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Synthesis of Nucleoside Dimers Bridged on Ribose with a Butadiynyl Group

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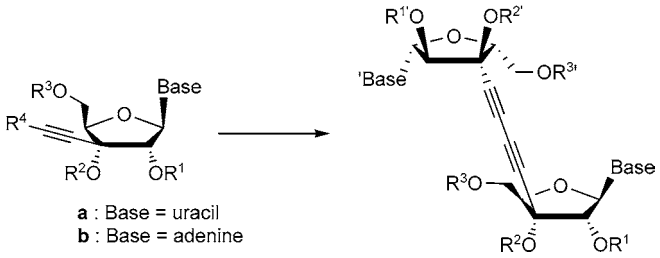
ABSTRACT



The nucleoside dimer linked by a butadiynediyl group at C-3′β may serve as a building block for the preparation of backbone-modified oligonucleotides for DNA repair or mutation in functional genomics. We prepared this type of dimer by an Eglington or Sonogashira coupling reaction. The unsymmetrical dimer was synthesized by coupling the acetylene with the bromoacetylene. Only marginal cytotoxicity was detected for one of the dimers.

Chemically modified oligonucleotides have potential applications as therapeutic agents or as biomolecular tools.[1] The backbone modification of the phosphodiester linkage is illustrative of the enduring interest in this area.[1,2] For instance, the phosphodiester bridge has been replaced by acetylenic links between the two furanose moieties at natural C-3′ and C-5′ positions[3] or between the furanose and the nucleobase at C-5′ and C-2, C-8, or C-5 positions.[4] However, acetylenic connection between both C-3′ positions has so far never been investigated.[5] We thus envisioned the preparation of the homo- and heterodimers of uridine and adenosine linked at C-3′β by a butadiynyl group and evaluated the lowest energy conformation of the methyl ether derivatives by molecular mechanics and dynamics.[6] Although the rotation barrier around the butadiynyl group is expected to be very low, CFF91 force field calculations revealed that the most stable conformation of the two homodimers has methyl ethers of both C-5′ positions and of C-3′ and C-5′ positions of each side oriented in the same direction (Figure 2). The lowest energy conformation of the heterodimer has the C-3′ and C-5′ methyl ethers of each side oriented in the same direction and both C-5′ methyl ethers pointed in the opposite direction

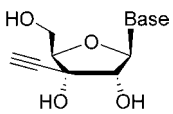


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Figure 1. 3′β-*C*-Ethynyl-nucleosides.

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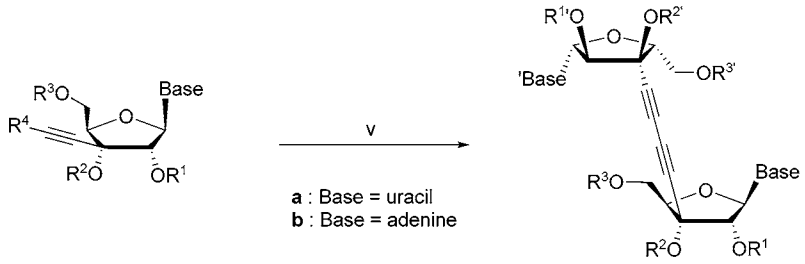
Scheme 1 [a]



i
- **4a,b** R^1 = TBDMS; R^2= R^3 = H; R^4 = TMS
- **5a,b** R^1 = TBDMS; R^2= R^3 = R^4 = H

ii
- **6a** R^1 = TBDMS; R^2= R^3 = Ac; R^4 = H

iii

iv
- **7a,b** R^1 = R^3= TBDMS; R^2 = H; R^4 = H
- **8a** R^1 = R^3= TBDMS; R^2 = H; R^4 = Br

- **3aa', 3bb', 3ab'** R^1= $R^{1'}$ =R^2= $R^{2'}$ =R^3= $R^{3'}$= H
- **9aa'** R^1= $R^{1'}$ = TBDMS; R^2= R^3 = $R^{2'}$= $R^{3'}$ = Ac
- **10aa',bb'** R^1= $R^{1'}$ = TBDMS; R^2= R^3 = $R^{2'}$= $R^{3'}$ = H
- **11bb'** R^1= $R^{1'}$ = R^3= $R^{3'}$ = TBDMS; R^2= $R^{2'}$ = H
- **12ab'** R^1= $R^{1'}$ = R^3= TBDMS; R^2= $R^{2'}$ = $R^{3'}$= H

[a] Reagents and conditions: (i) K_2CO_3, MeOH, **5a** (85%), **5b** (94%); (ii) Ac_2O, DMAP cat., Py, **6a** (68%); (iii) TBDMSCl, Py, **7a** (86%), **7b** (70%); (iv) NBS, $AgNO_3$, acetone, **8a** (quantitative); (v) for conditions, see Table 1.

