Nucleoside-metallacarborane conjugates: synthesis of a uridine-bearing 3,3,3-(CO)$_3$-*closo*-3,1,2-ReC$_2$B$_9$H$_{10}$ complex

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Abstract

A new type of nucleoside-metallacarborane conjugate is presented. 3,3,3-Tricarbonyl-*closo*-3,1,2-ReC$_2$B$_9$H$_{10}$ is used as a modifying unit. The method is based on the *de novo* formation of a metallacarborane complex via the reaction of [NEt$_4$]$_2$[ReBr$_3$(CO)$_3$] with the uridine-bearing carborane as a boron cluster ligand. The uridine-tricarbonyl rhenacarborane represents an example of a novel type of nucleoside-metallacarborane conjugate bearing a therapeutically important rhenium component.

Keywords: Nucleoside, metallacarborane, rhenium, radiopharmaceuticals

Introduction

Contemporary medical diagnostics take advantage of knowledge from different fields of science and practice. The crossroads of biology and materials engineering and of biological and inorganic chemistry represent areas of fruitful interconnections yielding new pharmaceuticals, diagnostic methods and materials such as biological/nonbiological conjugates. Herein, a method for the synthesis of 2′-*O*-(3,3,3-tricarbonyl-*closo*-3,1,2-ReC$_2$B$_9$H$_{10}$)methyluridine, a novel type of nucleoside-metallacarborane conjugate, is proposed. The availability of this type of molecule, along with the availability of previously published nucleoside conjugates containing bis-dicarba-dodecaborane complexes of cobalt, iron, and chromium[1-4], facilitates studies on the medical applications of nucleosides bearing metals.

The recent significant progress in the chemistry of rhenium and technetium supports their applications in diagnostic and therapeutic nuclear medicine.[5-7]

Rhenium is the third row congener of Tc, its chemistry is expected to be similar to that of Tc. This similarity in chemistry combined with availability of the rhenium radioisotopes [186]Re and [188]Re has led to the development of diagnostic/therapeutic pairs of Re and Tc isotopes. Both

radioisotopes of Re are beta emitters, making them suitable for radiotherapy applications. The radioisotopes of Re are available as per-metal tetra oxyanion (MO_4^-) salts, the metal must be reduced from the +7 oxidation state for the synthesis of radiopharmaceuticals with lower oxidation states: +1, +3, +4, and +5. All radiopharmaceutical syntheses require the presence of a ligand to complex the Re in a lower oxidation state.[7]

Carboranes have been investigated extensively as ligands for a range of different radionuclides, including isotopes of iodine (^{125}I, ^{131}I), astatine ([211]At), cobalt ([57]Co), and technetium (^{99m}Tc).[8-14] Rhenacarboranes were prepared several years ago.[11,12,14-20] ^{131}I-rhenacarborane is enzymatically stable and is able to cross the blood-brain barrier (BBB) by transmembrane diffusion, allowing this compound to accumulate in the brain in substantial amounts.[21]

The biomedical applications of icosahedral carboranes make use of their extraordinary hydrophobicities when employed as substituents in biomolecules, their apparent invisibility to know enzyme systems and their boron content, which is suitable for Boron Neutron Capture Therapy (BNCT). To date, the boron clusters and their derivatives that have been employed for radiolabeling, as ligands, include *nido*-7,8-$C_2B_9H_{12}^-$, *nido*-7,9-$C_2B_9H_{12}^-$, *closo*-$CB_{10}H_{11}^-$, *closo*-$B_{12}H_{12}^{2-}$, and *closo*-$B_{10}H_{10}^{2-}$.[8]

There is an entire area of radiopharmaceuticals devoted to the application of radiolabeled nucleic acids, and the use of the components of nucleic acids, nucleosides and nucleotides, may be advantageous. The labelling of single-stranded oligonucleotides with gamma, Auger electron, positron or single-photon emitters can yield valuable compounds. The applications of these compounds include the imaging of specific mRNAs, i.e., the visualisation of the expression of defined genes *in vivo*; the monitoring of antisense chemotherapy; gene therapy, i.e., the targeting of radiation damage to specific DNA sequences to destroy tumours; the imaging of protein targets using aptamer oligonucleotides; and pre-targeting based on hybridisation with the complementary sequence.[22-24] Often, radiolabeling can be achieved with complexes of radioactive metal isotopes. Encouraging results have been reported using chromium-51, gallium-57 and -68, indium-111, platinum-193 or technetium-99 attached to nucleic acids, usually in the form of complexes with a suitable chelating ligand.[24]

In this paper, I present a novel type of nucleoside/metallacarborane conjugate and a new method for nucleoside/metallacarborane synthesis. The proposed findings extend the range of nucleoside derivatives for studies as potential radiopharmaceuticals.

