SYNTHESIS AND CHARACTERIZATION OF HYBRIDS CARBORANE-CARBOHYDRATES FOR BNCT APPLICATIONS

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ABSTRACT

The synthesis of different families of compounds containing a carborane cage and a sugar moiety is described. Some of them are simple carboranyl glycosides, in which the carbohydrate is expected to give not only some hydrophilicity but also a selectivity for tumor cells; other derivatives could exploit active transport mechanisms for glucose. Finally, a third class is prepared from a scaffold, which allow to easily introduce a chemical diversity in order to get information on structure-activity relationship.

Introduction

Boron neutron capture therapy (BNCT) is a therapy for tumors treatment based on the selective irradiation with thermal neutrons of molecules containing ^{10}B atoms, which has a large capture cross section relative to the more abundant endogenous nuclei (^{1}H, ^{12}C, ^{31}P, ^{14}N).[1]

BNCT is referred to as a binary therapy because the individual components (i.e. the boron atoms and the thermal neutrons) themselves are not efficacious. In combination, however, they have the potential to create a highly selective therapy. The interaction between the nucleus of boron atom and the neutron produces an alpha particle and a ^{7}Li ion with about 2.4 MeV which dissipate their energy before travelling one cell diameter so that the destructive effect is highly localized to boron loaded tissues. In order to be therapeutically useful, an ideal boronated candidate should have:

- high tumor targeting selectivity: BNCT agents should accumulate preferentially into tumor tissues. They must also clear the blood rapidly to avoid inducing necrosis in the vasculature. The optimal tumor:blood ratio is around 5:1.
- low cytotoxicity: this is a challenging aspect when one considers the amount of agent that must be administered to achieve the requisite levels of boron in the tumor;
- high water solubility: required for intra-arterious administration of the BNCT agent;
- high uptake by cancer cells: the therapeutically useful amount of boron in tumor cells usually accepted is between 10 and 35 μg of ^{10}B for gram of tumor. This amount is substantially reduced if the boron is concentrated in or close to the cell nucleus.

As boron moiety, our attention has been focused mainly on carboranes, a cluster containing ten boron atoms. Such boron containing structures allow obtaining molecules that bring a large number of boron atoms per molecule. On the other hand, carboranes are highly hydrophobic compounds, which require their conjugation with hydrophilic compounds in order to have molecules with adequate water solubility for their administration. Among the different structures that can be exploited to give the desired solubility properties to the carborane containing derivatives, carbohydrates can be a useful class of natural compounds.

