Solution-Phase Segment Synthesis of Boron-Rich Peptides

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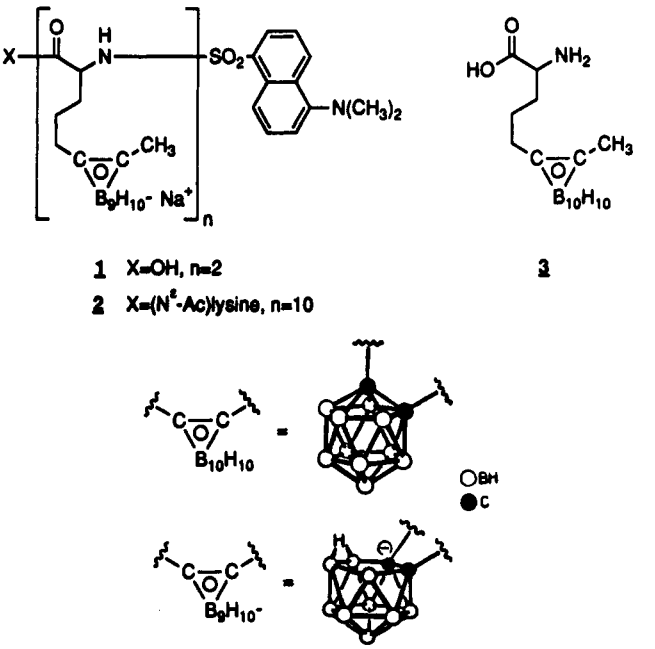
Summary: Small peptides containing up to 40 boron atoms were efficiently synthesized in solution.

The production of highly localized and cytotoxic radiation through thermal neutron capture by ^{10}B ($^{10}B(n,\alpha)^7Li$) is the basis for boron neutron capture therapy (BNCT).[1] Successful cancer therapy using this novel binary approach will require the selective accumulation of ~5–30 ppm ^{10}B in tumor.[2] A potential method for the delivery of boron to tumors is an antibody-based approach which requires each immunoprotein to deliver ~10^3 boron atoms in order to achieve the required ^{10}B concentrations. This requirement presents a chemical challenge which has led us to an interest in the precise chemical synthesis of hydrophilic ^{10}B-rich "trailer" molecules.[3] We recently described the synthesis of anionic *nido*-carborane[4] containing peptides **1** and **2** (derived from amino acid **3**, Figure 1), their attachment to monoclonal IgG antibodies (MAbs) against the CEA antigen, and the in vivo characterization of these immunoconjugates.[5] Because the synthesis of **1** and **2** under solid-phase conditions proved to be slow, we began to explore the synthesis of similar ^{10}B-rich peptides in solution using a "doubling" approach (segment synthesis), which is described herein.

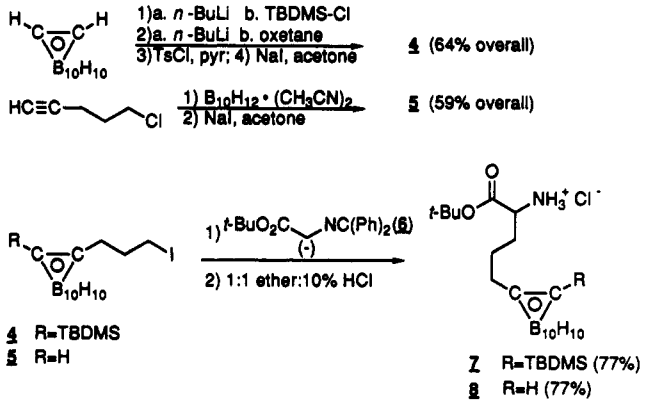


1 X=OH, n=2
2 X=(N$^{\epsilon}$-Ac)lysine, n=10
3

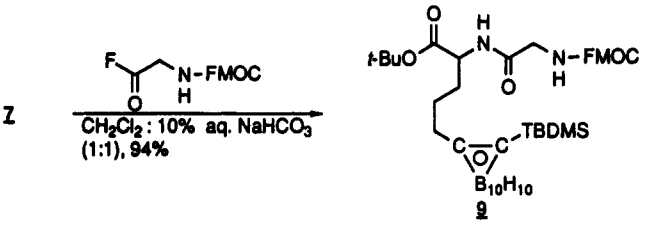
Figure 1.

