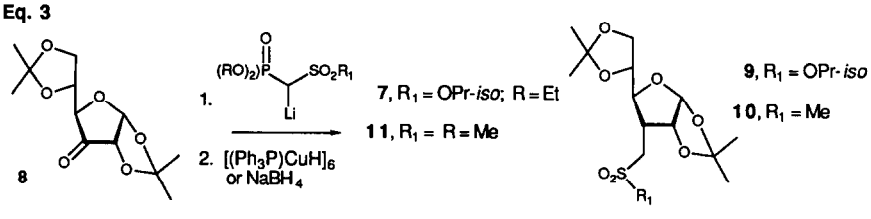
The addition of sulfonyl stabilized Horner-Emmons reagents to aldehydes also provides a useful method for coupling monosaccharides via a sulfonate linkage. For example, the 3-O-mesylate of 1,2-5,6-diacetone allose (**3**) was converted to the Horner-Emmons reagent (**4**) by reaction with diethyl phosphorochloridate in the presence of $KN[Si(Me)_3]_2$. Reaction of the phosphonate **4** with the aldehyde (**5**), followed by reduction of the resulting α,β-unsaturated sulfonate with $NaBH_4$ or $[(Ph_3P)CuH]_6$[15] (eq. 2) gave the disaccharide (**6**) in good yield. ($[(Ph_3P)CuH]_6$ (Aldrich) was superior to $NaBH_4$ for reduction of these conjugated sulfonates.)

Eq. 2



We have also found that contrary to a published report stating that sulfonate-stabilized Horner-Emmons (**7**) reagents do not react well with ketones,[13] such reagents react readily with 1,2-5,6-diacetone ulose (**8**) to give a mixture of *cis*- and *trans*-α,β-unsaturated sulfonates (80% yield) which were reduced with $NaBH_4$ (85% yield) to give the saturated sulfonate (**9**) (eq. 3). (The reaction of sulfonyl-stabilized phosphonate anions with carbohydrate ketones therefore provides simple access to non-hydrolyzable, isosteric and isoelectronic analogues of carbohydrates sulfated on secondary hydroxyl groups [e.g. heparin, heparan sulfate, dermatan sulfate, chondroitin sulfate, keratan sulfate and many others[16]]. To our knowledge, this is the first simple synthesis of such compounds.) Synthesis of the corresponding sulfone units (**10**) was accomplished in an analogous fashion, by using the sulfone-stabilized Horner-Emmons reagent (**11**)[17] instead of a sulfonate-stabilized phosphonate anion (56% yield), and adding *N,N'*-dimethylpropyleneurea to the reaction mixture. The resulting α,β-unsaturated sulfone was reduced with $[(Ph_3P)CuH]_6$ to give the saturated sulfone **10** in 88% yield (eq. 3).

Eq. 3



Access to nucleoside sulfonates may also be realized by glycosidation of a suitably protected ribose 5-sulfonate. As we have noted, the isopropyl sulfonate ester is stable toward a wide variety of reagents required to manipulate carbohydrate functionality, yet may be easily deprotected under the appropriate conditions.[12] Thus the protected ribose 5-sulfonate (**12**) was converted to a mixture of 1,2-diacetates and subjected to Hilbert-Johnson glycosidation[18-20] (eq. 4) under the conditions shown (fig. 1). In all cases reasonable yields of the sulfonated nucleosides were obtained.[21] As expected, glycosidation with silylated uracil and cytosine derivatives proved particularly effective. Cleavage of the isopropyl ester was effected by treatment of the sulfonates with Bu_4NI in boiling acetone. The salts were then purified by flash chromatography using mixtures of EtOAc/EtOH/Et_3N as eluent.

