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Synthesis and characterization of carborane-functionalized hyperbranched polyester for boron neutron capture therapy

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ABSTRACT

The purpose of this study is to synthesize new boron containing carrier compound for Boron Neutron Capture Therapy (BNCT) which is two-component radiation therapy method that especially is promising for the treatment of brain tumors and is being actively researched in many countries. For this research hyperbranched polyester (HBP), bifunctional p-carborane and carborane-functionalized HBP were synthesized. Dipentaerythritol was chosen as a core molecule of the HBP and esterified with dimethylol propionic acid. Observed characteristic ester bands and OH stretching band in the FTIR spectrum and the methyl & methylene peaks in the ^{1}H NMR spectrum of HBP indicated that hyperbranched polyester synthesis was done successfully. A bifunctionalized p-carborane containing an acid group and a benzyl ether protected alcohol was prepared in three-step reactions. As a result of these reactions, a bifunctionalized p-carborane compound was produced with 60% yield. Then, the HBP was esterified with the bifunctional p-carborane. The characterization of the synthesized compounds was determined by FTIR and NMR spectra. The synthesis of carborane-functionalized HBP was confirmed by disappearance of HBP's OH groups and B-H stretching band observed in the FTIR spectrum of carborane-functionalized HBP and in addition, appearance of proton signals of HBP core, carborane linker and peripheral-protecting groups in the ^{1}H-NMR spectrum. Finally, synthesized water-soluble carborane-containing HB carrying many boron atoms should be served as potential BNCT agents.

1. Introduction

Carboranes are polyhedral clusters consisting of carbon, hydrogen and boron. They have been widely used in many areas such as carboraneous nano-material in electrochemical, semiconductor, drug delivery, boron neutron capture therapy (BNCT), molecular imaging, molecular sensors, catalysis and gas storage applications. Carboranes have a highly electron-delocalized hydrophobic surface, spherical geometry and convenient molecular size. Carboranes can readily be reacted with other organic groups because of relatively acidic protons of the two carbon atoms of carborane. Besides, carboranes are notably charming because of their high stability and charge neutrality. In particular, use of carborane derivatives in the field of medicinal chemistry has become more attractive day by day. Carboranes are mainly used to design BNCT agents in medicinal applications [1-2].

Boron neutron capture therapy is a binary radiation therapy method which becomes a significant treatment for numerous types of tumors. Boron compound is the main component of BNCT method. The basis of BNCT is neutron capture and fission reactions. Nonradioactive boron-10 (^{10}B) compounds accumulated in tumor cells are irradiated with low energy thermal neutrons. As a result of neutron caption reaction, alpha particles (^{4}He) and lithium-7 (^{7}Li) nuclei are released. In order to be successful, boron compound containing approximately 20-50 μg/g (~109 atoms/cell) of ^{10}B must be selectively delivered to the tumor and adequate amount of thermal neutrons must be absorbed by ^{10}B atoms. Thus, a lethal ^{10}B(n,a)^{7}Li capture reaction is occurred. High LET particles (^{4}He) have limited path lengths in tissue, so destructive effects of these particles are restrained to boron in tumor cells.

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Boron delivery agents such as (L)-4-dihydroxy-boryl-phenylalanine (BPA) and sodium mercaptoundeca-hydro-closo-dodecaborate (BSH) are used in clinical BNCT trials. Lots of boron delivery agents have been designed and synthesized over the last few decades but only a few have been approved in preclinical trials. As a result of clinical experiences and outcomes in the development of BNCT delivery agents, many researchers have described five golden principles for ideal BNCT agents. These are: (1) ^{10}B atoms/tumor cell are required more than 10^9 (2) boron tumor/normal tissue and high tumor/blood concentration ratios ≥ 3 (3) low intrinsic toxicity (4) clearance rate from normal tissue and blood is fast and tumor retention is enhanced (5) good with hydrophobic and hydrophilic balance [2-5]. Functional delivery agents containing functional end groups which are associated with tumor targeting moieties such as receptor, peptides, folic acid, amino acids, monoclonal antibodies (MAbs), lipids, nucleosides, carbohydrates, liposomes have become the most interesting compounds among synthesized new BNCT agents.

Recently, the conjugation of carborane to dendritic molecule has been investigated to fulfill these requirements. The synthesis of dendrimer and hyperbranched polymers of dendritic molecules is one of the most studied topics because these highly branched polymers have a large number of branching points and functional end groups that can be easily modified with functional groups [6-14]. The dendrimers have well-defined shape and size. Multi-step reactions and complicated purifying processes are used in the preparation of dendrimers [15-18]. In contrast, their structures of the hyperbranched polymers are not organized as properly as dendrimers'. They contain randomly branched and some linear structures. They were firstly synthesized by simple one-pot polymerizations. Later, a chain growth mechanism e.g self-condensing vinyl polymerization and self-condensing ring-opening polymerization were used in the preparation of hyperbranched polymers [7,19-22]. Reaction strategies in the preparation of hyperbranched polymer resemble polymerization reactions of classical lineer polymers rather than dendrimers. The production of dendrimers is very complex for use as engineering materials and large scale industrial purposes and also costly. On the other hand, the preparation of large quantities of hyperbranched polymers is much easier and cheaper than dendrimers but control cost of their structure and molar mass is high [22]. However, hyperbranched polymers are very attractive for industrial applications such as blend, resin and additive components and also, high-tech fields like nanomaterials in electric and electronic device, catalysis, biomedicine, etc. [22]. Terminal functional groups of hyperbranched polymers can be easily modified with other compounds to obtain new materials for specific applications [23]. These modifications bring in extra dissolution, thermal, elec-trochemical and luminescence properties of synthesized new materials.

