Preparation of Synthons for Carborane Containing Macromolecules

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Summary: The modification of *para*-carborane with appropriate functionalities for incorporation within a dendrimer framework was accomplished by functionalizing the carbon centers with protected alcohol and free acid groups. These compounds are excellent candidates for utilization as functional linkers between two generations of an aliphatic polyester dendrimer structure. Future assembly of these structures will result in dendritic macromolecules containing carboranes within their interior and will be enveloped by hydrophilic groups (hydroxyls) to maintain their water solubility and biocompatibility. These structures have potential applications in Boron Neutron Capture Therapy and Synovectomy. Additionally, carboranes were coupled to polymerizable acrylate structures, and it was shown that the resulting carborane monomers could be polymerized using living free-radical polymerization techniques.

Keywords: atom transfer radical polymerization; carboranes; dendrimers; drug delivery; polymers

Introduction

Carboranes, icosahedral clusters with molecular formula $C_2B_{10}H_{12}$, have attracted a great deal of attention for more than 40 years. Their extraordinary thermal stability, hydrophobicity, and three dimensional "aromaticity" impart interesting properties to the molecules and materials that they comprise.[1] Through functionalization at the carbon centers, carboranes have been incorporated in a wide array of coordination compounds, catalysts, polymers, and pharmaceuticals.[2] Perhaps the most interesting aspect of boron-rich compounds is the nuclear reaction that results upon irradiation of ^{10}B nuclei with thermal neutrons, namely the $^{10}B(n,\alpha)^7Li$ reaction.[3] Due to the high neutron capture cross section of ^{10}B, and its high relative natural abundance (20%), this α-particle emitting reaction can be induced with reasonably high yields. The low relative penetration of α-radiation, equal to approximately the diameter of a single human cell, allows this reaction to cause highly localized damage to boron-containing environments. In 1936, Locher proposed that neutron irradiation of ^{10}B delivered to malignant cells could be used to treat

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diseases such as cancer, and this treatment was dubbed Boron Neutron Capture Therapy (BNCT).[4] More recently, a similar approach has been investigated for the treatment of refractory cases of rheumatoid arthritis (RA), in which the neutron capture reaction is used to destroy the inflamed synovial lining of a joint that causes debilitating pain to the patient.[5] This process is termed Boron Neutron Capture Synovectomy (BNCS). Despite the great potential of BNCT and BNCS in treating disease, clinical success with both of these therapies has been limited. The main challenge to these approaches has been the delivery of adequate amounts of boron (10-30 μg ^{10}B g^{-1} tumour) to the site of interest, and especially into the cells that are to be destroyed.[2] Carboranes, with their high B content have been the molecules of choice for B-delivery, but are themselves inadequate due to their hydrophobicity. Conjugation with various hydrophilic biological molecules, such as peptides, carbohydrates, nucleosides, and monoclonal antibodies has been investigated, but only limited success has been achieved due to rapid clearance of these structures from the circulation.[2] Conjugation with non-biological molecules, such as hydrophilic polyamines, has been shown to target specific intracellular sites (i.e., DNA) *in vitro*, but these studies have revealed substantial cytotoxicity and a general inability to deliver adequate quantities of boron to tumours *in vivo*.[6]

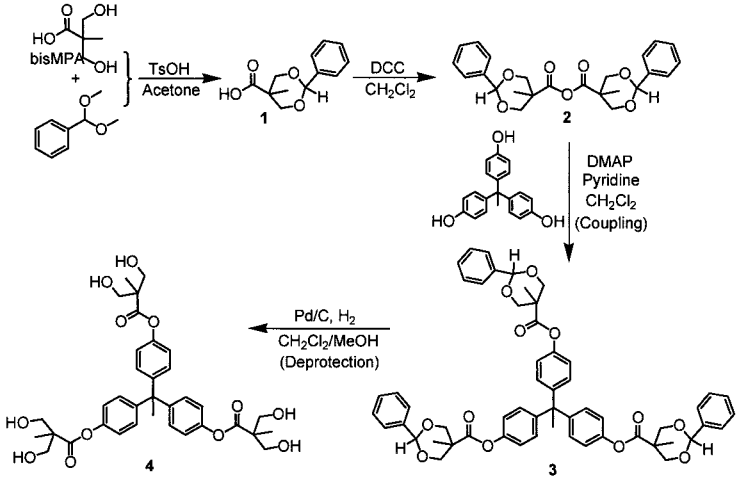
Dendrimers are a class of polymers that are precisely synthesized, monodisperse, and can exhibit a large number of functionalizable end-groups.[7] This multivalency of dendrimers makes them attractive scaffolds for drug delivery applications, as a high loading of pharmacophores can be achieved on each structure. In addition, if the pharmacophore is incorporated within the interior of the dendrimer, the periphery of the macromolecule can be tailored for solubility and specific targeting functions.[8]

