Synthesis of Novel Carborane-hybrids Based on a Triazine Scaffold for Boron Neutron Capture Therapy

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Received 12 December 2003

Abstract: Cyanuric chloride has been used as a scaffold for the synthesis of potential agents for BNCT containing a carborane cluster, a sugar moiety as hydrophilic arm and an amino acid for the conjugation with bioactive molecules; this approach is, in principle, particularly suitable for a combinatorial approach.

Key words: carborane cluster, glycosides, amino acids, boron, medicinal chemistry

Boron neutron capture therapy (BNCT) is a therapy for the treatment of tumors based on the selective irradiation of molecules containing ^{10}B atoms with a thermal neutrons beam.[1] This nuclide shows a large capture cross section relative to the more abundant endogenous nuclei (^{1}H, ^{12}C, ^{16}O, ^{31}P, ^{14}N).[2] In order to be therapeutically useful, an ideal boronated candidate should have the following properties: high tumor targeting selectivity; low cytotoxicity; high water solubility required for intra-arterious administration of the BNCT agent; high uptake by cancer cells.[3]

As boron moiety, our attention has been focused mainly on carboranes. Such boron containing structures allow to obtain chemical constructions that carry a large number of boron atoms per molecule.[4] On the other hand, carboranes are highly hydrophobic compounds, thus requiring them to be conjugated with hydrophilic counterparts in order to have structures with adequate water solubility for their administration. Among the different classes of molecules able to give the desired solubility properties to the carborane containing derivatives, carbohydrates are particularly interesting compounds[5] as they could also target specific receptors found on the surface of tumor cells, and usually show low toxicities. Moreover, the introduction of an amino acid unit could enable the incorporation of carboranes into peptides.[6] To date, only a few examples of carborane derivatives containing both a sugar and an amino acid have appeared in the literature, and their syntheses are usually quite laborious.[7]

We have chosen to exploit cyanuric chloride as a scaffold for the easy introduction of a carborane, a sugar and an amino acid onto the same molecule. Such a scaffold, due to the different reactivity of the chlorine atoms, allows a sequential introduction of various substituents, opening the way to introduce diversity into the products. For this reason, cyanuric chloride or triazine derivatives are often used in combinatorial synthesis.[8] It has to be noted that, to the best of our knowledge, only one example of triazine derived carboranes has appeared in the literature.[9] The authors showed that it is possible to directly join the carborane cage to a triazine core, but the other substituents reported were only simple secondary amines. It is remarkable that, in their reactions, the carborane cage seems to be quite resistant to the presence of secondary amines, as they did not observe the degradation of the *closo* form with formation of anionic *nido* derivatives.

In order to verify the possibility of generating differently functionalised triazines, we prepared 2-carboranyl ethylamine as well as protected glucose and lactose aminopropyl glycosides.

Among the different methods developed for the synthesis of aminoalkyl carboranes,[10] the preparation of the derivative **2** was performed according to the procedure suggested by Soloway and co-workers,[11] with a modification of the azide reduction step, carried out without acid added. Tosylation of 3-butyn-1-ol, carborane formation by treatment with decaborane-acetonitrile adduct and substitution with azide anion gave 2-carboranyl azido ethane **1**. The azido function was converted into the corresponding amino group with H_2-Pd/C in dry THF. Excess of cyanuric chloride and Hünigs' base were directly added into the reaction mixture containing the freshly prepared amine at room temperature,[12] affording smoothly the desired monosubstituted triazine **3** in good yields (Scheme 1).