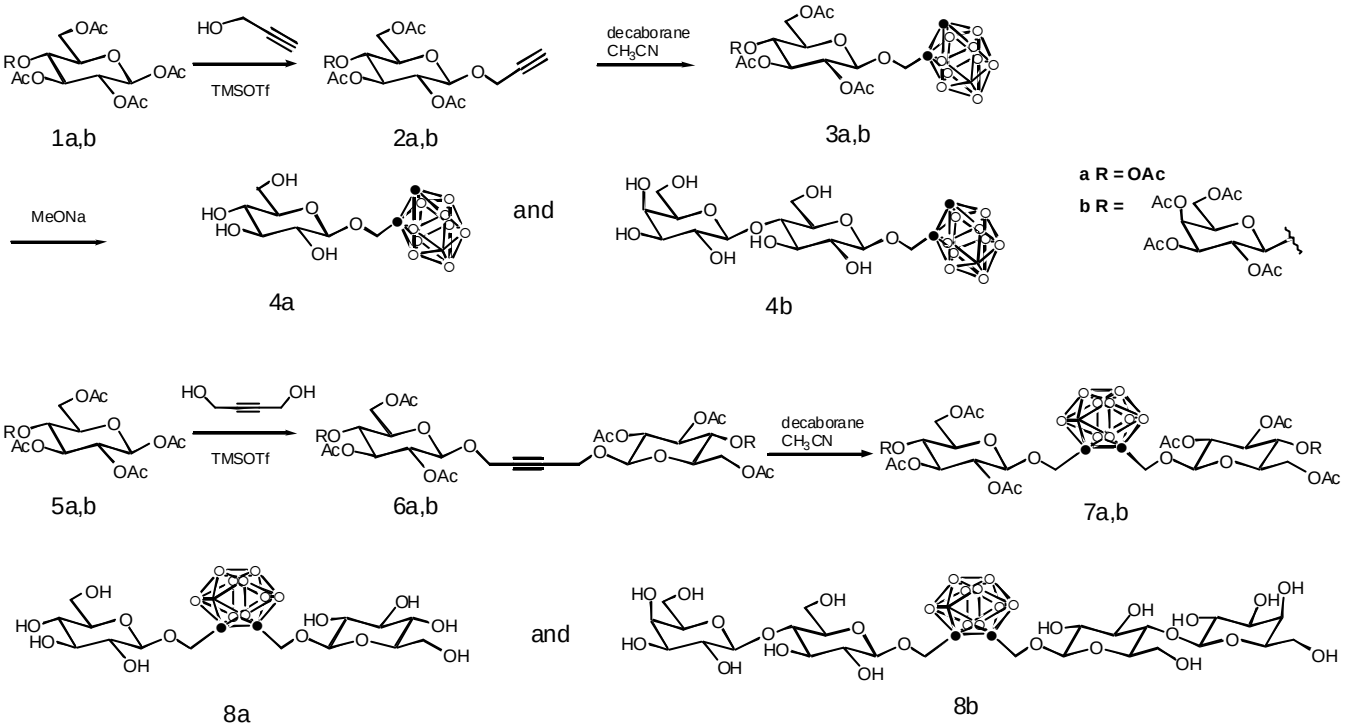
In fact, carbohydrate research has undergone a renaissance over the last decade because of the improvements in oligosaccharide synthesis[2] and carbohydrate biology.[3] One of the advantages of using carbohydrates as targeting agents for BNCT, beyond their ability to target specific receptors found on the surface of tumors, is that simple oligosaccharides usually show low toxicities. A further benefit of this particular class of targeting agent is their ability to compensate

for the hydrophobicity of the carborane cores, which could help limit non-specific protein binding and/or high liver uptake.

Results and discussion

Synthesis of glycosyl carboranes

There have been numerous reports on the synthesis of carborane-carbohydrate conjugates[4]. Among them, our research group prepared a series of carboranyl glycosides, from the corresponding alkynyl-glycosides which were in turn synthesized by glycosylation of propargyl alcohol or 3-butyn-1,4-diol with peracetylated glucose and lactose promoted by trimethyl sislyl trifluoromethanesulfonate (Scheme 1).



Scheme 1: Synthesis of glycosyl carboranes derived from propargylic alcohols

Reaction of the alkynes with decaborane, in the presence of acetonitrile, resulted in the desired products in 40-60% yields.

Further derivatives are in preparation, starting from glycosides of 4-amino-2-butyn-1ol to allow the functionalyzation of the amino group with various compounds, such as amino acids or appropriate probes to monitor the metabolic fate of such compounds.

Synthesis of glycosyl carboranes with different linkers between sugar and carborane moiety

