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Direct carborane-peptide conjugates: Synthesis and evaluation as non-natural lipopeptide mimetics

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ARTICLE INFO

This article is dedicated to the memory of Prof. Dr. Peter Welzel.

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ABSTRACT

Herein, we report the synthesis and characterization of direct carborane-peptide conjugates. Carboranes are non-natural and extremely hydrophobic compounds and turned out to be suitable pharmacophores for diverse biological applications. In this work, we established an efficient procedure for the coupling of carboranes to peptides on solid support. We identified the coupling of carborane-1-carboxylic acids to amino groups to be superior to those with hydroxy- or sulfhydryl-groups. The carborane-peptide conjugates showed remarkably prolonged, and carborane isomer dependent chromatographic retention times. This effect can be used to generate non-natural lipopeptides with fine-tuned properties.

1. Introduction

Dicarba-*closo*-dodecaboranes, also termed carb(ab)oranes, are non-natural cluster compounds that contain both, boron-hydrogen and carbon-hydrogen vertices in an electron delocalized icosahedral system (Grimes, 2011). Carboranes represent unique inorganic-organic scaffolds and stand out owing to their extraordinary geometric and electronic structure. Carboranes have already entered different fields of application such as catalysis, material design and medicine (Cabrera-Gonzalez et al., 2017; Gabel, 2015; Kirlikovali et al., 2016; Scholz and Hey-Hawkins, 2011; Zhou et al., 2016). Carboranes are obtained by reacting $B_{10}H_{14}$ with acetylene (Heying et al., 1963), yielding 1,2-carborane (*ortho*-), which can be converted into 1,7-$C_2B_{10}H_{12}$ (*meta*-) and 1,12-$C_2B_{10}H_{12}$ (*para*-) isomers by thermal rearrangement (Fig. 1) (Edvenson and Gaines, 1990; Grafstein and Dvorak, 1963).

Carboranes are slightly larger than adamantane and highly hydrophobic (Scholz and Hey-Hawkins, 2011). The hydrogen atoms at the boron vertices have a rather hydridic character, whereas the hydrogen atoms at the carbon vertices show a rather acidic behavior (Scholz and Hey-Hawkins, 2011). Unsubstituted carboranes are insoluble in water. However, the connection to polar molecules, such as peptides, makes the resulting carborane-conjugate water-soluble and in turn the peptide more hydrophobic. Here, we aim to use and evaluate this effect to generate synthetic lipopeptides.

Naturally expressed lipopeptides consist of a hydrophobic fatty acid connected to an often cyclic peptide, which represents the hydrophilic component (Hamley, 2015). These amphipathic molecules have received considerable attention owing to their outstanding properties. For instance, they are used as surfactants, and most importantly, they show promising antibiotic activity (Cochrane and Vederas, 2016; Wenzel et al., 2016). The latter feature is based on their amphiphilic structure resulting in high membrane activity (Hamley, 2015). Within this, it was already reported that the antimicrobial activity can be tuned by varying the attached aliphatic chain (Makovitzki et al., 2006). In general, lipidation of peptides is a suitable method to improve their biological activity and to develop drugs based on peptides (Zhang and Bulaj, 2012). A prominent example is the lipopeptide daptomycin featuring a capric acid amide (von Nussbaum et al., 2006). Daptomycin is a highly efficient last-resort antibiotic that targets the bacterial cell membrane. Recently it was found out that daptomycin inhibits cell envelope synthesis by interfering with fluid membrane microdomains (Müller et al., 2016).

Previously reported carborane-peptide conjugates applied carboranyl building blocks that had to be synthesized in a multi-step synthesis with the functional groups well separated from the cluster (Fig. 2) (Ahrens et al., 2015; Ahrens et al., 2011; Naeslund et al., 2005). This allowed the application of standard organic/peptide chemistry reactions.

In this work, we aimed to modify peptides on-resin with carborane-1-carboxylic acids. Carborane-1-carboxylic acids have the advantage,

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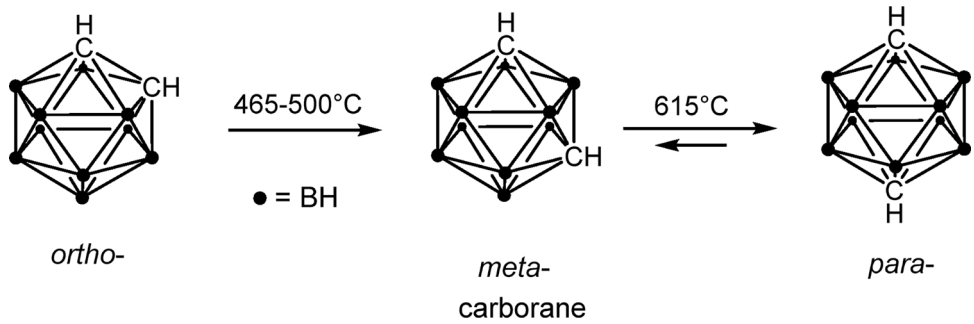

Fig. 1. Isomerization of carboranes (Heying et al., 1963).