Various hyperbranched polymers are obtained from different core and chain extender molecules. Hyperbranched polyester (HBP) based on 2,2 bis(hydroxymethyl)propanoic acid, which is one of the first synthesized hyperbranched polymers, has been commercially available over years [22,24]. Mishra et. al. [25] studied the modification of second generation aliphatic hyperbranched polyester polyol with isofo-ronediisocinate for coating film. Kutyreva et. al. [26] synthesized 2nd generation HBP polyol with succinic anhydride used as a chelating agent. Murillo and co-workers [27] synthesized hyperbranched alkyd resins from 4th generation HBP modified with tall oil fatty acids. Murillo and Mesias [28] also modified second generation HBP with polylactic acid to obtain a compatibilizer agent for plasticized tapioca starch/polylactic acid blends. Hyperbranched polyesters having end hydroxyl groups were combined with fatty acids or acrylate used as various resins in coating applications [6,29-31]. As most of the studies published in the literature have concentrate on the synthesis and characterization of aliphatic hyperbranched polyester for various applications, it can't be seen any literature study about modifying aliphatic hpyerbranced polyester with carborane as a biomaterial. But, many studies about use of dendrimers as boron carriers have been found in the literature. Firstly, Barth and coworkers [32] attached isocyanato polyhedral borane $[Na(CH_3)_3NB_{10}H_8NCO]$ to the outer shells of second- and fourth-generation poly(amidoamine) (PAMAM) dendrimers. In subsequent studies, dendrimers, aimed to attach it with the epidermal growth factor receptor, were designed by using boron compounds and PAMAM [33-35]. Carboranes modified to outermost groups of various dendrimers such as PAMAM [32], poly(propylene imine) [36], carbosilane [37], polylysine [38] and metallodendrimers [39] were investigated. On the other hand, these are not an ideal boron delivery agent because of their solubility and cytotoxicity. Newkome and co-workers reported that dendrimers functionalized with water-soluble carborane were synthesized [40]. Aqueous solubility was accomplished by using peripheral sulfate groups and thus, a unimolecular micelle-type structure was synthesized. Aliphatic polyester dendrimers synthesized from 2,2-bis(hydroxymethyl)propanoic acid [41] by Frechet and coworkers were biocompatible, nonimmunogenic, nontoxic and water-soluble as drug delivery agents [42-44]. Parrott et. al. studied the development of alike aliphatic polyester dendrimers for the incorporation of functionalized carborane compound [2]. In the study, the least amount of peripheral alcohol groups required per carborane was determined to evaluate the aqueous solubility of each of their synthesized structures for potential therapeutic applications of carborane-containing compounds. Carborane modified second generation dendron contain-

ing forty boron atoms from a 2,2 bis(hydroxymethyl) propanoic acid scaffold for BNCT were synthesized by Galie and coworkers. The outer shells of these dendrimers were designed to combine with targeting functionality which would be provided at the focal point or solubilizing groups [45]. Later, the tricarboranyl building block was also attached to 2,2-bis(hydroxymethyl) propionic acid with a succinimidyl linker and thus, a hexacarborane modified dendron containing 60 boron atoms was synthesized by Mollard and Zharov [46].

The terminal groups (such as peripheral alcohol) of dendrimers or hyperbranched polymers provide an attractive platform to modify these with other compounds for synthesizing new specific application materials like diagnostic and therapeutic modalities into tumors. Trifunctional theranostic agent which has diagnostic and therapeutic modalities was synthesized for targeting solid tumors by Dubey et.al. In this study, photophysical properties of dendritic molecule modified with carborane, a monomethine cyanine dye and an integrin ligand were evaluated. Thus, these dendritic molecule has been acquired modalities both diagnostic such as visible-light fluorescent imaging and therapeutic such as BNCT [47].

Studies about the synthetization of aliphatic hyperbranched polyesters containing carborane have not been enough although hyperbranched polymers are prepared much easier and cheaper than dendrimers [22]. Taking all of these factors into consideration, we described the synthesis and characterization of carborane-functionalized hyperbranched aliphatic polyester in this study. Firstly, aliphatic hyperbranched polyester was synthesized with dipentaerythritol as the core molecule that was twice esterifed with dimethylol propionic acid as the chain extender according to study of Bat et. al. [30]. Afterwards, this aliphatic hyperbranched polyester was modified with functionalized p-carborane. p-carborane was bifunctionalized with n-butyllithium and trimethylene oxide by three-step reaction taken into the consideration of previous study [2] in order to easily incorporation into the synthesized hyperbranched polyester. Finally, the peripheral alcohols of these synthesized hyperbranched were reacted with carboxylic acid of bifunctional p-carborane by esterification reaction to obtain carborane-functionalized hyperbranched polyester. All synthesized compounds were characterized by FTIR and NMR techniques.

Hydroxy functional aliphatic hyperbranched polyesters synthesized from a dimethylol propionic acid monomer and a dipentaerythritol core molecule have mostly been studied to be used as resin in coating applications [6,29-30] but not to evaluate as biomaterial carrier. The important part of our work is that the hydroxyl groups at the outer shells of aliphatic hyperbranched polyester were modified with functionalized p-carborane compound by esterification to be evaluated as a biomaterial. As a result of this study, carborane-containing hyperbranched polyester carrying many boron atoms and functional groups is successfully synthesized as a candidate BNCT agent.

2. Materials and Methods

2.1. Materials

Dipentaerythritol and dimethylol propionic acid (DMPA) (synomym: bis(hydroxymethyl)propanoic acid, bisMPA) were supplied by Aldrich. p-Carborane, (1,12-D-dicarbadodecaborane, $1,12-C_2B_{10}H_{12}$) (MA=144.23) was purchased by KATCHEM spol. s r.o., Czech Republic. Butyllithium solution (n-BuLi) (Aldrich) was used as 1.6°M solution in hexanes. N,N-Dimethylformamide (anhydrous-99.8%) (DMF), Ethyl acetate, TEMPO (2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical), Iodobenzene I,I-diacetate, pyridine, sodium hydride (NaH) and benzyl bromide were supported from Aldrich. Trimethylene oxide, magnesium sulphate anhydrous and metalic sodium were purchased from Fluka. 4-(Dimethylamino)pyridine (DMAP) and Tetrabutylammonium iodide, ≥99% were supplied by Sigma. p-toluene sulfonic acid and N-Ethyl-N-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) as a catalyst was supported from Aldrich-Sigma. All solvents and reagents were of analytical grade and used without purification.

