**MOLECULAR MEDICINE: SYNTHESIS AND
IN VIVO DETECTION OF AGENTS FOR
USE IN NEUTRON CAPTURE THERAPY**

Progress Report

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I. OBJECTIVE

The primary objectives of the D.O.E. Program at the University of Tennessee Biomedical Imaging Center are the development of new boron-neutron-capture (BNCT) agents and the technology to detect boron compounds *in vivo*. The detection technology focuses on the development of effective magnetic resonance imaging (MRI) and spectroscopy (MRS) techniques for verifying and measuring BNCT agents *in vivo*. A significant portion of the research effort is directed toward the design of boron-containing neutron-capture-therapy agents. The U.T. - D.O.E. program is unique in that it has access to two state-of-the-art multinuclear magnetic resonance imaging units housed in the Biomedical Imaging Center at the University of Tennessee Graduate School of Medicine at Knoxville. In addition, the U.T. - D.O.E. researchers actively collaborate with colleagues at other D.O.E. facilities (Brookhaven National Laboratory and Oak Ridge National Laboratory.)

An important goal of the D.O.E. program at U.T. is to provide training for students (predoctoral and postdoctoral). The University of Tennessee is one of the very few institutions in the world where students have "hands-on" access to both modern scientific equipment and medical imaging modalities such as clinical MRI units. The academic nature of the program facilitates collaborative interactions with other D.O.E. programs and helps to insure the continued availability of skilled scientists dedicated to the advancement of diagnostic medical procedures.

II. RESEARCH

I am pleased to report that this has been a productive year for the D.O.E. program at the University of Tennessee. A book chapter resulted from the work and five scientific presentations were made; they are compiled in Section III.

A. SYNTHESIS OF POTENTIAL NEUTRON CAPTURE THERAPY AGENTS

1. Carboranyl Precursors

Carboranes contain ten boron atoms in a three-dimensional space equivalent to a benzene ring; consequently, the carborane isomers can be utilized to prepare a variety of boron-rich agents for potential use in boron-neutron-capture therapy.

[Where $\circ$ = BH, $\bullet$ = CH]

We are currently developing synthetic methodology suitable for preparing amino acids and other physiologically active compounds of potential use in BNCT. The research protocols and targeted molecules are outlined in the following subsections.

2. Synthesis of 5-[4-(2-Methyl)-1,2-dicarbo-closo-dodecaboranyl-but-1-ynyl]uridine (1).

Recently, several boron-containing nucleosides have been synthesized and evaluated for BNCT *in vitro*.¹ During the initial year of this study, we synthesized carboranyl

substituted uridine **1**. We felt that separating the lipophilic carboranyl group from the uridine using a linear alkyne should permit **1** to maintain a conformation similar to that of uridine. A retrosynthetic analysis for the efficient synthesis of **1** is given below.

The synthetic strategy for the synthesis of **1** involves the preparation of the carboranyl alkyne **2** and subsequent coupling of **2** with readily available 5-iodouridine (**3**). The bromide **5** required for the synthesis of **2** was prepared from 3-butyne-1-ol (**6**) in four steps.

- a) Dihydropyran, PTS, CH_2Cl_2 . b) $n\text{-BuLi}$, dimethylhexylsilyl chloride, THF, -78°C .
c) PTS, MeOH. d) CBr_4 , Ph_3P , CH_2Cl_2 , 4 h.

The alcohol **6** was converted to the tetrahydropyranyl ether **7** in 82% yield using dihydropyran in dichloromethane and a catalytic amount of *p*-toluenesulfonic acid. The alkynyllithium obtained from **7** using *n*-butyllithium in tetrahydrofuran at -78 °C was quenched with dimethylhexylsilyl chloride to form **8** in 90% yield. Removal of tetrahydropyranyl group with a catalytic quantity of *p*-toluenesulfonic acid in methanol gave the alcohol **9** in 92% yield. Alcohol **9** was converted to the bromide **5** in 74% yield using carbon tetrabromide and triphenylphosphine in dichloromethane.

Alkylation of methyl-*o*-carboranyllithium with the bromide **5**, according to the reported procedure,² was not successful. The enyne **10** was isolated as the main product.

Fortunately, the lithium methyl-*o*-carboranylcyano cuprate also failed to react with the bromide **5**. Lithium iodide mediated alkylation of methyl-*o*-carboranyllithium with the bromide **5** proceeded smoothly to yield the desired product, **11**, in 95% yield.