The aminopropyl glycosides were obtained by conventional Lewis acid catalysed glycosylation of commercially available peracetylated β-D-glucose (**4a**) and β-D-lactose (**4b**), with 3-benzyloxycarbonylaminopropanol.[13] Although the yields were not very high, each product was obtained in a single step. Benzyloxycarbonyl protecting group of compounds **5a** and **5b** was removed by catalytic hydrogenolysis. The reaction mixture containing the free amino derivative **6a** (or **6b,** respectively) was added dropwise to a solution of compound **3** and Hünigs' base in THF at 0 °C and the resulting mixture was allowed to warm to room temperature. We were pleased to observe the clean formation of the disubstituted triazines **7a** and

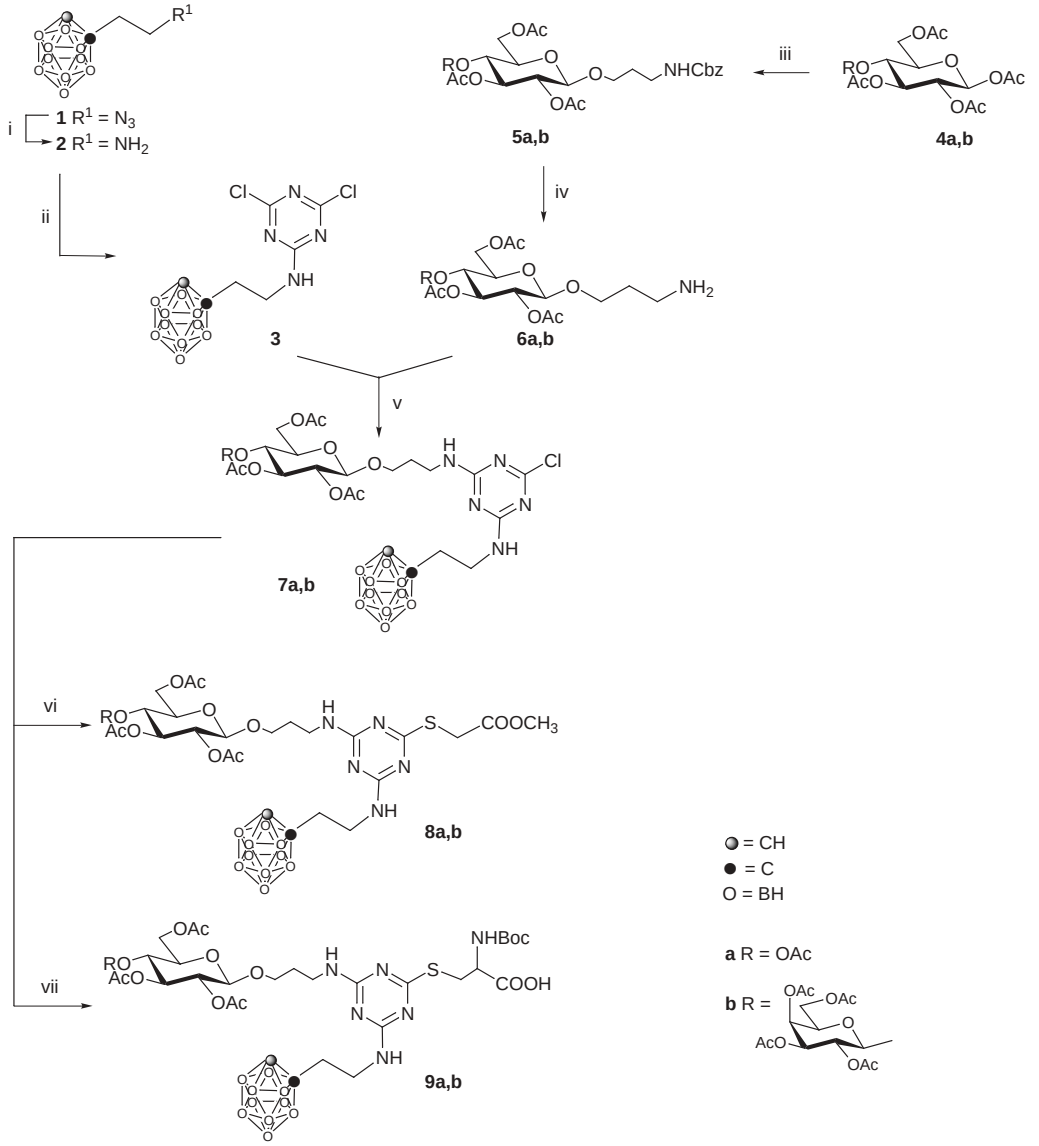
SYNLETT 2004, No. 6, pp 1007–1010
Advanced online publication: 25.03.2004
DOI: 10.1055/s-2004-822897; Art ID: G35303ST

7b both with glucose and lactose derivatives. Under these conditions the carborane cage demonstrated complete stability in the presence of free amino groups.

