Simple, High-Yield Synthesis of Polyhedral Carborane Amino Acids

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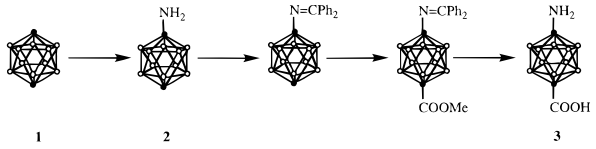
Boron neutron capture therapy (BNCT) is a form of binary cancer therapy that offers the potential of delivering spatially selective, high linear energy transfer radiation to the target cells while sparing surrounding normal tissue.[1] BNCT is based on the nuclear reaction that occurs when the stable isotope ^{10}B absorbs a thermal neutron to yield an α particle and a recoil ^{7}Li nucleus. These highly energetic particles have mean free paths of 9 and 5 μm, respectively, resulting in confinement of their kinetic energies to approximately one cell diameter. The neutrons used for activation of this process are of subionizing energy (<0.025 eV), and radiation damage to cells that do not contain ^{10}B is minimal. It has been estimated that a gross ^{10}B content of approximately 35 μg/g of cancerous tissue is sufficient to provide a therapeutic gain of about 4.[2]

The most formidable obstacle preventing the widespread use of BNCT has been the development of new boron-containing compounds that are truly selective for tumor cells. Despite the recent apparently successful treatments of several patients in the United States with glioblastoma multiforme and malignant melanoma, this goal remains essentially unattained as the boronated amino acid used in these early clinical trials, *p*-boronophenylalanine (BPA), is not highly tumor-selective. Typical tumor-to-blood and tumor-to-normal brain ratios in animals and humans are about 3:1.[3] Moreover, the single boron atom present in BPA produces a low weight percentage of boron in the molecule (∼5%) and very large doses must be administered in order to obtain therapeutically useful tumor boron concentrations. This is a particular disadvantage for BPA since it is poorly soluble at physiologic pH and is being administered as the fructose complex, further decreasing the weight percentage of boron. The ideal boron compound for BNCT would be water-soluble, nontoxic, and highly tumor-selective and would carry multiple boron atoms on each molecule. Hawthorne has recently published an excellent review on the role of boron chemistry in the development of agents for BNCT.[4]

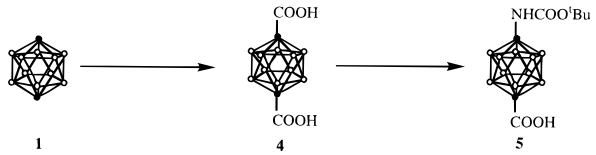
Heterobifunctional polyhedral carboranes appeared to us to be logical synthetic targets in our continuing investigations of compounds for BNCT. Carboranes bearing dissimilar polar functional groups on the two carbons of the icosahedral cage might be water-soluble and possible tumor localizers in themselves, but could be even more important synthons for attachment to known tumor seekers such as porphyrins.[5] Unfortunately, such compounds are exceedingly rare in the chemical literature and general methods for their preparation are nonexistent. Since peptides have long been recognized as an amazingly diverse class of biologically specific agents with numerous potential therapeutic applications, we decided to focus our synthetic work on carborane amino acids.[6]

Scheme 1[a]



[a] Reagents: (a) (i) *n*-BuLi; (ii) CH_3Li, CH_3ONH_2·HCl; (iii) H_2O; (b) benzophenone/PTSA; (c) (i) *n*-BuLi; (ii) $ClCOOCH_3$; (d) (i) aqueous KOH; (ii) Pd−C/H_2.

Scheme 2[a]



[a] Reagents: (a) (i) *n*-BuLi; (ii) CO_2; (iii) HCl; (b) $(PhO)_2PON_3$/TEA/*t*-BuOH/reflux.

