2018년 8월
석사학위 논문

Synthesis, Characterization and Biological Evaluation of Boronated Ionic Liquids for Potential BNCT Agents

조 선 대 학 교 대 학 원

화 학 과

박 샘 이

Synthesis, Characterization and Biological Evaluation of Boronated Ionic Liquids for Potential BNCT Agents

2018년 8월 24일

조 선 대 학 교 대 학 원

화 학 과

박 샘 이

Synthesis, Characterization and Biological Evaluation of Boronated Ionic Liquids for Potential BNCT Agents

지도교수 이 종 대

이 논문을 이학석사학위신청 논문으로 제출함.

2018년 8월

조 선 대 학 교 대 학 원

화 학 과

박 샘 이

박샘이의 석사학위논문을 인준함

위원장 조선대학교 교수 고 문 주 (인)

위 원 조선대학교 교수 김 호 중 (인)

위 원 조선대학교 교수 이 종 대 (인)

2018년 8월

조 선 대 학 교 대 학 원

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

초록

BNCT를 위한 붕소 화합물의 합성, 특성, 생물학적 평가

박샘이
지도교수 : 이종대 교수님
화학과
조선대학교

최근 homogeneous 와 biphasic 과정에서 유기용매에 대한 친환경적인 훌륭한 대안으로 상온이온성액체에 대한 관심이 높아지고 있습니다. 상온이온성액체는 알킬 사슬 길이에 크게 영향을 받는 항균활성을 나타내는 것으로 알려졌다. 이번 연구에서, imidazolium과 *N*,*N*-dimethylaminopyridinium에 기반을 둔 이온성 액체를 다양한 보론음이온 (BF_4^-, BOB^-, $BMLB^-$, and dicarbollide와 같은)과 합성하였고, FT-IR, ^1H, ^{11}B, and ^{13}C NMR을 통해 구조를 확인했다. 30μM와 다른 농도에서 이들의 생물학적평가를 진행하였다. 생체 외 연구는 붕소 이온성액체 유도체는 dicarbollide > $BMLB^-$ ≃ BOB^- > BF_4^- 와 같은 순으로 독성이 나타났다. 다양한 이온성액체 중, 1-butyl-3-methylimidazolium tetrafluoroborate $[C_4MIM]BF_4$ 이 다른 이온성액체에 비해 가장 낮은 독성을 보였다.

Abstract

Synthesis, Characterization and Biological Evaluation of Boronated Ionic Liquids for Potential BNCT Agents

Saem-I Park
Advisor : Prof. Lee Jong Dae, Ph.D,
Department of Chemistry,
Graduate School of Chosun University

Room temperature ionic liquids (RTILs), a class of neoteric solvent have attracted increasing interest over recent years as environmentally benign excellent alternatives to organic solvents in homogeneous and biphasic processes. RTILs are also found to display anti-microbial activities being greatly affected by alkyl chain lengths. In the present investigation, certain imidazolium and *N*,*N*-dimethylaminopyridinium based ionic liquids with various boronated anions (such as BF_4^-, BOB^-, $BMLB^-$, and dicarbollide) were synthesized and the structures were confirmed by elemental and spectral studies such as FT-IR, ^1H, ^{11}B, and ^{13}C NMR and they were screened for their biological evaluation against 30μM at different concentrations. In vitro studies of boronated ionic liquids derivatives could be summarized as decreasing in the order dicarbollide > $BMLB^-$ ≃ BOB^- > BF_4^-. Among the various ionic liquids, 1-butyl-3-methylimidazolium tetrafluoroborate $[C_4MIM]BF_4$ was found to be less toxic than others.

1. Introduction

The most well-known radiation types applied to radiotherapy are gamma ray (γ) and X-ray. They kill not only cancer cells but also normal cells. As appearance of gamma knife, conformal radiation therapy and intensity modulated radiation therapy (IMRT),[1] radiation dose can be increased to a higher level to the patients at the same time as minimizing healthy tissue destruction, such as the treatment for nasopharyngeal carcinoma (nose & throat cancer) and mastocarcinoma (breast cancer). However, at present, many cancers such as malignant brain tumors can not be treated effectively by conventional therapy (surgery, chemotherapy and radiotherapy). Boron neutron capture therapy (BNCT) has been born to aim at these tricky cancers.

Chadwick who was from the Cavendish Lab of the University of Cambridge, United Kingdom discovered the neutron in 1932. Three years later, in 1935, Taylor, Burcham and Chadwick showed that boron-10 (^{10}B) has the ability to capture slow neutron to release lithium-7, alpha particles and gamma rays. That is called the boron-10 neutron capture reaction. A year after, in 1936, the first person who proposed that this reaction can be utilised to treat cancer was Locher.[2] Boron neutron capture therapy (BNCT) is an ideal treatment to kill cancer cells selectively without harming healthy cells nearby. It is a targeted chemo-radiotherapy which utilizes boron-10 that is attached to a suitable tumor-seeking drug [Stable isotope boron-10 is used because of its high neutron capture cross-section (approx 4000 barns) which means that it is capable to capture slow neutron easily].[3] First of all, a boron-10 carrying drug is injected into the blood. Then, a tumor accumulates the drug through the blood transportation system. Thereafter, the tumor is irradiated by a thermal neutron ($E \approx 0.025$ eV) or an epithermal neutron source.[3] Finally, the boron-10 atoms inside the tumor capture the neutrons to produce highly energetic Helium-4 (^{4}He) nuclei [*i.e.*, alpha (α) particles] and recoiling Lithium-7 (^{7}Li) ions to kill the tumor cells. Figure 1 illustrates the sequence of nuclear events.

The range of the two particles - ^{4}He and ^{7}Li within a tumor cell are 5 − 9 μm (approx the diameter of a tumor cell) respectively. They each have high linear energy transfer (LET) values as well. Thus, all high energy is released in a tumor cell. As a result, the reactions bring the tumor cells high lethal probability, while normal cells outside the tumor survive.

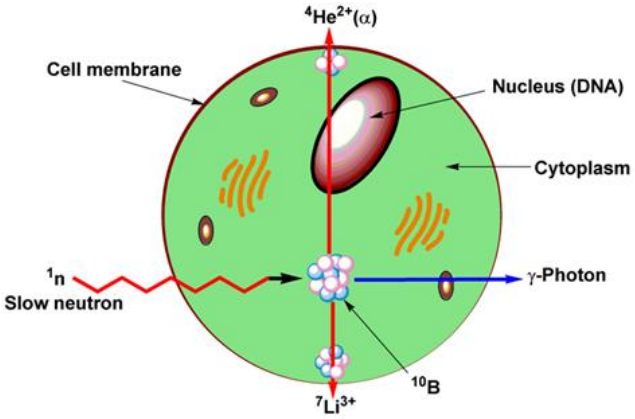


Figure 1. Schematic of B-10 with neutron interaction.

BNCT has been experimentally tested to treat malignant brain tumors called glioblasoma multiforme (GBM) and other tumors. There are a number of reports of successful trial examples, but these have not shown to be outstanding to other conventional therapies yet. Thus, as shown in Figure 2, BNCT is still at trial stage.