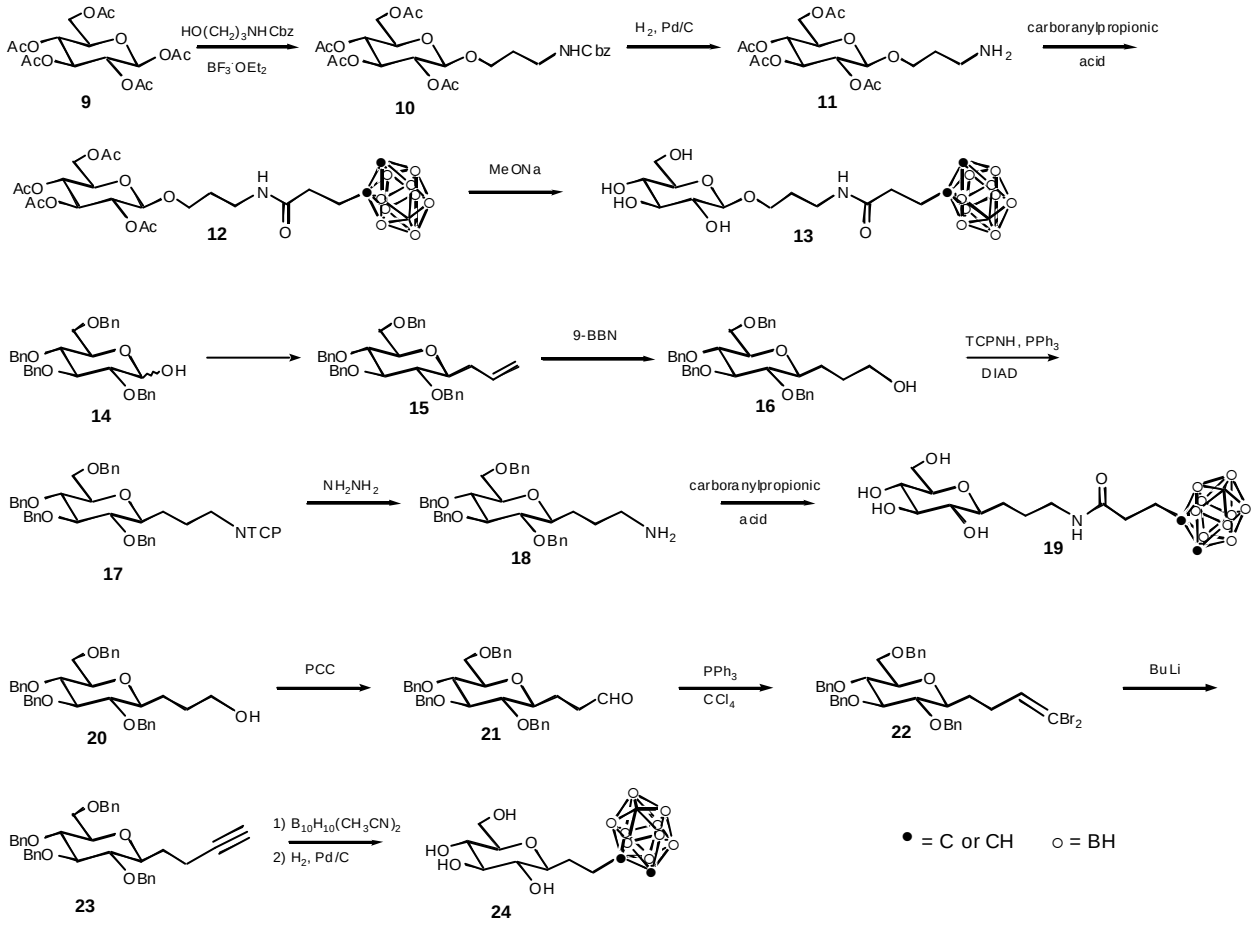
Recently, Tietze et al.[5] prepared and screened a series of carboranyl glycosides, which included glucoside, lactoside and maltoside conjugates. In following papers they planned to utilize these compounds as prodrugs, as glycosidases, either already present *in-vivo* or successively administred, could be able to cleave the sugar residues in proximity of tumor cells so allowing a selective uptake of the more lipophilic catabolite.[6]

We then decided to synthesize three different carborane-carbohydrate hybrids in which there are linkers between the two moieties containing functional groups of different type or no functional groups at all. The strategy exploited for two compounds involved the synthesis of a sugar with a linker containing an amino group, to be functionalized with a carboxylic acid containing carborane. Although the carborane derived from 4-pentynoic acid was already known, to the best of our knowledge it has never been used for the synthesis of carbohydrate-containing derivatives. This

approach opens the possibility to obtain any of such hybrids if a sugar moiety bearing a free amine is available.

In previous examples, carboranes have been joined to carbohydrates either through glycosidic linkages, in which the sugar structure is maintained, or by functionalization of hydroxyl groups as ethers or formation of carbon-carbon bonds by nucleophylic addition to aldehydo groups, with consequent modification of the carbohydrate moiety properties.

