Journal of Organometallic Chemistry 798 (2015) 46–50

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

A convenient route towards deoxygalactosyl-functionalised *ortho*-carbaborane: Synthesis of a building block for peptide conjugation

René Frank, Evamarie Hey-Hawkins*

Leipzig University, Institute of Inorganic Chemistry, Johannisallee 29, 04103 Leipzig, Germany

ARTICLE INFO

Article history:
Received 6 June 2015
Received in revised form
15 August 2015
Accepted 17 August 2015
Available online 20 August 2015
Dedicated to Prof. Russell N. Grimes on the occasion of his 80th birthday

Keywords:
Carbaboranes
Carboranes
Monosubstitution
Galactose
Water solubility
Peptide
Bioconjugation

ABSTRACT

In the treatment of cancer, boron neutron capture therapy (BNCT) is a mild and promising alternative to established harsh therapeutic methods such as chemo- and radiation therapy. However, successful BNCT procedures strongly depend on efficient, tumour-selective boron-delivery systems, and recently we have demonstrated that the breast tumour-selective peptide [F^7,P^{34}]-NPY is a promising boron-shuttle system after conjugation with three *ortho*-carbaborane clusters (1,2-*closo*-$C_2B_{10}H_{10}$). The extreme hydrophobicity of the latter, however, causes problems in medicinal applications in vivo. Therefore, we have elaborated a synthetic protocol towards a deoxygalactosyl-modified (and thus more hydrophilic) building block that can readily be conjugated with the shuttle peptide. A key intermediate, i.e., *ortho*-carbaborane functionalised with a bis-isopropylidene-protected deoxygalactosyl moiety, was synthesised by both a silyl protection strategy (which is generally recommended for monosubstitution) and direct reaction of metallated *ortho*-carbaborane in acceptable yield.

1. Introduction

Borane cluster compounds have increasingly been used in medicinal chemistry with *ortho*-carbaborane (**1**; 1,2-*closo*-$C_2B_{10}H_{12}$, *ortho*-$C_2B_{10}H_{12}$) being a widely used representative member. Advantages of **1** include *in-vivo* metabolic stability and facile synthetic chemistry. The high content of boron in **1** has stimulated applications as starting material for boron-delivery agents in BNCT (Boron Neutron Capture Therapy) [1] as a gentle alternative to classical approaches of cancer therapy. The strongly hydrophobic behaviour, the spherical shape and the hydridic shell found in **1** facilitate novel binding modes with biomolecules (in particular with enzymes and receptors), which are without precedent in common organic compounds, and thus derivatives of **1** have increasingly been studied as pharmacophores [2]. State-of-the-art biochemical research is focussed on receptor-dependent peptides due to high affinity and selectivity (e.g. for tumours) of numerous representative members [3]. Surprisingly, considering the vast number of peptides, reports on the interaction with peptides or incorporation of boron clusters into peptides are comparatively rare [4–6], although the integration of boron clusters into peptides had led to unexpected advantages in the past. Thus, positive impact upon modification with *ortho*-carbaborane, i.e. higher binding affinity and stability compared to the parent peptides, could be demonstrated for pyrokinin/PBAN (PBAN = pheromone biosynthesis activation neuropeptide) or FXPRLamide derivatives (FXPRL denotes the sequence of amino acids in a one-letter code) [7], and the introduction of boron clusters resulted in better affinity of a marker for antibody labelling than for conventional systems [8]. Recently, we demonstrated a change in affinity for hY_1 to hY_2/hY_4 receptor types when selected positions in parent neuropeptide Y (NPY) analogues were substituted by hydrophobic carbaboranyl moieties [9]. Derivatives of **1** incorporated into a modified, Y_1-selective NPY, namely, [F^7,P^{34}]-NPY, designed for BNCT applications showed selective accumulation of boron in isolated breast tumour cells exceeding the required amount of 10^9 boron atoms per cell [10]. However, the hydrophobic behaviour of boron clusters [11] caused problems in the past, i.e. *in-vivo* tumour selectivity of