Fourier transform infrared (FTIR-ATR) spectra were recorded using FTIR Nicolet 8700 spectrometer model in the 3700–600 cm^{-1} range. ^1H and ^{13}C NMR spectra were performed on a Bruker Avance (300 MHz) and Varian (400 MHz) spectrometer with Deutero dimethyl sulfoxide(DMSO-d_6) or Deutero chloroform (CDCl$_3$) as a solvent at 25°C.

2.2. Synthesis of Aliphatic Hyperbranched Polyester

Aliphatic hyperbranched polyester was synthesized from dipentaerythritol and DMPA according to the literature [30-31]. In this research, the aliphatic hyperbranched polymer was synthesized by a pseudo-one step polymerization. Dipentaerythritol as core molecule and dimethylol propionic acid as chain extender were used. Appropriate quantities of dipentaerythritol, dimethylol propionic acid and p-toluene sulfonic acid as a catalyst were placed in a five necked flask. The flask was placed in an oil bath preheated to 140°C. After all of materials were melted, the reaction was carried out under nitrogen atmosphere and the mechanical stirrer was started (Reaction condition: Mole of DMPA=50 mmol, Mole of Dipentaerythritol=5.55 mmol, m$_{catalyst}$=0.5 wt% of DMPA). After 2 h, the nitrogen gas stream was turned off, vacuum line was connected to the flask and vacuum was applied from time to time in an hour. After the pressure was increased to atmospheric, the required amount of DMPA (66.7
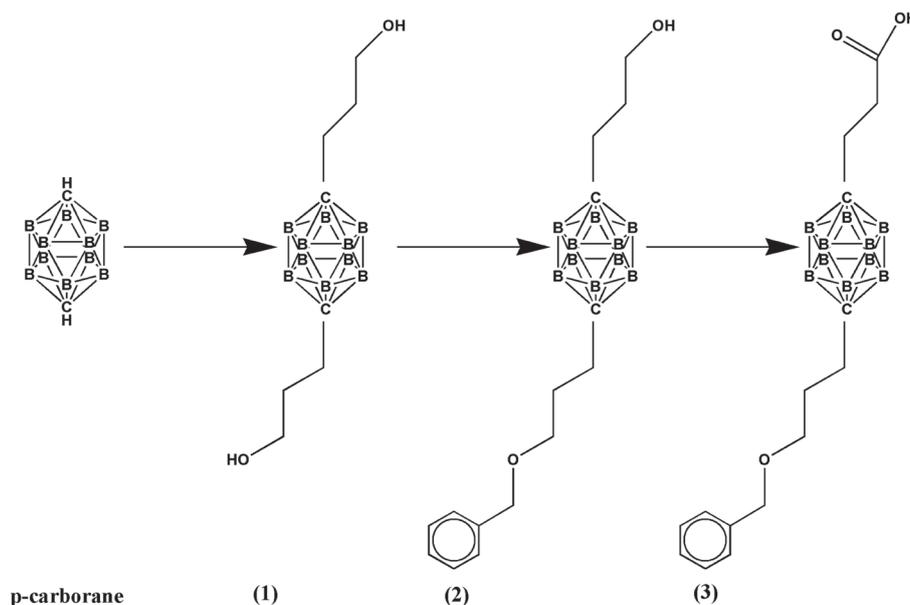
Figure 1. Synthesis of 2nd generation aliphatic HBP.

mmol) and p-TSA (44.8 mg) were added and the nitrogen gas flow was restarted for 2 h and vacuum was applied for an hour. Then prepared powder product was dried at 50°C under vacuum (Yield=83.75%). The reaction scheme of the aliphatic HBP synthesis is given in Figure 1. FTIR-ATR spectrum of aliphatic HBP, cm^{-1}: 3500-3200 (strong, broad) O-H stretching, 2960-2885 (weak, broad) C-H stretching vibration in CH_3 and CH_2, 1710 (very strong C=0 stretching vibration, 1459 (medium) C-H stretching vibration in CH_3, 1362 (medium) C-H deformation in CH_3 and 1227-1024 (medium) C-O-C stretching vibration.

2.3. Preparation of the Bifunctional Carborane

p-carborane was bifunctionalized as a carboxylic acid and a protected alcohol in order to incorporation of p-carborane cages with the peripheral alcohols of this synthesized aliphatic hyperbranched polyester. Then, the protected alcohols of carborane-functionalized hyperbranched polyester would subsequently be opened, and polyethylene glycol and targeting moieties should be attached to regeneration peripheral alcohol groups for use in BNCT.

The bifunctional p-carborane was prepared in three steps according to the literature procedures [2] (Figure 2).

2.3.1. 1st step: Synthesis of 1,12-bis(3-hydroxypropyl)-1,12-dicarbadodecaborane (compound 1):

Two carbon vertices of p-carborane were reacted with two equivalents of n-butyllithuim and trimethylene oxide to obtain long tails containing diol group. The reaction in THF (tetrahydrofurane) solution medium (50 mL) carried out in a well dried round bottom flask in a magnetic stirrer under argon atmosphere. The reaction mixture was stirred approximately 14 h at room temperature. THF was evaporated in vacuum at the end of reaction time and crude product was dissolved with 1M hydrochloric acid (HCl). The organic layer was separated, washed with 80 ml of acid and dried in vacuum. At the end of the 1st step procedure, product was obtained as white needles with 70% yield. ^{1}H-NMR (400 MHz, $CDCl_3$, 300 K): δ=1.3 and 1.4 (dd, 4H, CH_2-p-Carbor.), 1.6 and 1.7 (dd, 4H, CH_2CH$_2$OH), 1.9-3.2 (br., H, p-Carbor.) and 3.3 and 3.4 (s, 4H, CH_2OH) (Figure 3).

