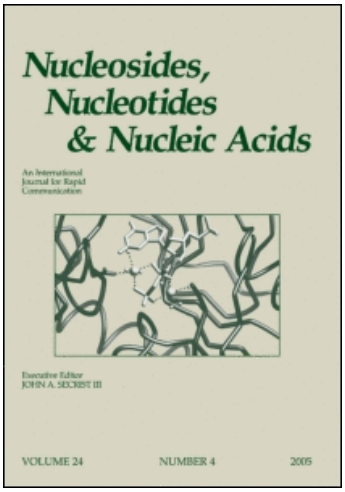

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PREPARATION OF 1-(3-C-(PROPA-1,2-DIENYL)-D-*RIBO*-PENTOFURANOSYL)URACIL, AN ALLENIC NUCLEOSIDE

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ABSTRACT

The Crabbé reaction was extended to the preparation of C-3′-allenyl-uridine. The effects of solvent and protecting group on the reaction were studied. The conversion in refluxing dioxan of disilyl either **3** proceeds to the corresponding allenic nucleoside **7**; whereas, in refluxing THF the Mannich base **5** was obtained. Fully deprotected Mannich base and allenic uridines **6** and **9** were tested for their antitumor activity.

C-3′-Ethynyl uridine and cytidine showed very potent anticancer activity (1). The interest to explore the activity of C-3′ substituted nucleosides induced us to prepare the C-3′-allenyl uridine (2). So far, 2′-desoxy-2′-ethenyliden nucleosides seem to be the only allenic nucleoside analogues described (3). However, the procedure based on palladium catalysed hydride reduction of propargylic −O-2′-carbonates was unsuitable to our purpose since the 3′-hydroxyl would be lost. By contrast, starting from ethynyl uridines **3** or **4** of controlled stereochemistry at C-3′, the Crabbé synthesis was the obvious choice beside the interest to extend this reaction to a multifunctional and crowded molecule. Crabbé and co-workers have described an easy two step homologation of an ethynyl group to an allenyl group with addition of one carbon atom (4).

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The reaction involves the formation of an intermediate, a Mannich base formed from acetylene and the Schiff base of formaldehyde with diisopropylamine. This intermediate undergoes a 1,5 hydrogen shift to the allene in the presence of cuprous salt (4). The reaction has been extended to one aliphatic aldehyde (5).

Two different preparations of C-3′-ethynyl uridine have been described: introduction of the ethynyl group to the nucleoside or introduction of the base into the modified ribose (6, 1a,b). The Crabbé homologation was studied on two protected nucleosides **3** and **4** prepared by standard procedures from known C-3′-trimethylsilylethynyl uridine **1** (6). When the disilyl nucleoside **3** was treated with formaldehyde and diisopropylamine in the presence of cuprous bromide in refluxing THF, the reaction stopped to the formation of the Mannich base **5**. Compound **5** was isolated in a yield of 81%. However, if the reaction was carried out in a higher boiling point solvent, dioxan at reflux for 20 hrs, the desired allenic nucleoside **7** was isolated in a yield of 41%. In refluxing N-methylpyrrolidone the yield dropped to 25%. In order to increase the yield in the synthesis of allenic compound we tried the homologation on the monosilyl ether **4**. We expected that the ethynyl group would be less crowded in derivative **4** than in the disilyl ether **3** and would thus undergo the homologation in higher yield. This was not borne out since the conversion to allene **8** went in a yield of 10%. During the Crabbé reaction, colour changes as described in (4) were observed. The silyl groups of base **5** were removed with tetrabutylammonium fluoride and gave in a yield of 95% the deprotected Mannich base **6**. Removal of the silyl groups of allenes **7** and **8** was realised under the same conditions to furnish the allenic nucleoside **9** in a yield of 72% and 66% respectively. The allenic nucleosides showed the expected spectroscopic properties of this functional group.

Antitumor activity was evaluated on RDM4 tumor cells with uridine analogues **6** and **9**. The Mannich base **6** showed an IC_{50} of 75 μM, close to that of 80 μM for C-3′β-prop-1-ynyluridine (7), whereas the allenic nucleoside **9** did not show any detectable activity in the usual antitumor activity test (1c).

EXPERIMENTAL SECTION

Melting points were measured on a Reichert microscope and are uncorrected. UV spectra were measured on a Hewlett-Packard 8451A. NMR spectra were recorded on a Bruker SY 200 or 400 MHz apparatus. MS were measured on a Trio 2000 (FISONS, UK) apparatus by chemical ionization (CI), or on a ZAB (FISONS, UK) apparatus by fast atom bombardment (FAB, matrix nitrobenzyl alcohol). Microanalyses were performed by the Service de Microanalyses de Strasbourg. Analytical thin-layer chromatography was performed on glass backed silica gel 60 F_{254} plates (Merck) and visualised by UV light. Products were isolated by flash chromatography on silica gel (Merck, 60, 230–400 mesh).

1-[2,5-di-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-[(trimethylsilyl)-ethynyl]-β-D-*ribo*-pentofuranosyl]uracil (2). *tert*-Butyldimethylsilyl chloride (0.87 g, 5.8 mmol) was added to a solution of monosilyl ether **1** (6) (2.19 g, 4.8 mmol) in dry pyridine (15 mL). The suspension readily obtained was stirred at rt for 4 h, and then the solvent was evaporated *in vacuo*. The residue was chromatographed (silica gel: ether-hexane 7:3) to give the nucleoside **2** (2.46 g, 90% yield). Mp 54 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.31 (bs, 1H), 8.06 (d, $J = 8.1$ Hz, 1H), 6.12 (d, $J = 6.9$ Hz, 1H), 5.72 (d, $J = 8.1$ Hz, 1H), 4.31 (d, $J = 6.9$ Hz, 1H), 4.16 (m, 1H), 3.95 (m, 2H), 3.34 (s, 1H), 0.96 (s, 18H), 0.18 (s, 18H), 0.00 (s, 3H); MS (CI) m/z 569 (MH^+, 100), 511 (23).

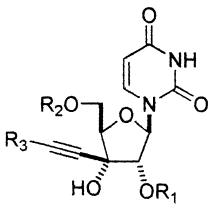
1-[2,5-di-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (3). Potassium carbonate (1.56 g, 11.3 mmol) was added to a solution of disilyl ether **2** (2.14 g, 3.75 mmol) in methanol (50 mL). The solution was stirred at rt for 1.5 h, and glacial acetic acid (0.66 mL, 11.5 mmol) was added. The mixture was poured in water (100 mL), and the aqueous phase was extracted with chloroform (2 × 200 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was chromatographed (silica gel: ether-hexane 7:3) to give the terminal alkyne **3** (1.71 g, 92% yield). Mp 61–64 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.16 (bs, 1H), 8.06 (d, $J = 8.2$ Hz, 1H), 6.13 (d, $J = 7.2$ Hz, 1H), 5.73 (dd, $J_1 = 8.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.34 (d, $J = 7.2$ Hz, 1H), 4.20 (m, 1H), 3.95 (m, 2H), 3.38 (s, 1H), 2.61 (s, 1H), 0.95 (s, 9H), 0.90 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), 0.10 (s, 3H), −0.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 163.6, 151.1, 141.0, 103.7, 86.7, 86.5, 82.3, 80.6, 78.3, 74.7, 64.4, 26.6, 26.1, 19.1, 18.4, −3.8, −4.3, −4.7, −5.2; MS (CI) m/z 514 (MNH_4^+, 100), 497 (MH^+, 60), 439 (4).

1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (4). Potassium carbonate (0.2 g, 1.43 mmol) was added to a solution of **1** (6) (0.43 g, 0.95 mmol) in methanol (50 mL). The solution was stirred at rt for 1.5 h, and an aqueous hydrochloric acid solution (C = 0.1 N, 16 mL, 1.6 mmol) was added. The aqueous phase was extracted with chloroform (100 mL, then 2 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was chromatographed (silica gel: ether-hexane 8:2) to give the terminal alkyne **4** (0.26 g, 72% yield) as a white solid: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.41 (bs, 1H), 7.71 (d, $J = 8.1$ Hz, 1H), 5.79 (d, $J = 7.1$ Hz, 1H), 5.78 (d, $J = 8.1$ Hz, 1H), 4.58 (d, $J = 7.1$ Hz, 1H), 4.22 (m, 1H), 3.98 (m, 2H), 3.36 (s, 1H), 2.69 (s, 1H), 2.64 (m, 1H), 0.90 (s, 9H), 0.14 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (CD_3OD, 50 MHz) δ 156.0, 152.6, 143.1, 103.5, 88.6, 82.8, 81.2, 77.8, 74.7, 63.0, 26.3, 19.0, −3.7, −4.7.