Results and Discussion

The synthesis of the uridine Re(I)-carborane conjugate is based on the reaction of carborane-nucleoside conjugate **1** with the rhenium complex [NEt_4]_2[ReBr_3(CO)_3] in the presence of aqueous solutions of tetraalkylammonium fluoride salts (Scheme 1) following the methodology

developed by Valliant and associates for carborane clusters without complex bioorganic substituents.[11,19]
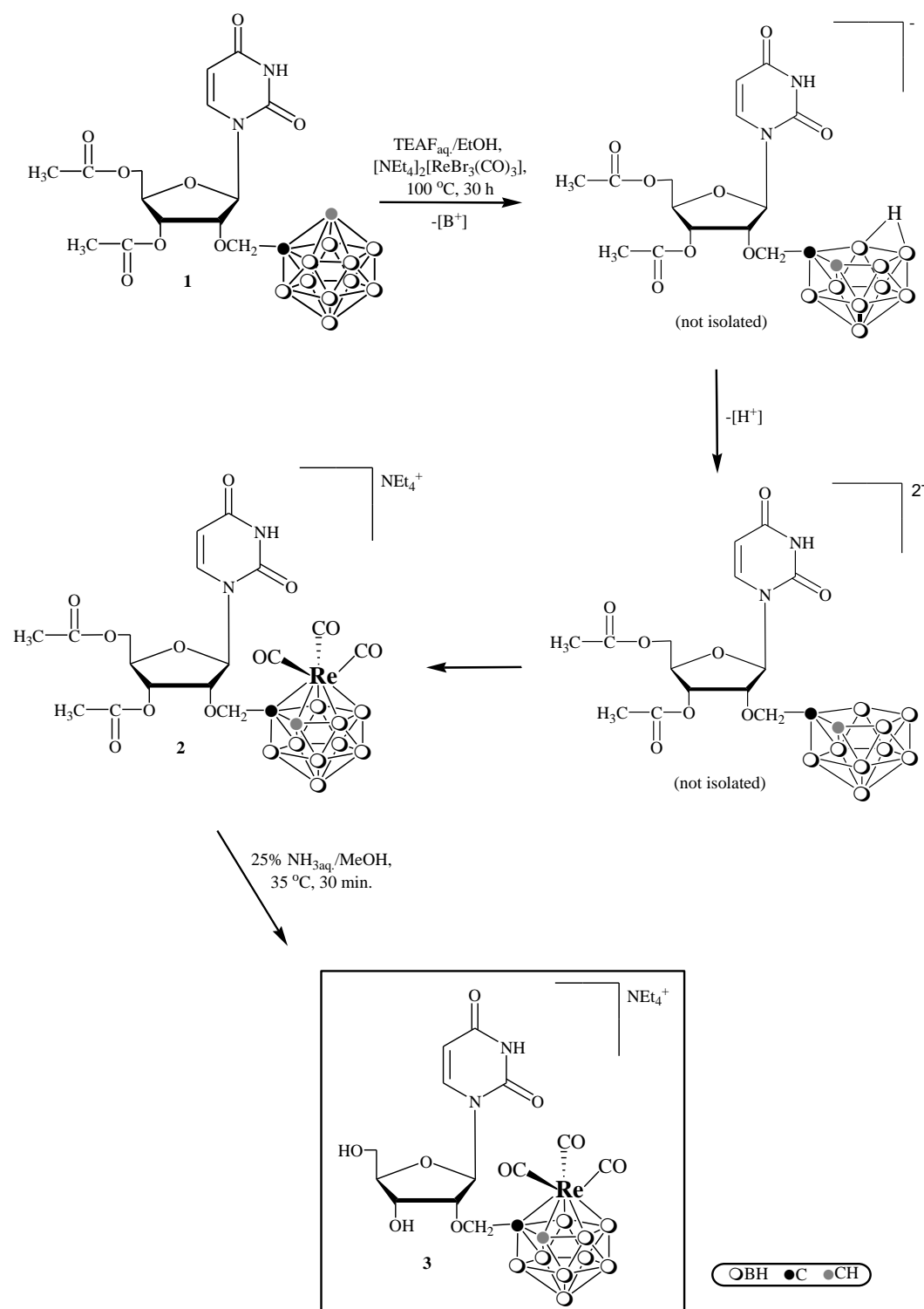
Scheme 1