Figure 2. Synthesis steps of bifunctional p-carborane.

2.3.2. 2ⁿᵈstep: Synthesis of 1-(3-(benzyloxy) propyl)-12-(3-hydroxypropyl)-1,12-dicarbadodecaborane (compound 2):

One hydroxyl group of the compound 1 was protected using NAH, Tetra-butylammonium iodide and benzyl bromide according to the literature [2]. The protected alcohol of the resulting product would be opened to re-build peripheral alcohol functionalities or attach to targeting moieties for its intended purpose. In this step, the reaction solution was firstly cooled down to 0°C and stirred for 2 h. All necessary chemicals were added,

the mixture was kept until it reached room temperature and stirred for an additional 12 h. After all volatiles were removed by vacuum, the residue was taken up in 80 mL of diethyl ether and transferred into a separatory funnel. Then, 80 mL of HCl was rigorously added to dissolve crude product. After drying procedure, 1-(3-(benzyloxy)propyl)-12-(3-hydroxypropyl)-1,12-dicarbadodecaborane (compound 2) was obtained as white solid with 50-55% yield. ¹H-NMR (400 MHz, CDCl₃, 300 K): δ=1.2 and 1.3 (dd, 4H, CH_2-p-Carbor.), 1.6 and 1.7 (dd, 4H, CH_2CH₂O), 2.0-3.1 (br., H, p-Carbor.), 3.2 (d, 2H, CH_2OH), 3.4 (d, 2H, CH_2O), 4.3 (s,
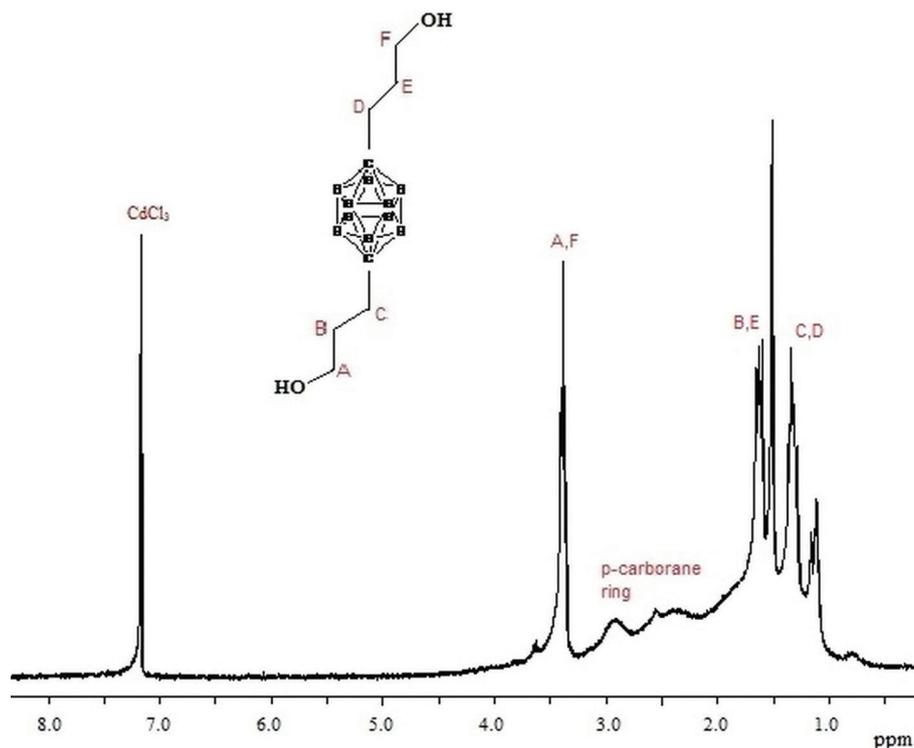
Figure 3. ¹H-NMR (400 MHz, CDCl₃, 300 K) spectra of 1,12-bis(3-hydroxypropyl)-1,12-dicarbadodecaborane.
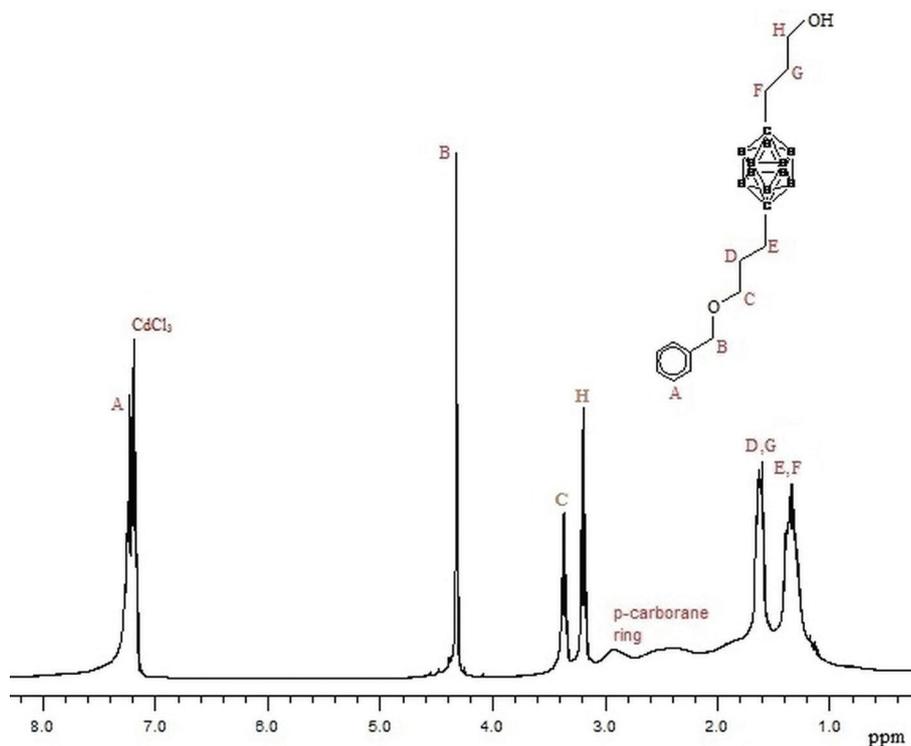
Figure 4. ¹H-NMR (400 MHz, CDCl₃, 300 K) spectrum of 1-(3-(benzyloxy)propyl)-12-(3-hydroxypropyl)-1,12-dicarbadodecaborane.

