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Carbometallation of 5-ethynyl-pyrimidine-2'-deoxy Nucleosides: Preparation of 5-(1-[E]-butenyl)- and 5-(3-[E]-hex-3-enyl)-2'-deoxyuridine.

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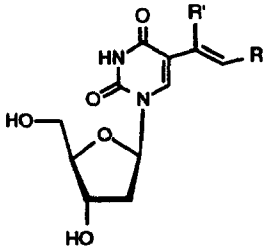
Abstract: *The regioselectivity of the carbometallation of 5-ethynyl-2'-deoxy-uridine with organocopper reagents is completely controlled in an anti-Markovnikov way by the oxygen atom at C-4. Hydrolysis or alkylation of the resulting vinyl-copper derivative afford respectively 5-(1-[E]-buenyl)- and 5-(3-[E]-hex-3-enyl)-2'-deoxy-uridine.*

Much efforts are devoted at increasing the stability of the complex with RNA or DNA and the penetration of these oligodeoxynucleotides in the cell. It has been demonstrated that replacement of 2'-deoxy-uridine and 2'-deoxy-cytidine in an oligonucleotide, respectively by thymidine and 5-methyl-2'-deoxy-cytidine stabilizes the triple-helix and double-helix complex.[1,2] The incorporation of an unsaturated side-chain (1-propynyl) at C-5 of uridine was recently shown to increase binding to both single strand RNA and double strand DNA.[3].

As a part of an antisense program devoted to modulation of gene expression of HIV, we decided to synthesize and evaluate a new class of pyrimidine nucleosides substituted at C-5 by a vinyl group bearing one or two alkyl groups (identical or not).

The feasibility of this project was examinated by the syntheses of 5-(1-[E]-butenyl)-2'-deoxy-uridine **1** and 5-(3-[E]-hex-3-enyl)-2'-deoxy-uridine **2** which are described in this letter.

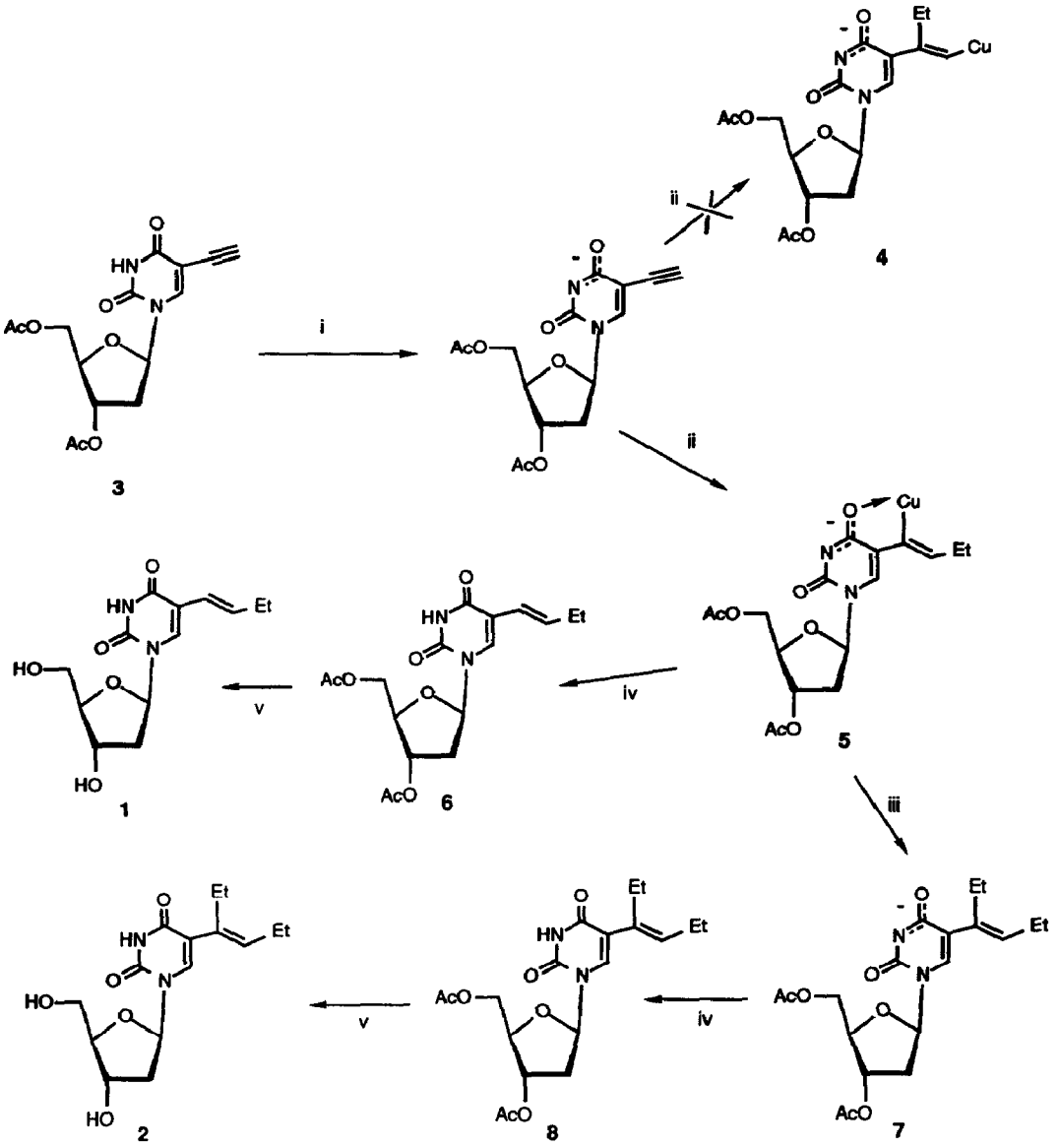


1 R= C_2H_5 , R'= H

2 R= R'= C_2H_5

Molecular modelling studies[4] have indicated that these substituents at C-5 of the pyrimidine moiety are planar with respect to the heterocycle and allow for increased stacking of the bases. Moreover oligodeoxynucleotides containing several of these modified nucleosides should exhibit enhanced hydrophobicity and consequentely promoted cell membrane penetration.[5]

SCHEME



Reagents and conditions: *i*; 1 equiv. EtCuBr - MgBr, THF, - 60°C. *ii*; further EtCuBr - MgBr, THF, - 60°C. *iii*; " coupling" with excess EtBr. *iv*; 5% aqueous HCl, - 15°C to room temperature. *v*; 1.15 equiv. NaOMe in MeOH, room temperature, then H^+ resin.

Pyrimidine nucleosides modified at the 5- position by an alkenyl residue have been prepared by a number of routes, most of them involving palladium catalyzed coupling between 5-iodouracil nucleosides and either activated olefins[6] or suitable alkenylmetal compounds.[7,8] In both of these methods a specific ethylenic synthon has to be prepared for each nucleoside. We decided to explore a more versatile methodology in which several modified nucleosides could be prepared from the same intermediate. We thought that C-metallation of the triple bond of protected 5-ethynyl-2'-deoxyuridine **3**[9] could be suitable for our purpose because the resulting vinyl-copper derivative could be hydrolyzed or alkylated, thus affording mono- or di- substituted vinyl group. Although the carbometallation of alkynes was extensively studied,[11] to the best of our knowledge this reaction was not previously used in nucleoside chemistry.

Addition of organocopper reagents to monosubstituted alkynes was demonstrated to be completely regio- and stereo-selective, occuring in a cis fashion and in the Markovnikov way.[11] However, taking in account the known sensitivity of the reaction to chelation effects,[11] we anticipated that the chelating character of the oxygen atom at position 4- of the uracil ring would be sufficiently enhanced by prior abstraction of the acidic 3-H to afford the "linear" vinylic copper derivative **5** rather than the "branched" Markovnikov adduct **4** (see scheme).

Due to the moderate solubility of nucleoside derivatives particularly at low temperature we had to use a rather polar solvent, *i.e.* THF. This fact turned us towards the methodology of Westmijze *et al.* involving the heterocuprate reagent EtCuBr.MgBr.[12] Consumption of one molar equivalent by the acidic H-3 led us to use an excess of cuprate. We found that 3 molar equivalents were necessary for the transformation to go to completion in 30 minutes at -60°C. Mild acidic work-up afforded a sirupy compound exhibiting a single spot on TLC (Merck 5554; silicagel F_{254}, eluted by petroleum ether / ethyl acetate : 1/3) although it was shown to be a mixture by ^{1}H.NMR spectroscopy. Conventionnal deacetylation was performed and reverse phase chromatography (Merck 5434; RP 18 F_{254s} , eluted by water / acetone : 20 / 13) allowed us to separate and identify the constitutents of the mixture as compounds **1** and **2**[13] (see scheme) in the respective amounts 9 to 1 (overall yield from **3** : 74%). No branched bisubstituted derivative arising from **4** was detected thus demonstrating that the carbometallation of the triple bond was effectively performed in the anti-Markovnikov way.

The presence of a small amount (≅ 10%) of **2** in the final product could be rationalized by a vinyl-alkyl coupling side reaction between the intermediate **5** and the ethyl cuprate reagent affording **8**. Such a reaction is known to take place under these conditions.[11] This was demonstrated by further experiment involving an excess of the ethyl heterocuprate in which the percentage of **2** (and **8**) was increased.

When excess of ethyl bromide (10 equiv.) was added after the carbometallation step, **2** was obtained (72% yield from **3**) after hydrolysis and deacetylation. It was not possible to completely transform **5** as shown by the presence of some **1** (≅ 10%) at the end of the sequence.

In conclusion, carbometallation of 5-ethynyl uracil nucleosides has proven to be a valuable alternate route for the synthesis of 5-alkenyl nucleosides with complete stereocontrol of the branched C=C bond. Further experiments are in progress in order to establish the versatility of this methodology, namely by taking in account the reactivity of vinylcopper intermediates such as **5**.

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13. The following physical data are representative: compound 1: ^{1}H-NMR (CD_3OD) δ 8.15 (1H, s, H-6) 6.55 (1H, m, J_{7-8} = 15.8 Hz, H-8), 6.4 (1H, m, $J_{1'-2'} = J_{1'-2''}$ = 6.7 Hz, H-1'), 6.2 (1H, d, H-7), 4.5 (1H, m, H-3'), 4.05 (1H, dd, H-4'), 4.0 and 3.8 (2H, 2 dd, $J_{5'-5''}$ = 12 Hz, H-5' and H-5"), 2.35 (2H, 2 dd, $J_{2'-2''}$ = 13.1 Hz, H-2' and H-2"), 2.25 (2H, m, J = 7.7 Hz, CH_2), 1.15 (3H, t,J = 7.7 Hz, CH_3); MS (CI NH_3) m/z 283 ($M+H^+$), 300($M+NH_4^+$); $[\alpha]_D^{20}$ + 7 (*c* 1.2 MeOH). compound 2: ^{1}H-NMR (CD_3OD) δ 7.9 (1H, s, H-6), 6.45 (1H, $J_{1'-2'} = J_{1'-2''}$ = 6.7 Hz, H-1'), 5.6 (1H, t, J = 7.2 Hz, H-8), 4.45 (1H, m, H-3'), 4.0 (1H, m, $J_{4'-3'}$ = 3.3 Hz, $J_{4'-5'}$ = 3.3 Hz, $J_{4'-5''}$ = 2.9 Hz, H-4'), 3.7 and 3.9 (2H, 2 dd, $J_{5'-5''}$ = 12.8 Hz, H-5' and H5") 2.40 (2H, q, J = 7.5 Hz, 7-CH_2), 2.30 (2H, m, $J_{2'-2''}$ = 12.8 Hz, H-2' and H-2"), 2.1 (2H, m, J = 7.5 Hz, 8-CH_2), 1.1 and 0.9 (2x3H, 2 t, 7-CH_3 and 8-CH_3); MS (CI NH_3) m/z 311 ($M+H^+$); $[\alpha]_D^{20}$ + 8 (*c* 0.67, MeOH).

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