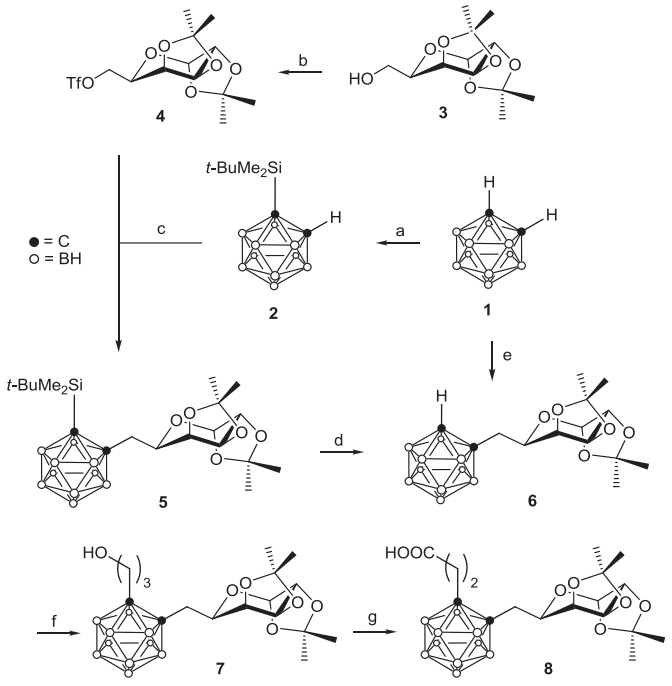
* Corresponding author.
E-mail address: hey@uni-leipzig.de (E. Hey-Hawkins).

http://dx.doi.org/10.1016/j.jorganchem.2015.08.011
0022-328X/

peptides was lost due to decreased water solubility and preferred interaction with hydrophobic blood proteins [1a,b]. Although attempts to conjugate the hydrophobic components with strongly hydrophilic polymers (e.g. dextran) led to high water solubility, the tumour affinity was again lost, and accumulation of the boronated peptides in kidneys and liver occurred [1a,b]. These examples demonstrate that besides other features the polarity and water solubility of boron-delivery systems play a key role and must be well balanced, which in turn requires a library of compounds displaying gradual changes in hydrophilicity. Among various strategies to increase water solubility of hydrophobic systems, we have focussed on monosaccharides, and in particular we have recently demonstrated approaches to water-soluble carbaboranes (1,2-, 1,7- or 1,12-*closo*-$C_2B_{10}H_{12}$) by using galactosyl or deoxygalactosyl moieties [12]. The existing carbaborane systems contain either at least two monosaccharide moieties per cluster unit or are of ionic nature. Our continuing research on breast tumour-selective boron-delivery NPY derivatives [10] necessitated a building block of moderate hydrophilicity (only one deoxygalactosyl moiety) and required the presence of a carboxyl group for covalent coupling to a peptide backbone. The synthesis of a suitable carbaborane derivative **8** is described here (Scheme 1).

2. Results and discussion

The synthesis of *C*-substituted *ortho*-carbaboranes can generally be achieved by lithiation (e.g., with *n*-BuLi) and subsequent reaction with electrophiles, but is often hampered in the case of monosubstituted or asymmetrically disubstituted derivatives. Due to the occurrence of an equilibrium in solution between the parent 1,2-*closo*-$C_2B_{10}H_{12}$ (**1**) and the mono- and dicarbometallated species 1-Li-1,2-*closo*-$C_2B_{10}H_{11}$ and 1,2-Li_2-1,2-*closo*-$C_2B_{10}H_{10}$, respectively [13], a mixture of mono- and disubstituted *ortho*-carbaborane occurs. Therefore, we employed the elaborated strategy of protection with *t*-BuMe_2Si groups [14] (Scheme 1). Following the established protocol, *ortho*-carbaborane (**1**) was silylated to give compound **2**. For the preparation of the galactosyl electrophile commercially available bis-isopropylidene-protected α-D-galactopyranose **3** was triflated to give compound **4** [15]. Treatment of the latter with the *C*-lithiated product of **2** gave compound **5**. The silyl protecting group was removed by the action of fluoride to give deoxygalactosylated carbaborane **6** in an acceptable overall yield of 48%. Although silyl protection is generally recommended as a reliable route towards monosubstituted derivatives of **1**, we investigated direct reactions of lithiated species of **1** with **4** and found an optimised yield of **6** (42%) with 0.7 equiv of *n*-BuLi and no indication for disubstituted *ortho*-carbaborane. Unconsumed starting material **1** (45%) could be recovered by sublimation. It appears that the excess of *ortho*-carbaborane (**1**) led to a maximal concentration of the monolithiated species in the metallation equilibrium, and the steric congestion of the galactosyl electrophile **4** kinetically inhibits the formation of the disubstituted species [16]. Crystals suitable for X-ray crystallography (Fig. 1) could be obtained from a solution of compound **6** in *n*-hexane/Et_2O (1/2, v/v) by slow solvent evaporation at ambient temperature, providing definitive proof of the structure. The ^{1}H (Fig. 2) and ^{11}B{^{1}H} NMR spectrum (Fig. 3) of compound **6** are also shown here as representative examples for the NMR spectroscopic studies of all compounds. The carboxyl group for conjugation to peptides was introduced by lithiation of compound **6** followed by ring opening of oxetane, resulting in alcohol **7**. The oxidation of the latter to give carboxylic acid **8** required mild conditions due to the presence of the isopropylidene protecting groups and was performed with periodate in the presence of a ruthenium catalyst.