The displacement of the third chlorine atom is usually more difficult: in our hand, any attempt to attach the primary ε-amino group of the side chain of the Z-α-*N*-L-lysine invariably failed, even after refluxing in acetonitrile for two days.[14] So we decided to exploit a better nucleophile such as a thiolate anion for this reaction. In order to verify the reaction conditions we initially employed the thiolate of methyl thioglycolate, generated with sodium hydride in dry acetonitrile. After addition of compound **7a** or **7b**, the reaction mixture was refluxed overnight. Aqueous work-up and purification by flash chromatography afforded the desired trisubstituted compounds **8a** and **8b**, respectively in 70–75% yield. The newly introduced carboxylic function would make this molecule ready for conjugation with biopolymers.

Encouraged by these results, we decided to try to perform the third substitution reaction with cysteine. This amino acidic moiety, allowed us to generate a compound, which could not only be easily bound to a protein, but also inserted into a synthetic peptide.

Thus, the reaction was repeated essentially under the same conditions employed for methyl thioglycolate, using *N*-Boc-L-cysteine and 2 equivalents of sodium hydride. After refluxing overnight in dry acetonitrile, a 5% HCl solution was added dropwise until pH 3 was reached, then the mixture was diluted with dichloromethane and extracted as previously. Again, we were pleased to observe



Scheme 1 (i) H_2, cat. Pd/C, dry THF; (ii) DIPEA, 3 equiv cyanuric chloride, 85% for two steps; (iii) 3-benzyloxycarbonylaminopropanol, $BF_3{\cdot}Et_2O$, dry CH_2Cl_2, 47% for **5a**, 41% for **5b**; (iv) H_2, cat. Pd/C, dry THF; (v) DIPEA, dry THF, 0 °C, then r.t., 88% for **7a,** 60% (not optimised) for **7b**; (vi) NaH, methyl thioglycolate, dry MeCN, 80 °C, 75% for **8a**, 71% for **8b**; (vii) 2 equiv NaH, *N*-Boc-cysteine, dry MeCN, 80 °C, 72% for **9a**, 70% for **9b**.

the formation of the expected compounds **9a** and **9b** in comparable yields with respect to the previous reaction. In the ^{11}B NMR spectra, compounds **8a**,**b** and **9a**,**b** showed signals in the range $\delta = -2$ to -15 ppm,[15] while no peaks were observed in the range $\delta = -30$ to -40 ppm, diagnostic for the *nido*-carborane.[16] MALDI-TOF mass experiments confirmed the presence of the intact carborane cage.

In conclusion, this paper describes a simple and effective way to introduce a carborane, a sugar and a carboxylic acid or an amino acid onto a triazine scaffold. Such compounds open the possibility to generate easily a remarkable diversity employing a combinatorial approach. It is in fact possible to introduce various oligosaccharidic structures as well as to use this class of derivatives for the synthesis of biologically relevant peptides.

Work is in progress to extend the scope of the procedure to more complex derivatives directly involved in tumor cell surface recognition and endocytosis phenomena.

Preparation of Compound 3

Compound **1** (516 mg, 2.42 mmol) was dissolved in 35 mL of dry THF. Catalytic hydrogenation (Pd/C, 20 mg) furnished compound **2** after 2.5 h. Disappearance of **1** was monitored by TLC (petroleum ether/EtOAc 8:2). Hünig's base (422 μL, 2.42 mmol) and cyanuric chloride (1339 mg, 7.26 mmol) were added directly to the reaction mixture at r.t. under nitrogen. After 14 h, the mixture was diluted with THF, filtered over celite and the solvent evaporated. The residue was purified by flash chromatography (petroleum ether–EtOAc, 85:15) giving 690 mg of **3** as a white solid (85% yield).

