Synthesis of acyclic nucleoside 5-Ocarbonyl uracil derivative

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Summary: The synthesis of 1-(2,2-dihydroxymethyl-3-hydroxypropoxymethyl)-5-o-carboranyl uracil compound **7** as potentially antiviral agent and a suitable candidate for Boron Neutron Capture Therapy (BNCT) is described starting from 5,5-dihydroxymethyl-1,3-dioxane **1**, an intermediate 2,2,2-triacetoxymethyl ethoxymethyl acetyl **2** was synthesized and coupled with 5-Iodouracil to get 1-(2,2,2-triacetoxymethyl ethoxymethyl)-5-iodouracil intermediate **3** which enabled us to obtain the final compound **7**

Keywords: Carboranes, Acyclic Nucleosides, Coupling reaction, cancer therapy

Syntèse d'un nucléoside acyclique: dérivé du 5-O-Carbonyle uracil

Résumé: La synthèse du 1-(2,2-dihydroxyméthyl-3-hydroxypropoxyméthyl)-5-o-carboranyl uracile **7**, un nucléoside acyclique pouvant être potentiellement antiviral et candidat la *boron neutron capture thérapy* (BNCT) est ici décrite. Partant du 5,5-dihydroxyméthyl-1,3-dioxane **1**, l'intermédiaire 2,2,2-triacétoxyméthyl éthoxyméthyl acétyle **2** est obtenu, puis il est couplé avec le 5-Iodouracile pour isolement du composé clé le 1-(2,2,2-triacétoxyméthyl éthoxyméthyl)-5-iodouracile **3** permettant la synthèse du produit final **7.**

Mots clés : Carboranes, Acyclo nucléosides, réaction de glycosilation, thérapie du cancer

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1. Introduction

Acyclic nucleosides[1,2] have long been sought for the broad spectrum of antiviral activities, since the discovery of Acyclovir (ACV) active against HSV. Nucleosides as well as their acyclic analogues exert antiviral activity into their triphosphate form[3]. In a recent report we described synthesis of lipophilic tethered 5-o-carboranyl-2'-deoxyuridine[4] as an intermediate for building block for oligonucleotides synthesis and as potential agent for Boron Neutron Capture Therapy (BNCT) which is a cancer therapeutic modality using the combination of boron containing compounds targeted to the tumor cells and neutron irradiation as initiator to produce microreactions confined mostly within the tumors[5-7].
Attentions have been paid to molecules containing carboranes[8-15] since these boron cages can provide a relatively high number of fissionable ^{10}B atom to tumor cells.
In this regard, we describe the synthesis of Acyclic -5-O-carboranyluracil potentially antiviral agent and a suitable candidate for BNCT.

1-(2,2-dihydroxymethyl-3-hydroxypropoxymethyl)-5-o-carboranyl uracil **7** was prepared as follow: 5,5-dihydroxymethyl-1,3-dioxolane **1** was allowed to react with acetic anhydride under acid catalyst to give tetra-o-acetylated intermediate **2** in good yield. Compound **2** reacted in one pot glycolysation reaction with 5-Iodouracil catalysed by $SnCl_4$/BSA and gave 1-(2,2,2-triacetoxymethyl ethoxymethyl)-5-iodouracil **3** in satisfactory yield (70%) after chromatography purification. Product **3** was then coupled with trimethylsilylacetylene in the presence of $Pd(PPh_3)Cl_2/CuI/Et_3N$[16-17] to produce 1-(2,2,2-triacetoxymethylethoxymethyl)-5-trimethylsilyl ethynyl uracil **4**, which on selective deprotection with TBAF gave the corresponding 5-end triple bound uraril **5** suitable for boron cluster building up using decaborane/CH_3CN under reflux in THF[18] allowed to isolate product **6**, which on deprotection with NaOMe gave the expected compound **7**. The final compound **7** was also obtained from the fully deprotected intermediate **8** in moderate yield (15%) from **4**. Figure 1.
The corresponding acyclic 1-(2,2-dihydroxymethyl-3-ydroxypropoxymethyl) purines derivatives have been prepared, the purine aglycon was silylated prior the glycolysation reaction was performed.