The fluoride ion (KF, TBAF, CsF) can be used to degrade the closed forms of *ortho*-carboranes (*closo*-carboranes) to yield the corresponding open forms of carboranes (*nido*-carborane monoanion that can be deprotonated to afford the corresponding dianion), which can then serve as ligands in metallacarborane complexes.[25-27] Reports on the direct formation of metallacarboranes from *closo*-carboranes were published by Hawthorne.[28] Re-carborane complexes can be prepared in aqueous solution using different sources of fluoride ions to degrade *closo*-carboranes to yield *nido*-carboranes to facilitate the formation of the desired Re-carborane complexes directly from the *closo* forms. This direct synthesis of Re-carborane complexes allows the number of steps necessary to prepare the desired compound to be reduced.[11,12,19,28]

Briefly, 3′,5′-diacetyl-2′-*O*-(*o*-carboran-1-yl)uridine (**1**) was synthesised from uridine in a five-step procedure, as described by Soloway's group.[29]

Compound **1** was combined with a slight excess of $[NEt_4]_2[ReBr_3(CO)_3]$ in a solution of 500 mM TEAF containing a small quantity of absolute ethanol, which was needed to solubilise compound **1**. The heterogeneous suspension was heated at 100 °C, and after a 30 h extraction followed by chromatography, compound **2** was isolated in 57% yield.

Next, compound **2** was dissolved in MeOH followed by the addition of 25% NH_{3aq} to remove the acetyl groups. The reaction was conducted at 35 °C without stirring. After 30 min., the TLC analysis revealed the complete consumption of **2**. Chromatographic purification of the crude product led to the isolation of compound **3** with an 80% yield.

The purity of both new compounds was assessed by HPLC. Compounds **2** and **3** were characterised by IR; ^1H, ^{13}C, and ^{11}B NMR; MS; UV; TLC; and HPLC. The IR spectra for **2** and **3** exhibited characteristic BH stretches at 2526 and 2524 cm^{-1}, respectively. CO stretches from the carbonyl metallacarborane unit were also clearly visible at 1904 and 2000 cm^{-1} for compound **2** and 1901 and 2000 cm^{-1} for compound **3**. In addition compound **2** exhibited CO stretches from two acetyl groups at 1691 and 1743 cm^{-1}, as expected. All IR spectra featured the characteristic OH stretch at 3429 cm^{-1}.

The ^1H NMR spectra of **2** and **3** revealed the presence of two diastereomers. Two diastereomers are present because the degradation of the substituted *closo*-carborane into *nido*-carborane yields two diastereomeric species.[30] Double signals for the uridine and CH moieties from the carborane were detected using ^1H NMR. The ^{13}C NMR spectra for **2** and **3** also indicated a mixture of diastereomers.

FAB-MS spectra for compounds **2** and **3** contained the molecular ion and other fragment ions corresponding to the loss of three CO groups and Re ion. The parent peak in both spectra exhibited the expected isotopic distribution patterns.

UV spectra for compounds **2** and **3** showed maximum peaks characteristic of the nucleobase part of the nucleoside residue at 260 and 263 nm. The RP-HPLC retention time (t_R) was higher for uridine-Re-carborane (16.06 min) conjugates than for unmodified nucleosides (2.52 min) under the same conditions.

Research on synthesis of other known conjugates such as thymidine is in progress.

Conclusions

A new type of uridine-Re-carborane conjugate was developed using an aqueous solution of TEAF. The primary product was fully characterised by means ^{1}H, ^{13}C, and ^{11}B NMR; UV; IR; and TLC. The RP-HPLC retention time was sixfold higher for the uridine-Re-carborane than for the unmodified compound under the same conditions. The reported Re-compounds are attractive as potential organometallic radiopharmaceuticals because of the inertness of nucleosides and rhenacarboranes.

Experimental Section

General. Uridine was purchased from Avocado Research Chemicals Ltd (Karlsruhe, Germany). Acetic anhydride was from Avocado Research Chemicals Ltd (Karlsruhe, Germany). Propargyl bromide was from Lancaster (Mühlheim am Mein, Germany). Decaborane was purchased from KATCHEM spol. s r. o. (Reź/Prague, Czech Republic). [NEt$_4$]$_2$[ReBr$_3$(CO)$_3$] was prepared following the published procedure.[31] Tetraethylammonium fluoride hydrate (98%, TEAF) was from Sigma-Aldrich (Sp. z o.o. Poznań, Poland). Ethanol (99.8%, EtOH) was from POCH (Gliwice, Poland). Potassium carbonate was purchased from POCH (Gliwice, Poland).