Methyl-*o*-carboranyllithium was alkylated with alkyl bromides in the presence of lithium iodide (0.2 equivalent) to form the alkylated carboranes under mild conditions.³ Bromide **5** required 1.2 equivalent of lithium iodide for the reaction to proceed. (There was no reaction with less than 1.0 equivalent of lithium iodide.) When the silyl protecting group in the bromide **5** was replaced by trimethylsilyl group, alkylation of the carboranyllithium was not successful.

Deprotection of **11** was achieved by adding tetrabutylammonium fluoride to **11** in tetrahydrofuran at -78°C and then raising the temperature to 0°C over a period of 2 hours to yield carboranyl alkyne **2** in 74% yield.

Carboranyl alkyne **2** (2 mmol) and 5-iodouridine (**3**) (1 mmol) were coupled using tetrakis(triphenylphosphine)palladium(0) (10 mol%), a cuprous iodide (20 mol%) and triethylamine (2 mmol) in dimethylformamide at room temperature for 24 hours to yield the desired product, **1**, in 64% (based on 5-iodouridine).

When tetrakis(triphenylphosphine)palladium(0) (5 mol%) and cuprous iodide (10 mol%) were used, the product **1** was isolated in 48% yield. Currently, carborane containing uridine derivatives with different chain lengths are being synthesized and will be evaluated for potential use in BNCT.

3. Synthesis of 3-[2-(2-Methyl)-1,2-dicarbo-*closo*-dodecaboranyl-1-ethyl]cyclobutanone 14.

The retrosynthetic analysis for the synthesis of a carborane substituted aminocyclobutane carboxylic acid is outlined below.

We synthesized cyclobutanone **13**, a key intermediate for the synthesis of aminocyclobutanecarboxylic acid **12**. Methyl-*o*-carboranyllithium was reacted with 4-bromo-1-butene in the presence of 0.2 equivalents of lithium iodide at 0 °C to prepare the alkylated product **14** in 94% yield.

Dichloroketene, generated from trichloroacetyl chloride in the presence of zinc-copper couple and phosphorous oxychloride,⁴ was reacted with the alkene **14** to prepare the dichlorocyclobutanone **15** in 75% yield.

Reduction of **15** with zinc dust in 80% aqueous acetic acid at 45 °C yielded the required cyclobutanone **13** in 94% yield.

At present, aminocyclobutancarboxylic acid **12** is being synthesized from **13**. Further efforts will be directed to synthesise carborane containing aminocyclobutanecarboxylic acids with different chain lengths and will be evaluated for potential use in BNCT.

B. NUCLEAR MAGNETIC RESONANCE IMAGING

We pioneered the application of MRI and MRS to the detection and quantitation of BNCT agents *in vivo*.^{5,6} In the present study, we used a 3D gradient-echo pulse sequence (Figure 1) with a Time to Echo (TE) of 750 μ seconds and a Repetition Time (TR) of 0.9 second for data collection. A 64 X 32 X 32 acquisition matrix was used and the data processed to a 32 X 32 X 32 matrix, With a 75 mm field of view (FOV) the effective volume element was 2.3 mm. This methodology allowed us to view the BSSB in all three planes, which has not been possible using other methods.⁷

Our initial studies were carried out using the 0.1 M solution of BSSB in 0.9% Saline. The images of the 0.1 M BSSB phantom (in all three planes) are depicted in Figure 2a. This data set was acquired in less than 5 minutes (10 scans) and gave a signal to noise (S/N) ratio of 112:1. To approximate the conditions and concentrations of BSSB found in the body another phantom was prepared with 2% agarose in 0.9% saline and 80 μ g of ^{11}B . The resultant 3D ^{11}B image is shown in Figure 2b. The image was acquired in 55 minutes (150 scans) and gave a S/N ratio 27:1.

The *in vitro* boron-11 MRI and MRS study of the boronated porphyrin 2,4-bis-vinyl-*o*-nidocarboranyl-deuteroporphyrin (VCDP) in a female BALB/c tumor-bearing white mouse was carried out. The mouse was dosed with VCDP via intraperitoneal (ip) injection for a total dose of 275 μ g/gbw. The mouse was sacrificed and examined 18 hours after dosing, the tumor was found to have 180 ppm of VCDP via necroscopic examination.