Recent reports of the synthesis of rod-like molecules containing two to five *p*-carborane cages[7,8] led us to consider first the preparation of 1-amino-12-carboxy-*p*-carborane (**3**). The synthetic strategy is shown in Scheme 1. Initially our approach was to synthesize 1-amino-*p*-carborane (**2**) from the corresponding mono acid under Curtius conditions. The monoacid has reportedly been prepared by stepwise lithiation and carboxylation of *p*-carborane (**1**),[9] but in our hands this method consistently produced mixtures of the mono- and diacids. Application of the method of Michl *et al*. (lithiation under high-dilution conditions followed by treatment with methyl chloroformate and base cleavage of the ester)[8] gave the desired monoacid in 83% yield, but we were unable to convert this acid into the amine **2** using Curtius conditions. Direct amination of organolithium compounds has been reported using methoxyamine,[10] and this method was successfully applied to the synthesis of 1-amino-*p*-carborane **2**, albeit in low yield and accompanied by significant amounts of the diamine. Protection of the amine group as the diphenylimine followed by lithiation, treatment with methyl chloroformate, and stepwise deprotection gave the desired compound **3**. However, the overall yield of amino acid **3** was unacceptably low, and we turned our attention to other potential routes.

A far simpler route, as shown in Scheme 2, successfully avoided the complications imposed by attempts to monolithiate the carborane. *p*-Carborane (**1**) was dilithiated with *n*-butyllithium (2.4 equiv) and converted to 1,12-bis(hydroxycarbonyl)-*p*-carborane (**4**) in almost quantitative yield by reaction with carbon dioxide followed by acidification. Shiori and co-workers have reported a simple, one-step conversion of carboxylic acids to urethanes using 1 equiv each of the acid, diphenylphosphoryl azide (DPPA), and triethylamine in the presence of an alcoholic solvent.[11] When this modified Curtius reaction is carried out

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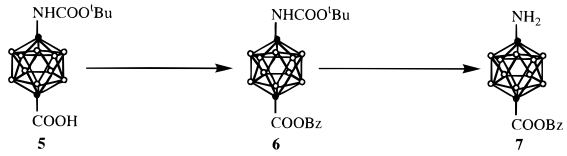
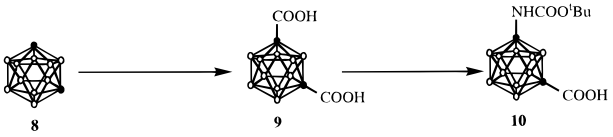
Scheme 3[a]



[a] Reagents: (a) Cs_2CO_3/$PhCH_2Br$/CH_3CN/reflux; (b) (i) TFA/CH_2Cl_2; (ii) TEA/CH_2Cl_2.

Scheme 4[a]



[a] Reagents: (a) (i) *n*-BuLi; (ii) CO_2; (iii) HCl; (b) $(PhO)_2PON_3$/DIEA/*t*-BuOH/reflux.

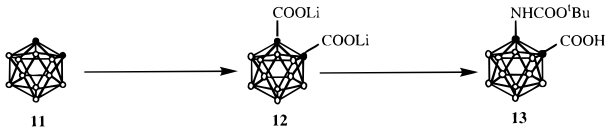
in *tert*-butyl alcohol, the intermediate isocyanate collapses to a Boc-protected amine group, giving a protected amino acid ideally suited for peptide synthesis. Thus the diacid **4** was reacted with DPPA/TEA and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in refluxing *tert*-butyl alcohol under high-dilution conditions to afford the N-protected amino acid **5** in an overall yield of 87% from *p*-carborane. If desired, the free amino acid **3** can be obtained easily by treatment of this compound with acid.

It is also possible to invert the functional group protection to expose the amine group as shown in Scheme 3. Treatment of N-protected amino acid **5** with benzyl bromide and cesium carbonate in refluxing acetonitrile protects the carboxyl group as its benzyl ester **6**. Acid-induced cleavage of the Boc carbamate followed by neutralization provides the C-protected amino acid **7**. In this manner, both the N- and C-protected amino acids become readily available for linkage to tumor-seeking functions or for peptide synthesis.