The current goals of our research program are the synthesis, characterization, and application of well-defined carborane-functionalized macromolecules. This paper describes the preparation of synthons for aliphatic polyester dendrimers, as well as linear block copolymers, in which the location and loading of carboranes can be easily controlled. The application of living radical polymerization techniques to the synthesis of carborane containing block-copolymers has the potential to yield boron-rich macromolecules with tuneable properties. In addition, aliphatic polyester dendrimers are water soluble in the neutral state, non-toxic, bio-compatible, and easily derivatized with a wide range of functionality, making them ideal for drug-delivery

applications.[9] We have explored the use of functionalized carborane cages as linkers between two generations on the interior of the dendrimer, so as to effectively encapsulate them within the hydrophilic scaffold. This strategy will allow us to maximize water solubility and biocompatibility, control the degree of carborane incorporation, and possibly attach active targeting agents to the periphery of the final macromolecule.

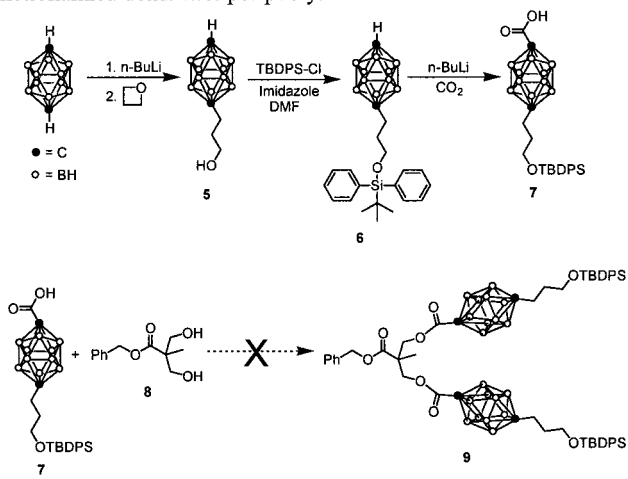
Results and Discussion

Dendrimer Synthesis. The synthetic methodology for the preparation of aliphatic polyester dendrimers, based on 2,2-bis(hydroxymethyl) propanoic acid (bisMPA) as the monomer unit, has been documented in recent literature and involves standard esterification chemistry.[10-13] This family of dendrimers poses a number of key advantages, including the low cost of the starting materials, the possibility of utilizing convergent or divergent growth strategies, and the high-yields of the synthetic steps.[14] We have chosen to utilize the divergent growth strategy involving a highly active anhydride form of the protected bisMPA monomer. The steps involved in the preparation of a first-generation (G-1) hydroxyl terminated dendrimer are outlined in Scheme 1. Higher generation dendrimers were obtained through repetition of the coupling and deprotection steps.



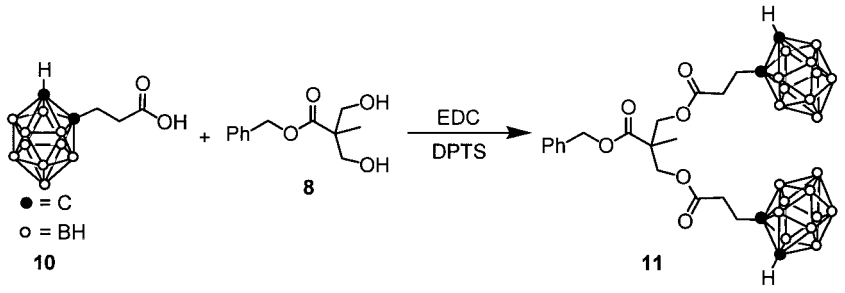
Scheme 1.

