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Synthesis of α-carboranyl-α-acyloxy-amides as potential BNCT agents

Subash C. Jonnalagadda [a,*], Jonathan S. Cruz [a,†], Ryan J. Connell [b,†], Patricia M. Scott [c], Venkatram R. Mereddy [b,*]

[a] Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ 08051, USA
[b] Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN 55812, USA
[c] Department of Biochemistry and Molecular Biology, University of Minnesota Duluth, Duluth, MN 55812, USA

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ABSTRACT

Novel α-carboranyl-α-acyloxy-amides were prepared as potential BNCT agents utilizing three-component Passerini reaction. Preliminary cytotoxicity of the representative compounds on two brain tumor cell lines (U-87 and A-172) showed no effect on cell viability; an essential requirement for utility as potential BNCT agents.

1. Introduction

Boron Neutron Capture Therapy (BNCT) is a binary treatment method in which, the cancer cells are loaded with[10] B atoms, followed by bombardment with low-energy neutrons. The resulting excited[11] B nuclei produce high-linear energy transfer species causing cell death. Since the range of these particles is one cell diameter, the neighboring healthy cells are usually spared of damage. The success of this modality depends on the preferential accumulation of boron atoms into the cancer cells. Hence it is very important to synthesize polyboronated molecules that could be selectively delivered to tumor site.[1]

Multicomponent coupling is an extremely important tool in organic and medicinal chemistry toward the synthesis of structurally diverse scaffolds of biological interest. The isocyanide-based Passerini and Ugi coupling reactions offer an easy access to a diverse range of peptidomimetic analogs under mild reaction conditions (Fig. 1).[2]

Low-density lipoprotein (LDL) contains about 1500 molecules of cholesterol esters per LDL particle and functions as a main carrier of cholesteryl esters in blood circulation. Several cancers such as malignant human *gliomas* overexpress LDL receptors and thus consume high levels of LDL-derived cholesteryl esters for the cell membrane biosynthesis via receptor-mediated endocytosis.[3] The evidence that rapidly dividing cancer cells have an elevated requirement for cholesterol can be observed by the 100-fold increase in cholesteryl ester concentration as well as by the increased LDL receptor-related apoprotein in the vicinity of the *glioma* cells.[3]

Liposomes have been extensively studied as carrier molecules due to their ability to deliver a wide range of substrates to tumor sites in a targeted way. The biphasic nature of liposomes facilitates the transportation of lipophilic and hydrophilic compounds readily. This procedure also offers advantages in terms of therapeutic efficacy at a lower dosage, minimal side effects and protection of the structural integrity in blood.[4] Currently there are several drugs in clinical usage which are being delivered to the cancer cells via liposomes.[5]

Carboranes and other polyhedral boranes have been extensively studied as BNCT agents.[6] Our continued interest on the functionalization of carboranes[7] prompted us to utilize the three-component Passerini reaction to synthesize α-carboranyl-α-acyloxy-amides as valuable intermediates for BNCT applications. Since the success of

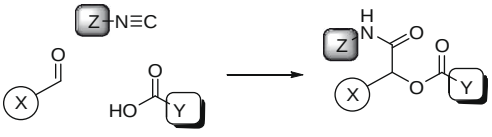

Figure 1. Passerini reaction template.

* Corresponding authors. Tel.: +1 856 526 5452; fax: +1 859 256 4478 (S.C.J.).
E-mail addresses: jonnalagadda@rowan.edu (S.C. Jonnalagadda), vmereddy@d.umn.edu (V.R. Mereddy).
† Undergraduate Research Participant, Departments of Chemistry and Biochemistry, Rowan University and University of Minnesota Duluth.