2H, OC*H₂*-Ph), 7.3 (m, 5H, Ph) (Figure 4).

2.3.3. 3ⁿᵈ step: Synthesis of 12-(benzyloxy)pro-pyl-1,12-dicarbadodecaboranyl-1-(3-propionic acid) (Compound 3):

The remaining free alcohol of compound 2 was react-ed with TEMPO and iodobenzene diacetate accord-ing to the steps in the literature [2]. At the end of this procedure, the product was crystallized from ethanol and p-carborane containing carboxyl group (Com-pound 3) was obtained as colorless crude product with 60% yield. ¹H-NMR (400 MHz, CDCl₃, 300 K): δ=0.8-2.3 (br., H, p-Carbor., one above the other), 1.29 (t, 2H, C*H₂*-p-Carbor.), 1.47-1.51 (dt, 4H, C*H₂*-p-Carbor

and C*H₂*COOH), 1.72-1.76 (q, 2H, C*H₂*CH₂-p-Carbor.), 3.30 (t, 2H, C*H₂*O), 4.43 (s, 2H, OC*H₂*-Ph), 7.30 (m, 5H, Ph), 9.62 (s, H, COO*H*) (Figure 5).

2.4. Synthesis of Carborane-functionalized Hyperbranched Polyester

The peripheral alcohols of the synthesized aliphatic hy-perbranched polyester were reacted with bifunctional p-carborane by esterification reaction to obtain carbo-rane-functionalized hyperbranched polyester. All syn-thesis was carried out under airless medium. In this re-action, a small excess of bifunctional p-carborane (the carborane acid) (1.25 equiv. per alcohol) was used to complete functionalization. Appropriate quantities of
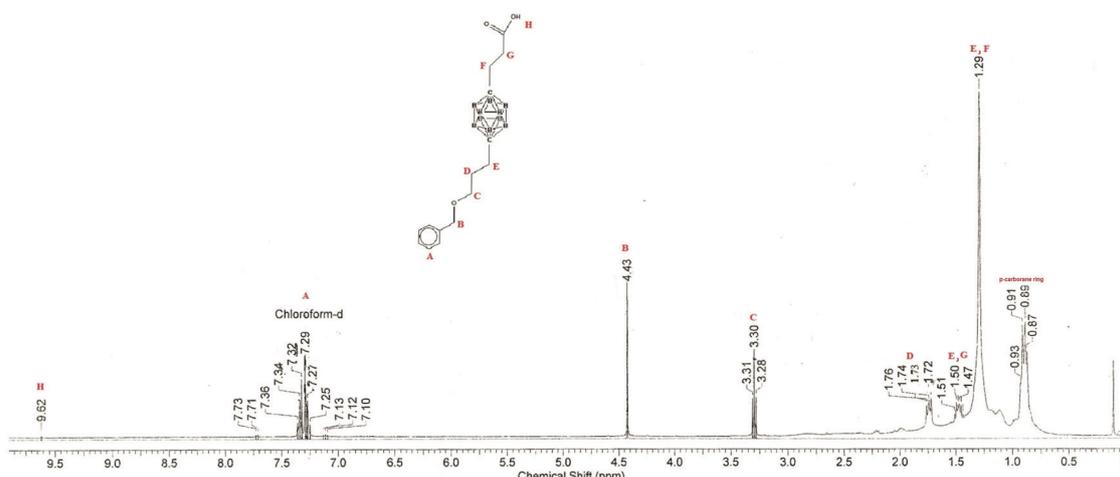

Figure 5. ¹H-NMR (400 MHz, CDCl₃, 300 K) spectrum of 12-(benzyloxy)propyl-1,12-dicarbadodecaboranyl-1-(3-propionic acid).

aliphatic HBP and compound 3 were placed in a flask and vacuum was applied to remove air and humidity. 4 mL of dichloromethane (CH_2Cl_2) and 0.4 mL of dried pyridine were added to this mixture, respectively. Solutions of EDAC and DMPA in CH_2Cl_2 were prepared into dried tapped bottom flask equipped. These solutions were added into reaction mixture drop by drop under argon atmosphere. Reaction solution was mixed for 40 hours. After all volatiles were removed by vacuum, residue colorless solid was washed by distilled water three times in order to provide neutral solid product and thus, pyridine was removed. 8 mL of cooled pentane was added to the residue and unreacted organic impurity was removed by filtration. The product was crystallized from hexanes at -30°C and the colorless needles crystal product was obtained with 60% yield. ^{1}H and ^{13}C NMR spectra of final product were performed with $CdCl_3$-d_1 as a solvent at 25°C.