Eq. 4

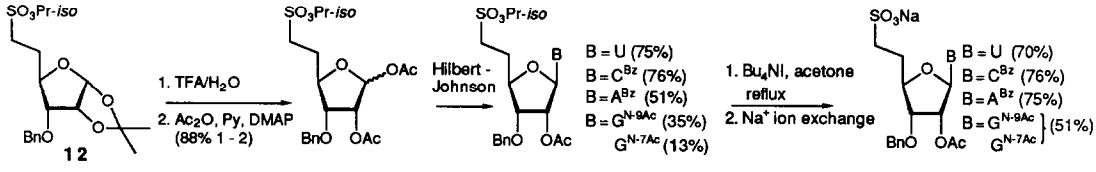
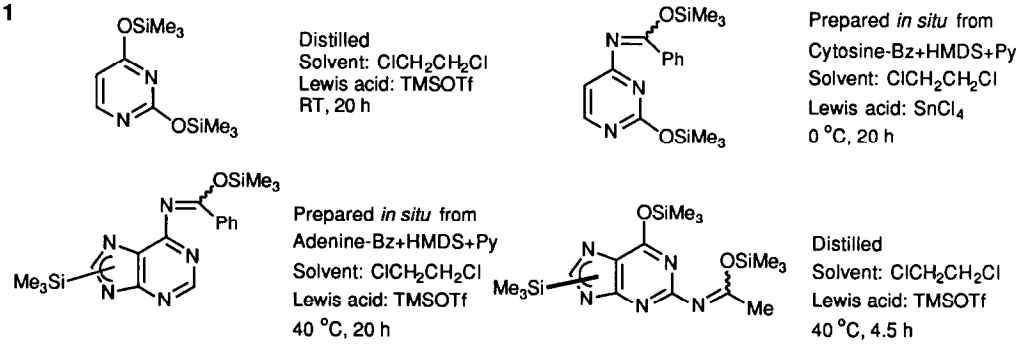


Fig. 1

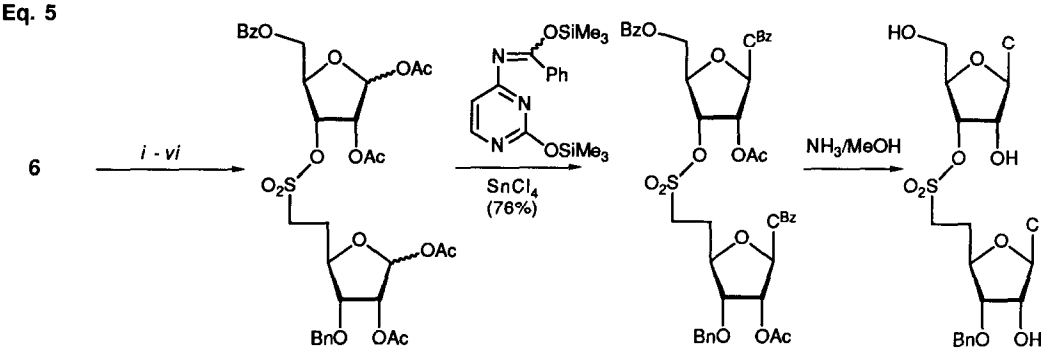


The Hilbert-Johnson glycosidation of carbohydrate sulfonates can easily be extended to disaccharide sulfonates to give dinucleosides as shown (eq. 5). The primary acetonide of the disaccharide sulfonate **6** was selectively hydrolyzed, followed by periodate cleavage, borohydride reduction of the resulting aldehyde and finally protection of the 5-OH as a benzoate ester. Removal of the two remaining acetonides of the disaccharide followed by conversion to a mixture of tetraacetates proved straightforward. The Hilbert-Johnson glycosidation of this disaccharide with silylated cytosine benzoate gave the dinucleotide in 76% yield. Deprotection of the ester protecting groups by treatment with 10% methanolic ammonia (50 °C, 12 hours) was virtually quantitative. Importantly, no evidence of 3'-cyclonucleoside formation was observed under these conditions. In fact, no cleavage of the sulfonate linkage occured even after boiling of the dinucleotide with saturated methanolic ammonia for a 24 hour period. This suggests that oligomers linked via a 3'-O-sulfonate group may enjoy high chemical stability.

This paper presents methodology useful for the synthesis of both carbohydrate and nucleoside sulfonates. The three previously unreported nucleoside sulfonates are described, and coupling chemistry suitable for the synthesis of short oligomers is detailed. In addition, the first synthesis of a carbohydrate sulfonate isosteric to a carbohydrate

sulfated on a secondary hydroxyl group is demonstrated. The synthesis of sulfonate, sulfone and sulfonamide homopolymers, and an evaluation of their helix forming capacity with both RNA and DNA is currently underway.

Eq. 5



i, THF/MeOH/H_2O/HCl (76%); *ii*, $NaIO_4$; *iii*, $NaBH_4$; *iv*, BzCl (87% *ii* - *iv*); *v*, TFA/H_2O; *vi*, Ac_2O/Py (89% *v* - *vi*).

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