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Insight into lipophilicity of deoxyribonucleoside-boron cluster conjugates

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ABSTRACT

Lipophilicity was investigated for 20 2′-deoxyribonucleoside derivatives modified with electron-neutral 1,2-dicarba-*closo*-dodecaborane, 1,12-dicarba-*closo*-dodecaborane, 7,8-dicarba-*nido*-undecaborate anion, and metallacarborane containing Co, Fe, or Cr. The partition coefficient (*P*) for neutral conjugates and the distribution coefficient ($D_{7.4}$) for ionic compounds were determined as a lipophilicity descriptor using a shake-flask method. All modified nucleosides had $P/D_{7.4}$ values higher than those of an appropriate unmodified 2′-*closo*-dodecaborane and metallacarborane was found to be three orders of magnitude higher than that of its unmodified counterpart. The lowest impact on the $P/D_{7.4}$ values of the conjugates was observed for the 7,8-dicarba-*nido*-undecaborate anion. A preliminary molecular modeling study of a thymidine-carborane conjugate with β-cyclodextrin confirmed the ability of the components to form an inclusion complex.

1. Introduction

One of the major goals of physicochemical screening is the prediction of human absorption, for example, the transport of a molecule through cellular membranes. There are several absorption routes for a molecule, and the most frequent is a passive transport through the cellular membranes, a process that strongly depends on the lipophilicity of the absorbed molecules (Hartmann and Schmitt, 2004).

Lipophilicity is a key physicochemical property that contributes to the absorption, distribution, metabolism, and elimination (ADME) characteristics of drugs, thus impacting their metabolism and pharmacokinetics as well as their pharmacodynamics and toxicological profile (Testa et al., 2000; Arnott and Planey, 2012). According to the IUPAC Gold Book lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behaviour in a biphasic system, either liquid–liquid (such as 1-octanol/water) or solid–liquid (such as RP-HPLC or TLC) (Van de Waterbeemd et al., 1997).

Conventionally, lipophilicity is expressed by the logarithm of the ratio of the concentration of the uncharged form of a compound in a nonpolar phase, traditionally water-saturated 1-octanol (simulates a lipid membrane), to that in water (log *P*) or by the logarithm of the ratio of the total concentration (neutral and charged species) of a compound in a nonpolar phase, frequently water-saturated 1-octanol solution, to that in a buffer at a given pH (log D_{pH}, usually pH 7.4; the model for cytoplasm) (Sangster, 1997; Martin et al., 2016). The value of *P* varies slightly with temperature and solute concentration, but for neutral molecules in dilute solutions (< 0.01 M) and small temperature changes (± 5 °C), variations in *P* are minor.

There exists a variety of different computational methods for the prediction of log *P* and log *D*. A potential drawback of using the calculated lipophilicity is that the methods of calculation predict the measured log *P* or log *D* values with a reasonable degree of error, and often, the error is systemic (Waring, 2010).

A common finding when comparing commercial oral drugs with compounds in earlier stages of development is that high lipophilicity (> 5) frequently leads to compounds with rapid metabolic turnover, low solubility, poor absorption, and strong nonspecific binding, which reduces the available free concentration at the site of action. Very lipophilic compounds may partition into the membrane, but they do not cross it. If their lipophilicity is too low, the compounds show poor permeability if active transport is not included. The average log *P* value for synthetic drug molecules is 2.4, whereas natural compounds are less lipophilic (0.7). The optimum range of lipophilicity of drug discovery

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compounds is between 1 and 3 log *P* (Arnott and Planey, 2012; Valko, 2013).

Boron clusters belong to the family of boron-containing caged compounds (Grimes, 2011; Farràs et al., 2012). Of this, the icosahedral dicarba-*closo*-dodecaborane (carborane, $C_2B_{10}H_{12}$) is used the most for the synthesis. The dicarba-*closo*-dodecaboranes exist in three isomeric forms: 1,2-$C_2B_{10}H_{12}$ (*ortho*-), 1,7-$C_2B_{10}H_{12}$ (*meta*-), and 1,12-$C_2B_{10}H_{12}$ (*para*-), depending on the mutual orientation of the carbon atoms within the boron cage (Grimes, 2011). One of the most important features of *ortho*-carborane is high lipophilicity and susceptibility to the transformation of a closed-cage cluster (*closo*-carborane) into the ionic, open cage form *nido*-7,8-$C_2B_9H_{11}$(–1) (*nido*-carborane) with amphiphilic properties, followed by the ability to form "sandwich"-type metal complexes (metallacarboranes, 3,3′-$[M(C_2B_9H_{11})_2]^-$), e.g., M = Co, Cr, Fe, Ni) with amphiphilic properties (Farràs et al., 2012). The high hydrophobicity of many boron clusters and their derivatives can be explained by the presence of a partial negative charge located on the boron-bound hydrogen atoms in the BH groups (density of the negative charge differs for different hydrogen atoms and depends on the type of cluster) and their "hydride-like" character. This prevents boron clusters from forming classical hydrogen bonds and leads to their lipophilic character (Lesnikowski, 2011).

The biomedical application of carboranes has been reviewed over the years (Issa et al., 2011; Scholz and Hey-Hawkins, 2011; Ban and Nakamura, 2015; Kahlert et al., 2013; Leśnikowski, 2016a; Leśnikowski, 2016b), focusing mainly on their application as boron carriers in boron neutron capture therapy (BNCT) and as pharmacophores in medicinal chemistry. Metallacarboranes are known inhibitors of Human Immunodeficiency Virus (HIV) protease (Cigler et al., 2005; Řezáčová et al., 2009). Enhanced lipophilicity can facilitate the transport of such compounds across biological membranes such as the blood–brain barrier.

The high lipophilicity of carboranes has advantages and disadvantages and can limit the therapeutic bioavailability of their derivatives in some cases. Cyclodextrins are often used to solubilize lipophilic drugs by forming complexes in which a lipophilic compound or the lipophilic part of the compound interacts with the hydrophobic part of cyclodextrin.

Preparation of carborane–cyclodextrin complexes has been described for unsubstituted *ortho*-, *meta*-, and *para*-carborane with β-cyclodextrin (Sadrerafi et al., 2015), simple substituted 1,2-dicarba-*closo*-dodecaborane with α-cyclodextrin (Ohta et al., 2009), β-cyclodextrin (Frixa et al., 2002), or platinum(II)-*ortho*-, and *para*- and *nido*-carborane with β-cyclodextrin (Ching et al., 2012a; Ching et al., 2012b; Ching et al., 2013).

The main goal of the present work was to study the effects of boron cluster modification, its type (carborane or metallacarborane), and its location within the nucleoside structure on the conjugates' lipophilicity. To that end, a library of pyrimidine and purine 2′-deoxyribonucleoside–boron cluster conjugates was synthesized, the partition/distribution coefficient was measured by the shake-flask method, and the structure–lipophilicity relationship was assessed. Further, the possibility of forming a stable inclusion complex between β-cyclodextrin and a nucleoside conjugate was evaluated using the molecular modeling approach.

2. Materials and methods

2.1. General

Nucleosides (2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxycytidine, 2′-thymidine) were purchased from Pharma-Waldhof (Düsseldorf, Germany). Others chemicals were obtained from Sigma Aldrich (Sigma-Aldrich Sp. Z o.o. Poznan, Poland) used without further purification unless otherwise stated. Carborane was purchased from KATCHEM spol. s r.o. (Rež/Prague, Czech Republic). Flash column chromatography was performed on silica gel 60 (230–400 mesh, Sigma-Aldrich). R_f 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 indicator. Compounds were visualized by use of UV light (254 nm) or a 0.5% acidic solution of $PdCl_2$ in HCl/methanol by heating with a heat gun for boron-containing derivatives. The yields are not optimized.