3. Results and Discussion

Aliphatic hyperbranched polyester was synthesized according to esterification reaction with acid catalyst and by azeotropic distillation method with toluene in the literature [30,31]. The FTIR and ^{1}H NMR spectra of aliphatic HBP are given in Figure 6 and 7, respectively. In the FTIR spectrum of aliphatic HBP, the characteris-

tic ester bands (C=0 and C-O-C stretching vibrations) are observed at 1710 cm^{-1} and 1227-1024 cm^{-1}, respectively. Also, the broadest and the most intense OH stretching band is observed at 3350 cm^{-1} in Figure 6.

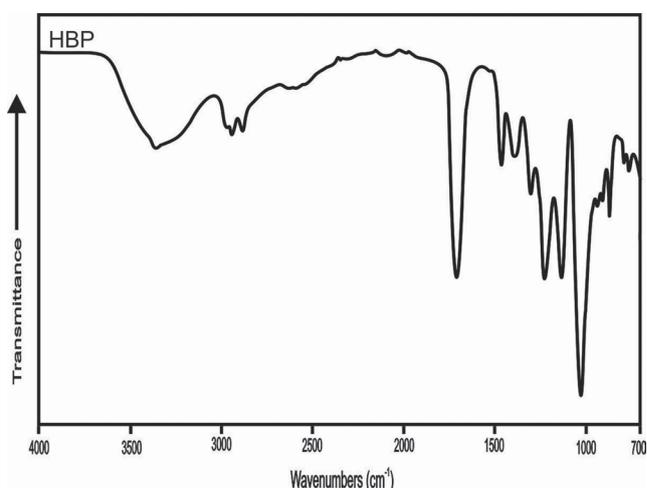**Figure 6.** FTIR spectrum of aliphatic hyperbranched polyester.

As it was seen from ^{1}H NMR spectrum of aliphatic HBP, observed characteristic peaks of CH_3 and CH_2 at 1.1-1.3 ppm and 3.4-4.3 ppm (a, b, c and d, e, f, respectively) show a clear indication of aliphatic hyperbranched polyester synthesis in Figure 7.
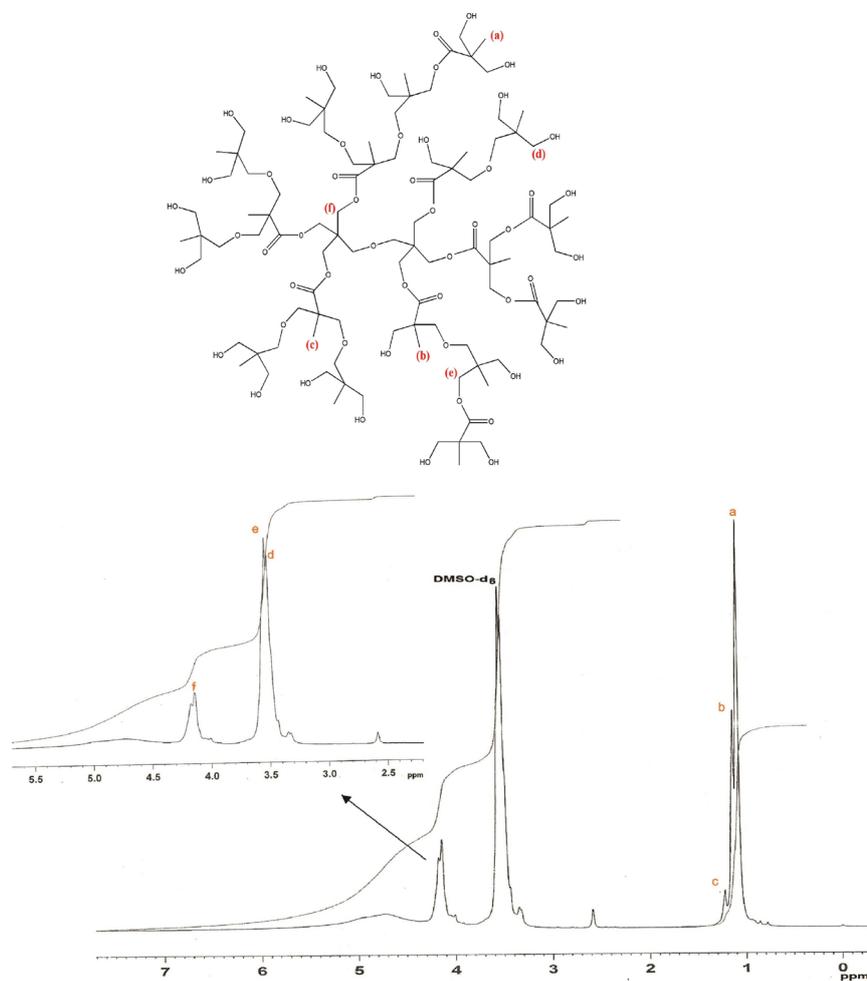

Figure 7. ^{1}H NMR (in DMSO-d$_6$) spectrum of aliphatic HBP.

The most important point in the modification of carbo-rane cages into the aliphatic hyperbranched polyester is the preparation of a bifunctionalized carborane containing a carboxylic acid and a protected alcohol. The bifunctional p-carborane was synthesized in three steps according to the literature procedures [2].

Amounts of used acids (HCl) and the crystallizati-on medium in the synthesis of compounds 1, 2 and 3 were slightly different from the literature [2]. At first, p-carborane was reacted with two equivalents of *n*-butyllithium and trimethylene oxide to produce the long tails compound 1 containing two -OH groups with 70% yield. Then, benzyl bromide was attached to one of two -OH groups in compound 1 by protonation un-der basic condition to obtain compound 2. Finally, the remaining free alcohol of compound 2 was oxidized with TEMPO and IBDA to yield compound 3 contai-ning carboxylic acid in 60% yield. The synthesis of 12-(benzyloxy)propyl-1,12-dicarbadodecaboranyl-1-(3-propionic acid) (bifunctionalized p-carborane, com-pound 3) is confirmed by the evaluation of ^{1}H NMR spectrum (Figure 5). The observed proton signals of phenly group at 7.29 and 7.73 ppm, -CH$_2$O- at 4.43 ppm, -OCH$_2$CH$_2$ at 3.31-3.28 and -CH$_2$ at 1.76-1.72 ppm are confirmed the formation of bifunctionalized p-carborane cages.