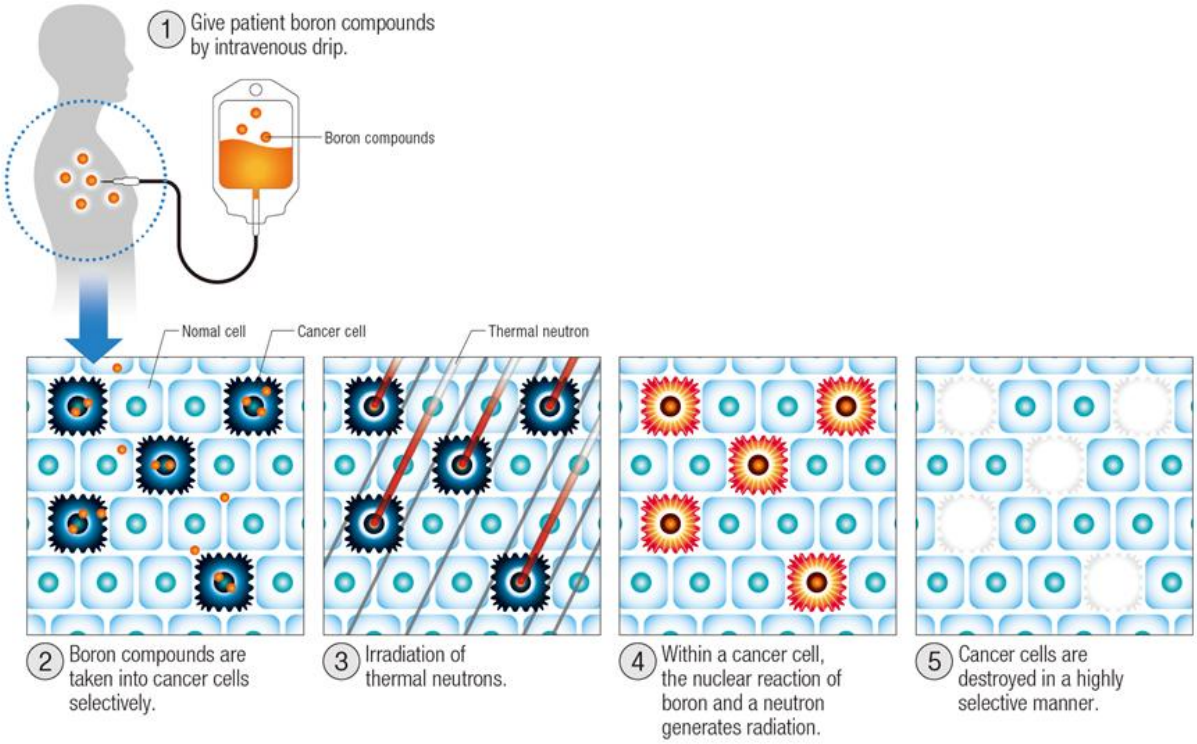


Figure 2. Schematic depicting concept of BNCT.

The primary factor for successful BNCT relies on the boron-10 delivery agents. These

are the types of tumor cell-finding boron-10 containing agent which are injected into the human body, which then accumulates in the tumor through blood transportation system within a period of time. There are seven aspects should be considered for a useful boron-10 delivery agent: (1) the most important aspect is strong selectively accumulative ability to achieve high ratios of (concentration of boron-10 in tumor cells)/(concentration of boron-10 in normal cells); (2) low or even none systemic toxicity; (3) to achieve at least ≃30 μg ^{10}B/g of tumor boron-10; (4) rapid clearance from blood and normal tissues and persistence in tumor during BNCT; (5) chemical stability; (6) water solubility and (7) lipophilicity.[4]

At the beginning of BNCT usage, sodium borate and boric acid and its derivatives were utilized in the 1950s, but boron concentrations in the tumor were not satisfactory.[5] Then, BPA (4-dihydroxy-L-boronophenylalanine) was reported.[6] The compound was shown to give much higher ratios of (concentration of boron-10 in tumor cells)/(concentration of boron-10 in normal cells) than the other compounds. In 1980s, it was reported to have a greater potential to treat melanomas.[7] Later on, it was indicated to be applied in BNCT for treatments of malignant brain tumors.[8] BPA can be considered to be the representative of the first generation of B-10 compound as a type of B-10 delivery agent for BNCT.

Polyhedral boranes $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$ were discovered with cage structures which have very impressive properties of hydrolytic and chemical stabilities.[9] Later on, sodium decahydrodecaborate $Na_2B_{10}H_{10}$ was indicated to be a promising B-10 delivery agent.[10] It was observed to show high ratios of (concentration of boron-10 in brain tumor cells)/(concentration of boron-10 in normal cells). Unluckily, the drug was slightly toxic to the human body. Finally, BSH ($Na_2B_{12}H_{11}SH$) with lower toxicity was developed.[11] Afterward, BSH had been applied to most of the clinical trials in the USA, Europe and Japan, and then adequate results were obtained. Therefore, BSH can be considered to be the representative of the second generation of B-10 compound as a type of B-10 delivery agent for BNCT.

The development of the third generation of the boron-10 delivery agents is the most crucial factor to directly affect the destiny of BNCT. The third generation of delivery agents has been developing due to unsatisfactory clinical results by using BPA or BSH for BNCT. There is a major difference between the third generation agents and the previous two. The third generation agents adopt third party agents to deliver boron-10 compounds. They mainly

consist of a stable boron group or cluster attached via a hydrolytically stable linkage to a tumor targeting moiety.[12] There are a few strategies for targeting the third generation agents to the tumor cells, such as conjugating to recognition factors, entrapping in vesicles or incorporation in vital compounds.[13] So far, there are a number of third generation agents have been investigated, such as monoclonal antibodies,[14] biochemical precursors,[15] polyamines,[16] DNA-binding agents,[17] peptides,[18] antisense agents,[19] polyhedral borane,[20] porphyrins,[21] carbohydrates,[22] amino acids(Kabalka & Yao, 2003),[23] and liposomes(Carlsson *et al.*, 2003; Justus *et al.*, 2007).[24] Organelles in the tumor cells such as golgi bodies, endoplasmic reticulums, lysosomes, mitochondrion and nuclei are suitable for targeting; thereinto, nuclei are particularly good for targeting, because less B-10 nuclei will be needed to kill a tumor cell if the B-10 nuclei are located at/near the tumor cell centers(Gabel *et al.*, 1987).[25] A useful boron-10 delivery agent must be soluble in water, which is to be systemically administered; also, lipophilicity enables it to cross the blood-brain barrier (BBB) and diffuse into the tumor.[12]

Ionic liquids (ILs) have been described as molten salts that are entirely ionic in nature, comprising both cationic and anionic species and having a melting point below 100 °C.[26] If they are liquid at room temperature then they are termed as room temperature ionic liquids (RTILs). The choice of cations and anions has a large influence on their properties. Usually, ionic liquids consist of a large organic cation and an organic or inorganic anion. The structural modification of ionic liquids can be made either to the anion, cation, or to the substituents on the cation or anion, so that an almost limitless number of ionic liquids are possible. Hence, by changing the cation or anion of ionic liquids, their physical properties can be modified according to the requirements of a process. These properties include melting point, density, viscosity, solubility, hydrophobicity etc. Ionic liquids have a special place in the current scientific literature due to their special properties, which distinguish ionic liquids from conventional organic solvents. These are very low vapour pressure, wide liquid range, large electrochemical window, low flammability etc. Some commonly used ionic liquid systems are presented in Figure 3.