(Figure 3). Therefore, one can take advantage of such dimers for the synthesis of backbone-modified oligonucleotides in a preorganized conformation. The nucleic acid chains could be introduced on the C-5′ and C-3′ hydroxyls of each side using either the homo- or heterodimer core. These constructions, as bifunctional or chimeric RNA/DNA oligonucleotides, may find applications in DNA repair or mutation in functional genomics.[7]

Herein we present the preparation and characterization of dimers **3aa′**, **3ab′**, and **3bb′** (**a** = uracil, **b** = adenine). The coupling of monosubstituted acetylenes to diacetylenes is a simple reaction to build molecules and is compatible with a large number of functional groups.[8] Since the coupling of acetylenes may be carried out in a symmetrical or unsymmetrical way, we embarked upon the synthesis of dimers **3** from known 3′-C ethynyl nucleosides **4a**,**b**.[9] Uridine and adenosine were chosen as representatives of pyrimidine and purine nucleosides (Scheme 1).[10]

Selective deprotection of the trimethylsilyl group for compounds **4a** and **4b** was achieved with K_2CO_3 in methanol to afford the corresponding monoacetylenic derivatives **5a** and **5b** in 85 and 94% yields, respectively. Esterification of the 3′-C and 5′-C hydroxyls of **5a** with acetic anhydride in the presence of catalytic DMAP in pyridine furnished compound **6a** in 68% yield. 2′,5′-Di(*tert*-butyldimethylsilyl) ethers **7a** and **7b** were obtained after treatment of compounds **5a** and **5b** with TBDMSCl in pyridine in 86 and 70% yields, respectively. For solubility reasons, the bromoacetylene **8a**

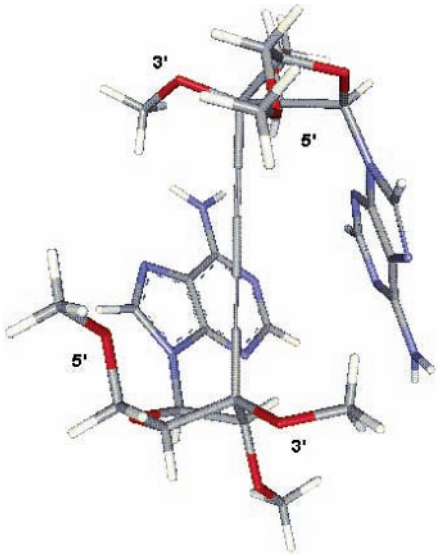


Figure 2. Lowest energy conformation of the adenosine−adenosine dimer linked by a butadiynyl at 3′β-C.

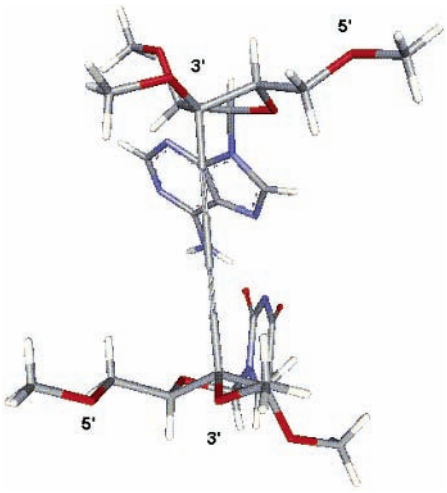


Figure 3. Lowest energy conformation of the uridine−adenosine dimer linked by a butadiynyl at 3′β-C.