Carborane Functionalization. In order to incorporate the carborane cage structure into the polyester dendrimer synthesis, it was necessary to introduce an acid functionality for coupling to the terminal hydroxyls of the dendrimer, as well as a protected alcohol functionality that could be subsequently utilized to react with **2** for further dendrimer growth. In this way, the functionalized carborane acts as an inter-generation linker, allowing its incorporation at any desired stage of dendrimer synthesis. Preparation of functionalized *para*-carboranes was accomplished using literature procedures,[15] and is illustrated in Scheme 2. Deprotonation of *para*-carborane with n-butyllithium (n-BuLi) followed by addition of oxetane produced **5** in 50% yield. Protection of the alcohol with TBDPS was accomplished in quantitative yield under standard conditions and was followed by a second n-BuLi deprotonation and reaction with CO_2. The desired acid (**7**) was obtained in 42% yield over the three steps. The benzylated bisMPA (**8**) was prepared in a single step through the reaction of bisMPA with benzyl bromide under basic conditions,[13] and was used as a model compound to investigate the esterification chemistry between acid **7** and the eventual hydroxyl functionalized dendrimer periphery.



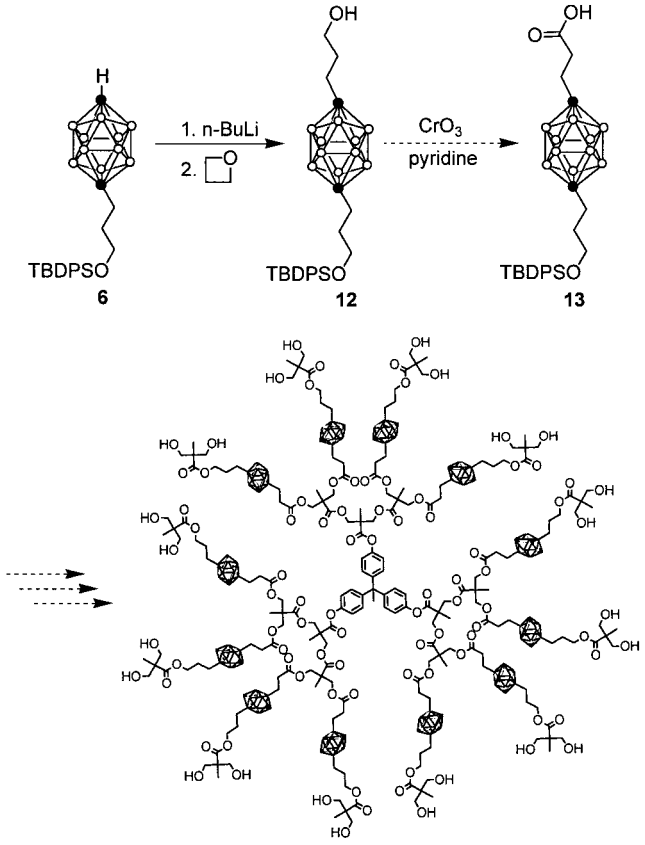
Scheme 2.

Unfortunately, all attempts to couple **7** and **8** using carbodiimide chemistry were unsuccessful, presumably due to deactivating electronic and steric effects of the proximal carborane cluster. It was hypothesized that increasing the distance between the acid group and the carborane of compound **7** should greatly improve the coupling efficiency. This was tested with model compound **10**,[16] the acid derivative of *ortho*-carborane having a two carbon spacer between the acid and the carborane. Coupling of **10** with **8** proceeded smoothly using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and the 1:1 salt of 4-dimethylaminopyridine and toluene sulfonic acid (DPTS), yielding **11** in quantitative yield.



Scheme 3.