2. Conclusion

The carboranyl compounds are very lipophilic, and then compound **7** having three hydroxylmethyl groups can relatively moderate the lipophilicity effect of the carboranyl cluster presuming that, one could expect a better cell uptake of the compound .

3. Experimental section.

Starting materials and chemicals were purchased from Aldrich company. Characterization and analysises were performed on the intermediates and final compounds. Liquid chromatography purifications were done on gel sylice 60 mesh; HPLC analysis were performed using Whatman A C18 RP column; UV spectra were obtained on Beckman DU 640 spectrophotometer; ^1H NMR performed on Varian 400 MHz; mass spectra were conducted on Finnigan TSQ 7000.

2,2,2-triacetoxymethyl ethoxymethyl acetyl 2

Concentrated sulphuric acid 8 µl was added to an ice bath cooled mixture of 5,5-Hydroxymethyl-1,3-dioxolane 2g (13.5 mmol) and acetic anhydride 5 ml, the solution was stirred at room temperature for 5 hrs, and pored into saturated 100 ml

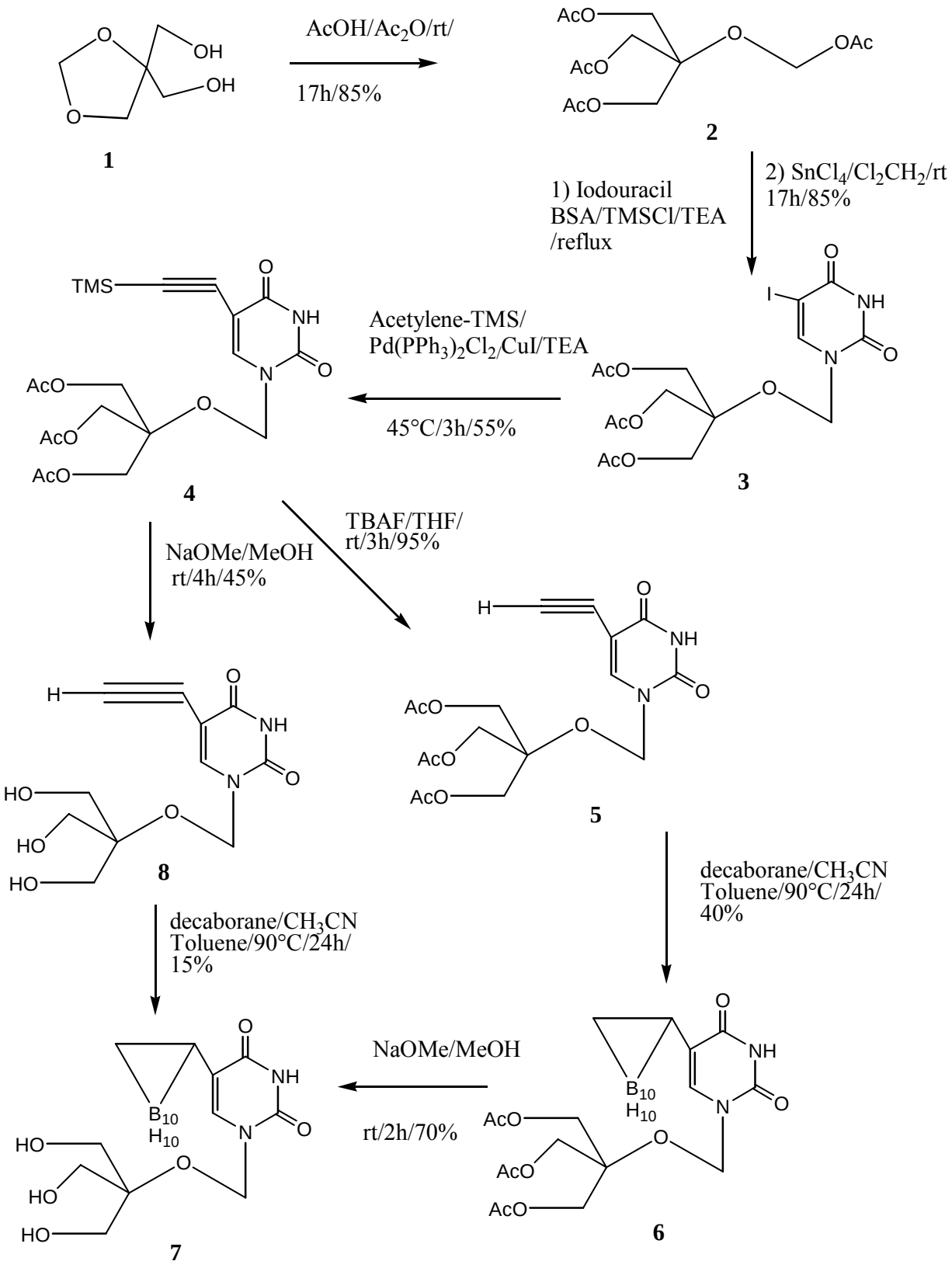

Figure 1 : scheme synthesis of compound **7**

of $NaHCO_3$, extracted with 2x150 ml of CH_2Cl_2, the combined organic extracts were washed with 100 ml of H_2O, 100 ml of $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated under reduce pressure.
Product **2**, an oil, was used as such without further purifications. 89 % yield. ^1H NMR: ($CDCl_3$)δppm. 4.6 (s, 2H, CH_2); 3.45 (s, 6H, $3CH_2$); 3.30 (m, 12H, $4CH_3$).

1-(2,2,2-triacetoxymethyl ethoxymethyl)-5-iodouracil 3

5-Iodouracil 1g (4.20 mmol) and compound 2 1.70g (5.04 mmol, 1.2 eq) in suspensionin anhydrous 52 ml of CH_2Cl_2 was stirred under a nitrogen atmosphere at room temperature, then BSA 2.59 ml (10.5 mmol, 2.5 eq) was added, stirring was maintained until complete dissolution, then $SnCl_4$ 0.400 ml (4.2 mmol) was added. The reaction solution was stirred at room temperature overnight, TLC monitoring in Hexane/Ethyl acetate 7/3 (v/v) as mobile phase revealed complete transformation of 5-Iodo uracil to a fast moving spot. The solution was pored into a mixture of 75 ml of saturated $NaHCO_3$/ CH_2Cl_2, organic layer separated, the aqueous phase extracted with 25 ml of CH_2Cl_2, combined organic extracts were washed with 30 ml H_2O, 30 ml brine, then dried over $MgSO_4$, filtered and the solvent removed under reduce pressure. The residue was purified by flash chromatography on silica elution with Hexane/Ethyl acetate 7/3 (v/v), and then recrystallized from ether to give compound **3,** 70% yield, 1.6g. Mp: 119.7°C. ^1H NMR: ($CDCl_3$)δppm. 10.8 (s, 1H, NH); 7.9 (s, 1H, H6); 5.9 (s, 2H, CH_2); 4.0 (s, 2H, CH_2); 3.7 (s, 6H, $3CH_2$); 3.31 (m, 9H, $3CH_3$). MS: for $C_{16}H_{21}IN_2O_9$: calcd m+/z = 512.55, found 512.51.

1-(2,2,2-triacetoxymethyl ethoxymethyl)-5- trimethylsilylethynyl uracil 4

To the product **3**, 1.06g (2.07 mmol) in solution in anhydrous THF 12 ml, was added trimethylsilylacetylene 0.380 ml (2.7 mmol) and Et_3N 0.600 ml (4.14 mmol) followed by $Pd(PPh_3)Cl_2$ 0.145g (0.207 mmol) and CuI 0.039g (0.207 mmol). The reaction was stirred under inert atmosphere at 50°C for 4 hrs. TLC monitoring showed complete conversion of the starting material to a new spot, the solution was diluted with Ethyl acetate 30 ml, washed with a mixture of H_2O/EDTANa 30 mml, with Na_2SO_4 1N (30 ml) then water. The organic phase was dried over MgSO4, filtered solvent evaporated to dryness under reduce pressure, the residue was purified by flash chromatography, elution with Ethyl acetate to give 0.400 g of **4** (42% yield). ^1H NMR: ($CDCl_3$)δppm. 10.8 (s, 1H, NH); 7.9 (s, 1H, H6); 5.9 (s, 2H, CH_2); 4.0 (s, 2H, CH_2); 3.7 (s, 6H, $3CH_2$); 3.31 (m, 9H, $3CH_3$).