Phosphate-buffered saline (PBS, pH 7.4) was obtained by dissolving NaCl (400 mg), KCl (10 mg), Na_2HPO_4 (72 mg), KH_2PO_4 (12 mg) in H_2O (40 mL). The pH was adjusted to 7.4 with HCl and NaOH then H_2O was added to 50 mL.

^{1}H NMR, ^{13}C NMR, ^{11}B NMR spectra were recorded mostly on a Bruker Avance III 600 MHz spectrometer equipped with a direct ATM probe. The spectra for ^{1}H, ^{13}C, and ^{11}B were recorded at 600.26 MHz, 150.94 MHz, and 192.59 MHz, respectively. ^{1}H NMR spectrum of compound **29** was recorded with a Bruker Ultra Shield 700 MHz spectrometer. The spectrum was recorded at 699.725 MHz. Deuterated solvents were used as standards. All chemical shifts (δ) are quoted in parts per million (ppm) relative to the external standards. For NMR following solvents were used: acetone-d_6 (δ_H = 1.96, δ_C = 30.60, 205.87 ppm) DMSO-d_6 (δ_H = 2.50, δ_C = 39.70 ppm), CD_2Cl_2 (δ_H = 5.35, δ_C = 54.25 ppm). ^{1}H, ^{11}B, and ^{13}C NMR spectra of compound **22** 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 $BF_3/(C_2H_5)_2O$ were used as standards for ^{1}H/^{13}C and ^{11}B, respectively. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, dt = doublet of triplets, q = quartet, quin = quintet, bs = broad singlet, m = multiplet. *J* values are given in Hz.

IR spectra were recorded with Thermo Scientific Nicolet™ 6700 FT-IR spectrometer equipped with a Smart Orbit Attenuated Total Reflectance (ATR) accessory, and diamond crystal.

Fast atom bombardment (FAB) mass spectra were recorded with a Finnigan MAT 95 spectrometer (Bremen, Germany) with glycerin (Gly) as the matrix. The *m/z* was measured in a positive and negative modes. MALDI-TOF MS spectrum of compound **22** was recorded on a Voyager Elite spectrometer (PerSeptive Biosystem, Framingham Inc., MA) equipped with a nitrogen laser (337 nm). Spectrum was obtained in the linear, negative ion mode at the acceleration voltage of 20 kV. The energy of the laser beam was set slightly above threshold level. Calculation of the theoretical molecular mass for compounds was performed using the "Show Analysis Window" option in the ChemDrawUltra 12.0 program. The calculated *m/z* corresponds to the average mass of the elements consisting of natural isotopes.

UV measurements were performed using a GBC Cintra10 UV–Vis spectrometer (Dandenong, Australia). The samples used for the UV experiments, ca. 0.5 A_{260} optical density units (ODUs) of each compound, were dissolved in CH_3OH, DMSO or CH_3CN. The measurement was performed at room temperature.

Partition coefficient measurements were performed using a Thermo Scientific™ Varioskan™ Flash Multimode Reader equipped with UV-Star® 96-well plates (Greiner Bio-One GmbH, Germany).

Electronic Supporting Information (ESI) available: ^{1}H, ^{13}C, ^{11}B {H BB} NMR, IR, MS spectra of 8-{[(*ortho*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxyadenosine (**9**), and 8-{[(*para*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxyadenosine (**10**). ^{1}H NMR spectrum of N^3-(1*N*-1,2,3-triazol-4-yl)methylenethymidine (**29**) and N^3-[(propyl)-1*N*-1,2,3-triazol-4-yl]methylenethymidine (**30**).

2.2. Chemistry

Synthesis of N^3-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl} methylenethymidine (**1**), N^3-{[(7,8-dicarba-*nido*-undecaboranyl)propyl]-1*N*-1,2,3-triazol-4-yl}methylenethymidine (**2**), 5-{[(*o*-carboran-1-yl)propyl]-1*N*-1,2,3-triazol-4-yl}-2′-deoxycytidine (**3**), 5-