Column chromatography was performed on 230-400 mesh silica gel obtained from Sigma-Aldrich (Steinheim, Germany). An Econosil C(18) reverse-phase column (5 μm, 4.6 × 250 mm) was obtained from Grace Davison Discovery Sciences Headquarters (Deerfield, IL, USA). ^{1}H, ^{11}B, and ^{13}C NMR spectra were recorded with a Bruker Avance DPX 250 MHz spectrometer. The spectra were recorded at 250.13, 80.25, and 62.90 MHz, respectively. Tetramethylsilane and BF$_3$/(C$_2$H$_5$)$_2$O were used as standards for ^{1}H/^{13}C and ^{11}B, respectively. All chemical shifts are reported in ppm (δ) relative to the external standards. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, quin = quintet, bs = broad singlet, m = multiplet. UV measurements were performed with a GBC Cintra 10 UV-Visible spectrometer (Dandenong, Australia). Samples for UV experiments were dissolved in 95% ethanol. The measurements were performed at ambient temperature. Fast atom bombardment (FAB, Gly) mass spectra (MS) were recorded with a Finnigan MAT spectrometer (Bremen, Germany) with glycerin as the matrix. Calculation of the theoretical molecular mass for compounds was performed using the "Analyze Structure" option in the ChemDraw program. Infrared absorption spectra (IR) were recorded using a Nexus Fourier-transform infrared spectrometer (Thermo-Nicolet) equipped with a silicon carbide (SiC) air-cooled source, a caesium iodide beam splitter, and DTGS (deuterated triglycine sulphate) detectors. Samples were prepared by diluting compounds with potassium bromide (70 - 140 mg of KBr per sample) and then pressing in a stainless-steel die to form disks of 0.8 cm diameter. TLC analysis was performed on F254 silica gel plates purchased from Sigma-Aldrich (Steinheim, Germany). All solvents were purchased in the highest available quality. RP-HPLC

analysis was performed on a Hewlett-Packard 1050 system equipped with a UV detector and Econosil C(18) column (5 μm, 4.6 × 250 mm). UV detection was conducted at λ = 268 nm. The flow rate was 1 ml/min. All analyses were run at ambient temperature. The gradient elution profile was as follows: 20 min. from 0% to 100% D, 5 min. at 100% D, and 5 min. from 100% to 0% D. Buffer A contained 0.1 M TEAB, pH 7.0, in acetonitrile:H_2O 2:98, v/v, and buffer D contained 0.1 M TEAB, pH 7.0, in acetonitrile:H_2O 60:40, v/v.

Synthesis of 3',5'-di-*O*-acetyl-2'-(*o*-carboran-1-yl)methyl uridine (1). This synthesis was performed according to the literature procedure.[29]

Synthesis of 3′,5′-di-*O*-acetyl-2′-*O*-(3,3,3-tricarbonyl-3-rhenium-7,8-dicarba-*closo*-dodeca-boran-1-yl)methyluridine (2). Compound **1** (0.23 g, 0.48 mmol) and $[NEt_4]_2[ReBr_3(CO)_3]$ (0.42 g, 0.55 mmol) were placed in round-bottom flask that was subsequently sealed with a rubber septum and flushed with argon for 10 min. A solution containing 500 mM $TEAF_{aq}$/99.8% EtOH (4 ml, 9:1, v/v) was added, and the resulting suspension was heated at 100 °C. After 30 h the heat was removed, and the mixture was acidified by the addition of 1 M HCl (final pH ~ 3). Next, the mixture was dissolved in CH_2Cl_2 (20 ml) and was extracted with H_2O (3 × 10 ml). All organic fractions were combined and dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude product **2** was purified by flash silica gel column chromatography (230-400 mesh, 14 g) using gradient elution: 0-6% CH_3OH.

White solid, yield: 57%, 202.85 mg; IR (KBr) (ν_{max}, cm^{-1}): 1692 and 1743 (C=O of nucleobase), 1904 and 2000 (C=O of metallacarborane), 2526 (BH); UV (95% C_2H_5OH) (λ, nm): λ_{min} = 233, 290, λ_{max} = 260; TLC (CH_3OH:CH_2Cl_2, 1:9, v/v): R_f = 0.37; ^1H NMR (250 MHz, CDCl$_3$, 25 °C)*: δ_H 1.00-3.00 (9H, m, BH of metallacarborane), 1.38 (2H, t, N$\underline{CH_2}$CH$_3$), 2.21 (3H, s, CH$_3$CO-), 2.28 (3H, s, CH$_3$CO-), 3.26 (3H, q, NCH$_2$$\underline{CH_3}$), 3.59-5.34 (8H, m, H-3′a, H-3′b, H-4′a, H-4′b, H-2′a, H-2′b, H-5′a, H-5′b, H-5″a, H-5″b, OCH$_2$a, OCH$_2$b, CHa of metallacarborane, CHb of metallacarborane), 5.84-5.87 (1H, m, H-5a, H-5b), 6.04-6.12 (1H, 2 × d, H-1′a, H-1′b), 7.60-7.69 (1H, 2 × d, H-6a, H-6b); ^{13}C NMR (62.90 MHz, CDCl$_3$, 25 °C): δ_C 7.61 (1C, NCH$_2$CH$_3$), 20.86 and 21.20 (2C, CH$_3$CO-), 52.79 (C-5′), 63.89 and 64.05 (2C, OCH$_2$), 70.46 and 70.79 (2C, CH of metallacarborane), 76.80 and 77.64 (2C, C-3′), 80.47 (C-4′), 80.71 (C-2′), 87.83 (C-1′), 103.35 (C-5), 140.47 (2C, 2 × C-6), 150.40 (C-2), 164.03, 164.32, 170.13, 170.49 and 170.63 (5C, C=O); ^{11}B NMR (80.250 MHz, CDCl$_3$, 25 °C): δ_B 5-(-7.2), -10.73, -15.00, -17.52, -35.14, -37.11 (9B, BH of metallacarborane); MS (FAB, Gly, -Ve): m/z (%) = 743.3 [M-1]$^-$ ($C_{19}H_{27}B_9N_2O_{11}Re$, calculated 744.2 (100%)); HPLC: t_R = 18.25 min.

*a, b – signals from diastereomers

Synthesis of 2′-*O*-(3,3,3-tricarbonyl-3-rhenium-7,8-dicarba-*closo*-dodecaboran-1-yl)methyl-uridine (3). Compound **2** (0.198 g, 0.266 mmol) was dissolved in MeOH (12 ml). NH_{3aq}. (25%, 17.7 ml) was added. The resulting mixture was heated to 35 °C, without stirring. After 30 min. the heat was removed, and the mixture was cooled to room temperature. Next, the mixture was evaporated. The product **3** was purified by flash column silica gel chromatography (230-400 mesh, 6 g) using gradient elution: 5-15% CH_3OH/CH_2Cl_2.

White solid, yield: 80%, 139.71 mg; IR (KBr) (ν_{max}, cm^{-1}): 1683 (C=O of nucleobase), 1901 and 2000 (C=O of metallacarborane), 2524 (BH); UV (95% C_2H_5OH) (λ, nm): λ_{min} = 234, 294, λ_{max} = 263; TLC (CH_3OH:CH_2Cl_2, 2:8, v/v): R_f = 0.33; ^1H NMR (250 MHz, CD_3OH, 25 °C)*: δ_H 0.5-3.00 (9H, m, BH of metallacarborane), 1.25 (2H, t, N$\underline{CH_2}CH_3$), 3.25-3.33 (5H, m, H-5′a, H-5′b, H-5″a, H-5″b, N$CH_2\underline{CH_3}$), 3.33-3.67 (1H, m, H-4′a, H-4′b), 3.67-3.80 (3H, m, H-3′a, H-3′b, OCH_2a, OCH_2b), 4.01 (1H, m, H-2′a, H-2′b), 4.15-4.42 (1H, m, CH of metallacarborane), 5.74-5.77 (1H, 2 × s, H-5a, H-5b), 5.95-5.99 (1H, t, H-1′a, H-1′b), 8.03-8.08 (1H, 2 × d, H-6a, H-6b); ^{13}C NMR (62.90 MHz, CD_3OH, 25 °C): δ_C 53.34 (C-5′), 62.70 (OCH_2), 70.31 (CH of metallacarborane), 78.53 and 79.05 (2C, C of metallacarborane), 80.74, (C-3′), 81.05 (C-4′), 87.30 and 87.27 (2C, C-2′), 88.82 and 88.94 (2C, C-1′), 103.39 (C-5), 142.99 and 143.11 (2C, C-6), 152.35 (C-2), 166.29 (C-4); ^{11}B NMR (80.250 MHz, CD_3OH, 25 °C): δ_B -9.59, -14.38, -17.07, -20.25, -31.44, -35.81 (9B, BH of metallacarborane); MS (FAB, Gly, -Ve): m/z (%) = 659.3 [M-1]$^-$ ($C_{15}H_{23}B_9N_2O_9Re$, calculated 660.2 (100%)); HPLC: t_R = 16.06 min.
*a, b – signals from diastereomers

Acknowledgements

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