In our approach the structure of the carbohydrate is conserved; however, the tree compounds synthesized contain different types of linkages between sugar moiety and carborane. In particular they contain a glycosidic linkage, hydrolysable by glycosidases and an amide sensitive to proteases, only an amido function or a C-glycosidic bond, not hydrolysable at all (Scheme 2).[7]



Scheme 2: Synthesis of carborane–carbohydrate conjugates with different linkages between the two moieties.

It has to be noted that recently Tietze and Dondoni reported the synthesis of a series of C-glycosides derived carboranes.[8]

From NMR studies on compound **4a** it was possible to deduce the presence of a very unusual intramolecular hydrogen bond between the carborane CH and the anomeric oxygen of the sugar moiety, even using ethanol as solvent. Such an observation was confirmed by the fact that the same intramolecular hydrogen bond was absent on C-glycosyl carborane **24** as an obvious consequence of the absence of the anomeric oxygen.[9]

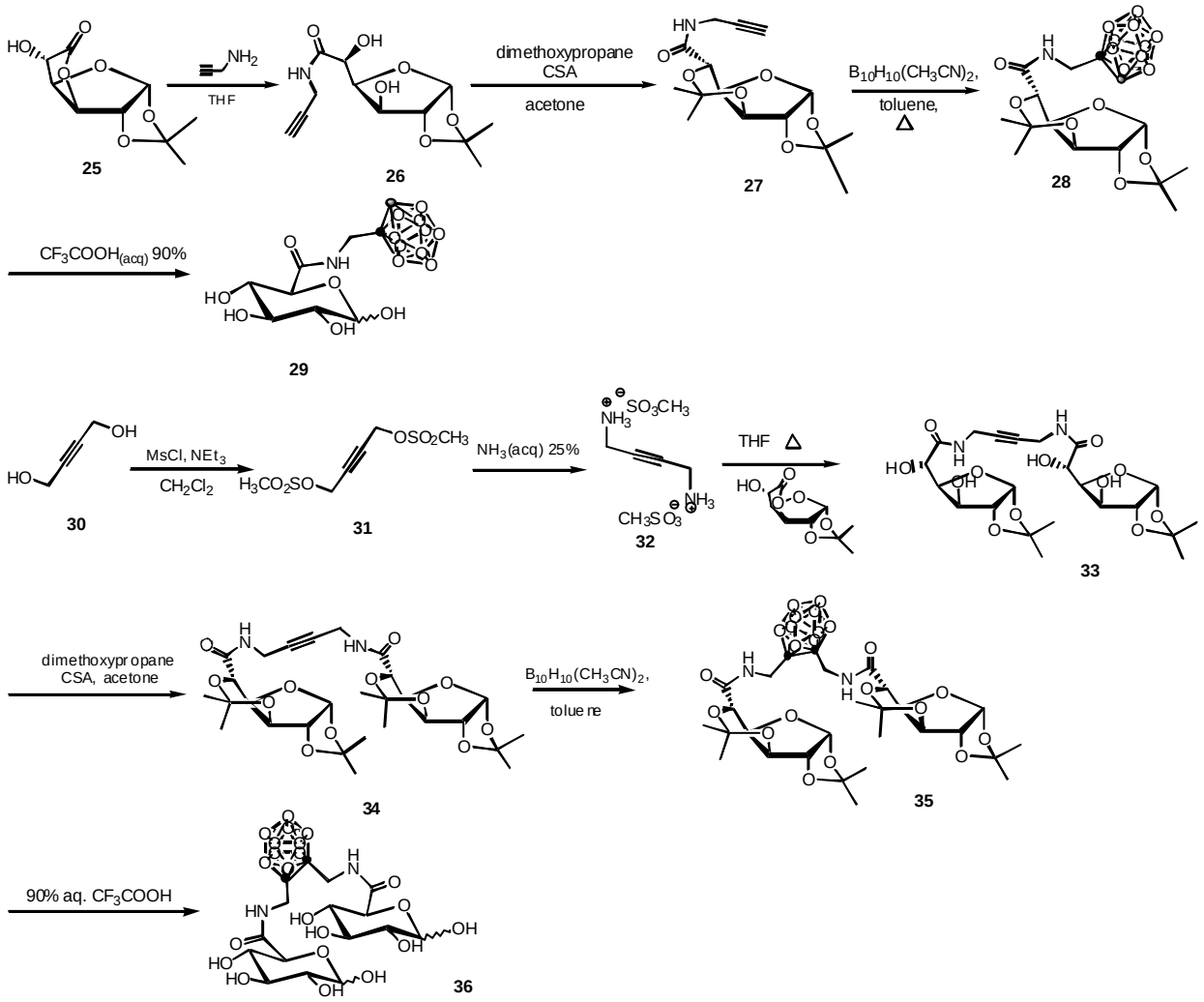
Synthesis of carborane-carbohydrate conjugates other than glycosyl derivatives

