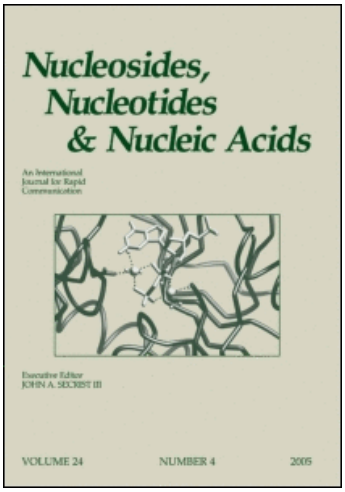

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2'-*C*-Alkylribonucleosides: Design, Synthesis, and Conformation

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2'-*C*-ALKYLRIBONUCLEOSIDES: DESIGN, SYNTHESIS, AND CONFORMATION

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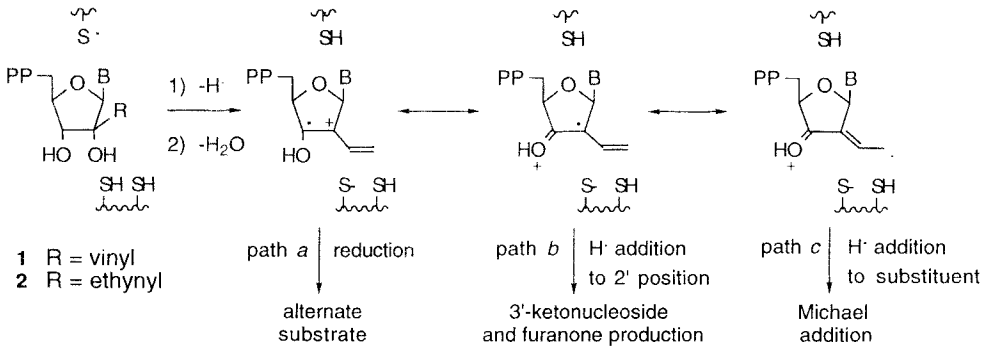
ABSTRACT: Certain 2'-*C*-alkylribonucleotides have been designed as potential mechanism-based inactivators of ribonucleotide reductases. A short, flexible route toward the corresponding nucleosides and NMR evidence concerning their preferred solution conformations are discussed.

The incorporation of a carbon substituent into the 2'β position of nucleosides has provided a number of analogs with promising anticancer and antiviral activities. Along these lines, considerable effort has gone into the design, synthesis, and evaluation of 2'-*C*-branched 2'-deoxyribonucleosides. In particular, the chemistry developed by the Matsuda laboratory over the past decade toward analogs of this type have led to notable nucleosides with potent antileukemic[1] and antiherpetic[2] activities, including one agent with a unique DNA strand-breaking mechanism.[3]

In contrast, synthetic methodologies toward and biological effects of 2'-*C*-branched ribonucleosides have been relatively unexplored. There are few reported examples of such compounds,[4] and these have been virtually limited to methyl substitutions. 2'-*C*-Branched ribonucleosides are potential antiviral agents: 2'-*C*-methyladenosine inhibited vaccinia virus in a rat tail vein assay.[4c] In addition, they are of interest as potential building blocks for antisense drug design: incorporation of a methyl group into the 2'*C* position of adenosine led to the predominance of Northern-type sugar conformations,[4b] similar to the sugar conformations of A-form double-stranded RNA and RNA-DNA heteroduplexes.[5] Despite the potential of 2'-*C*-branched ribonucleosides as chemotherapeutic agents and as tools for controlling the conformation of oligonucleotides, to date there are no synthetic methodologies toward molecules of this type which allows easy variation of the alkyl substituent.

