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Cyclization by Free Radical Addition of Stannyl or Thiyl Radical to 3'-β-Ethynyl Uridine. Is the 3'-β-Ethynyl Group a Spin Trap in Ribonucleotide Reductase?

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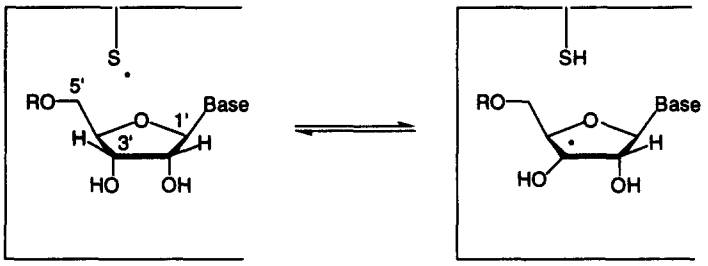
Abstract: Reaction of a stannyl or a thiyl radical, generated *in situ* with AIBN, on (2',5'-di-O-tert-butyldimethylsilyl-3'-C-ethynyl-β-D-ribofuranosyl)uracil (**2**) gave in 60% and 17% yield respectively cyclized adducts **3** and **4** where the vinylic radical, formed by addition of stannyl or thiyl radical to the triple bond, reacted with the base. The 3'-β-ethynyl uridine di- and triphosphates could be a spin trap of the transient thiyl radical of ribonucleotide reductases. © 1997 Elsevier Science Ltd.

The conversion of nucleotides into 2'-deoxynucleotides by ribonucleotide reductases is the rate determining step in DNA biosynthesis. The importance of ribonucleotide reductases has led to intensive studies on the reaction mechanism.[1] A common complex radical mechanism has been proposed for all ribonucleotide reductases. It is supported by studies with labelled substrates and substrate analogues,[2] by site directed mutagenesis[3] and by determination of X-ray crystal structures of the two subunits (R1 and R2) of ribonucleotide reductase from *Escherichia coli*.[4] The first step involves the formation of a C-3' radical by abstraction of the 3' hydrogen atom of the substrate. A transient thiyl radical of a cysteine in the active site is proposed to initiate the reaction (scheme 1).[1a,5]



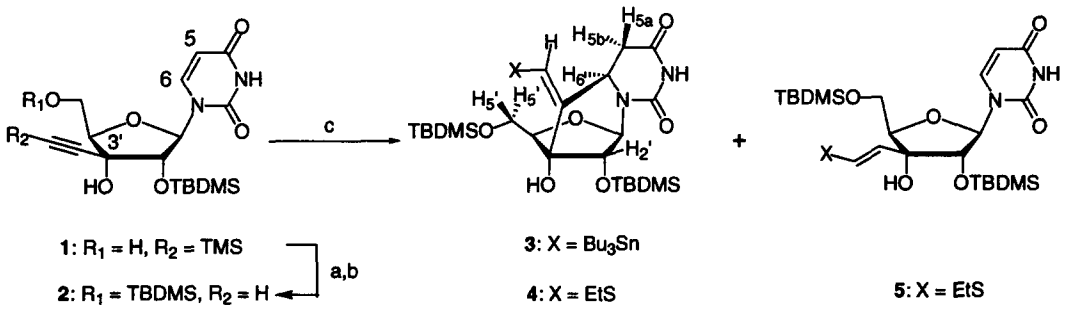
Scheme 1 : Base: U, C, A, G; R: $P_2O_6^{3-}$ or $P_3O_9^{4-}$

This elegant proposal would be supported if the thiyl radical could be trapped.[6] With this in mind, we have designed and synthetized analogues of uridine and adenosine where the 3'-hydrogen has been replaced by an ethynyl and a vinyl group.[7] The stereospecific synthesis of these compounds has been described elsewhere.[8]

In the meantime Matsuda and coll.[9] described a different route to 3'-β-ethynyl nucleosides and the high potent antitumour activity against human tumour cells *in vitro* and *in vivo* in particular of 3'-β-ethynyl uridine. In their publication, they pointed out that the ethynyl nucleoside analogues could act as inactivators of ribonucleotide reductase. Their comments prompted us to publish our results on model reactions with 3'-β-ethynyl-uridine in the presence of stannyl or thiyl radicals.