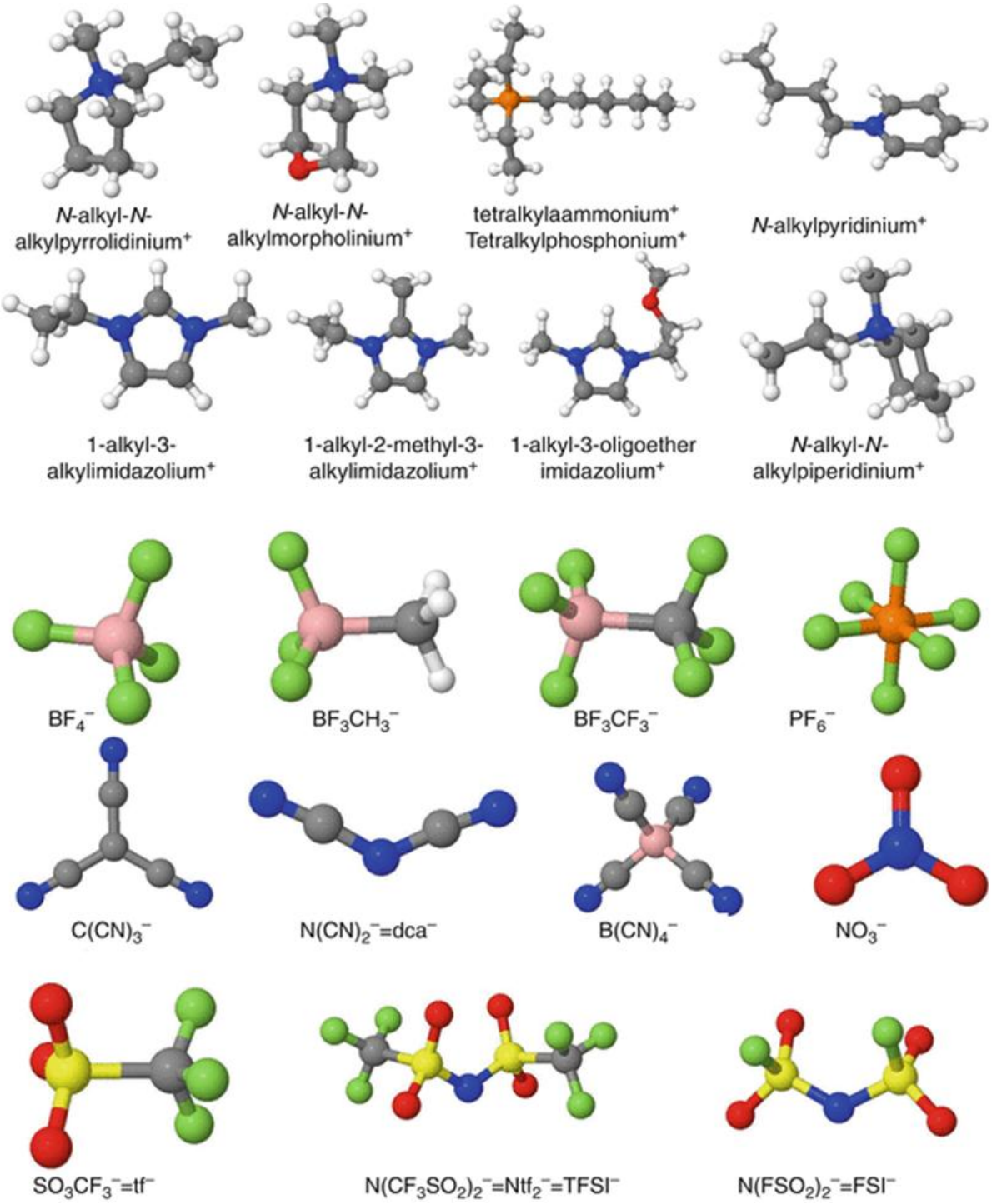


Figure 3. Some commonly used ionic liquids systems: cations and anions

Ionic liquids have been known for a long time. They made their first appearance in the scientific literature in 1914 by the report about the physical properties of $[EtNH_3][NO_3]$, which

has a melting point of 12 °C.[26−28] However their extensive use as solvents in chemical processes for synthesis,[29−31] separation processes[32−34] and catalysis[35−39] has recently become significant. Aluminium chloride based molten salts were used for high temperature electroplating during the 1940s. In 1951 Hurley *et al*. reported the synthesis of an ionic liquid by warming a mixture of 1-ethyl pyridinium chloride with aluminium chloride for low temperature electroplating of aluminium.[40,41] In the 1970s and 1980s, a thorough investigation on organic chloride–aluminium chloride ambient temperature ionic liquids was carried out by Robinson *et al*.[42,43] and Hussey et al.[44−46] In the 1970s, Wilkes *et al*. developed electrolytes with lower melting temperature to tackle the temperature related problems associated with the molten salt electrolytes.[47] In 1983, Hussey wrote the first major review on room temperature ionic liquids.[48] In the mid 1980s, low melting point ionic liquids were used as solvents for organic synthesis.[49,50] Following their work, ionic liquids became one of the most important classes of solvent systems. Initially, the applications of aluminium chloride based ionic liquids were limited, because of their highly hygroscopic nature. Moreover, they were not inert towards various organic compounds.[51] The first report on air and water stable ionic liquids based on the 1-ethyl-3-methylimidazolium cation and different anions such as tetrafluoroborate and hexafluorophosphate appeared in 1992.[52] After this report, the number of air and water stable ionic liquids started to increase rapidly. In 1998, a new class of ionic liquids called "functionalized ionic liquids" were prepared by Davis and co-workers,[53] based on cations derived from the antifungal drug miconazole (Figure 4).

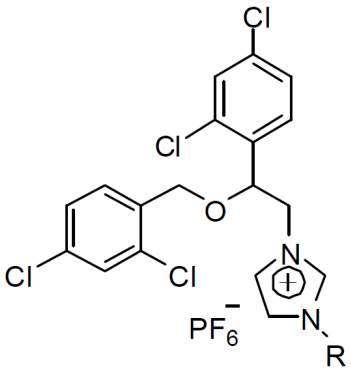


Figure 4. First 'task specific ionic liquid' based on the miconazole cation.

Functionalized ionic liquids may be defined as ionic liquids in which a functional group is covalently attached either to the cation or to the anion or even to both. The advantage of

introducing a functional group into ionic liquids is the fine-tuning of their properties for a particular application.

Nowadays, the field of ionic liquids is one of the most popular areas of research both in academia and industry.[54] In the last decade, over 8000 paper have been published on this subject. It was reported that over one million ionic liquids can be prepared by the simple combination of different catins and anions.[55] Currently, only about 300 ionic liquids have been commercialized. In the light of the above facts, one can imagine the opportunities still to be uncovered in ionic liquids. Therefore, the therapeutic dosage of the drug may be reduced, thereby decreasing side effects. Herein, a new efficient methodology for the preparation of boronated-ILs for use in BNCT is presented and discussed.

2. Experimental Section

2.1. General considerations

[C*n*mim]Br,[21] [C*n*mim][BF4],[22] [C*n*mim][BOB],[23] and [C*n*mim][BMLB][24] were synthesized as previously reported. Organic solvents were purchased as reagent grade and used as received. All metathetic reactions were carried out under standard laboratory conditions. Nevertheless, $[C_4mim]Br$, $[C_5mim]Br$ and $[C_6mim]Br$ were stored and weighted inside an inert atmosphere (Ar) glovebox (O_2, H_2O < 1 ppm). The elemental analyses were performed using a Carlo Erba Instruments CHNS-O EA 1108 analyzer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-JNM-AL300 spectrometer operating at 300.1 and 75.4 MHz, respectively. ^{11}B NMR spectra were recorded on a Bruker Ascend 400 spectrometer in KBSI Ochang Center (operating at 128.4MHz). All ^{11}B chemical shifts were referenced to $BF_3{\cdot}O(C_2H_5)_2$ (0.0ppm) with a negative sign indicating an upfield shift. All ^{1}H, ^{11}B and ^{13}C chemical shifts were measured relative to internal residual peaks from the lock solvent (99.5% DMSO-d_6). All melting points were uncorrected. $NaBF_4$, butyl bromide, pentyl bromide, and hexyl bromide were purchased from Aldrich Chemicals and o-carborane were purchased from Katchem.

2.2. Cell viability assay (MTT assay)

The boron compound (20 mg) was dissolved in DMSO (1.0 mL) and the resulting

solution was either diluted with MEM (Modified Eagle Medium) (10% FCS) or BPA was directly dissolved in the same medium. In a Falcon 3072, 96-well culture plate, HCT116 cancer cells (1×10^3 cells/well) were cultured in five wells with the medium containing boron compounds at various concentrations (1 – 100ppm) and incubated for 72 h at 37 °C in a CO_2 incubator. DMSO is typically non-toxic at the concentrations less than 0.5% and control experiments confirmed that DMSO was non-toxic at the concentrations used in the present experiments. After incubation, the medium was removed, the cells were washed three times with phosphate-buffered saline [PBS(–)], and the CellTiter 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay [MTT, 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used for counting cells on a Microplate reader. The results are presented in Table 3 as the concentration of agent that resulted in a cell culture with 50% of the cell number of the corresponding untreated group (IC_{50}).