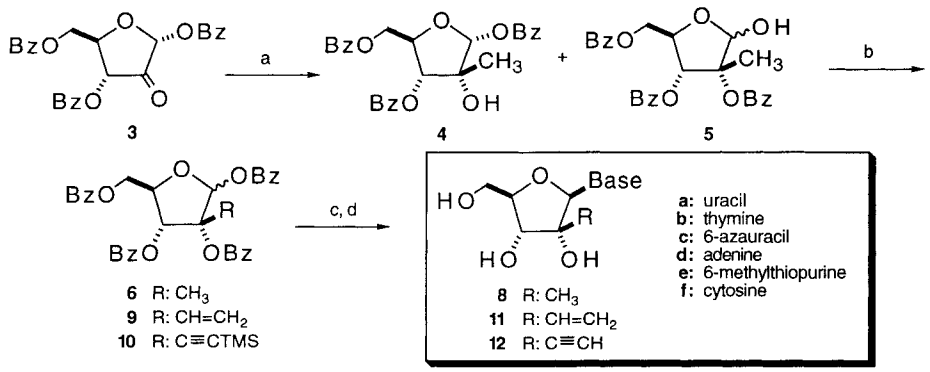
It was with this backdrop that we first designed 2'-*C*-vinyl- and 2'-*C*-ethynylribonucleotides **1** and **2** (scheme 1) as potential mechanism-based



Scheme 1

inactivators of ribonucleotide diphosphate reductases (RDPR).[6] Human RDPR is an important target for anticancer chemotherapy, and selective inhibition of viral RDPRs has become a recent goal toward developing antiviral agents.[6a] The mechanism of action of RDPRs involves removal of the 3'-H via a putative thiyl radical followed by cysteine-assisted elimination of the 2'-OH. The resultant radical cation is then reduced by the thiolate/thiol combination; the hydrogen atom is returned to the 3' position, generating the product 2'-deoxynucleotides.[6] Similar turnover of a nucleotide such as **1** and **2** would result in a radical cation that could be converted to a reactive α,β-unsaturated ketone within the active site of RDPR (scheme 1, path *c*; illustrated for **1**). The nearby thiolate anion formed in the process should be ideally positioned for alkylation by the reactive intermediate. Other paths (*a* and *b*) include alternate substrate activity and turnover to a 3'-ketonucleoside (which subsequently dissociates and fragments to a reactive furanone species).[6]

In designing an ideal synthetic route toward the nucleoside counterparts of **1** and **2**, we desired brevity, efficiency, stereoselectivity, and the flexibility to incorporate a variety of alkyl substituents and nucleobases. The general route illustrated in scheme 2 meets these criteria. Ketosugar **3**[7] was treated with organometallic reagents selective for ketones in the presence of esters. The reaction between **3** and $MeTiCl_3$[8] resulted in the simple 1,2-adduct **4** and an anomeric mixture of its transesterification product **5** in an overall yield of 66%. The **4/5** mixture was benzoylated to provide **6** in 72% yield (α/β 1:4). Methylsugar **6** was coupled to pyrimidines (uracil, thymine, 6-azauracil) and



(a) $MeTiCl_3$, $RMgBr/CeCl_3$, or $RLi/CeCl_3$; (b) BzCl, DMAP, Et_3N; (c) bis(trimethylsilyl)acetamide, pyrimidine, $SnCl_4$, acetonitrile, reflux 3 h; or bis(trimethylsilyl)acetamide, purine,TMSOTf, acetonitrile, reflux overnight; (d) NH_3/MeOH

Scheme 2

to purines (N^6-benzoyladenine, 6-methylthiopurine) under Vorbrüggen conditions[9] in yields ranging from moderate (6-methylthiopurine, 47%) to excellent (thymine, 94%). Deprotection with methanolic ammonia then afforded 2'-*C*-methylribonucleosides **8a-e**.[10]

Reaction between ketosugar **3** and organometallic reagents in the presence of $CeCl_3$[11] proceeded in a similar fashion. Tetrabenzoylated 2-*C*-branched ribofuranosides **6**, **9**, and **10** were obtained in 60%, 84%, and 75%, respectively, by (1) treatment with $MeMgBr/CeCl_3$, CH_2=$CHMgBr/CeCl_3$, or $TMSC{\equiv}CLi/CeCl_3$, and (2) subsequent benzoylation. Vinyl- and ethynylsugars **9** and **10** were each coupled to uracil and to N^4-acetylcytosine in 62-73% yield and deprotected to provide 2'-*C*-vinyl- and 2'-*C*-ethynyluridine (**11a** and **12a**), and 2'-*C*-vinyl- and 2'-*C*-ethynylcytidine (**11f** and **12f**).[12] We are currently working toward converting these new nucleosides to their corresponding 5'-diphosphates for evaluation as inhibitors of recombinant RDPRs.

