An efficient method for the synthesis of 2'-O-modified nucleosides via double alkylation using cyclic sulfates

Allister S. Fraser, Andrew M. Kawasaki, Michael E. Jung and Muthiah Manoharan

Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc., 2292 Faraday Ave., Carlsbad, CA 92008, USA
Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA

Received 1 December 1999; accepted 14 December 1999

Abstract

The alkylation of N-3-benzyloxymethyl-5-methyluridine with 1,3,2-dioxathiolane 2,2-dioxide or 1,3,2-dioxathiane 2,2-dioxide resulted in a 2'-O versus 3'-O selectivity of 3:1, respectively. The resulting product has a built-in sulfate leaving group at the terminal end of an ethyl or propyl carbon chain, which can be displaced with sulfur and nitrogen nucleophiles to produce modifications at the 2'-O or 3'-O positions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: cyclic sulfate; alkylation; 2'-modified nucleosides.

2'-O Modifications of ribonucleotides have shown the ability to enhance the antisense properties of oligonucleotides (e.g., nuclease resistance and binding affinity for the target RNA) (Scheme 1).1-3 Several 2'-O-aminoalkyl and 2'-O-alkythioalkyl groups have demonstrated very favorable antisense properties.4 In order to create an efficient synthesis of 2'-O-modified oligonucleotides, one needs a facile method to produce the corresponding monomers. Alkylation of the 2'-O position using a five- or six-membered cyclic sulfate followed by nucleophilic displacement would create a modified nucleoside in only two or three synthetic steps. There has been an appreciable amount of work reported on the use of cyclic sulfates5-7 in general organic synthetic transformations.5 However, their use in novel nucleoside synthesis is a totally unexplored area. In view of the fact that the five- and six-membered cyclic sulfates (namely, 1,3,2-dioxathiolane 2,2-dioxide and 1,3,2-dioxathiane 2,2-dioxide) A and B (Scheme 2), respectively, are commercially available, we wanted to exploit their use in modified nucleoside synthesis. As a test case, we decided to synthesize 2'-O-[(N,N-dimethylaminoalkyl) and 2'-O-[(methylthio)ethyl] nucleosides (Fig. 1).

Treatment of the nucleoside 19 with NaH in DMF at −45°C followed by addition of the cyclic sulfates A or B afforded compounds 2a and 2b in 63 and 50% yields, respectively (Scheme 2). The selectivity for 2' over 3' alkylation was about 3:1 in both cases. Due to the stability of these sodium...
Scheme 1.

Scheme 2. Reagents and conditions: (i) NaH, DMF, A or B, −45°C to rt; (ii) 3a: NaSCH₃, DMF, 80°C; 3b: dimethylamine, THF, autoclave; (iii) Pd(OH), EtOH, AcOH, H₂ at 55 psi; (iv) pyridine, dimethoxytrityl chloride; (v) CH₂Cl₂, disopropylamine tetrazolide salt, 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite. *The BOM group was deprotected under the same conditions as in (iii) before nucleophilic displacement with NaSCH₃.
sulfate salts as leaving groups, subsequent displacement with nucleophiles required somewhat vigorous reaction conditions. In the case of dimethyamine as a nucleophile an autoclave was used, while in the case of sodium methylmercaptide as a nucleophile, reflux temperatures were needed to obtain nucleophilic displacement of the sulfates to give compounds 3a and 3b in moderate to good yields. Intramolecular displacement of sulfate salts by nucleophiles has been reported, but to our knowledge intermolecular displacement has not been reported.\(^7,8\) Reductive cleavage of the N-3-benzyloxymethyl (BOM) protecting group using catalytic hydrogenation over a palladium hydroxide catalyst proceeded normally for compound 3b to give the 2′-modified nucleoside 4b\(^10\) in 67% yield. This compound was then protected at the 5′-hydroxyl with a dimethoxytrityl (DMT) group to give compound 5b (56%) which was then converted to the phosphoramidite 6b (65%).

In the case of methylthio substitution of the cyclic sulfate, the N-3-BOM group of compound 3a proved to be more difficult to deprotect due to poisoning of the palladium hydroxide catalyst by the sulfide functionality. Therefore, the BOM group of the sulfate derivative 2a was removed via catalytic hydrogenation over a palladium hydroxide catalyst (product not purified) before nucleophilic displacement with sodium methylmercaptide to give compound 3c (product not purified). The 5′-hydroxyl group of the crude compound 3c was converted to the DMT-protected compound (5a)\(^11\) in 34% yield from 2a. Conversion of 5a into the 3′-phosphoramidite 6a proceeded in 63% yield.

In conclusion, we have found that alkylation of N-3-benzyloxymethyl-5-methyluridine with the five- and six-membered cyclic sulfates and subsequent nucleophilic displacement of the sulfate salt leaving group is an efficient, facile method to produce 2′-O-modified 5-methyluridine nucleoside monomers for incorporation into oligonucleotides. We have used this methodology to synthesize nucleosides and their phosphoramidites containing 2′-O-(N,N-dimethylaminopropyl) and 2′-O-[(methylthio)ethyl] substituents. The synthesis and evaluation of the properties of the modified oligonucleotides containing these modifications are in progress and will be reported in due course.

Acknowledgements

We are grateful to Robert H. Springer for largescale synthesis of Compound 1.

References


10. Compound 4b: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.65 (bs, 2H), 1.78 (s, 3H), 2.17 (s, H), 2.29 (m, 2H), 3.56 (m, 4H), 3.88 (m, 2H), 4.17 (t, 1H), 4.86 (bs, 1H), 5.19 (bs, 1H), 5.84 (d, 1H), 7.80 (s, 1H), 10.24 (bs, 1H). Anal. calcd for C$_{15}$H$_{25}$N$_3$O$_6$: C, 51.79; H, 7.39; N, 12.08. Found: C, 51.94; H, 7.40; N, 11.80. LRMS (ES) [MH$^+$] $m/z$ calcd: 344. Found: 344.

11. Compound 5a: $^1$H NMR (CDCl$_3$) $\delta$ 1.42 (s, 3H), 2.03 (s, 3H), 2.74 (m, 2H), 3.13 (d, 1H), 3.46 (dd, 2H), 3.81 (bs, 7H), 4.13 (m, 2H), 4.48 (m, 1H), 5.97 (s, 1H), 6.83 (d, 2H), 7.29 (m, 13H), 7.68 (s, 1H), 8.19 (bs, 1H). Anal. calcd for C$_{34}$H$_{38}$N$_2$O$_8$: C, 62.56; H, 6.18; N, 4.29. Found: C, 62.87; H, 6.00; N, 4.10. LRMS (ES) [MH] $m/z$ calcd: 633. Found: 633.