2.3. In vitro boron incorporation into HCT116 cells

HCT116 cells were cultured in Falcon 3025 dishes (150 mm). When the cell population had increased to fill the dish (3.6×10^7 cells/dish), the ionic liquid compounds and BPA (10 μM) were added. The cells were incubated for 3 h at 37 °C in a medium of MEM and 10% FBS (20 mL). The cells were washed thrice with Ca/Mg-free PBS(–), collected by a rubber policeman, digested with a mixture of 60% $HClO_4$–30% H_2O_2 (1:2) solution (2 mL), and then decomposed for 1 h at 75 °C. After filtration through a membrane filter (Millipore, 0.22 mm), the boron concentration was determined by ICP-AES (Shimadzu, ICPS–1000–III). Each experiment was performed in triplicate.

2.4. Synthesis of ionic liquids based on 1-methylimidazole and 4-dimethylaminopyridine

2.4.1. Synthesis of 1-alkyl-3-methylimidazolinium tetrafluoroborate

General procedure: 1-Butyl-3-methylimidazolinium bromide (5.00 g, 22 mmol) was dissolved in 30 mL of acetone and sodium tetrafluoroborate (2.50 g, 22 mmol) was added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off with aluminum oxide (basic), washed with cold acetone (10 mL × 3) and dried under reduced pressure. The target compound was obtained as slight yellow oil.

2.4.1.1. 1-Butyl-3-methylimidazolinium tetrafluoroborate: Yield: 80% (4.17 g). ^{1}H NMR (DMSO-d_6) δ 0.86-0.91 (m, 3H), 1.23-1.26 (m, 2H), 1.73-1.75 (m, 2H), 3.83 (s, 3H), 4.12-4.17 (t, 2H), 7.67 (s, 1H), 7.74 (s, 1H), 9.07 (s, 1H). ^{13}CNMR(DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.1.2. 1-Pentyl-3-methylimidazolinium tetrafluoroborate: Yield: 57% (1.70 g). ^{1}H NMR (DMSO-d_6) δ 0.83-0.87 (m, 3H), 1.20-1.29 (m, 4H), 1.75-1.80 (m, 2H), 3.84 (s, 3H), 4.12-4.16 (t, 2H), 7.66 (s, 1H), 7.74 (s, 1H), 9.06 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.1.3. 1-Hexyl-3-methylimidazolinium tetrafluoroborate: Yield: 96% (3.78 g). ^{1}H NMR (DMSO-d_6) δ 0.83-0.87 (m, 3H), 1.26 (m, 6H), 1.74-1.81 (m, 2H), 3.89 (s, 3H), 4.12-4.17 (t, 2H), 7.69 (s, 1H), 7.76 (s, 1H), 9.09 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.2. Synthesis of 1-alkyl-3-methylimidazolinium bis(oxalato)borate

General procedure: 1-Butyl-3-methylimidazolinium bromide (4.38 g, 20 mmol) was dissolved in 40 mL of acetone and sodium bis(oxalate)borate (5.04 g, 24 mmol) was added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off with aluminum oxide (basic), washed with cold acetone (10 mL × 3) and dried under reduced pressure. The target compound was obtained as slight yellow oil.

2.4.2.1. 1-Butyl-3-methylimidazolinium bis(oxalato)borate: Yield: 12.3% (0.80 g). ^{1}H NMR (DMSO-d_6) δ 0.84-0.89 (m, 3H), 1.23 (m, 2H), 1.75-1.80 (m, 2H), 3.92 (s, 3H), 4.22-4.27 (t, 2H), 7.87 (s, 1H), 7.96 (s, 1H), 9.50 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.2.2. 1-Pentyl-3-methylimidazolinium bis(oxalato)borate: Yield: 55.0% (3.74 g). ^{1}H NMR

(DMSO-d_6) δ 0.80-0.85 (m, 3H), 1.24 (m, 4H), 1.77-1.82 (m, 2H), 3.93 (s, 3H), 4.22-4.25 (t, 2H), 7.88 (s, 1H), 7.98 (s, 1H), 9.53 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.2.3. 1-Hexyl-3-methylimidazolinium bis(oxalato)borate: Yield: 66.6% (4.72 g). ^{1}H NMR (DMSO-d_6) δ 0.83-0.85 (m, 3H), 1.24 (m, 6H), 1.76-1.81 (m, 2H), 3.92 (s, 3H), 4.21-4.26 (t, 2H), 7.86 (s, 1H), 7.95 (s, 1H), 9.48 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.3. Synthesis of 1-alkyl-3-methylimidazolinium bis(malonato)borate

General procedure: 1-butyl-3-methylimidazolinium bromide (4.38 g, 20 mmol) was dissolved in 40 mL of acetonitrile and sodium bis(malonato)borate (5.71 g, 24 mmol) was added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off with aluminum oxide (basic), washed with cold acetonitrile (10 mL × 3) and dried under reduced pressure. The target compound was obtained as slight yellow oil.

2.4.3.1. 1-Butyl-3-methylimidazolinium bis(malonato)borate: Yield: 49.8% (3.52 g). ^{1}H NMR (DMSO-d_6) δ 0.83-0.88 (m, 3H), 1.21-1.23 (m, 2H), 1.69-1.77 (m, 2H), 3.87 (s, 3H), 4.17-4.21 (t, 2H), 7.79 (s, 1H), 7.87 (s, 1H), 9.37 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.3.2. 1-Pentyl-3-methylimidazolinium bis(malonato)borate: Yield: 51.4% (3.78 g). ^{1}H NMR (DMSO-d_6) δ 0.81-0.86 (m,3H), 1.18-1.27 (m,4H), 1.74-1.79 (m,2H), 3.86 (s,3H), 4.15-4.20 (t,2H), 7.77 (s,1H), 7.85 (s,1H), 9.32 (s,1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.3.3. 1-Hexyl-3-methylimidazolinium bis(malonato)borate: Yield: 34.5% (2.64 g). ^{1}H NMR (DMSO-d_6) δ 0.81-0.86 (m, 3H), 1.19-1.27 (m, 6H), 1.74-1.79 (m, 2H), 3.86 (s, 3H), 4.15-4.20 (t, 2H), 7.77 (s, 1H), 7.85 (s, 1H), 9.32 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 22.2, 25.6, 29.8, 30.8, 36.0, 49.8, 122.1, 123.6, 135.9.

2.4.4. Synthesis of 1-alkyl-4-dimethylaminopyridinium bis(oxalato)borate

General procedure: 1-butyl-3-dimethyaminopyridinium bromide (2.59 g, 10 mmol) was dissolved in 30 mL of acetone and potassium bis(oxalate)borate (2.71 g, 12 mmol) was added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off with aluminum oxide (basic), washed with cold acetone (10 mL × 3) and dried under reduced pressure. The target compound was obtained as slight yellow oil.