In order to test the generality of this method, it was applied to *m*- and *o*-carborane, **8** and **11**, respectively. The diacids of these compounds are readily prepared by treatment with excess butyllithium followed either by carbon dioxide or by methyl chloroformate and base. Yields for these reactions are generally in excess of 95%. Reaction of *m*-carborane dicarboxylic acid (**9**) with DPPA and diisopropylethylamine (DIEA) in the presence of a catalytic amount of DMAP in *tert*-butyl alcohol gave the desired amino acid as a mixture of the *tert*-butyl and phenyl carbamates. These products can be separated by silica gel chromatography, but pure Boc-protected amino acid **10** can be obtained by further modification of the reaction sequence, as shown in Scheme 4. The diacid **9** was reacted with 1 equiv of ethyl chloroformate in the presence of diisopropylethylamine in acetone at high dilution at room temperature. Further treatment *in situ* with aqueous sodium azide afforded the mono acyl azide. After removal of the acetone *in vacuo* at room temperature followed by acidification, the product was extracted with ethyl acetate−toluene (30:70). The extraction solution was refluxed in the presence of *tert*-butyl alcohol overnight to give the Boc-protected amino acid **10** in more than 80% yield from *m*-carborane (**8**).

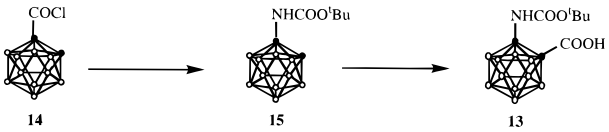
Application of the DPPA/TEA method to *o*-carboranedicarboxylic acid produced only the phenyl carbamate in quantitative yield. However, modification of the reaction sequence as shown in Scheme 5 gave the desired Boc-protected amino acid **13**.

Scheme 5[a]



[a] Reagents: (a) (i) *n*-BuLi; (ii) CO_2; (b) $(PhO)_2PON_3$/TEA/*t*-BuOH/PTC.

Scheme 6[a]



[a] Reagents: (a) (i) $(CH_3)_3SiN_3$; (ii) *t*-BuOH; (b) (i) *n*-BuLi; (ii) CO_2; (iii) HCl.

Treatment of *o*-carborane (**11**) with excess *n*-butyllithium gave the dilithio salt **12** which, instead of being converted into the diacid as with *m*-carborane, was treated with DPPA and diisopropylethylamine in the presence of a phase transfer catalyst in refluxing *tert*-butyl alcohol to afford the desired Boc-protected amino acid **13** in 54% yield.

However, the yields in this reaction varied widely, and another, more reproducible approach was sought. The reaction sequence shown in Scheme 6 proved useful. *o*-Carboranecarbonyl chloride **14**, easily prepared from the monoacid, was refluxed with trimethylsilyl azide followed by subsequent addition of *tert*-butyl alcohol to give quantitatively the Boc-protected amine **15**. Treatment of **15** with *n*-butyllithium followed by carbon dioxide reproducibly gave the desired amino acid **13** in 70−80% yield.

In summary, we have demonstrated a versatile, general method for the conversion of *o*-, *m*-, and *p*-carborane to their corresponding Boc-protected amino acids. Heterobifunctional polyhedral carboranes are exceedingly rare in the literature, and the amino acids prepared by this general method may prove to be valuable synthons for use in the synthesis of tumor-seeking compounds for BNCT or PDT. Moreover, these conformationally constrained amino acids should be particularly interesting for use in peptide synthesis. The dihedral angle between the carbon atoms of these polyhedra increases in the order 60° (ortho), 110° (meta), and 180° (para), allowing the peptide chemist to select a desired conformation. Efforts are currently underway in our laboratory to demonstrate both uses and will be reported at a later date.

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Supporting Information Available: Experimental details for the synthesis and characterization of **2**, **4**, **5**, **7**, **9**, **10**, **12**, **13**, and **15** (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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