Scheme 1. a) *n*-BuLi, toluene/Et_2O (2/1), rt, 2 h, then *t*-BuMe_2SiCl, rt, overnight, 84%; b) Tf_2O (Tf = CF_3SO_2), collidine, CH_2Cl_2, rt, 2 h, 90%; c) **2**, *n*-BuLi, Et_2O, rt, 1 h, then **4**, rt, overnight, 76%; d) N(*n*-C_4H_9)$_4$F·3H_2O, THF, −78 °C → 0 °C, 30 min, 75%; e) *n*-BuLi (0.7 equiv), Et_2O, rt, 1 h, then **4**, rt, overnight, 42% (76% based on consumed starting material **1**); f) *n*-BuLi, THF, rt, 1 h, then oxetane, rt, 48 h, 63%; g) $NaIO_4$, cat. $RuCl_3$·3H_2O, $CHCl_3$/MeCN/water (2/2/3), rt, 2 h, 49%.

3. Experimental

3.1. Syntheses

Reactions requiring inert conditions were carried out under

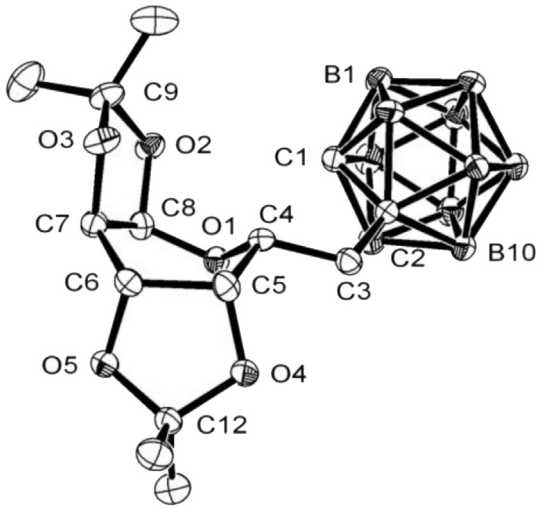


Fig. 1. Molecular structure of compound **6**. Thermal ellipsoids are drawn at the 50% level of probability. Hydrogen atoms are omitted for clarity. The pyranose ring (O1–C4–C5–C6–C7–C8) adopts a twist-boat conformation. Selected bond lengths (pm) and bond angles (°): O1–C8 141.3(3), O1–C4 142.9(3), O2–C8 141.9(3), O3–C7 142.3(3), O4–C5 142.7(3), O5–C6 143.1(3), C1–C2 165.1(3), C1–B1 169.1(3), C4–C5 152.0(3), C5–C6 155.0(3), C6–C7 151.7(3), C7–C8 154.3(3), C8–O1–C4 113.1(2), C8–O2–C9 109.4(2), C7–O3–C9 106.1(2), C5–O4–C12 108.8(2), C12–O5–C6 106.6(2), C2–C1–B1 112.4(2), C3–C2–C1 120.6(2), C4–C3–C2 115.9(2), O1–C4–C(3) 107.9(2), O1–C4–C5 108.5(2), C3–C4–C5 112.5(2), O4–C5–C4 108.6(2), O4–C5–C6 104.1(2), C4–C5–C6 111.5(2), O5–C6–C7 106.2(2), O5–C6–C5 104.1(2), C7–C6–C5 115.4(2), O3–C7–C6 109.6(2), O3–C7–C8 103.8(2), C6–C7–C(8) 113.5(2), O1–C8–O2 108.9(2), O1–C8–C7 114.1(2), O2–C8–C7 104.3(2).