2.4.4.1. 1-Butyl-4-dimethylaminopyridinium bis(oxalato)borate: Yield: 50.59 % (1.85 g). ^{1}H NMR (DMSO-d_6) δ 0.82-0.86 (t, 3H), 1.19-1.22 (m, 2H), 1.68-1.73 (m, 2H), 3.17 (s, 6H), 4.16-4.21 (t, 2H), 6.99-7.02 (d, 2H), 8.32-8.35 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.8, 21.9, 25.1, 29.3, 30.5, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.4.2. 1-Pentyl-4-dimethylaminopyridinium bis(oxalato)borate: Yield: 45.4% (1.72 g). ^{1}H NMR (DMSO-d_6) δ 0.79-0.84 (t, 3H), 1.16-1.25 (m, 4H), 1.71-1.76 (m, 2H), 3.17 (s, 6H), 4.13-4.18 (t, 2H), 6.99-7.02 (d, 2H), 8.29-8.31 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.8, 21.9, 25.1, 29.3, 30.5, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.4.3. 1-Hexyl-4-dimethylaminopyridinium bis(oxalato)borate: Yield: 54.4% (2.14 g). ^{1}H NMR (DMSO-d_6) δ 0.83-0.88 (t, 3H), 1.15-1.26 (m, 6H), 1.74-1.79 (m, 2H), 3.20 (s, 6H), 4.16-4.19 (t, 2H), 7.04-7.06 (d, 2H), 8.33-8.36 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.8, 21.9, 25.1, 29.3, 30.5, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.4.4. 1-Heptyl-4-dimethylaminopyridinium bis(oxalato)borate: Yield: 60% (1.22 g). ^{1}H NMR (DMSO-d_6) δ 0.82-0.86 (t, 3H), 1.13-1.23 (m, 8H), 1.69-1.79 (m, 2H), 3.18 (s, 6H), 4.12-4.17 (t, 2H), 7.02-7.04 (d, 2H), 8.30-8.32 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 21.9, 25.1, 29.3, 30.5, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.5. Synthesis of 1-alkyl-4-dimethylaminopyridinium bis(malonato)borate

General procedure: N-butyl-3-dimethylaminopyridinium bromide (1.30 g, 5 mmol) was dissolved in 30 mL of acetone and potassium bis(malonato)borate (1.51 g, 6 mmol) was added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off with aluminum oxide (basic), washed with cold acetone (10 mL × 3) and dried under reduced pressure. The target compound was obtained as slight yellow oil.

2.4.5.1. 1-Butyl-4-dimethylaminopyridinium bis(malonato)borate: Yield: 7.2% (0.14 g). ^{1}H NMR (DMSO-d_6) δ 0.88-0.92 (t, 3H), 1.15 (m, 2H), 1.75 (m, 2H), 3.20 (s, 6H), 4.18-4.23 (t, 2H), 7.04-7.06 (d, 2H), 8.34-8.36 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.7, 21.5, 27.6, 29.0, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.5.2. 1-Pentyl-4-dimethylaminopyridinium bis(malonato)borate: Yield: 0.6% (0.0125 g). ^{1}H NMR (DMSO-d_6) δ 0.81-0.86 (t, 3H), 1.12 (m, 4H), 1.71-1.76 (m, 2H), 3.17 (s, 6H), 4.11-4.16 (t, 2H), 7.00-7.02 (d, 2H), 8.28-8.30 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.7, 21.5, 27.6, 29.0, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.6.3. 1-Hexyl-4-dimethylaminopyridinium bis(malonato)borate: Yield: 4.2% (0.0879 g). ^{1}H NMR (DMSO-d_6) δ 0.82-0.87 (t, 3H), 1.24 (m, 6H), 1.72-1.77 (m, 2H), 3.18 (s, 6H), 4.12-4.17 (t, 2H), 7.02-7.04 (d, 2H), 8.30-8.32 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.7, 21.5, 27.6, 29.0, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.5.4. 1-Heptyl-4-dimethylaminopyridinium bis(malonato)borate: Yield: 60% (1.22 g). ^{1}H NMR (DMSO-d_6) δ 0.84-0.87 (t, 3H), 1.13-1.24 (m, 8H), 1.69-2.11 (m, 2H), 3.18 (s, 6H), 4.12-4.17 (t, 2H), 7.01-7.04 (d, 2H), 8.29-8.32 (d, 2H). ^{13}C NMR ($CDCl_3$) δ13.7, 21.5, 27.6, 29.0, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.6. Synthesis of 1-alkyl-3-methylimidazolinium dicarbollide

General procedure: 1-butyl-3-methylimidazolinium bromide (0.57 g, 2.4 mmol) was dissolved in 30 mL of acetone and potassium dicarbollide (0.42 g, 2 mmol) was added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off, washed with cold acetone

(10 mL × 3) and dried under reduced pressure. The target compound was obtained as slight yellow oil.

2.4.6.1. 1-Butyl-3-methylimidazolinium dicarbollide: Yield: 54% (0.35 g). ^{1}H NMR (DMSO-d_6) δ 0.87-0.92 (m, 3H), 1.23-1.26 (m, 2H), 1.73-1.75 (m, 2H), 3.84 (s, 3H), 4.13-4.18 (t, 2H), 7.70 (s, 1H), 7.77 (s, 1H), 9.13 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.2, 18.7, 31.3, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.6.2. 1-Pentyl-3-methylimidazolinium dicarbollide: Yield: 48% (0.33 g). ^{1}H NMR (DMSO-d_6) δ 0.84-0.89 (m, 3H), 1.21-1.32 (m, 4H), 1.73-1.80 (m, 2H), 3.84 (s, 3H), 4.11-4.16 (t, 2H), 7.69 (s, 1H), 7.76 (s, 1H), 9.09 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.7, 21.5, 27.6, 29.0, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.6.3. 1-Hexyl-3-methylimidazolinium dicarbollide: Yield: 52% (0.37 g). ^{1}H NMR (DMSO-d_6) δ 0.83-0.87 (m, 3H), 1.26 (m, 6H), 1.74-1.81 (m, 2H), 3.84 (s, 3H), 4.12-4.17 (t, 2H), 7.70 (s, 1H), 7.77 (s, 1H), 9.12 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 13.8, 21.9, 25.1, 29.3, 30.5, 35.7, 48.7, 122.2, 123.6, 136.4.

2.4.7. Synthesis of 1-alkyl-4-dimethylaminopyridinium dicarbollide

General procedure: N-butyl-dimethylaminopyridinium bromide (1.54 g, 6 mmol) was dissolved in 30 mL of acetone and potassiun dicarbollide (1.04 g, 5 mmol) was added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off, washed with cold acetone (10 mL × 3) and dried under reduced pressure. The target compound was obtained as slight yellow oil.

2.4.7.1. 1-Butyl-4-dimethylaminopyridinium dicarbollide: Yield: 54% (1.01 g). ^{1}H NMR (DMSO-d_6) δ 0.86-0.91 (t, 3H), 1.19-1.26 (m, 2H), 1.71-1.75 (m, 2H), 3.18 (s, 6H), 4.13-4.17 (t, 2H), 7.01-7.04 (d, 2H), 8.28-8.31 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.3, 18.7, 32.3, 56.4, 107.6, 141.9, 155.7.

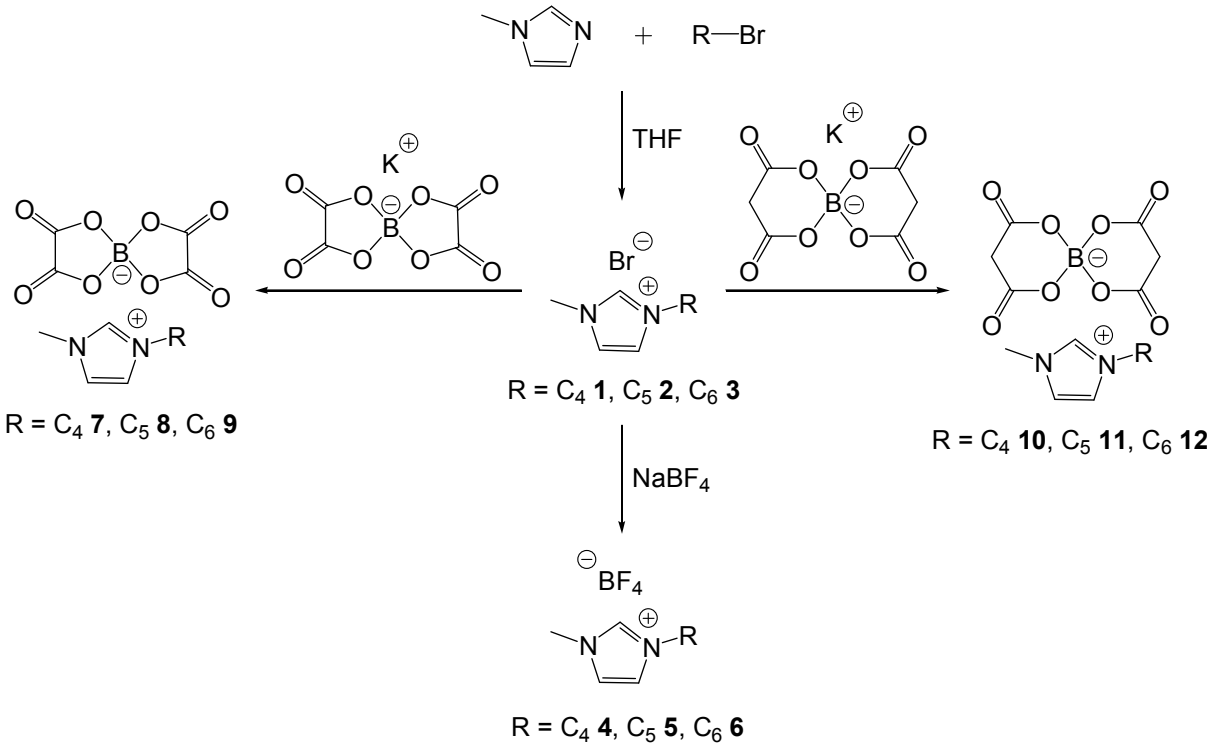
2.4.7.2. 1-Pentyl-4-dimethylaminopyridinium dicarbollide: Yield: 47% (0.92 g). ^{1}H NMR (DMSO-d_6) δ 0.82-0.87 (t, 3H), 1.17-1.31 (m, 4H), 1.72-1.75 (m, 2H), 3.17 (s, 6H), 4.11-4.16 (t, 2H), 7.01-7.04 (d, 2H), 8.28-8.31 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.8, 21.5, 27.5, 29.9, 56.6, 107.6, 141.9, 155.7.