In our model studies we first examined the reactivity of 3'-β-ethynyl uridine for the following considerations. The addition of the relatively stable stannyl and thiyl radicals (BDE Bu_3Sn-H= 308 kJ mol^{-1}, CH_3S-H= 371 kJ mol^{-1})[10] to a double or a triple bond[11] is well known and gives a less stable carbon centred radical, an alkyl and a vinyl radical respectively, that are of different reactivity : the C-sp^2 radical being more reactive than the C-sp^3 radical (BDE CH_3CH_2-H= 419.5 kJ mol^{-1}, CH_2=CH-H= 444 kJ mol^{-1}).[10,12] Besides the C5-C6 double bond of the uracil heterocycle is sensitive to the attack of carbon centred radicals[13] to give a more stabilized C-5 radical.[13c] Therefore, if a vinyl radical is produced enzymatically one can envision that the reactive C-sp^2 radical will further react either with an aminoacid of the active site or with the base by intramolecular trapping.[14] The result could be irreversible inactivation of the ribonucleotide reductase.

The (2',5'-di-O-tert-butyldimethylsilyl-3'-C-ethynyl-β-D-ribofuranosyl)uracil (**2**) was prepared starting from compound **1**[8] with an overall yield of 73% by successive treatment with TBDMSCl in pyridine and potassium carbonate in methanol.



Scheme 2: (a) TBDMSCl/Py. (b) K_2CO_3/MeOH. (c) Bu_3SnH or EtSH/AIBN/toluene/Δ.

Our first reaction was conducted with the ethynyl **2** and the stannyl radical formed *in situ* by reaction of 1.2 eq tributyltin hydride (0.2 M) and 0.3 eq AIBN in toluene at 80°C under argon.[15] The formation of a cyclized product as the major compound was evidenced by TLC and ^1H NMR of the crude extract. The UV transparent compound **3** was isolated as a single diastereomer with a 60% yield. Why was the 6-exocyclization so efficiently promoted by the stannyl radical? Molecular modelling showed that when a vinyl radical is produced from the ethynyl compound **2** and is in a C-3' endo orientation,[16] the radical carbon is close to the C-6 carbon of the base in its anti conformation[16] and that its orbital is favourably aligned to overlap with the Π orbital of the C-6 carbon. This result is remarkable because Broka and Reichert[15b] described that oct-7-en-1-yne did not give any cyclized product in the presence either of stannyl or thiyl radicals. The first example of a 6-exocyclization of a 7-en-1-yne system has been reported by Ichinose et al..[15c] The reaction was promoted by the triphenylstannyl radical to furnish a pyranyl derivative with a 23% yield.

The reactivity of our compound in the presence of a thiyl radical was then investigated. Reaction of the ethynyl **2** with 1.2 eq ethanethiol (0.1 M) and 0.3eq AIBN in toluene under argon afforded the cyclized product **4** (yield 17%) along with the trans thiovinyl adduct **5** (44%) and a complex mixture of other products.

The ^{1}H, ^{13}C NMR (1D and 2D ^{1}H-^{13}C, COSY, HMBC, NOESY) and mass spectral dataof products **3**[17] and **4**[18] were consistent with the proposed structures. The stereochemistry of the double bond (Z) and of the C-6 position (S) was assigned from 2D NOESY NMR experiments. Marked NOE effects were observed for the olefinic proton, and H-6 of compound **3** after irradiation of H-5b and H-5' respectively; and of H-5' and H-6, and H-5a of compound **4** after irradiation of the olefinic proton and H-2' respectively.

In conclusion, we have shown that 3'-β-ethynyl uridine yielded cyclonucleosides by reaction with a stannyl and a thiyl radical. Preparation of 3'-β-ethynyl uridine di- and triphosphates and their evaluation as a spin trap of the thiyl radical of ribonucleotide reductases are currently in progress.