We began by improving the synthesis of the *closo*-carborane amino acid monomer, as the synthesis of **3** required nine steps from propargyl bromide (~30% overall).[5a] The TBDMS-protected carboranylpropyl iodide **4** efficiently monoalkyl imine anion **6** under standard conditions, affording amino ester·HCl **7** after mild hydrolysis (Scheme I).[6] The silyl-protected iodide **4** was utilized because a previous report from our laboratories suggested that unprotected iodide **5** was unsuitable for imine–anion alkylation, presumably due to the relatively acidic carborane C–H bond.[5a] We were surprised to discover that the readily available unprotected iodide **5** efficiently alkylates imine anion **6**, affording amino ester **8** in good yield after mild hydrolysis (77% for two steps). Although the remainder of this paper describes the preparation of derivatives of protected amino ester **7**, unprotected analogue **8** is being used in our current work, as it is expected to exhibit very similar reactivity.

Scheme I



Scheme II



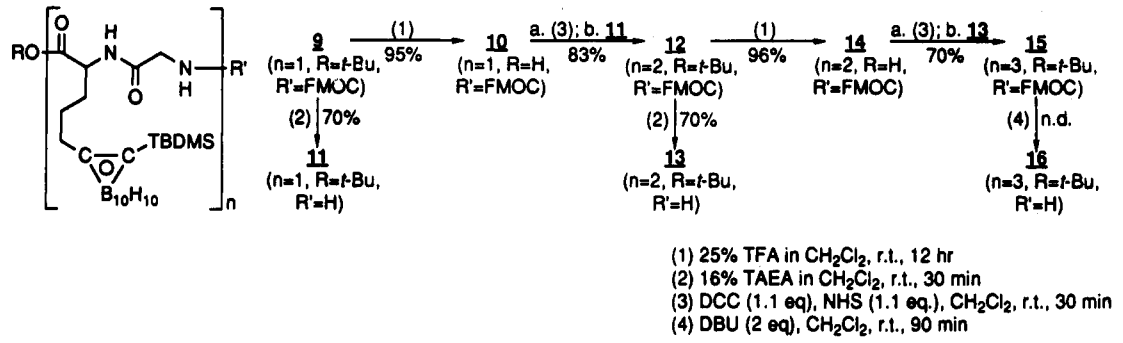
9

The *tert*-butyl ester in **7** is used as the carboxyl-terminus protecting group for segment peptide synthesis. Coupling of this amino ester salt with *N*-fluorenylmethoxycarbonyl (FMOC) glycyl fluoride under biphasic conditions[7] proceeds efficiently, affording the orthogonally protected dipeptide **9** in good yield (Scheme II). Glycine was incorporated into this peptide (and larger derived peptides) for a number of reasons. Glycine is an available source of a *N*-FMOC-protected amino acid, which allows the conversion of **7** to the *N*-FMOC-protected derivative while serving as a "spacer", reducing unfavorable steric inter-

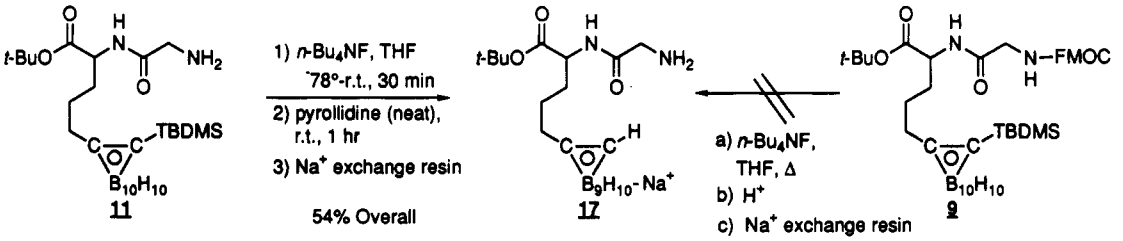
(1) Barth, R. F.; Soloway, A. H.; Fairchild, R. A. *Cancer Res.* **1990**, *50*, 1061–1070.
(2) Fairchild, R. G.; Bond, V. P. *Int. J. Radiat. Oncol. Biol. Phys.* **1985**, *11*, 831.
(3) Hawthorne, M. F. *Pure Appl. Chem.* **1991**, *24*, 327–334.
(4) Throughout this paper *closo*-carborane, *o*-carborane, or carboranyl refer to derivative of the *closo*-1,2-$C_2B_{10}H_{12}$ cage, while *nido*-carborane refers to derivatives of the [*nido*-7,8-$C_2B_9H_{11}$]$^-$ cage fragment.
(5) (a) Varadarajan, A.; Hawthorne, M. F. *Bioconjugate Chem.* **1991**, *2*(4), 242–253. (b) Paxton, R. J.; Beatty, B. G.; Varadarajan, A.; Hawthorne, M. F. *Bioconjugate Chem.* **1992**, *3*(3), 241–247.
(6) All new compounds reported in this communication have been appropriately characterized (HRMS, HPLC, multinuclear NMR, etc.). Yields are reported for material homogeneous by NMR, TLC, and/or HPLC.
(7) Carpino, L. A.; Mansour, E.-S. M. E.; Sadat-Aalee, D. *J. Am. Chem. Soc.* **1991**, *56*, 2611–2614.