2.4.7.3. 1-Hexyl-4-dimethylaminopyridinium dicarbollide: Yield: 60% (1.22 g). ^{1}H NMR (DMSO-d_6) δ 0.84-0.87 (t, 3H), 1.18-1.24 (m, 6H), 1.72-1.76 (m, 2H), 3.18 (s, 6H), 4.12-4.17 (t, 2H), 7.01-7.03 (d, 2H), 8.28-8.31 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 13.8, 21.9, 25.0, 30.2, 30.6, 56.6, 107.6, 141.9, 155.7.

3. Results and Discussion

3.1. Synthesis of boronated room temperature ionic liquids based on 1-methylimidazole.

The synthetic route used to prepare 1-alkyl-3-methylimidazolium salts is depicted in Scheme 1. The imidazolium bromides [C_nMIM]Br (C_n: n = 4 butyl, n = 5 pentyl, n = 6 hexyl) are prepared in high yield from 1-methylimidazole and the appropriate alkyl bromide in a modification to the literature procedure for the related 1-alkyl-3-methylimidazolium chlorides. The synthesis of [C_nMIM]Br has been describe previously using a somewhat more complicated method. The relatively electron withdrawing effect of the bromide activates alkyl bromide, $CH_3(CH_2)_n$Br, to such an extent that it reacts smoothly with 1-methylimidazole in the acetone solvent to give target compound. However, as the alkyl chain in the alkyl bromide precursor increases in length, the temperature and reaction time required to complete the reaction also increases.



Scheme 1. Preparation of boronated ionic liquids based on 1-alkyl-3-methylimidazolium cations (**1** – **12**).

Reaction of 1-methylimidazole with a molecular equivalent of alkyl bromide in THF affords the 1-alkyl-3-methylimidazolium bromide salts $[C_nMIM]^+Br^-$ [C_n = C_4 (butyl) **1**, C_5 (pentyl) **2**, C_6 (hexyl) **3**]. The imidazolium tetrafluoroborate salts $[C_nMIM]^+BF_4^-$ (**4** – **6**), bis(oxalate)borate salts (**7** – **9**), and bis(malonate)borate salts (**10** – **12**) are prepared from **1** – **3** with various borate anions such as sodium tetrafluoroborate, potassium bis(oxalato)borate, and potassium bis(malonato)borate using an analogous method. For imidazolium salts were filtered off with aluminum oxide (basic) in order to remove the NaBr or KBr salts formed during the anion exchange reaction, then washed with cold acetone solvent. The imidazolium salts were then dried under vacuum for 2 days. The salts **4** – **12** are liquid form at room temperature and were further purified by filtration through silica and left at 60 °C for 2 days. All the imidazolium salts were obtained to high yields (up to 80%). They are stable in air and showed no signs of decomposition up to 100 °C.

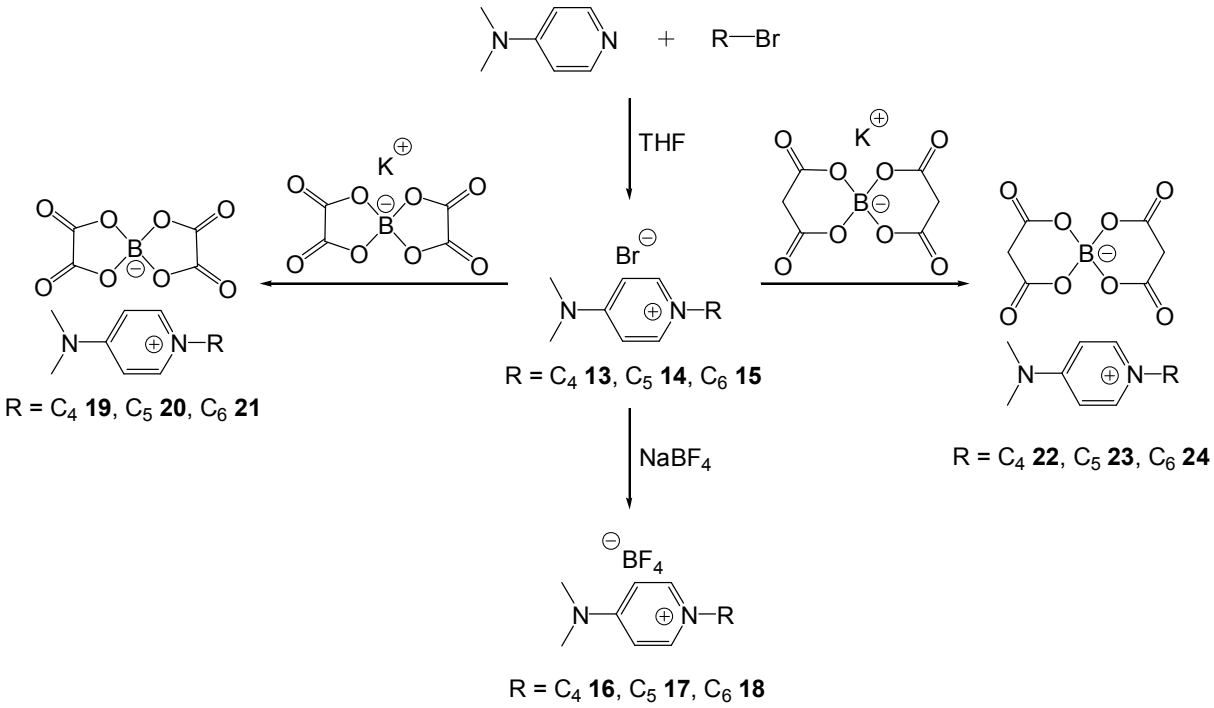
The imidazolium salts were characterized using infrared (IR), ^1H, ^{13}C, and ^{11}B nuclear magnetic resonace (NMR) spectroscopy. The IR spectra exhibit C–H bond stretches between

3150 – 2950 cm^{-1} and weaker C–H bond stretches between 2850 – 2460 cm^{-1}, possibly arising from the formation of hydrogen bonding with the anion. The most noteworthy feature of the ^{1}H NMR spectra of the imidazolium salts is the characteristic resonance for the acidic proton in the 2-position. In most compounds this proton is observed at around 8.45 – 9.99 ppm, but no clear trends are present. It is noteworthy that H–D exchange takes place at the acidic 2-position in all the ILs described, and is fastest where the alkyl chain is short and the protons interact most strongly with the anion.