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REFERENCES AND NOTES

1. For reviews, see: Stubbe, J.; van der Donk, W.A., *Chem. Biol.* **1995**, *2*, 793-801. Reichard, P. *Science*, **1993**, *260*, 1773-1777. Stubbe, J. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1990**, *63*, 349-419.
2. For reviews, see: Ashley, G.W.; Stubbe, J. in *Inhibitors of Ribonucleotide Diphosphate Reductase Activity*,. International Encyclopedia of Pharmacology and Therapeutics, Section 128, Cory, J.G.; Cory, A.H.; Eds : Pergamon Press, New-York, **1989**. Robins, M.J.; Samano, M.C.; Samano, V. *Nucleosides Nucleotides* **1995**, *14*, 485-493.
3. Rova, U.; Goodtzova, K.; Ingermarson, R.; Behravan, G.; Gräslund, A., Thelander, L. *Biochemistry* **1995**, *34*, 4267-4275. Mao, S.S.; Holler, T.P.; Yu, G.X.; Bollinger, J.M.; Booker, S.; Johnston, M.I.; Stubbe, J. *Biochemistry* **1992**, *31*, 9733-9743. Mao, S.S.; Holler, T.P.; Bollinger, J.M.; Yu, G.X.; Johnston, M.I.; Stubbe, J. *Biochemistry* **1992**, *31*, 9744-9751. Mao, S.S.; Yu, G.X.; Chalfoun, D.; Stubbe, J. *Biochemistry* **1992**, *31*, 9752-9759.
4. Uhlin, U.; Eklund, H. *Nature* **1994**, *370*, 533-539. Nordlund, P.; Sjöberg B.-M; Eklund, H. *Nature* **1990**, *345*, 593-598.
5. Strong evidence for a thiyl radical in B12-reductase has been reported, see: Licht, S.; Gerfen, G.J.; Stubbe, J. *Science* **1996**, *271*, 477-481. Gerfen, G.J.; Licht, S.; Hoffman, B.M., Stubbe, J. *J. Am. Chem. Soc.* **1996**, *118*, 8192-8197.
6. Stubbe, J. *Nature* **1994**, *370*, 502.
7. 3'-C-Methyladenosine and 3'-C-methyl uridine diphosphates have been prepared and have been shown to function as inhibitors of ribonucleotide reductase from *Corynebacterium nephridii*, see: Ong, S.P.; Nelson, L.S., Hogenkamp, H.P.C. *Biochemistry* **1992**, *31*, 11210-11215. A series of 3'-C modified nucleosides has been prepared as candidates as radical traps of ribonucleotide reductase, see: Auguste, S. P.; Young, D. W. *J. Chem. Soc. Perkins Trans I* **1995**, 395-404.
8. Jung, P. M. J.; Burger, A.; Biellmann, J.-F. *Tetrahedron Lett.* **1995**, *36*, 1031-1034.