General Procedure for the Preparation of Compounds 7a and 7b

(*Z*)-aminopropyl glycoside **5a** or **5b** (0.90 mmol) was dissolved in dry THF (25 mL) A catalytic amount of Pd on activated charcoal was added and the amino group was deprotected under hydrogen atmosphere (3 h). The mixture containing the free amine **6a** (or **6b** respectively) was transferred into a solution of **3** (300 mg, 0.90 mmol) and Hünig's base (156 μL, 0.90 mmol) in THF (15 mL) at 0 °C. The mixture was allowed to warm to r.t. and stirred overnight, then diluted with THF and filtered over celite. The solvent was evaporated and the brown solid purified by flash chromatography (petroleum ether–EtOAc, 1:1 for **7a** and 3:7 for **7b**). Compound **7a** and **7b** were obtained as white solids in 88% (554 mg) and 60% (533 mg, not optimised) yields, respectively.

General Procedure for the Preparation of Compounds 8a and 8b

95% NaH (7 mg, 0.28 mmol) was added to a solution of methyl thioglycolate (27 μL, 0.28 mmol) in 2 mL of dry MeCN in an ice bath. After the addition, the ice bath was removed and the mixture was allowed to warm to r.t. for 15 min. A solution of **7a**,**b** (0.14 mmol) in dry MeCN (3 mL) was added. The resulting mixture was refluxed overnight under argon, then diluted with CH_2Cl_2 and H_2O and extracted. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified flash chromatography (petroleum ether–EtOAc, 1:1 for **8a** and 4:7 for **8b**), giving **8a** and **8b** in 75% (82 mg) and 71% (76 mg) yields, respectively.

General Procedure for the Preparation of Compounds 9a and 9b

95% NaH (15 mg, 0.57 mmol) was added to a solution of *N*-Boc-cysteine (63 mg, 0.28 mmol) in 2 mL of dry MeCN in an ice bath. After the addition, the ice bath was removed and the mixture was allowed to warm to r.t. for 15 min. A solution of **7a**,**b** (0.14 mmol) in dry MeCN (3.5 mL) was added. The resulting mixture was refluxed overnight under argon, then cooled to r.t. and concentrated to 1 mL volume. To this mixture H_2O (5 mL) was added followed by 5% HCl until pH 3 was reached. The mixture was diluted with CH_2Cl_2 (15 mL) and extracted. The organic layer was washed with H_2O, then dried over Na_2SO_4, filtered and concentrated. The crude product was purified flash chromatography (EtOAc–MeOH, 9:1), furnishing **9a** and **9b** in 72% (91 mg) and 70% (124 mg) yields, respectively.

Acknowledgement

We thank MIUR (COFIN 2001), Università del Piemonte Orientale and CNR for financial support.

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(14) Reaction conditions: Z-α-*N*-L-lysine, 2 equiv DIPEA, MeCN, 15 min, r.t.; then, **7a** or **7b**, 48 h, 80 °C.
(15) Selected data for final compounds. MALDI-MS: **8a** calcd for $C_{27}H_{47}B_{10}N_5O_{12}S$: 773.9; found: 796.0 $[M + Na]^+$, 812.8 $[M + K]^+$; **8b** calcd for $C_{39}H_{63}B_{10}N_5O_{20}S$: 1062.1; found: 1085.2 $[M + Na]^+$, 1101.2 $[M + K]^+$; **9a** calcd for $C_{32}H_{56}B_{10}N_6O_{14}S$: 889.0; found: 928.44 $[M + K]^+$; **12b** calcd for $C_{44}H_{72}B_{10}N_6O_{22}S$: 1177.2; found: 1216.3 $[M + K]^+$. ^{11}B NMR (CD_3OD): **8a** –3.62 (2 B), –10.54 (4 B), –12.36 (4 B); **8b** –3.59 (2 B), –10.47 (4 B), –12.22 (4 B); **9a** –3.55 (2 B), –10.50 (4 B), –12.25 (4 B); **9b** –3.64 (2 B), –10.45 (4 B), –12.18 (4 B).
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