3.2. Synthesis of boronated room temperature ionic liquids based on 4-dimethylaminopyridine.

A similar protocol was applied to the preparation of 4-dimethylamino-1-alkylpyridinium bromide salts and its anion exchange reaction as shown in Scheme 2. The required starting material 1-alkyl-4-dimethylaminopyridinium bromide (**13** – **15**) were prepared by the reaction of 4-dimethylaminopyridine with alkyl bromide (alkyl = butyl **13**, pentyl **14**, hexyl **15**) at 0 °C in THF. The 1-alkyl-4-dimethylaminopyridinium tetrafluoroborate $[C_nDMAP]BF_4^-$ (**16** – **18**), bis(oxalate)borate (**19** – **21**), bis(malonate)borate salts prepared from **13** – **15** with various borate anions such as sodium tetrafluoroborate, potassium bis(oxalato)borate, and potassium bis(malonato)borate using an analogous method. For 1-alkyl-4-dimethylaminopyridinium salts were filtered off with aluminum oxide (basic) in order to remove the NaBr or KBr salts formed during the anion exchange reaction, then washed with cold acetone solvent. The 1-alkyl-4-dimethylaminopyridinium salts were then dried under vacuum for 2 days. The salts **16** – **24** are liquid or oil form at room temperature and were further purified by filtration through silica and left at 60 °C for 2 days. All the 1-alkyl-4-dimethylaminopyridinium salts were obtained to high yields (up to 80%). They are stable in air and showed no signs of decomposition up to 100 °C.

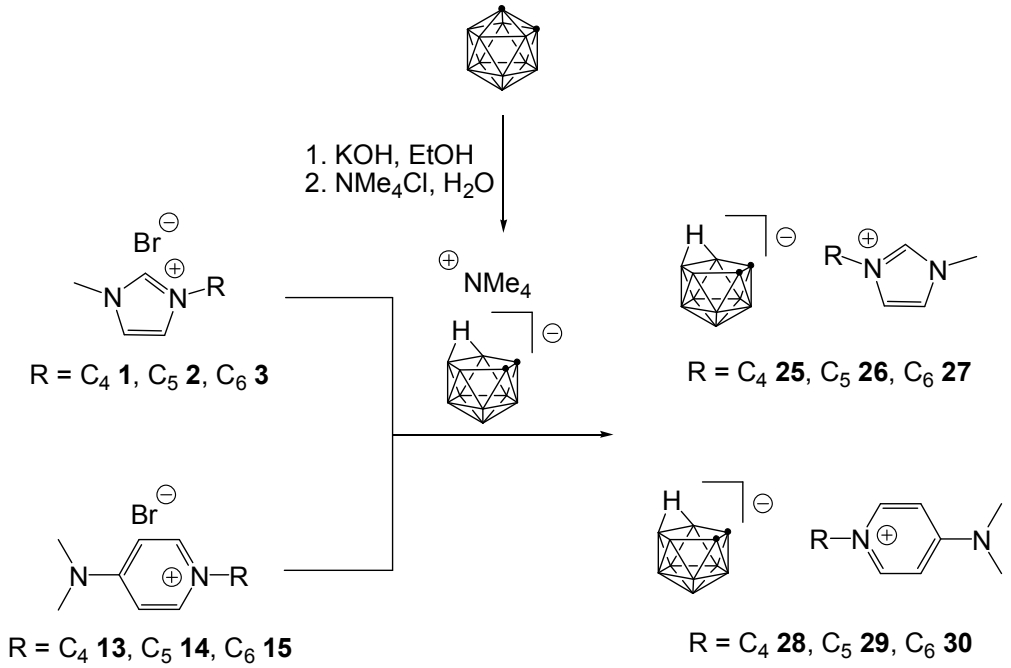
The 1-alkyl-4-dimethylaminopyridinium salts were characterized using infrared (IR), ^{1}H, ^{13}C, and ^{11}B nuclear magnetic resonace (NMR) spectroscopy. The IR spectra exhibit C–H bond stretches between 3150 – 2950 cm–1 and C=N bond stretches between 2850 – 2460 cm^{-1}. The most noteworthy feature of the ^{1}H NMR spectra of the 1-alkyl-4dimethylaminopyridinium salts is the characteristic resonance for the acidic proton in the 2-position. In most compounds this proton is observed at around 8.45 – 9.99 ppm, but no clear trends are present.



Scheme 2. Preparation of boronated ionic liquids based on 1-alkyl-4-dimethylaminopyridinium cations (**13** – **24**)

3.3. Synthesis of boronated room temperature ionic liquids based on carborane anion (dicarboliide).

As shown in Scheme 3, the salts were prepared by conventional metathetic reactions. The tetramethylammonium precursor used was $[NMe_4][C_2B_9H_{12}]$. Solvent was selected appropriated to its solubility and those of the 1-alkyl-3-methylimidazolium or 1-alkyl-4-dimethylaminopyridinium bromides. 1-Alkyl-3-methylimidazolium salts of the dicarbollide $[NMe_4][C_2B_9H_{12}]$ could be obtained in excellent yields by reaction of $[C_nMIM]Br$ and $[NMe_4][C_2B_9H_{12}]$ in THF. Reed and coworkers previously reported different reaction conditions (dichloromethane/propanone, 6:1 mixed solvent) to be very efficient for the preparation of related imidazolium monocarborane salts (monocarborane = $[CB_{11}H_{12}]^-$). The solid NMe_4Br formed was removed by a simple filtration. After removal of volatiles under reduced pressure, $[C_nMIM][C_2B_9H_{12}]$ were obtained as pale yellow liquids for n = 4 and oil for n = 5 and 6.



Scheme 3. Preparation of dicarbollyl ionic liquids based on 1-alkylated imidazolium and 4-dimethylaminopyridinium cations (**25** – **30**).

For the cation 1-alkyl-4-dimethylaminopyridinium, where bromide was the countercation, THF was used as the solvent. The 1-alkyl-4-dimethylaminopyridinium nido-carborane dissolved in solvent, but not quantitatively in all cases: especially the shortest alkyl chain 4-dimethylaminopyridinium salts were observed to be to soluble, and concentration under reduced pressure was needed prior to filtration, without observing a decrease in purity. Yields were good to high, the longest alkyl chain 1-hexyl-4-dimethylaminopyridinium giving the lowest yield. The tetramethylammonium salts were used because of its ease of purification and handling, but a procedue in aqueous solution starting from an potassium salt of the nido-carborane also proved convenient. So, if the metathetic reaction is carried out immediately after the partial degradation of 1,2-closo-$C_2B_{10}H_{12}$, where the potassium salt $K[C_2B_9H_{12}]$ is produced, yields increase to high and the tetramethylammonium salt isolation step is avoided. The prepared salts were dried in vacuo at 60 °C. Nevertheless, they are not especially hygroscopic and, for most purposees, can be handled and stored in air. This property makes them attractive for application purposes.

An extensive study on ^{1}H, 13C, and ^{11}B NMR nuclear magnetic resonance of the imidazolium

and 4-dimethylaminopyridinium dicarbollide anion salts has been undertaken. The ^{11}B NMR spectra recorded for the novel salts agree with the structures of the clusters. Assigned ^{11}B NMR chemical shifts for selected salts used in this work are listed in Experimental section. ^{1}H NMR measurements have been taken for salts with the two cations studied. ^{1}H NMR chemical shifts show changes as a function of all these parameters.