9. Hattori, H.; Tanaka, M.; Fukushima, M.; Sasaki, T., Matsuda, A. *J. Med. Chem.* **1996**, *39*, 5005-5011.
10. "Strengths in Polyatomic Molecules" in *Handbook of Chemistry and Physics*, Weast, R.C., Ed.: CRC Press, Inc., Boca rato, Florida,**1990**, pp F124-125.
11. Chatgilialoglu, C.; Ferreri, C. *The Chemistry of Triple-Bonded Functionnal Groups*, Supplement C2, Patai, S.; Ed.: John Wiley and Sons, Chichester, **1994**, pp 917-944.
12. Stork, G.; Baine, H.N. *J. Am. Chem. Soc.* **1982**, *104*, 2321-2323.
13. (a) For a review of cyclonucleosides bridged by carbon, see : Ueda, T. in *Chemistry of Nucleosides and Nucleotides*, Vol 1, Townsend, L.B.; Ed.: Plenum Press, New-York, 1988, pp 71-73. (b) Samano V.; Robins, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 4007-4008. (c) Fenick, D.J.; Falvey, D.E. *J. Org. Chem.* **1994**, *59*, 4791-4799.
14. Vinyl radicals are much more reactive towards hydrogen abstraction and cyclization than alkyl radicals. Hydrogen abstraction from Bu_3SnH by vinyl radical is about 100 times faster than abstraction involving the analogous alkyl radical, see : Johnston, L.J.; Lustyk, J.; Waymer, D.D.M., Abeywickrema, A.N.; Beckwith, A.L.J.; Scaiano, J.C.; Ingold, K.U. *J. Am. Chem. Soc.* **1985**, *107*, 4594-4596. 5-Exo cyclizations by vinyl radicals are ≥ 1000 times faster than cyclizations involving analogous alkyl radicals, see: Beckwith, A.L.J.; O'Shea, D.M. *Tetrahedron Lett.* **1986**, *27*, 4525-4528. Jasperse, G.P., Curran, D.P.; Fevig, T.L. *Chem. Rev.* **1991**, *91*, 1237-1286.
15. (a) Stork, G.; Mook, R. Jr. *Tetrahedron Lett.* **1986**, *27*, 4529-4532. (b) Broka, C.A.; Reichert, D.E.C. *Tetrahedron Lett.* **1987**, *28*, 1503-1506. (c) Ichinose, Y.; Oshima, K.; Utimoto, K. *Chem. Lett.* **1988**, 1437-1440.
16. Saenger, W. in *Principles of Nucleic Acid Structure*, Cantor, C.R.; Ed.: Springer-Verlag, New-York, **1984**, pp 17-23 and 55-78.
17. **(6S,Z)-1-(2',5'-di-O-tert-butyldimethylsilyl-6,3'-(2-(tributylstannyl)ethylidene)-β-D-ribofuranosyl) -5,6-dihydrouracil (3).** mp 153-155°C. IR (KBr, cm^{-1}): 3549, 3452, 3205, 2955, 1701. ^1H NMR ($CDCl_3$) δ: 7.76 (bs, 1H), 6.12 (s, 1H), 5.91 (dd, J = 2 Hz, J = 45 Hz, 1H), 4.48 (bd, J = 13 Hz, 1H), 4.09 (dd, J = 7.8 Hz, J = 2 Hz, 1H), 3.80 (ABX, J_{AB} = 11.8 Hz, J = 2 Hz, 1H), 3.68 (ABX, J_{AB} = 11.8 Hz, J = 7.8 Hz, 1H), 3.58 (s, 1H), 3.12 (dd, J = 16.5 Hz, J = 4 Hz, 1H), 2.69 (s, 1H), 2.60 (dd, J = 16.5 Hz, J = 13 Hz, 1H), 1.50-1.42 (m, 6H), 1.34-1.24 (m, 6H), 0.96-0.80 (m, 33H), 0.26 (s, 3H), 0.21 (s, 3H), 0.06 (s, 6H). ^{13}C NMR ($CDCl_3$) δ: 168.2 (q), 151.6 (q), 146.0 (q), 127.9 (t), 84.6 (t), 83.3 (t), 80.6 (q), 78.6 (t), 61.7 (s), 51.7 (t), 36.3 (s), 29.0 (s), 27.4 (s), 26.0 (p), 25.7 (p), 18.4 (q), 18.1 (q), 13.8 (p), 12.7 (s), -4.4 (p), -4.9 (p), -5.1 (p), -5.3 (p). MS *m/z* (%): 805 (MNH_4^+, 1), 789 (MH^+, 1), 516 (100).
18. **(6S,Z)-1-(2',5'-di-O-tert-butyldimethylsilyl-6,3'-(2-(ethylsulfanyl)ethylidene)-β-D-ribofuranosyl)-5,6-dihydrouracil (4).** mp 131-133°C. IR (KBr, cm^{-1}): 3286, 2934, 1708. ^1H NMR ($CDCl_3$) δ: 7.33 (bs, 1H), 6.59 (d, J = 2.4 Hz, 1H), 5.95 (s, 1H), 4.44 (ddd, J = 12.8 Hz, J = 3.1 Hz, J = 2.4 Hz, 1H), 4.05 (dd, $J_1 = J_2$ = 6.1 Hz, 1H), 3.84 (dd, J = 17.0 Hz, J = 3.1 Hz, 1H), 3.72 (s, 1H), 3.60 (d, J = 6.1 Hz, 2H), 2.81 (s, 1H), 2.80 (q, J = 7.3 Hz, 2H), 2.37 (dd, J = 17.0 Hz, J = 12.8 Hz, 1H), 1.34 (t, J = 7.3 Hz, 3H), 0.94 (s, 9H), 0.89 (s, 9H), 0.18 (s, 3H), 0.16 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 169.0 (q), 151.1 (q), 126.8 (q), 125.9 (t), 84.4 (t), 81.3 (t), 80.9 (q), 77.3 (t), 62.6 (s), 49.9 (t), 36.3 (s), 29.7 (s), 25.9 (p), 25.7 (p), 18.3 (q), 18.1 (q), 15.5 (p), -4.6 (p), -4.7 (p), -5.3 (p), -5.4 (p). MS *m/z* (%): 576 (MNH_4^+, 100), 559 (MH^+, 5), 501 (M^+ - tBu, 1).

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