1-[2,5-di-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-[(di-*iso*-propylamino)-prop-1-ynyl]-β-D-*ribo*-pentofuranosyl]uracil (5). Diisopropylamine (0.36 mL, 2.58 mmol) and cuprous bromide (0.09 g, 0.65 mmol) were added to a solution of disilyl ether **3** (0.64 g, 1.29 mmol) in dry THF (5 mL). Paraformaldehyde (0.10 g, 3.23 mmol) was added to the green suspension. The mixture was stirred at reflux for 0.5 h, and a saturated aqueous ammonium chloride solution (25 mL) was added. The mixture was extracted with dichloromethane (25 mL), and the organic phase was washed successively with a saturated aqueous ammonium chloride solution (3 × 25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was chromatographed (silica gel: ether-hexane 6:4) to give the Mannich base **5** (0.64 g, 81% yield). Mp 63–65 °C; UV (MeOH) δ_{max} 264 nm (ε 9600 M^{-1} cm^{-1}); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.52 (bs, 1H), 8.04 (d, $J = 8.1$ Hz, 1H), 6.10 (d, $J = 7.0$ Hz, 1H), 5.72 (d, $J = 8.1$ Hz, 1H), 4.44 (d, $J = 7.0$ Hz, 1H), 4.14 (m, 1H), 3.94 (m, 2H), 3.41 (s, 2H), 3.24 (s, 1H), 3.18 (spt, $J = 6.5$ Hz, 2H), 1.06 (d, $J = 6.5$ Hz, 12H), 0.95 (s, 9H), 0.89 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), 0.10 (s, 3H), −0.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 163.1, 150.5, 140.5, 102.9, 87.6, 86.5, 86.1, 80.3, 80.1, 74.4, 63.9, 48.3, 34.4, 26.0, 25.6, 20.6, 18.5, 17.9, −4.5, −4.8, −5.2, −5.6; MS (FAB+) m/z 611 (MH^+, 100), 595 (2), 553 (3). Anal. Calcd for $C_{30}H_{55}N_3O_6Si_2$: C, 59.07; H, 9.09; N, 6.89. Found: C, 59.00; H, 9.10; N, 6.71.

1-[3-*C*-[(Diisopropylamino)-prop-1-ynyl]-β-D-*ribo*-pentofuranosyl]uracil (6). *n*-$Bu_4NF \cdot 3H_2O$ (0.35 g, 1.19 mmol) was added to a solution of disilyl ether **5** (0.33 g, 0.54 mmol) in dry THF (10 mL). The solution was stirred at rt for 2 h, and then directly transferred to be chromatographed (silica gel: ethyl acetate) to give the deprotected Mannich base of nucleoside **6** (0.20 g, 95% yield). Mp 148–149 °C (decomp.); ^{1}H NMR (Acetone-d_6, 200 MHz) δ 9.98 (bs, 1H), 8.07 (d, $J = 8.1$ Hz, 1H), 5.99 (d, $J = 6.4$ Hz, 1H), 5.64 (d, $J = 8.1$ Hz, 1H), 4.39 (d, $J = 6.4$ Hz, 1H), 4.06 (m, 1H), 3.91 (m, 2H), 3.45 (s, 2H), 3.19 (spt, $J = 6.5$ Hz, 2H), 1.06 (d, $J = 6.5$ Hz, 12H); ^{13}C NMR (Acetone-d_6, 50 MHz) δ 163.5, 151.8, 141.8, 102.8, 88.6, 88.0, 87.4, 81.7, 79.9, 74.3, 63.1, 49.1, 34.8, 20.9; MS (FAB+) m/z 382 (MH^+, 100), 366 (2).

1-[2,5-di-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-(propa-1,2-dienyl)-β-D-*ribo*-pentofuranosyl]uracil (7). Diisopropylamine (0.28 mL, 2.0 mmol) and cuprous bromide (0.07 g, 0.5 mmol) were added to a solution of **3** (0.50 g, 1.0 mmol) in dry 1,4-dioxane (5 mL). Paraformaldehyde (0.08 g, 2.5 mmol) was added to the green suspension. The mixture was stirred at reflux for 20 h, and a saturated aqueous ammonium chloride solution (25 mL) was added. The mixture was extracted with dichloromethane (25 mL), and the organic phase was washed successively with a saturated aqueous ammonium chloride solution (3 × 25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was chroma-