Two main phenomena have been supposed to affect these shifts: hydrogen bonding and π -stacking. It is assumed that hydrogen bonding causes a low field chemical shift of the proton. This effect is observed for the aromatic acidic hydrogen atoms in imidazolium cations, that is, H4, H5 and especially H2. At identical concentrations in the same solvent, their chemical shifts increase with anion hydrogen bond acceptor ability. Thus, whereas H2 chemical shifts fall in the range of 9.5 – 11.0 ppm for salts with good hydrogen bond acceptor anions, such as halides or carboxylateds, they are below 9.1 ppm for all the newly synthesized salts presented here. This is accounted for by the low basicity of dicarbollide anion. This is behavior previously observed for salts of poor hydrogen bond acceptor anions in polar solvents. Ths chemical shifts of C–H and bridged hydrogen (B–H–B) of cluster show noticeable upfield shifts with increasing concentration, indicating that close contacts are probably being established between the open face of the nido-carborane anion and the molecular plane of the imidazolium and 4-dimethylaminopyridinium rings.

3.4. Biological activity of ionic liquids

All the synthesized ionic liquids were screened for their in vitro cytotoxicity. All tested 1-alkyl-3-methylimidazolium and 1-alkyl-4-dimethylaminopyridinium ionic liquids in the present study with a chain length of C_4, C_5, and C_6 at the 1-position consistently show higher cytotoxicity−*i.e.* lower IC_{50} values−than the corresponding BOB^-, $BMLB^-$, and $C_2B_9H_{12}^-$ salts of the BF_4^- anion. Among the ionic liquids only the BF_4^- anion is less cytotoxicity within the tested concentration range with an IC_{50} value around 1000 μM. A relatively high cytotoxicity was found for the $C_2B_9H_{12}^-$. In particular the boron containing anions BOB^- and $BMLB^-$ exhibited a moderate cytotoxicity. This suggests an intrinsic cytotoxicity effect of the imidazolium and 4-dimethylaminopyridinium cations and large borate anions. Additionally the well known side chain length effect of the cations headgroup was supported by our results when looking at ionic liquids of which all three different side chains were tested with the

same anion.

The influence of the anion moiety in ionic liquids on their cytotoxicity is identified by comparing the results obtained from one headgroup with one specific side chain length but different anions.

In order to identify significant anion effects for every single ionic liquid cation, we propose the concept of the anion effect ration for ranking the relative influence of the anions in all tested ionic liquids on cytotoxicity. In Figures 5 − 12 the side chain effect observed for the 1-alkyl-3-methylimidazolium and 1-alkyl-4-dimethylaminopyridinium ionic liquids and the anion effects are illustrated in parallel to provide an overview of all ionic liquids, in which the anions exhibit an significant effect according to the anion effect ratio.

The cytotoxicity of the ionic liquid is normalized on the cytotoxicity of the ionic liquids with various boron containing anions. The results obtained in our study are discussed along to the following questions: (i) Can the impact of the anions be attributed to physical and/or chemical properties of the anions and if so, which mode of action might be responsible for the observed cytotoxic effect? (ii) Can the impact of the anions in ionic liquids be explained by the intrinsic cytotoxicity of the anion? (iii) Is a simple model of mixture toxicity helpful in predicting the cytotoxicity of ionic liquids that have not been investigated yet?

The presented results clearly demonstrate that anions can influence the cytotoxicity of ionic liquids. In the following section the anions exhibiting a significant effect are analyzed in detail using the various programs to elucidate physical and/or chemical properties that are responsible for the observed anions and cations cytotoxicity.

4. Conclusion

In this study, we have reported the synthesis and biological activities of a series of halogen-free borate-ionic liquids such as BF_4^-, $B(oxalate)_2^-$, $B(malonato)_2^-$, or $[C_2B_9H_{11}]^-$ with either imidazolium or dimethylaminopyridinium cations in straightforward metathetic reactions, which can easily produce highly active biological molecules for BNCT. Highly pure and non-hygroscopic borate and boron cluster anion ionic liquids can be obtained by combining anions.

Using the HCT116 cancer cells as test system we could demonstrate that most of the

commercially available anion or synthetic anions investigated showed no or only marginal cytotoxic effects. However, for certain anions a significant influence on the ionic liquid cytotoxicity for BNCT is demonstrated for the first time.

Therefore the term ‘anion effect’ is proposed for some anions comparable to the side chain effect established for specific cations. In particular, anionic moieties with lipophilic and hydrolysable structural elements seem to be of considerable relevance with respect to the observed cytotoxic effects.

In general, the model-based prospective estimation of the cytotoxicity combined with experimentally derived cytotoxicity data for anions and cations complemented by consideration of structure-property relationships opens up the opportunity to overcome the above mentioned dilemma of an unmanageable pool of possible ionic liquids.

In this iterative approach chemists creativity is guided by the structural properties of cation and anion species, which leads to a reduced number of task specific and intrinsically safer ionic liquids. Through such a process more sustainable ionic liquids can be realized.

5. References

1. Treatment Choices for Men With Early-Stage Prostate Cancer, online, accessed 22/06/09, http://nci.nih.gov/cancertopics/prostate-cancer-treatment-choices/allpages, National Cancer Institute.

2. Sweet W. H. *J. Neurooncol.*, **1997**, *33*, 19.

3. Walker S. J. *Radiography.* **1998**, *4*, 211.

4. (a) Yamamoto T.; Nakai K.; Matsumura A. *Cancer Letters*. 2008, *262*, 143. (b) Barth R. F.; Coderre J. A.; Vicente M. G. H.; Blue T. E. *Clin. Cancer Res*. 2005, *11*, 3987.

5. Farr L. E.; Sweet W. H.; Locksley H. B.; Robertson J. S. *Trans. Am. Neurol. Assoc*. **1954**, *13*, 110.

6. Snyder H. R.; Reedy A. J.; Lennarz W. *J. Am. Chem. Soc*. **1958**, *80*, 835.

7. Ichihashi M.; Nakanishi T.; Mishima Y. *J. Invest. Dermatol*. **1982**, *78*, 215.

8. Coderre J. A.; Glass J. D.; Packer S.; Micca P.; Greenberg D. *Pigment Cell Res*. **1990**, *3*, 310.

9. Hawthorne M. F.; Pitochelli A. R. *J. Am. Chem. Soc*. **1959**, *81*, 5519.

10. Soloway A. H.; Wright R. L.; Messer J. R. *J. Pharmacol. Exp. Ther*. **1961**, *134*, 117.

11. Soloway, A. H.; Hatanaka H.; Davis M. A. *J. Med. Chem*. **1967**, *10*, 714.

12. Barth R. F.; Coderre J. A.; Vicente M. G. H.; Blue T. E. *Clin. Cancer Res*. **2005**, *11*, 3987.

13. Azab A. K.; Abu Ali H.; Srebnik M. *Studies in Inorg. Chem*. **2006**, *22*, 337.

14. Olsson P.; Gedda L.; Goike H. et al., *Anticancer Drug Des*. **1998**, *13*, 279.

15. Tjarks W. *J. Organomet. Chem*. **2001**, *614-615*, 37.

16. El-Zaria M. E.; Doerfler U.; Gabel D. *J. Med. Chem*. **2002**, *45*, 5817.

17. Tietze L. F.; Griesbach U.; Bothe U.; Nakamura H.; Yamamoto Y. *ChemBioChem*. **2002**, *3*, 219.

18. Ivanov D.; Bachovchin W. W.; Redfield A. G. *Biochemistry* **2002**, *41*, 1587.

19. Olejiniczak A. B.; Koziolkiewcz M.; Lesnikowski Z. J. *Antisense Nucleic Acid Drug Dev*. **2002**, *12*, 79.

20. Surewein W.; Moss R.; Wittig A. (Eds.) 2002, *Research and Development in Neutron Capture Therapy*, Monduzzi Ed. Bologna, p. 993, pp. 1107.

21. Isaac M. F.; Kahl S. B. *J. Organomet. Chem*. **2003**, *680*, 232.

22. Tietze L. F.; Griesbach U.; Schuberth I.; Bothe U.; Marra A.; Dondoni A. *Chem. Eur. J*. **2003**, *9*, 1296.

23. Kabalka G. W.; Yao M. L. *Synthesis* **2003**, *18*, 2890.

24. Carlsson J.; Kullberg E. B.; Capala J. *et al*., *J. Neurooncol.* **2003**, *62*, 47.

25. Gabel D.; