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Synthesis and *in vitro* antiviral activity of lipophilic pyrimidine nucleoside/carborane conjugates

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ABSTRACT

A series of novel conjugates of *para*-carborane with 5-ethynyl-uridine or 2′-deoxyuridine were synthesized. The conjugates were prepared by Sonogashira coupling of *para*-carborane terminal alkynes and 5-iodo-nucleoside. The designed compounds demonstrated low to moderate cytotoxicity in several cell lines. The antiviral activities of the agents were measured against a panel of DNA and RNA viruses. The most potent compound reported is 5-[(1,12-dicarba-*closo*-dodecaboran-2-yl)ethyn-1-yl]-2′-deoxyuridine (**8**), with an IC50 value of 5.5 μM and a selectivity index higher than 180. This compound is unusual in that it exhibits antiviral activity against HCMV and is not active against HSV-1, HPIV-3 or EMCV.

1. Introduction

The synthesis of analogs and modifications of natural nucleosides has attracted significant interest due to their crucial roles in biochemical systems and the search for therapeutically useful agents [1]. As a result, several nucleoside-derived drugs have been identified and approved for the therapy of viral infections and cancers. C-5 substituted pyrimidine nucleosides form a unique class of nucleoside derivatives due to their compatibility with nucleosides and nucleic acids metabolizing enzymes. Consequently, these compounds play an important role as components of nucleotide-derived tools for molecular genetics [2]. Some of these agents are also clinically used drugs [3], with Brivudine®, 5-(2-bromovinyl)-2′-deoxyuridine (BVDU), a potent and selective inhibitor of herpes viruses [4], as a representative example.

Recently, bicyclic furano pyrimidine 2′-deoxynucleoside derivatives of C-5 alkynyl uridine, were synthesized and demonstrated to be potent and selective inhibitors of certain herpes viruses without noticeable cytotoxicity [5,6]. An unusual structure-activity relationship was noted, with the requirement of a long alkyl side chain attached to the furanosyl ring for antiviral activity. The bicyclic systems without chains were noted to be inactive against herpes simplex virus type 1 (HSV-1) and 2 (HSV-2), human cytomegalovirus (HCMV) and varicella zoster virus (VZV) [7,8]. Also, longer chain homologs of the bicyclic compounds were reported to exhibit no antiviral activity [9,10], narrowing the optimal length of the side chain to C_8–C_{10} alkyl units. These homologs were all rather non-cytotoxic [6] and exhibited high lipophilicity with improved membrane permeation [11].

These observations prompted us to explore the lipophilic properties of carboranes and to design novel C-5 modified uridine derivatives as potential antiviral agents. In this manuscript, we describe the synthesis of several uridine and 2′-deoxyuridine derivatives bearing a *para*-carborane (1,12-dicarba-*closo*-dodecaboran-1-yl) modification attached at the carbon or boron atom of the cage through an alkynyl linker tethered at C-5 of uracil. The evaluation of the cytotoxicity and antiviral activity of these compounds is also reported.

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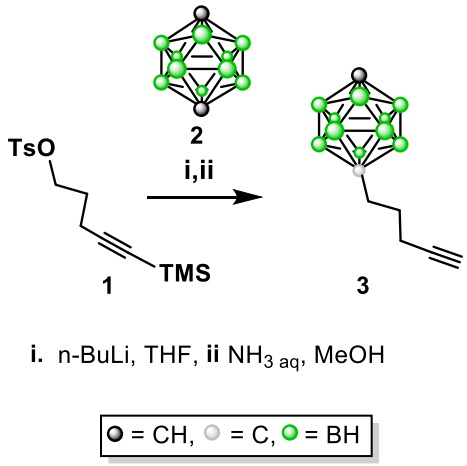

Fig. 1. Synthesis of 1-[1-(pent-4-ynyl)]-1,12-dicarba-*closo*-dodecaborane (**3**) boron cluster donor.

2. Materials and methods

2.1. Materials

If not otherwise stated chemicals and solvents were obtained from Sigma–Aldrich Chemical Company (St. Louis, USA) and used without further purification. *para*-Carborane was purchased from KatChem (Prague, Czech Republic), 5-iodouridine and 5-iodo-2′-deoxyuridine were purchased from Carbosynth (Berkshire, United Kingdom), pent-4-yn-1-ol was from Sigma–Aldrich Chemical Company (St. Louis, USA). Flash chromatography was performed on silica gel 60 (230–400 mesh, ASTM, Sigma–Aldrich Chemical Company) (St. Louis, USA). R_f values refer to analytical TLC performed using pre-coated silica gel 60 F254 plates purchased from Sigma–Aldrich (Steinheim, Germany) and developed in the solvent system indicated. Compounds were visualized by use of UV light (254 nm) or 0.5% acidic solution of $PdCl_2$ in HCl/methanol for boron containing derivatives. The yields are not optimized.

2.2. Methods

2.2.1. Nuclear Magnetic Resonance (NMR)

^{1}H NMR, ^{13}C NMR and ^{11}B-NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer equipped with a direct BBO ATM probe, the spectra for ^{1}H, ^{13}C, and ^{11}B nuclei were recorded at 600.26 MHz, 150.94 MHz and 192.59 MHz, respectively. All chemical shifts are reported in ppm (δ) relative to residual signal of the solvent. For NMR following solvents were used: $CDCl_3$ ($\delta_H = 7.25$, $\delta_C = 77.00$ ppm), CD_3OD ($\delta_H = 3.35$, $\delta_C = 50.00$ ppm), DMSO-d_6 ($\delta_H = 2.50$, $\delta_C = 39.70$ ppm). *J* values are given in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, bs = broad singlet, m = multiplet.