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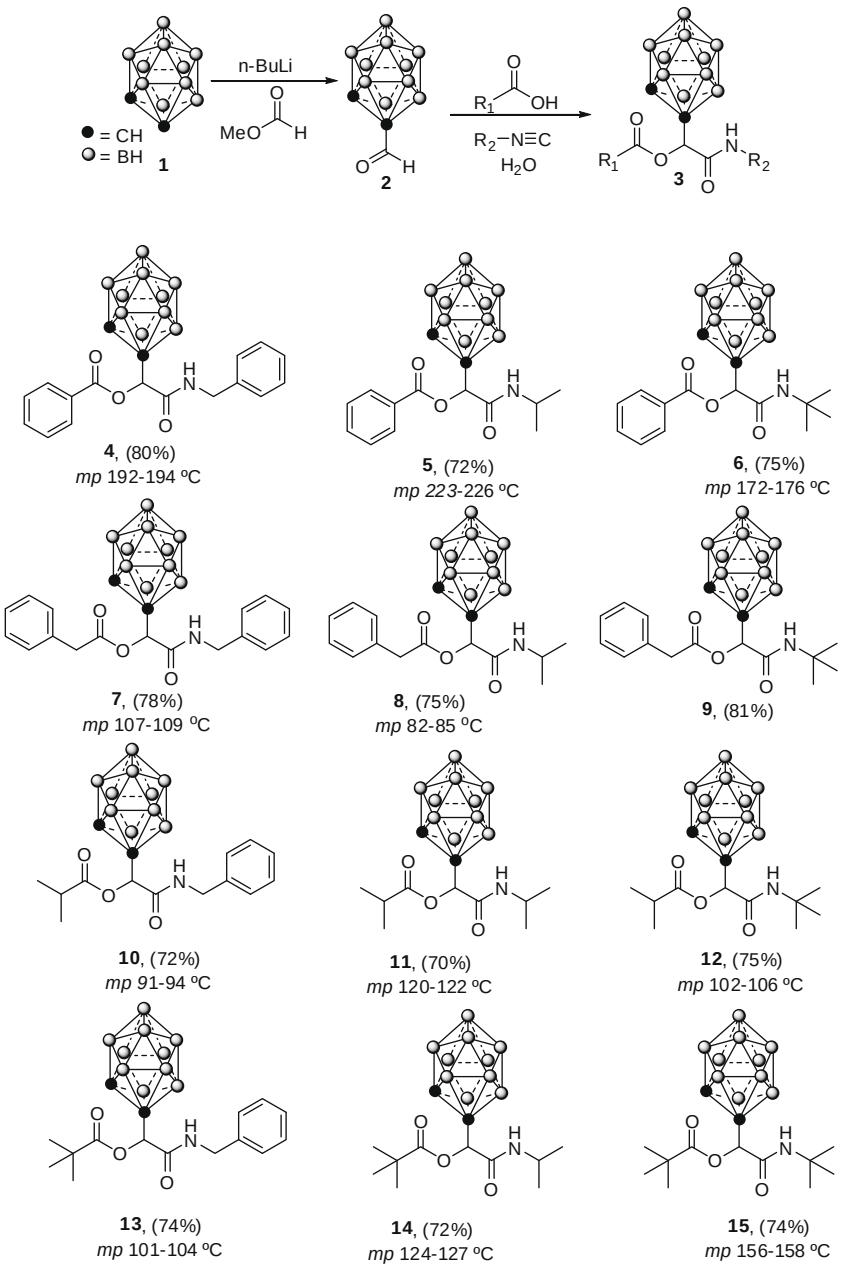
BNCT requires transporting boronated molecules in a targeted way into the cancer cells, we also synthesized lipophilic carboranes based on cholesterol and long-chain fatty acids as substrates for LDL reconstitution and liposomal encapsulation.

2. Results and discussion

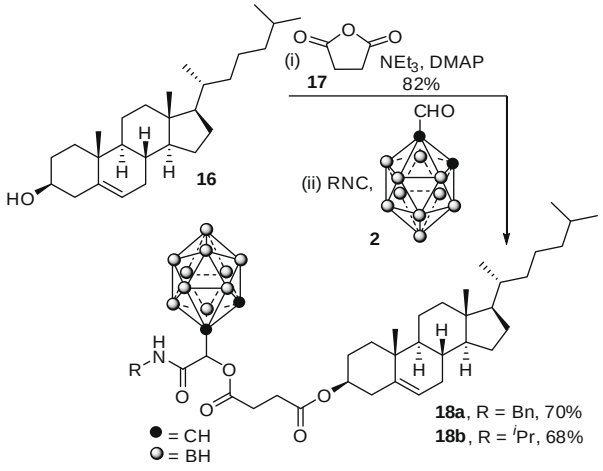
We initiated the synthesis of acyloxyamide-carborane conjugates via the formylation of *o*-carborane **1**. Lithiation of **1** with *n*-BuLi followed by the addition of methyl formate led to the formation of *o*-carborane aldehyde **2**.[8] Three-component Passerini reaction of the aldehyde **2** with benzoic acid and benzyl isonitrile in water provided *N*-benzyl-α-carboranyl-α-benzoyloxy-acetamide **4**.[9] The reaction mixture was worked up with ethyl acetate and the crude product was purified by trituration with hexane and diethyl ether. Similarly, the multicomponent reaction of *o*-carborane aldehyde with four carboxylic acids (benzoic acid, phenyl acetic acid, isobutyric acid, and pivalic acid) and three isonitriles (benzyl, isopropyl, and *t*-butyl isonitriles) afforded the carboranyl acyloxyamides **4–15** in good yields (Scheme 1).