It is well known that glucose allow recognition by the glucose transport proteins GLUTs provided linkage by a non binding region, such as the non reducing end of D-glucose.[10](RIF vedi lavoro Morin)

To introduce a glucose unit, it was taken advantage of the reactivity at C-6 of the readily available glucofuranono-γ-lactone acetal **25** as it is known that lactone ring opening by amines occur easily.

A first reaction was performed using propargyl amine, which gave very easily the corresponding amide **26**. Protection of the 3,5-diol as isopropylidene and cycloaddition with decaborane in the presence of acetonitrile gave smoothly the desired derivative **28**. More difficult was the deprotection of the acetals. Many different conditions were tested and the best results were obtained with 90% aqueous trifluoroacetic acid for a short time, as prolonged reaction time gave complex mixtures. Careful examination of such mixtures suggested that the acid could induce an unexpected hydrolysis of the amide bond, probably due to the electron withdrawing effect of the carborane cage.

The same synthetic scheme was applied to the synthesis of simmetrically substituted derivatives, in which 1,4-but-2-yn-diamine was employed.

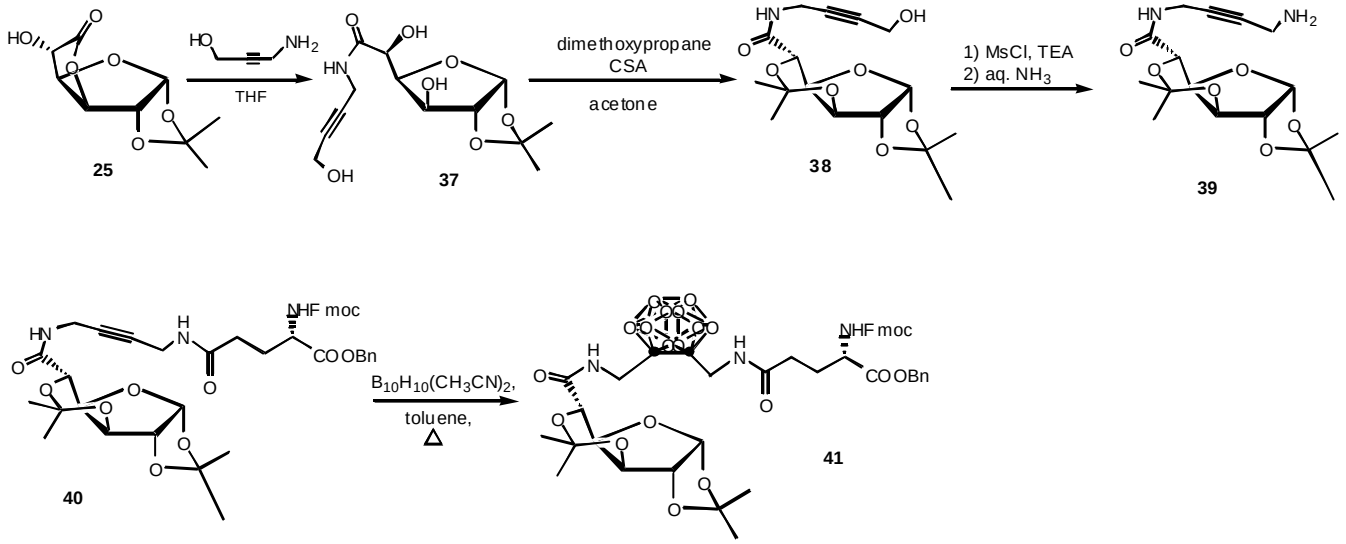


Scheme 3: carborane derivatives of D-glucuronic acid.