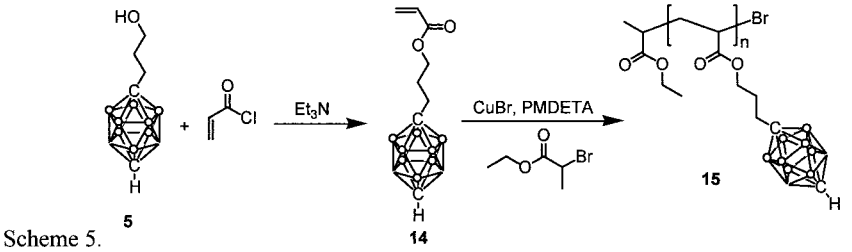
This successful model chemistry indicates that extending the distance between the acid group and the carborane in **7** by two methylene groups should restore its reactivity toward esterification. Preparation of the required carborane synthon, starting with protected alcohol **6**, involved a repetition of the n-BuLi/oxetane reaction, which was carried out in 61% yield, followed by oxidation to the acid (Scheme 4). The final step of this sequence has not yet been completed, but once accomplished, will provide the necessary structures for incorporation of *para*-carboranes into the aliphatic polyester dendrimer synthesis. The G-3 target structure is illustrated in Scheme 4.



Scheme 4.

Carborane-Containing Polymers. The carborane derivatization strategy described above was easily adapted to the preparation of a polymerizable carborane-containing acrylate monomer. This was accomplished by esterifying alcohol **5** with acryloyl chloride in triethyl amine to produce monomer **14** (Scheme 5). It was found that this monomer readily polymerizes by atom transfer radical polymerization, using CuBr/N,N,N',N'',N''-pentamethyldiethyltriamine (PMDETA) as the catalyst/ligand and ethyl-2-bromopropionate (2-EBP) as the initiator, resulting in narrow-polydispersity living polymers (Scheme 5). The polymerization product was

precipitated in MeOH to yield a white powder with Mw = 11,600 and PDI = 1.18 (Figure 1). The isolated polymer retained its living character and could be chain extended with various monomers, such as t-butyl acrylate. The formation of block copolymers, where one block contains carborane side chains, opens up the possibility for the preparation of a wide array of carborane-containing macromolecules, including amphiphilic structures capable of self-assembly in aqueous and/or organic media to produce micelles and vescicles. Investigation of the self assembly properties of block copolymers incorporating carboranes is currently underway in our laboratory.



Scheme 5.

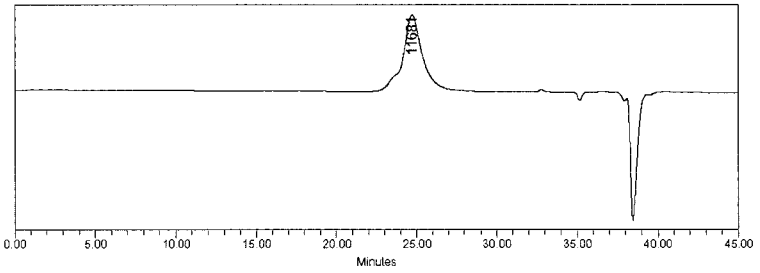


Figure 1. Size exclusion chromatogram for polymer 15.

Conclusions and Outlook

The preparation of appropriate synthons for the incorporation of carborane cages within a dendritic structure was accomplished. It was found that an acid group attached directly to the carborane was not active toward esterification, but one separated by a two carbon linker could be esterified in good yield. Once the final carborane synthon is prepared, it will be possible to produce a series of dendrimers in which the carborane loading can be controlled by varying the

generation at which it is incorporated. The solubility and biocompatibility properties of the dendrimers will be controlled by the hydroxyl groups present at the dendrimer periphery after deprotection, which can be further derivatized with biomolecules to impart active targeting characteristics to the structures. Once made, the effectiveness of these molecules in BNCT and BNCS will be evaluated. In addition, it was found that functionalization of carboranes with acrylate groups resulted in carborane-containing monomers that could undergo atom transfer radical polymerization. The resulting polymer had low polydispersity and could be chain extended with t-butyl acrylate, indicating that the polymerization of the initial block was living. Therefore, it will be possible to utilize this monomer to prepare a variety of block copolymers that will differ in their carborane loading, solubility, and mechanical properties, depending on the identity of the comonomers. Due to the unusual properties of carboranes, a number of new and interesting materials will likely result from this initial investigation.