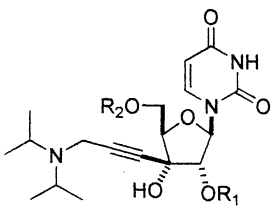


1, R_1 = TBDMS; R_2 = H
R_3 =TMS

2, R_1 = R_2 =TBDMS;
R_3 =TMS

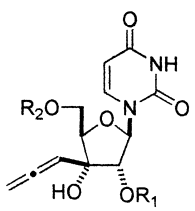
3, R_1 = R_2 =TBDMS;
R_3 =H

4, R_1 = TBDMS;
R_2 = R_3 =H



5, R_1 = R_2 = TBDMS

6, R_1 = R_2 = H



7, R_1 = R_2 = TBDMS

8, R_1 = TBDMS; R_2 = H

9, R_1 = R_2 = H

tographed (silica gel: ether-hexane 5:5) to give the allene **7** (0.21 g, 41% yield). Mp 66–68 °C; UV (MeOH) λ_{max} 261 nm (ε 10600 M^{-1} cm^{-1}); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.06 (bs, 1H), 7.95 (d, $J = 8.1$ Hz, 1H), 6.19 (d, $J = 7.4$ Hz, 1H), 5.73 (d, $J = 8.1$ Hz, 1H), 5.38 (dd, $J_1 \approx J_2 = 6.6$ Hz, 1H), 5.05 (d, $J = 6.6$ Hz, 1H), 4.95 (d, $J = 6.6$ Hz, 1H), 4.13 (m, 1H), 4.06 (d, $J = 7.4$ Hz, 1H), 3.85 (m, 2H), 2.97 (s, 1H), 0.97 (s, 9H), 0.87 (s, 9H), 0.16 (s, 6H), 0.01 (s, 3H), −0.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ208.9, 162.7, 150.4, 140.3, 102.9, 92.2, 86.1, 86.0, 79.4, 79.3, 77.7, 63.4, 26.0, 25.4, 18.3, 17.8, −4.8, −4.9, −5.5, −5.6; MS (FAB+) *m/z* 511 (MH^+, 100), 495 (6), 453 (75).

1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-(propa-1,2-dienyl)-β-D-*ribo*-pentofuranosyl]uracil (8). Diisopropylamine (0.17 mL, 1.2 mmol) and cuprous bromide (0.04 g, 0.3 mmol) were added to a solution of **4** (0.23 g, 0.6 mmol) in dry 1,4-dioxane (1 mL). Paraformaldehyde (0.05 g, 1.5 mmol) was added to the green suspension. The mixture was stirred at reflux for 72 h, and a saturated aqueous ammonium chloride solution (25 mL) was added. The mixture was extracted with dichloromethane (25 mL), and the organic phase was washed successively with a saturated aqueous ammonium chloride solution (3 × 25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The residue was chromatographed (silica gel: ether) to give the allene **8** (0.02 g, 10% yield). ^1H NMR ($CDCl_3$, 200 MHz) δ 8.40 (bs, 1H), 7.46 (d, $J = 8.1$ Hz, 1H), 5.78 (d, $J = 8.1$ Hz, 1H), 5.58 (dd, $J_1 \approx J_2 = 6.6$ Hz, 1H), 5.51 (d, $J = 7.4$ Hz, 1H), 5.04 (d, $J = 6.6$ Hz, 1H), 4.94 (d, $J = 6.6$ Hz, 1H), 4.67 (d, $J = 7.4$ Hz, 1H), 4.16 (m, 1H), 3.93–3.42 (m, 3H), 2.93 (s, 1H), 0.86 (s, 9H), 0.05 (s, 3H), −0.05 (s, 3H).

1-[3-*C*-(Propa-1,2-dienyl)-β-D-*ribo*-pentofuranosyl]uracil (9). *n*-$Bu_4NF \cdot 3H_2O$ (0.18 g, 0.56 mmol) was added to a solution of disilyl ether **7** (0.13 g, 0.25 mmol) in dry THF (2.5 mL). The solution was stirred at rt for 4 h, and then directly transferred to be chromatographed (silica gel: ethyl acetate). After evaporation of the solvents, the oily compound was dissolved in acetone. To the cooled solution ($-30\,^{\circ}C$), hexane was added to precipitate the deprotected allenic nucleoside **9** (0.05 g, 72% yield). Mp 95–98 °C; UV (MeOH) λ_{max} 261 nm (ε 9600 M^{-1} cm^{-1}); ^1H NMR (CD_3OD, 200 MHz) δ 7.93 (d, $J = 8.1$ Hz, 1H), 5.98 (d, $J = 7.6$ Hz, 1H), 5.69 (d, $J = 8.1$ Hz, 1H), 5.45 (dd, $J_1 \approx J_2 = 6.6$ Hz, 1H), 4.91 (m, 2H), 4.29 (d, $J = 7.6$ Hz, 1H), 3.99 (m, 1H), 3.73 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ 209.7, 166.1, 152.9, 143.2, 105.2, 103.1, 92.8, 89.1, 88.9, 78.9, 78.3, 62.6; MS (FAB+) m/z 283 (MH^+).

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This article is dedicated by the senior author to the memory of Pierre Crabbé with whom he did his first publication: Biellmann, J.F.; Crabbé, P.; Ourisson G. Le Diptérocarpol-II. Stéréochimie en C-13 et en C-17. *Tetrahedron*, **1958**, *3*, 303–309.

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