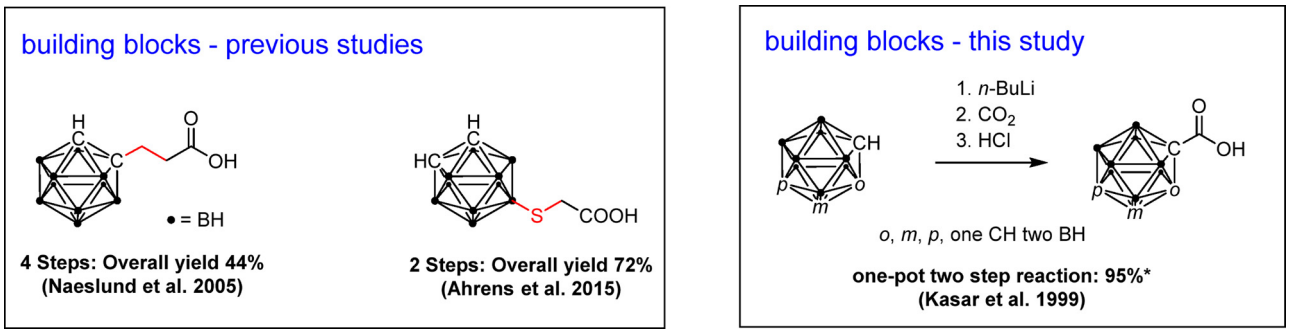

Fig. 2. Overview of carboranyl building blocks suitable for modification with peptides. Spacers are highlighted in red. *Quantitative yield upon educt recovery (Tribovane and Scholz, 2018).

that they are commercially available or easily and quantitatively accessible by reaction of unsubstituted clusters with CO_2 after lithiation (Kasar et al., 1999). Functional groups directly at the cluster core are strongly influenced by the steric and electronic properties of the cluster (Bregadze, 1992; Serino et al., 2017). Therefore, they are more challenging to be used in further reactions, as already pointed out earlier (Scholz and Wingen, 2017; Wingen and Scholz, 2016). Furthermore, we designed carborane-peptide conjugates by connecting carborane-1-carboxylic acids directly on resin to functional groups present in peptides. In addition, we aimed to study the impact of the clusters on the properties of the resulting conjugate.

2. Experimental

2.1. Carborane synthesis

1,7-dicarba-*closo*-dodecarbaborane was commercially available from Katchem Ltd. (Prague, Czech Republic). All other chemicals were purchased from Acros, Alfa Aesar, Fluka, Merck, Sigma-Aldrich and Carbolution and used without further purification. *meta*-Carborane-1-carboxylic acid was synthesized according to literature (Kasar et al., 1999).

2.2. Peptide synthesis

All N_α-Fmoc protected amino acids were purchased from IRIS Biotech (Marktredwitz, Germany). Other chemicals and consumables including (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), 1-[bis(dimethylamin)methylen]-1*H*-1,2,3-triazol[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU), *N*,*N*-diisopropylethylamine (DIPEA), acetonitrile (ACN), and trifluoroacetic acid (TFA) were derived from Fluka (Taufkirchen, Germany), Merck (Darmstadt, Germany), Sarstedt (Nümbrecht, Germany), Sigma-Aldrich (Taufkirchen, Germany) and VWR (Darmstadt, Germany).

All peptides shown were synthesized using a combination of standard Fmoc/*t*-Bu solid-phase peptide synthesis (SPPS) on a Syro I peptide synthesizer (MultiSynTech, Bochum, Germany) and manual coupling protocols according to previous works (Gronewold et al., 2017; Horn et al., 2016; Knyphausen et al., 2016). Peptides were generated on a Rink amide resin yielding *C*-terminally amidated molecules. Identification was done using HPLC-ESI mass spectrometry (LTQ XL, Thermo Scientific).

2.3. Carboranyl-peptide synthesis

Synthesis of carborane-peptide conjugates was performed directly on solid support (loading 0.5 mmol/g resin), using standard coupling procedures including activation of 2 eq carborane-1-carboxylic acid with 2 eq HATU/DIPEA for 2 h, and 5 eq carborane-1-carboxylic acid with 5 eq Oxyma/DIC overnight, respectively, at room temperature. Carboranyl-peptides were cleaved from the resin with trifluoroacetic acid/trisisopropylsilane/water (95/2.5/2.5, v/v/v), identified via HPLC-ESI (Chromolith® Performance RP-18e, 100–4.6 mm, Merck; 10–60% ACN in water (incl. 0.1% formic acid) over 15 min; 0.6 mL/min flow rate) mass spectrometry and afterwards purified using preparative HPLC (Nucleodur C18ec; 100-5; Macherey-Nagel; 10–60% ACN in water (incl. 0.1% TFA) over 45 min, 1.5 mL/min flow rate).

For analytical data, see Table 1. The conversion after the coupling was determined by HPLC UV-chromatogram as the ratio of the peak area of the coupled product to the areas of uncoupled starting material and coupled product.

Carborane-peptide conjugates were stored in aqueous solution at −20 °C for several weeks. After that, HPLC-ESI mass spectrometry analysis was performed again, to study the stability of the compounds.

2.4. Fatty acid-peptide synthesis

Synthesis of fatty acid-peptide conjugates was performed on solid support (loading 0.5 mmol/g resin), using standard coupling procedures including activation of 2 eq fatty acid with 2 eq HATU/DIPEA for 2 h, and 5 eq fatty acid with 5 eq Oxyma/DIC overnight at room