Experimental

Preparation of 1-Hydroxypropyl-p-carborane (5)

Para-carborane (300 mg, 2.08 mmol) was dissolved with 60 mL of dry tetrahydrofuran and placed in a flame dried round bottom flask with an argon atmosphere. The reaction mixture was cooled to 0°C, and butyllithium (945 μL, 2.08 mmol) was added slowly to the reaction mixture. After 1 hour, trimethylene oxide (135 μL, 2.08 mmol) was added and allowed to react for an additional 2 hours at room temperature. The excess butyllithium was quenched with 20 mL of HCl (0.2 M) for 30 minutes. After quenching, 50 mL of dichloromethane and 50 mL of water were used to extract the product. The organic layer was separated, dried over $MgSO_4$, and evaporated to yield a white glass: 0.211 g, 1.04 mmol (50%). IR (cm^{-1}): 3620, 2610. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.29 (s) 1.43 (m), 1.72 (m), 2.63 (s), 3.47 (t , 2, J = 6). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 32.25, 35.32, 58.07, 61.85, 84.20. ^{11}B NMR (160 MHz, $CDCl_3$, proton-decoupled): δ = -14.29, -11.78.

Silyl Protection of 1-Hydroxypropyl-p-carborane (6)

Compound 5 (1.00 g, 4.94 mmol) and imidazole (0.673 g, 9.89 mmol) were dissolved in 60 mL of DMF. The mixture was allowed to cool to 0°C, and tert-butylchlorodiphenylsilane (2.04 g, 7.41 mmol) was added to the mixture and allowed to react for 3 hours. The reaction mixture was

quenched with 20 mL of water and was washed ether. The ether layers were collected and additionally washed with 2 M HCl, 10% NaOH, and brine. The product was isolated by column chromatography (9:1 hexane : ethyl acetate), yielding a white glass: 2.12 g, 4.82 mmol (98%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.04 (s, 9), 1.43 (m), 1.72 (m), 2.63 (s), 3.50 (t , 2, J = 6), 7.44(m, 6), 7.62 (d, 4, J = 8) . ^{13}C NMR (125 MHz, $CDCl_3$): δ = 19.15, 26.81, 32.25, 35.51, 57.95, 62.76, 84.59, 127.63, 129.62, 133.72, 135.51. ^{11}B NMR (160 MHz, $CDCl_3$, proton-decoupled): δ = -14.29, -11.72.

Carboxylation of the Silyl Protected 1-Hydroxypropyl-p-carborane (7)

Compound 6 (0.199 g, 0.452 mmol) was dissolved in 9 mL of dry THF. The reaction vessel was cooled to 0°C and n-butyl lithium (0.224 mL, 0.477 mmol) was added and allowed to react for 1 hour. The mixture was then cooled to –78°C and CO_2 was bubbled into the mixture. The formation of a precipitate was observed after ca. 15- 30 min. After 3 hrs of bubbling, the reaction was quenched using 30 ml of HCl (0.2 M). This was followed by the addition of 50 ml of H_2O and extraction of the resulting mixture using CH_2Cl_2 (2 x 50 mL). The organic phases were collected, dried (Na_2SO_4), filtered, and evaporated to dryness in vacuo to yield a white crystalline solid (184 mg, 84%). ^{1}H NMR (200 MHz, $CDC1_3$): δ = 1.05 (s, 9 H), 1.43 (m, 2 H), 1.76 (t, 2 H), 3.50 (t, 2 H), 7.42 (m, 5), 7.61 (m, 5), 9.41 (s, 1).

Hydroxypropylation of the Silyl Protected 1-Hydroxypropyl-p-carborane (12)

Compound 6 (0.219 g, 0.498 mmol) was dissolved in 100 mL of dry THF. The reaction vessel was cooled to 0°C and n-butyl lithium (0.553 mL, 0.996 mmol) was slowly added and allowed to react for 4 hours. The reaction mixture was cooled to 0°C once more and the trimethylene oxide (0.0486 mL, 0.747 mmol) was added and allowed to react overnight. After the reaction was completed the THF was removed by roto-evaporation and the product was extracted with 2 M HCL and ether. The product was isolated by column chromatography using 100% DMC (to remove staring material) and then 8:2 (DCM : Ethyl Acetate). The product was isolated as clear oil: 0.1503 g, 0.301 mmol (61%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.02 (s, 9), 1.41 (m, 4), 1.73 (m, 4), 3.47 (t , 2, J = 6), 7.41 (m,

6), 7.59 (d, 4, J = 8) . ^{13}C NMR (125 MHz, $CDCl_3$): δ = 19.07, 26.60, 32.36, 34.10, 61.86, 62.72, 78.46, 78.98, 127.55, 129.53, 133.65, 135.43. ^{11}B NMR (160 MHz, $CDCl_3$, proton-decoupled): δ = -11.97.