0022-3263/93/1958-0991$04.00/0

Scheme III



Scheme IV



actions. Additionally, the incorporation of glycine demonstrates the viability of incorporating natural amino acids into boron-rich peptides. We are presently investigating the incorporation of hydrophilic amino acids (or amino acid derivatives) into carborane containing peptides.

Dipeptide **9** is equipped to participate in repetitive segment condensation by virtue of the orthogonal conditions available for removal of the carboxyl and amino protecting groups. The *tert*-butyl ester protecting group was selectively removed upon treatment of **9** with TFA, affording the *N*-FMOC amino acid **10** in 95% yield (Scheme III). The amine protecting group could also be selectively removed. Initially, treatment of the *N*-FMOC amino ester **9** with DBU[8] afforded the amino ester **11** in 61% yield. These conditions were chosen to avoid the conversion of the *closo*-carborane to its anionic *nido* derivative,[9] a potential side reaction of FMOC deprotection using 1° or 2° amines.[10] Since our discovery that FMOC removal is sufficiently rapid that 1° or 2° amines can be utilized without *nido*-carborane formation, we have begun to use tris(aminoethyl)amine (TAEA) as this reagent facilitates the removal of the dibenzylfulvene formed in the deprotection reaction (CH_2Cl_2, room temperature, 30 min, 70%).[11]

The coupling of acid **10** with amino ester **11** was efficiently accomplished using in situ activation (DCC/*N*-hydroxysuccinimide), producing tetrapeptide **12** in 83% yield. This demonstrates one cycle of "segment synthesis", effectively doubling the length of peptide **9** in three reactions. A second cycle of deprotection/coupling reactions again doubled the size of the peptide, affording octapeptide **15** in reasonable yield (~50%). In order to facilitate characterization the amino terminus of **15** was deprotected.

After demonstrating the solution-phase segment synthesis, attention was turned to the manipulation of the carborane cages. Removal of the TBDMS carborane protecting group of **11**, using 1 equiv of tetrabutylammonium fluoride in THF, was followed by conversion of the resulting *closo*-carborane-containing dipeptide to the anionic dipeptide **17** in 54% yield (Scheme IV). The general conversion of *closo*-carborane containing peptides to the corresponding anionic *nido*-derivatives had been described in a previous communication from our laboratories.[5a] An attempt to convert protected amino ester **9** directly to the *nido* amino **17** by extended treatment with tetrabutylammonium fluoride was less successful and resulted in the formation of a complex mixture of products.[12]

In conclusion, peptides derived from carborane-containing amino acids were rapidly and efficiently synthesized in solution. The segment condensation strategy employed is amenable to scale up and provides a means for the incorporation of alternating "natural" amino acids. Current work is concerned with the incorporation of hydrophilic amino acids (glutamic acid or phosphorylated serine, for example, rather than glycine) in order to increase the hydrophilicity of the derived peptides. These boron-rich compounds should prove to be useful whenever large homogeneous boron-rich compounds are needed, especially in immunoprotein-mediated BNCT.

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Supplementary Material Available: Full experimental details and compound characterization data (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(8) Kitas, E. A.; Wade, J. D.; Johns, R. B.; Perich, J. W.; Tregear, G. W. *J. Chem. Soc., Chem. Commun.* **1991**, 338–339.
(9) Wiesboeck, R. A.; Hawthorne, M. F. *J. Am. Chem. Soc.* **1964**, *86*, 1643–1644.
(10) Hawthorne, M. F.; Wegner, P. A.; Stafford, R. C. *Inorg. Chem.* **1965**, *4*, 1675–1678.
(11) Carpino, L. A.; Sadat-Aalee, D.; Beyermann, M. *J. Org. Chem.* **1990**, *55*(5), 1673–1675.
(12) TBAF was expected to remove the FMOC protecting group (Happ, E.; Scalfi-Happ, C.; Chládek, S. *J. Org. Chem.* **1987**, *52*(24), 5387–5391) and convert the *closo*-carborane to its *nido* derivative (Tomita, H., Luu, H.; Onak, T. *Inorg. Chem.* **1991**, *30*(4), 812–815). concurrently with removal of the silyl group.