The peripheral alcohols of this aliphatic hyperbranched polyester were reacted with bifunctional p-carborane by esterification reaction under airless medium to synthesize carborane-functionalized hyperbranched polyester. The FTIR spectra of aliphatic HBP, bifunc-tionalized p-carborane and carborane-functionalized hyperbranched polyester are given in Figure 8. Com-parative analysis of FTIR of these compounds indi-cates that the broad band of OH group of aliphatic hyperbranched polyester at 3361 cm^{-1} disappearance in the spectrum of carborane-functionalized hyperb-ranched polyester. However, B-H stretching band of bifunctionalized p-carborane and C=O stretching in ester groups of aliphatic HBP are observed at 2597

cm^{-1} and 1739 cm^{-1}, respectively in the spectrum of carborane-functionalized hyperbranched polyester.
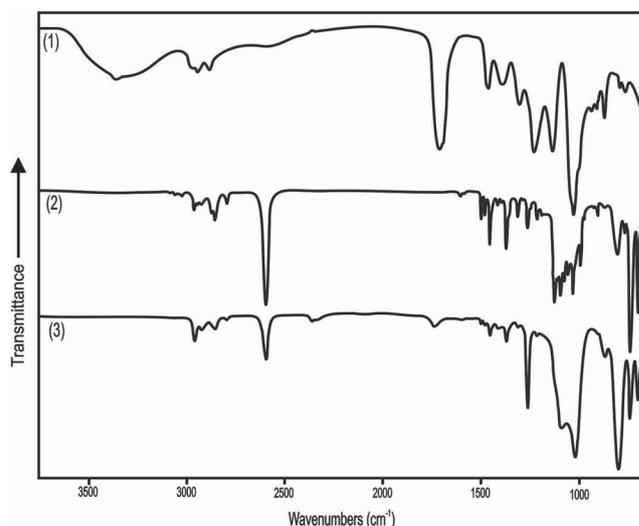
Figure 8. FTIR spectra of (1) aliphatic HBP (2) bifunctional p-car-borane and (3) carborane-functionalized hyperbranched polyester.

The synthesis of carborane-functionalized hyperbranc-hed polyester is confirmed by ^{13}C and ^{1}H NMR spectra (Figure 9 and 10). As it was seen from ^{13}C NMR spect-rum of carborane-functionalized hyperbranched pol-yester o-, m- and p-C atoms of phenyl group at 128.4 ppm and 127.6 ppm as overlapping and also ipso C atom at 138.2 ppm are observed in the aromatic area. The peaks of -O-CH$_2$ in bifunctionalized p-carborane group are observed at 72.8 ppm, 69.2 ppm and 61.9 ppm (C$_B$, C$_H$ and C$_C$), respectively. The five peaks bet-ween 34.5 ppm and 29.6 ppm indicate that 34.5 and 34.2 ppm for O-CH$_2$ (C$_H$ ve C$_B$), 32.5 ppm for carbon resonances in methyl groups (CH$_3$) and 29.7-29.6 ppm for CCH$_2$CH$_2$ group (C$_D$ ve C$_G$) are observed in the ^{13}C NMR spectrum of carborane-functionalized hyperb-ranched polyester. However, the peaks of carbon atom in the carborane cage and C-atom bound to branched oxygen atoms have not been exactly identified in ^{13}C NMR spectrum, carbon atoms of carbonyl are obser-ved as a weak peak at ~168.8 ppm.
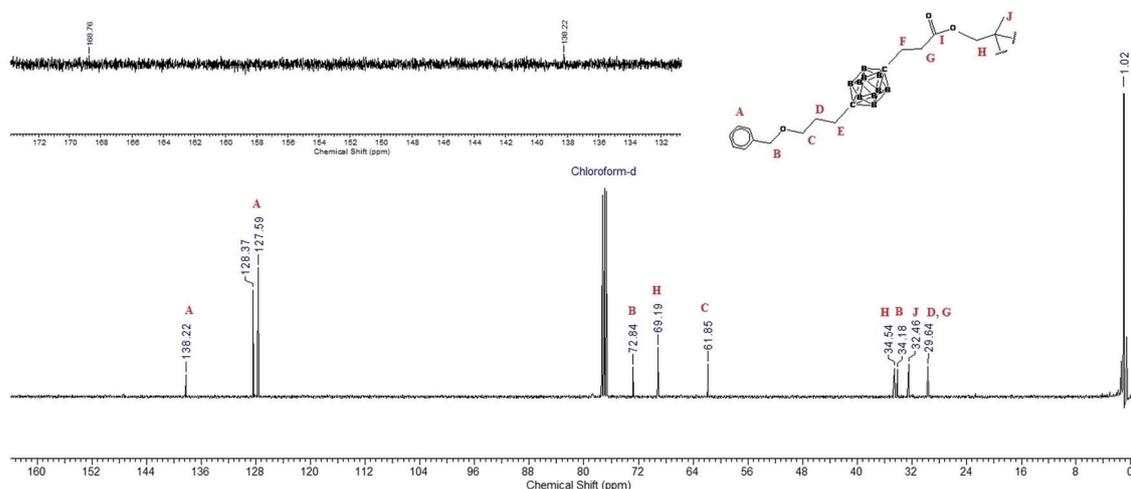
Figure 9. ^{13}C NMR (100 MHz, CDCl$_3$, 300 K) spectrum of carborane-functionalized hyperbranched polyester.
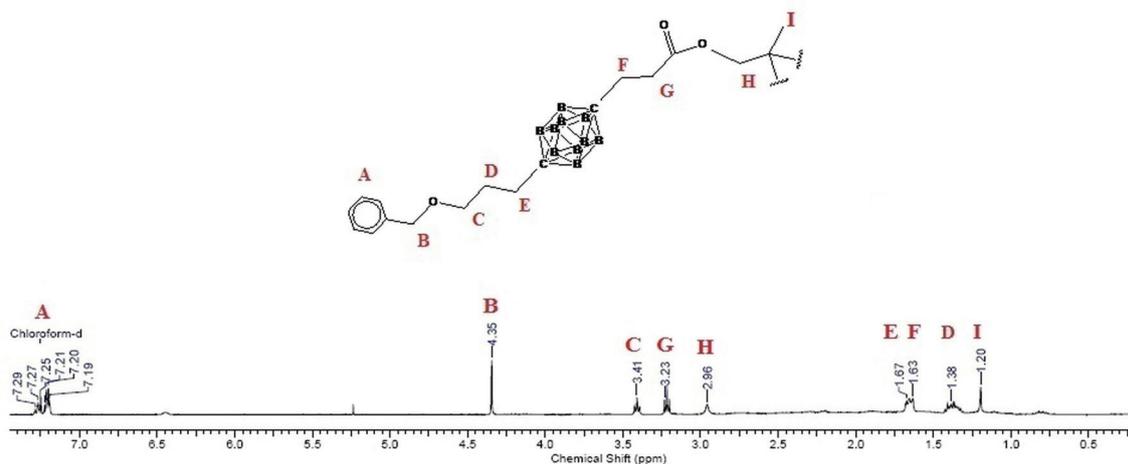
Figure 10. ¹H NMR (400 MHz, CDCl₃, 300 K) spectrum of carborane-functionalized hyperbranched polyester.