After synthesizing the carborane conjugates **4–15**, we ventured into the synthesis of few biologically relevant carrier-linked carborane conjugates as potential substrates for LDL reconstitution, and liposomal encapsulation for targeted delivery to tumor sites. In this regard, we synthesized cholesterol and long-chain alkyl carborane conjugates using Passerini reaction. Succinic acid moiety was chosen as a linker because of its relative non-toxic nature and also to create structural mimics of the native cholesteryl esters.

The cholesterol carborane conjugates **18a–b** were prepared in two steps starting from cholesterol. Succinylation of cholesterol afforded the monosuccinate ester, which upon reaction with carborane aldehyde **2** and benzyl or isopropyl isonitrile provided the cholesterol carborane conjugates **18a**[10] and **18b**, respectively (Scheme 2).



Scheme 1. Preparation of α-carboranyl-α-acyloxy-amides.



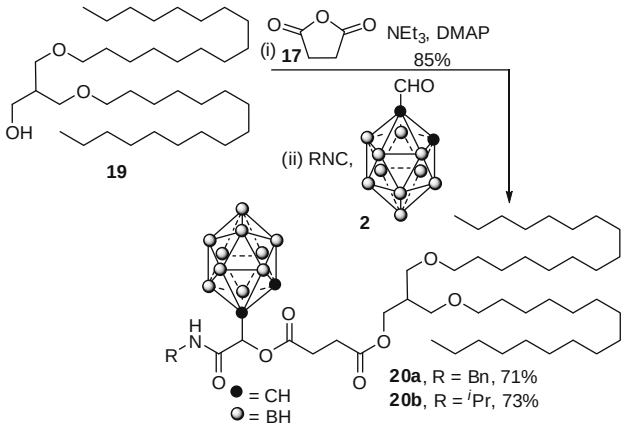
Scheme 2. Preparation of cholesterol carborane conjugates.

Similarly, the long-chain alkyl carborane conjugates **20a–b** were envisioned as the potential substrates for liposomal encapsulation. Thus the reaction of bishexadecyl-substituted alcohol **19** with succinic anhydride provided the monosuccinate ester which upon reaction with carborane aldehyde **2** and benzyl or isopropyl isonitrile provided the lipophilic carboranes **20a**[11] and **20b**, respectively (Scheme 3).

After synthesizing various carboranyl acyloxy amides, we carried out the cytotoxicity studies of the representative molecules **4–15**. Since the BNCT modality works better on localized cancers (such as brain tumors) than systemic treatment, we chose two human brain cancer cell lines A-172 and U-87 for the current studies. Cells were treated with compounds at a high concentration (50 μM), dissolved in DMSO for 18 h. Cell viability was determined using a colorimetric MTS assay. All the compounds tested were found to be non-toxic[12] to both the cancer cell lines thus fulfilling the primary criteria as potential BNCT agents. Future studies would include advanced biological studies especially involving LDL and liposomal encapsulation studies to determine the efficacy of these molecules as potential BNCT agents.

3. Conclusions

In conclusion, we have synthesized several α-carboranyl-α-acyloxy-amides as valuable intermediates for potential BNCT applications. We have also prepared cholesterol and bishexadecyl-oxyglyceryl carboranes as targeted molecules for LDL receptor and liposomal encapsulation. Some of these molecules were evaluated for cytotoxicity in two brain tumor cell lines, and were found to be non-cytotoxic even at high concentration (50 μM), thus fulfilling the basic requirement for utility as BNCT agents. The present work should be of interest to organic, inorganic, and medicinal chemists due to the flexibility of the multicomponent coupling reactions in providing wide array of carboranyl structural entities.



Scheme 3. Preparation of lipophilic carborane conjugates.

Acknowledgments

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9. *Preparation of N-benzyl-α-carboranyl-α-benzoyloxy-acetamide* **4:** To a stirred suspension of *o*-carborane aldehyde (0.34 g, 2.0 mmol), and benzoic acid (0.25 g, 2.1 mmol) in 2.0 mL water was added benzyl isonitrile (0.3 mL, 2.4 mmol), and it was stirred overnight. Upon completion (TLC), the reaction mixture was worked up with ethyl acetate and satd $NaHCO_3$. The combined organic layers were dried ($MgSO_4$), concentrated in vacuo, and triturated with hexane and ether to obtain 0.65 g (80% yield) of **4** as a white solid. Mp 192–194 °C ($C_{18}H_{25}B_{10}O_3N$ requires: C, 52.54; H, 6.12; N, 3.40. Found: C, 52.96; H, 6.22; N, 3.31); ^1H NMR (500 MHz, $CDCl_3$): 7.34–7.33 (m, 3H), 7.20–7.25 (m, 5H), 7.08–7.10 (m, 2H), 6.15 (t, *J* = 5.5 Hz, 1H), 5.60 (s, 1H), 4.25 (dd, *J* = 6.0, 15.0 Hz, 1H), 4.16 (dd, *J* = 5.5. 14.5 Hz, 1H), 4.15 (br s, 1H), 3.72 (s, 2H), 1.74–2.90 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): 168.5, 164.5, 136.6, 132.6, 129.4, 129.3, 129.2, 128.3, 128.2, 128.0, 71.8, 71.3, 59.2, 43.8, 41.3; ESI-MS: 401 [$(M-BH)^+$, 100%].
10. *Preparation of cholesteryl carborane conjugate* **18a:** Procedure similar to that of **4** (70% yield). Mp 98–100 °C ($C_{42}H_{69}B_{10}O_5N$ requires: C, 64.99; H, 8.96; N, 1.80. Found: C, 64.74; H, 8.99; N, 1.84); ^1H NMR (400 MHz, $CDCl_3$): ^1H NMR (500 MHz, $CDCl_3$): δ 7.44 (t, *J* = 7.3 Hz, 1H), 7.23–7.34 (m, 5H), 5.64 (s, 1H), 5.41 (dd, *J* = 2.5, 6.0 Hz, 1H), 4.28–4.54 (m, 4H), 2.61–2.74 (m, 4H), 2.19–2.31 (m, 3H), 1.96–2.04 (m, 3H), 1.74–2.90 (m, 10H), 1.66–1.90 (m, 4H), 1.03–1.62 (m, 18H), 1.00 (s, 3H), 0.92 (d, *J* = 8.0 Hz, 3H), 0.87 (d, *J* = 2.0 Hz, 3H), 0.85 (d, *J* = 2.0 Hz, 3H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 169.9, 164.7, 139.5, 137.2, 128.9, 128.1, 128.0, 123.2, 77.4, 75.6, 72.0, 71.0, 59.1, 57.0, 56.4, 50.4, 43.8, 42.6, 40.0, 39.8, 38.2, 37.1, 36.8, 36.4, 36.0, 32.2, 32.1, 29.6, 28.5, 28.2, 27.9, 24.5, 24.0, 23.0, 22.8, 21.3, 19.5, 19.0, 12.1; ESI-MS: 799 [$(M+Na)^+$, 100%].
11. *Preparation of long alkyl chain carborane conjugate* **20a**: Procedure similar to that of **4** (71% yield). Low-melting waxy solid ($C_{51}H_{97}B_{10}O_7N$ requires: C, 64.85; H, 10.35; N, 1.48. Found: C, 64.72; H, 10.44; N, 1.55); ^1H NMR (500 MHz, $CDCl_3$): ^1H NMR (500 MHz, $CDCl_3$): δ 7.33 (t, *J* = 7.0 Hz, 1H), 7.18–7.28 (m, 5H),

5.58 (s, 1H), 4.41 (dd, J = 7.0, 18.0 Hz, 1H), 4.40 (br s, 1H), 4.27 (dd, J = 7.0, 18.0 Hz, 1H),3.94 (d, J = 7.5 Hz, 2H), 3.24–3.36 (m, 8H), 2.54–2.70 (m, 4H), 2.05–2.11 (m, 1H), 1.74–2.90 (m, 10H), 1.43–1.50 (m, 4H), 1.13–1.23 (m, 52H), 0.75–0.85 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.4, 169.8, 164.7, 137.2, 128.9 (2C), 128.1 (2C), 127.9, 71.9, 71.7, 71.0, 68.8 (2C), 68.7 (2C), 64.6, 59.1, 43.8, 39.5, 32.2 (2C), 31.8, 29.9 (6C), 29.89 (4C), 29.88 (2C), 29.73 (2C), 29.6 (2C), 29.26 (2C), 29.22 (2C), 26.4 (2C), 22.9 (2C), 22.8 (2C), 14.3 (2C); ESI-MS: 967 [$(M+Na)^+$, 100%].

12. *Method for cytotoxicity experiments*: Cancer cells were grown in 5% CO_2 at 37 °C in DMEM containing 10% fetal bovine serum and 1% primocin. Cells were plated in 96-well plates at 2000 cells per well and were allowed to adhere for 18 h. Cells were then treated with each compound (50 μM) or with 0.3% DMSO alone for 18 h. The MTS tetrazolium salt assay was used for determining the number of remaining viable cells after exposure to compounds. Twenty microliters of MTS were added to 100-μl culture medium in each well. After incubation at 37 °C for three hours, absorbance at 490 nm was measured using an ELISA plate reader. MTS is converted to a formazan dye by the enzyme dehydrogenase found in the active cells. The quantity of formazan product measured by absorbance at 490 nm is directly proportional to the number of living cells in culture. Percent survival represents the ratio of viable cells remaining in compound-treated cells to viable cells remaining in DMSO-treated cells. As mentioned earlier, all the compounds that were tested under these conditions proved to be non-toxic at 50 μM.