Coupling of O-Carborane-1-propionic acid with Benzyl-2-2-bis(oxy methyl) Propionate (11)

O-carborane-1-propionic acid (0.150 g, 0.694 mmol), benzyl-2-2-bis(oxy methyl) propionate (0.071 g, 0.315 mmol) and 4-(dimethylamino)-pyridine/p-toluene sulphonic salt (0.037 g, 0.126 mmol) were dissolved in 10 mL of dichloromethane. EDC (0.151 g, 0.788 mmol) was added to the reaction and stirred for 18 hours at room temperature. The reaction mixture was diluted with 40 mL of DCM and washed with distilled water. The organic layers were collected and removed by roto-evaporation. The product was isolated through column chromatography using 100% DCM as the solvent system. The coupling product was isolated as a glass: 0.1795 g, 0.290 mmol (92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.28 (s, 3), 2.38 (m), 2.48 (m), 3.66 (s, 2), 4.24 (q, 4, J = 11), 5.17 (s, 2) 7.34 (m, 5). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.81, 32.45, 33.05, 46.27, 61.55, 65.89, 67.03, 73.72, 128.27, 128.59, 128.66, 135.40, 170.81, 172.05. ^{11}B NMR (160 MHz, $CDCl_3$, proton-decoupled): δ = -11.98, -11.32, -10.80, -8.48, -4.57, -1.17.

1-p-Carborane Propyl Acrylate (14)

Compound 5 (1.00 g, 4.943 mmol) and triethylamine (1.00 g, 9.886 mmol) were dissolved in 100 mL of dry DCM. After 10 minutes, acryloyl chloride (0.671 g, 7.414 mmol) was added and allowed to react for two hours. The reaction mixture was diluted with 50 mL of ether and washed with distilled water. The organic layers were collected and removed by roto-evaporation. The product was isolated through column chromatography using 100% DCM as the solvent system. The product was isolated as white crystals: 1.22 g, 4.764 mmol (96%). IR (cm^{-1}): 2610, 1732. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.54 (m, 2), 1.70 (m, 2), 2.64 (s, 1), 3.97 (t, 2, J = 6), 5.80 (d, 1, J = 1.3), 6.06 (q, 1, J = 10.5, J = 17) 6.36 (d, 1, J = 1.3). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.36, 35.33, 58.22, 63.26, 83.65, 128.21, 130.76, 165.89. ^{11}B NMR (160 MHz, $CDCl_3$, proton-decoupled): δ = -14.25, -11.82.

Poly(1-p-Carborane Propyl Acrylate) via ATRP (15)

Compound 14 (1.00 g, 3.900 mmol), pentamethyldiethylenetriamine (14 mg, 78 μmol), and ethyl 2-bromopropionate (14 mg, 78 μmol) were dissolved in 1 mL of DMF. Copper (I) bromide (11 mg, 78 μmol) was placed in a separate reaction vessel. After 30 minutes of evacuation with argon on both reaction vessels, the monomer/ligand/initiator mixture was added to the copper bromide catalyst. The reaction mixture was heated to 60°C and allowed to react for 18 hours. Polymeric products were separated by precipitation in methanol and water (1:1). The isolated product was a light blue glass: 0.91 g (91% conversion). GPC: Mw = 11,681g/mol, Mn = 10,285 g/mol. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.51 (s, 145), 1.70 (s), 2.69(s, 141), 2.88 (s, 3), 2.96 (s, 4), 3.84 (s, 100). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.33, 35.20, 41.27, 58.27, 63.50, 83.70, 173.88.^{11}B NMR (160 MHz, $CDCl_3$, proton-decoupled): δ = -14.20, -11.77.

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