2.2.2. Mass spectrometry

Fast atom bombardment (FAB) mass spectra were recorded with a Finnigan MAT 95 spectrometer (Bremen, Germany) using glycerin (Gly) as the matrix. The *m*/*z* was measured in a positive and negative mode. Calculation of the theoretical molecular mass for compounds was performed using the "Show Analysis Window" option in ChemDraw Ultra 12.0 program. The calculated *m*/*z* corresponds to the average mass of the elements consisting of natural isotopes.

2.2.3. Ultraviolet spectroscopy measurements (UV)

UV measurements were performed on GBC Cintra10 UV–VIS spectrometer (Dandenong, Australia). Samples for UV experiments, ca. 0.5 A_{260} Optical Density Unit (ODU) of each compound were dissolved in 95% C_2H_5OH. The measurement was performed at ambient temperature.

2.2.4. Infrared spectroscopy (FT-IR) measurements

Infrared absorption spectra were recorded with a Fourier-transform, infrared spectrometer Thermo Scientific Nicolet 6700, equipped with an ETC EverGlo* source for the IR range, Ge-on-KBr beam splitter, and DLaTGS/KBr detector with Smart Orbit Attenuated Total Reflectance (ATR) accessory and diamond window. Samples were placed directly on the diamond crystal and pressure was added to conform the surface of the sample to the surface of the diamond crystal.

2.2.5. HPLC analysis

Analyses were performed on a Hewlett–Packard 1050 system equipped with a UV detector. A Hypersil Gold C18 RP, 250 × 4.6 mm column, 5 μm particle size (Thermo Scientific, Runcorn, UK) was used, the flow rate was 1 ml/min. All analyses were run at ambient temperature. The gradient elution profile was as follows: 0 min–100% A, 0–20 min 0–100% D, 20–25 min 100% D, 25–30 min 0–100% A. Buffer A = 2% CH_3CN/0.1 M triethylammonium bicarbonate (TEAB), buffer D = 60% CH_3CN/0.1 M TEAB. UV detection was performed at λ = 268 nm.

5-Trimethylsililpent-4-yn-1-tosylate (***1***) was synthesized as described [12].

(1,12-Dicarba-closo-dodecaboran-2-yl)ethyne (***6***) was obtained according to the described procedure [13].

2.2.6. Synthesis of 1-[1-(pent-4-ynyl)]-1.12-dicarba-closo-dodecaborane (*3*)

The procedure was performed under anhydrous conditions, with a positive pressure of argon. To 1,12-dicarba-*closo*-dodecaborane (**2**) (0.050 g, 0.350 mmol) dissolved in anhydrous tetrahydrofurane (THF) (2.0 ml) cooled to 0 °C n-BuLi (1.6 M, 0.220 ml) was added then the mixture was stirred 1 h at 0 °C. Next 5-trimethylsililpent-4-yn-1-tosylate (**1**) was added (0.109 g, 0.350 mmol) and the mixture was stirred 1.5 h at the room temperature. Next the solvent was removed in vacuum, the residue was dissolved in the CH_2Cl_2 (2.5 ml) and the obtained solution was washed with water (3 × 1.0 ml). Organic layer was dried over $MgSO_4$ and evaporated to dryness to give 0.105 g of the crude product. The crude product was purified by silica gel column chromatography using a hexane as eluting solvent to afford 0.042 g of pure, protected with trimethylsilyl-group **3** as a pale oil. Yield 43%. Next, the protected **3** was dissolved in THF (2.0 ml), cooled to 0 °C then tetrabutylammonium fluoride (TBAF) (0.200 ml) was added. The reaction mixture was stirred 2 h at the room temperature then the solvent was evaporated to dryness to give 0.088 g of the crude **3** which was purified by silica gel column chromatography using hexane as an eluting solvent to afford 0.030 g of pure product **3** as a pale oil. Yield 97%. TLC (hexane): $R_f = 0.48$; ^{1}H NMR (600.26 MHz, $CDCl_3$) δ (ppm): 1.362–1.407 (m, 2H, $-CH_2$-carborane), 1.717–1.745 (m, 2H, $-CH_2$-), 1.914 (t, 1H, H–C≡, $J = 3$ Hz), 2.037 (dt, 2H, CH_2-alkyne group), 2.634 (m, 1H, carborane-C-H); ^{13}C NMR (150.94 MHz, $CDCl_3$) δ (ppm): 17.93 (CH_2-carborane), 27.91 ($-CH_2-$), 37.78 ($-CH_2-$alkyne group), 53.38 (C-carborane), 58.20 (C-carborane), 69.03(H–C≡), 82.02 (-C≡); ^{11}B NMR (192.59 MHz, $CDCl_3$) δ (ppm): −15.10 (d, 5B, $J = 165.6$ Hz), −12.61 (d, 5B, $J = 165.6$ Hz); MS (FAB, Gly, +VE): *m*/*z* (%) = 211.2 (100%), 212.2 (93%), 210.2 (62%), 213.2 (50%), 209.2 (32%), 208.2 (11%), 214.2 (9%), 207.2 (4%) $[M]^+$, calcd for $C_7H_{18}B_{10} = 211.24$.