The ¹H NMR spectrum for carborane-functionalized hyperbranched polyester indicates all of the expected resonances that show the aliphatic hyperbranched polyester center, the carborane bond and outer-protecting groups (Figure 10). ¹H-NMR (400 MHz, CDCl₃, 300 K): δ=0.7-2.8 (br., H, Carbor., one above the other), 1.20 (s, 3H, CH_3, polyester), 1.38 (t, 2H, CH_2-CH_2-p-Carbor.), 1.63-1.67 (m, 4H, CH_2CH₂-p-Carbor), 2.96 (br., 2H, OCH_2), 3.23 (t, 2H, CH_2COO), 3.41 (t, 2H, CH₂O), 4.35 (s, 2H, OCH_2-Ph), 7.30 (m, 5H, Ph) respectively in Figure 10 (A, B, C, G, H, E, F, D and I, respectively in Figure 10).

The ¹H-NMR of carborane characteristically exhibits a broad signal between -0.75 and 3.00 ppm coming from the protons next to the boron atoms of the carborane cage. The observed broad B-H resonances related to carborane in this spectrum inhibit accurate integration of signals between 0.7 ppm and 2.8 ppm. However, the appearance of proton signals of phenly group at 7.29-7.25 ppm, -OCH₂-Ph at 4.35 ppm, -OCH₂ at 3.41 ppm, -CH₂CH₂ at 1.67-1.63 ppm and -CH₃ at 1.20 ppm is also confirmed the synthesis of carborane-functionalized hyperbranched polyester.

As a result of this study, the functionalization of the p-carborane compound and the synthesis of the aliphatic hyperbranched polyester structure were made according to the literature. The characterization results (FITR and NMR data) closely matched those in the literature [2,6,24,29]. A compound containing hydrophilic and multi-functional groups should be chosen as a carrier in order to use p-carborane as a biomaterial. There are many studies in the literature showing that the water solubility of carborane, which has a hydrophobic surface, is increased by modification of dendrimer structure [2,32,36-39,45-48]. These studies indicate that aliphatic hyperbranched polyester structures are synthesized more easily, cheaply and in large quantities than dendrimers. Also, aliphatic hyperbranced polyester provides good hydrophobic and hydrophilic balance, low viscosity, solubility etc. to structure which is modified [22-31]. For these reasons, aliphatic hyperbranched polyester was chosen as the carrier for p-carborane used as a biomaterial. The peripheral -OH groups of the synthesized aliphatic hyperbranched polyester were reacted with carboxylic acid group of bifunctionalized p-carborane by esterification reaction to get a water-soluble material containing many boron atoms. Synthesized carborane-functionalized hyperbranched polyester can be evaluated for use in many different areas (flame-retardant material, radiation shielding material, biosensor), especially biomedicine.

4. Conclusions

This work presents the synthesis and characterization of aliphatic hyperbranched polyester, bifunctionalized p-carborane and carborane-functionalized hyperbranched polyester. The synthesized water-soluble aliphatic hyperbranched polyester containing 24 hydroxyl groups at the outer of its periphery can be modified with different functional materials for different purposes. In this study, water-soluble aliphatic HBP was used as boron carrier agent. It is necessary to overcome steric hindrance of carborane compound in order to easily incorporation of p-carborane cages with peripheral alcohols of carrier agent. A space between the carborane cage and the acid functionality is formed by bifunctional process to reduce this hindrance. The peripheral alcohols of the synthesized aliphatic hyperbranched polyester were reacted with carboxylic acid group of bifunctionalized p-carborane, thus carborane-containing hyperbranched polyester carrying many boron atoms was synthesized to fulfill the required boron amounts for a successful BNCT application. Future studies will focus on determining the amount of boron in the carborane-functionalized hyperbranched polyester compound, deprotonation of this compound and the attachment of polyethylene glycol and targeting moieties such as folic acid for use in BNCT and the evaluation of its toxicity by cell culture studies. This study will contribute to the synthesis and use of end products containing boron compounds in our country.

Acknowledgements

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