1-(2,2,2-triacetoxymethyl ethoxymethyl)-5-ethynyl uracil 5

Product **4**, 0.300g (0.62 mmol) was dissolved in anhydrous THF 15 ml and TBAF 0.5 ml was added and the solution stirred at room temperature with TLC monitoring for 1 hr. The solvent was evaporated under reduce pressure, the residue dissolved in CH_2Cl_2 35 ml and washed with water 20 ml, brine 20 ml, the organic phase dried over Na_2SO_4, filtered solvent removal under vacuum gave crude product 5, which was purified by chromatography elution with Ethyl acetate/methanol 95/5 (v/v). 230 mg (90%) of expected material 5 was obtained. IR (KBr) 2140 cm^{-1}, 3270 cm^{-1} and 690 cm^{-1} (acetylenic C-H). ^1H NMR: ($CDCl_3$))δppm. 11.0 (s, 1H, NH); 8.2 (s, 1H, H6); 5.9 (s, 2H, CH_2); 5.0 (s, 1H, H); 4.0 (s, 2H, CH_2); 3.7 (s, 6H, $3CH_2$); 3.31 (m, 9H, $3CH_3$).

1-(2,2,2-triacetoxymethyl ethoxymethyl)-5-o-carboranyl uracil 6

Compound **5**, 0.200g (0.48 mmol) was dissolved in anhydrous Toluene 4 ml and a

freshly distilled CH3CN 0.042ml (0.8 mmol, 2eq) was added followed by decaborane 0.06g (0.48 mmol). The mixture stirred with reflux under nitrogen atmosphere for 24 hrs, then decaborane 0.06g and CH_3CN 0.048 ml were added, refluxing was continued for an additional 24 hrs. The reaction solution was allowed to cool to room temperature, then evaporated to dryness and purified by chromatography elution with Hexane/Ethyl acetate 7/3 (v/v). Fractions containing the fast moving spots were combined, concentrated to dryness, the residue lyophylized to give compound **6** (58mg). ^1H NMR: ($CDCl_3$))δppm. 10.7 (s, 1H, NH); 7.7 (s, 1H, H6); 5.9 (s, 2H, CH_2); 5.1 (broad, 1H, C-H); 4.0 (s, 2H, CH_2); 3.7 (s, 6H, $3CH_2$); 3.31 (m, 9H, $3CH_3$); 2.98-1.6 (broad, 10H, carboranyl). MS (ESI M-1) : m+/z = 527.2 for $C_{18}H_{32}B_{10}N_2O_9$.

1-(2,2-dihydroxymethyl-3-hydroxypropoxymethyl)-5-o-carboranyl uracil 7

Compound **6**, 0.040g (0.075 mmol) in solution in anhydrous methanol was treated with sodium methoxyde (3.5 eq) at room temperature, the reaction was neutralized with Dowex 50 H+ resine, filtered, solvent was removed under reduce pressure, the residue was purified by chromatography elution with CH_2Cl_2/MeOH 8/2 (v/v). ^1H NMR: (DMSO-d_6))δppm. 10.6 (s,1H, NH); 7.6 (s, 1H, H6); 5.8 (s, 2H, CH_2); 5.1 (broad, 1H, C-H); 4.0 (s, 2H, CH_2); 3.4 (s, 6H, $3CH_2$); 2.98-1.6 (broad, 10H, carboranyl). MS (ESI M-1) : 401.2 for $C_{12}H_{25}B_{10}N_2O_6$. Pyrrolidine test to get *nido* form [4]: ESI (M-1) for $C_{12}H_{23}B_9N_2O_6$: calcd $m^+\backslash z$ = 389.2, found $m^+\backslash z$ = 388.7. Analysis. C: 35.80%; H: 6.21%; N: 6.96%; O: 23.86%; B: 26.87%. found C: 35.90%; H: 6.26%; N: 6.95%; O: 23.90%; B: 26.92%.

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