Table 1. Conditions Assayed for Dimerization

entry	starting material	reagent or catalyst	other reagents	product	% yield
1	**6a**	$Cu(OAc)_2$	Py	**9aa′**	60
2	**5a**	$Pd(PPh_3)_2Cl_2$	CuI, O_2, i-Pr_2NH	**10aa′**	67
3	**5b**	$Pd(PPh_3)_2Cl_2$	CuI, O_2 i-Pr_2NH	**10bb′**	42
4	**7b**	$Pd(PPh_3)_2Cl_2$	CuI, O_2, i-Pr_2NH	**11bb′**	80
5	**8a**, **5b**	$Pd(PPh_3)_2Cl_2$	CuI, Et_3N	**12ab′**	20
6	**8a**, **5b**	$Pd_2(dba)_3$	CuI, LiI, pentamethylpiperidine	**12ab′**	40

was prepared as its di(*tert*-butyldimethylsilyl) ether at 2′-C and 5′-C. The bromine was introduced on compound **7a** with *N*-bromosuccinimide−silver nitrate in acetone[11] to quantitatively yield the 3′β-C bromoethynyl **8a**.

Various reaction conditions have been described for the dimerization of acetylenes: Glaser,[8] Eglington,[12] Hay,[13] and Sonogashira couplings.[14] When the reaction conditions of Hay (copper(I) chloride-pyridine) or Eglington (copper(II) acetate-pyridine) were applied to the monoprotected nucleoside **5a**, formation of the dimer **10aa′** was not observed and only the starting nucleoside was recovered. When the hydroxyl groups at 3′-C and 5′-C were protected as an acetate, the Eglington coupling of **6a** gave the dimer **9aa′** in 60% yield (entry 1, Table 1). The Sonogashira coupling was then assayed for homocoupling[15] of monoprotected nucleosides **5a** and **5b** (entries 2 and 3) to afford dimers **10aa′** and **10bb′** in 67 and 42% yields, respectively. When the same conditions were used for the disilyl ether **7b**, dimer **11bb′** was obtained in 80% yield (entry 4).

Since the Sonogashira heterocoupling gives higher yields than the Cadiot−Chodkiewicz heterocoupling,[16] we first assayed this method for the preparation of the dimer starting from 3′β-C bromoethynyl uridine disilyl ether **8a** and 3′β-C ethynyl monosilyl ether adenosine **5b**. When the reaction was carried out with bis(triphenylphosphine) palladium(II) dichloride in the presence of copper(I) iodide and diethylamine or triethylamine in THF under exclusion of oxygen, the yield was low and many side products were present (entry 5). Similar problems have been described for the coupling of ethynyl sugars, but appropriate conditions for the heterocoupling were found.[17] Compounds **8a** and **5b** were treated under these reaction conditions ($Pd_2(dba)_3$, CuI, LiI) to give the dimer **12ab′** isolated in 40% yield (entry 6).

The removal of the protecting groups in homo- and heterodimers **10aa′**, **10bb′**, and **12ab′** was achieved with ammonium fluoride in methanol[18] or with tetrabutylammonium fluoride in THF in 50−80% yields. The low solubility of the fully deprotected dimeric nucleosides (**3aa′**, **3bb′**, and **3ab′**) in organic solvents was a problem for their purification and seems to be the reason for some of the lower yields.

The cytotoxicity of dimers **3** was determined in the laboratory of Dr. P. Bischoff of our university using described tests on the RDM4 cell line.[19] The homodimer of 3′β-C ethynyl uridine **3aa′** had an IC_{50} of 40 μM. For comparison, this dimer is 1600 times less toxic than 3′β-C ethynyl uridine (IC_{50} 0.025 μM).[19,20] The other dimers showed no activity even at the highest concentration used.

Building blocks for the synthesis of polynucleotides incorporating a dinucleoside linked at 3′β-C by a butadiynyl group are now available by Eglington or Sonogashira coupling reaction.

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Supporting Information Available: Experimental procedure for calculations of the methyl ethers of dimers **3aa′**, **3ab′**, and **3bb′** and synthesis and physical data of compounds **5a**, **5b**, **6a**, **7a**, **7b**, **8a**, **9aa′**, **10aa′**, **10bb′**, **11bb′**, **12ab′**, **3aa′**, **3bb′**, and **3ab′**. This material is available free of charge via the Internet at http://pubs.acs.org.

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