Information concerning the solution conformation of 2'-*C*-methyl-ribonucleosides **8b** and **8d** was obtained via ^{1}H-NMR experiments. The 2'-*C*-substituent precludes gleaning such information from $J_{1'2'}$ and $J_{2'3'}$ values, leaving only $J_{3'4'}$ as a clue to the sugar puckering. For **8b** in D_2O, the H3' peak is not resolved from those of H4', H5', or H5"; however, preirradiation of either the 2'-methyl or H-6 peak resulted in selective enhancement of H3' (9.2% and 11.1%, respectively). Thus, $J_{3'4'}$ of 9.7 Hz was determined. Using $J_{3'4'}$ of 10.3 Hz

as 100% N-pucker,[13] **8b** appears to be essentially locked in the Northern conformation. In addition, the large NOE effect between H3' and H-6 indicates that, like other pyrimidine nucleosides, **8b** spends considerable time in the *anti* conformation. This aqueous solution conformation of **8b** is therefore essentially the same as that of a reported x-ray structure of **8a**.[4d] With $J_{3'4'}$ = 9.1 Hz (DMSO-d_6 + D_2O), **8d** was also predominantly N-puckered, as previously reported for this compound in C_5D_5N.[4b] Pre-irradiation of H-8 enhanced H-1' 4.6%, which, based on correlations reported by Rosemeyer *et al.*,[14] translates into 40% population of *syn* conformers. For vinyl- and ethynylnucleosides **11a**, **12a**, **11f**, and **12f**, the H-3' peaks were well-resolved, with $J_{3'4'}$ = 9-10 Hz (i.e., the sugars are N-puckered). The high proportion of Northern conformations of these 2'-*C*-branched nucleosides should make compounds of this general type attractive monomers for antisense design.

REFERENCES AND NOTES

1. Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 234.
2. Awano, H.; Shuto, S.; Baba, M.; Kira, T.; Shigeta, S.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 367.
3. Matsuda, A; Azuma, A. *Nucleosides Nucleotides* **1995**, *14*, 461.
4. (a) Walton, E.; Jenkins, S. R.; Nutt, R. F.; Zimmerman, M. Holly, F. W. *J. Am. Chem. Soc.* **1966**, *88*, 4524. (b) Jenkins, S. R.; Arison, B.; Walton, E. *J. Org. Chem.* **1968**, *33*, 2490. (c) Walton, E.; Jenkins, S. R.; Nutt, R. F.; Holly, F. W; Nemes, M. *J. Med. Chem.* **1969**, *12*, 306. (d) Beigelman, L. N.; Ermolinsky, B. S.; Gurskaya, G. V.; Tsapkina, E. N.; Karpeisky, M. Y.; Mikhailov, S. N. *Carbohyd. Res.* **1987**, *166*, 219. (e) Wolf, J.; Jarrige, J.-M.; Florent, J.-C.; Grierson, D. S.; Monneret, C. *Synthesis* **1992**, 773.
5. In *Nucleic Acids in Chemistry and Biology, 2nd Ed.*; G. M. Blackburn and M. J. Gait, eds.; Oxford University Press: Oxford, UK, 1996; pp.62-65.
6. (a) Stubbe, J. *Adv. Enzymol.* **1990**, *63*, 349. (b) Robin, M. J.; Samano, M. C.; Samano, V. *Nucleosides Nucleotides* **1995**, *14*, 485.
7. Cook, G. P.; Greenberg, M. M. *J. Org. Chem.* **1994**, *59*, 4704.
8. Reetz, M. T.; Kyung, S. H.; Hüllman, M. *Tetrahedron* **1986**, *42*, 2931.
9. Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279.
10. A preliminary account of this work has been reported: Wolfe, M. S.; Harry-O'kuru, R. E. *Tetrahedron Lett.* **1995**, *36*, 7611.
11. Imamoto, T.; Takiyama, N.; Nakamura, K.; Hatajima, T.; Kamiya, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4392 and references therein.
12. A full account of this synthetic methodology is in preparation.
13. Davies, D. B. *Progress in NMR Spectroscopy*, **1978**, *12*, 135.
14. Rosemeyer, H; Tóth, B. G, Kazimierczuk, Z.; Bourgeois, W., Kretschmer, U.; Muth, H.-P.; Seela, F. *J. Org. Chem.* **1990**, *55*, 5784.

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