For the MRI study a 64 X 32 X 32 acquisition matrix was used and the data processed to a 64 X 64 X 64 matrix. With a 75 mm field of view (FOV) this gave an effective volume

Figure 1. The modified gradient echo pulse sequence used for the acquisition of the Boron-11 imaging data.

element of 1.2 mm, with a S/N ratio of 7:1. The reconstructed image data set is shown in Figure 3a-c. The corresponding proton image of the studied mouse is shown in Figure 3d.

The MRS study produced and the spectrum of the 80 ppm BSSB phantom which is presented in Figure 4. The spectrum was acquired in 20 minutes and the two borons attached to sulfur are resolved from the rest of the carborane. A S/N ratio of 36:1 was achieved. The MRS study of the examined mouse is shown in Figure 5. The spectrum was acquired with the same acquisition parameters as the phantom and gave a S/N ratio of 9:1

These preliminary results clearly demonstrate the potential of NMR microscopy for the development of clinical BNCT MRI protocols. The use of high field NMR spectrometers and stronger gradients permit greater resolution and sensitivity than is possible with a low field clinical imaging unit. High field spectrometers also provide an alternative for the development of new protocols minimizing the time required for research and development on clinical instruments.

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Figure 2. The three different planes of two Boron-11 data sets are shown. The images represent A) a 0.1 M BSSB solution in saline and B) an 80 ppm BSSB phantom in agarose. The above images were acquired using a modified gradient-echo pulse sequence with an effective time to echo of 750 μ sec and

Figure 3. a-c: The 3 deminsional boron-11 image of a BALB/c white mouse dosed with VCDP and sacrificed 18 hours after infusion. Figure 3a clearly shows liver and kidney clearly resolved. Figure 3d shows the corresponding proton image. The S/N ratio for the Boron-11 image was 7:1.

Figure 4. The boron-11 spectrum of an 80 ppm BSSB phantom made from 0.9% normal saline augar, which gave a S/N ratio of 22:1 after 10,000 scans in eighteen minutes.

Figure 5. The boron-11 spectrum of a white mouse dosed with VCDP and sacrificed 18 hours after infusion. Ten thousand scans in eighteen minutes gave a S/N ratio 9:1.

III. BIBLIOGRAPHY OF PUBLICATIONS

A. PUBLICATIONS

1. Kabalka, G. W.; Cheng, -Q. C.; Anderson, C. L.; Bendel, P.; Micca, P.; Slatkin, D. N. Proceedings of The Fifth International Symposium on BNCT., "*In Vivo* Pharmacokinetics of a Boron Neutron Capture Agent in a Tumor-Bearing Rat Via Boron-11 MRS and MRI", *Plenum Press* NY, in press.

B. PRESENTATIONS

1. "Synthesis of 5(4'-Carboranyl-1'-Butynyl)Uridine", G. W. Kabalka, N. K. Reddy, and C. Narayana, Eighth International Meeting on Boron Chemistry, Knoxville, Tennessee (July 1993).
2. "The Boron-11 MRS and MRI Study of the Boron Neutron Capture Agent BSSB at 9.4 Tesla: A New Application of NMR Microscopy", G. W. Kabalka, C. L. Anderson, P. Bendel, and G. -Q. Cheng, Eighth International Meeting on Boron Chemistry, Knoxville, Tennessee (July 1993).
3. "The Boron-11 MRS and MRI Studies of the Boron Neutron Captures Agents BSSB and VCDP at 9.4 Tesla. A New Application of NMR Microscopy", G. W. Kabalka, C. L. Anderson, P. Bendel, and G. -Q. Cheng, American Chemical Society Annual Meeting, Nucl 156, Chicago, Illinois (August 1993).
4. "Boron MRI for Use in BNCT", G. W. Kabalka, NIH Workshop on Biological Evaluation of Compounds for BNCT:, Washington, D.C. (September 1993).
5. "Diastereoselective Synthesis of Amino Alcohols Via Crotylboration of α -Amino Carbonyl Compounds", G. W. Kabalka, and R. David Pace, Southeast Regional American Chemical Society Annual Meeting, John City, Tennessee (October 1993).

IV. GRADUATE AND POSTDOCTORAL STUDENTS

Two postdoctoral students and one graduate student were supported during this period by the Department of Energy Nuclear Medicine Program.

A. Postdoctoral Students

Dr. Kesavulu N. Reddy
Dr. Charles L. Anderson

B. Graduate Students

George Hondrogiannis (Ph.D. Candidate)

*Budget removed.
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