In this context a new and simple synthesis of such an amine was developed. The final compounds, in this case have a carborane cage in between two glucuronic acid moieties.

Carboranes containing both sugars and amino acids

Exploiting the previous approach a derivative containing both a glucuronic acid and a glutamic acid has been synthesized (Scheme 4).[11] Glucofuranono-γ-lactone acetal **25** was allowed to react with 4-amino-but-2-in-1-ol, which was prepared by treatment of monotosylate of 2-butin-1,4-diol with aqueous ammonia, to give compound **39**. Protection of the 3,5-diol as isopropylidene, mesylation of the propargylic alcohol and treatment with aqueous ammonia gave product **39**. It was treated with Fmoc glutamic acid benzyl ester and DDC to give the derivative **40**, containing both a sugar and a amino acid. Reaction with decaborane-acetonitrile complex afforded eventually product **41**.

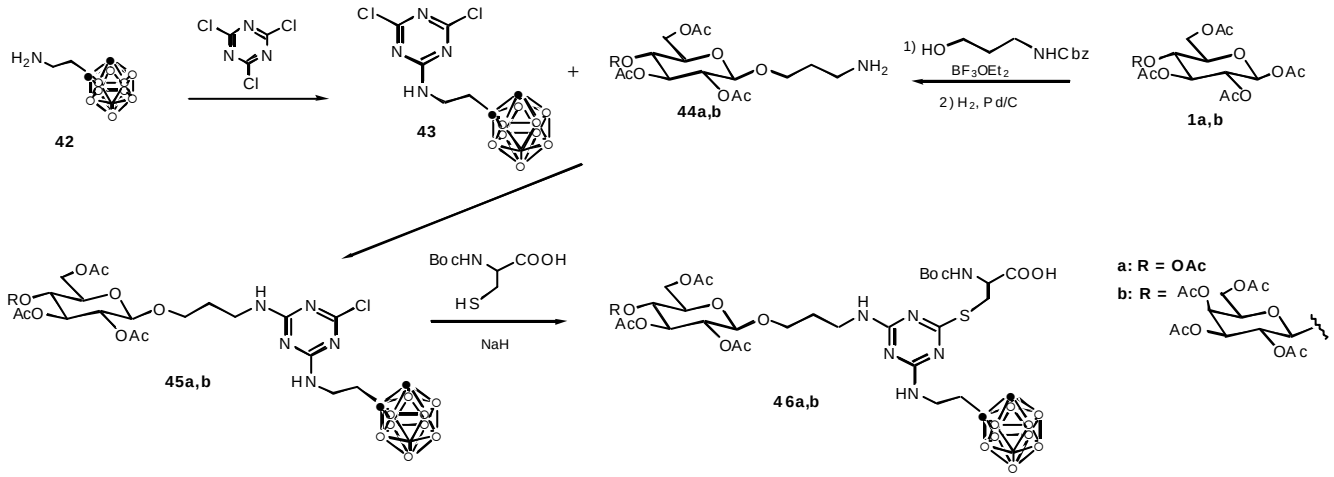


Scheme 4: synthesis of derivative containing a sugar and an amino acid

The final compound is properly protected for its use in peptide synthesis exploiting Fmoc technique.

Use of scaffolds in carborane containing hybrids

Trichlorotriazine is often used as scaffold in solution combinatorial chemistry as the three chlorine atoms can be easily substituted sequentially. We decided to exploit such a feature not only to generate a family of compounds, but also mainly to verify the feasibility of this approach in carborane chemistry. The nucleophiles we chose were 2-aminoethyl-o-carborane, 3-aminopropylglycosides of peracetylated glucose and lactose and thiol containing derivatives such as methyl thioglycolate and Boc-L-cysteine (Scheme 5).



Scheme 5: Scaffold constructs containing carboranes

In this way we demonstrated that it is possible to introduce the carborane cage, a sugar and a carboxylic acid or an amino acid on the triazine scaffold but, in principle, the method allow to introduce any derivative containing a suitable nucleophilic functional group.

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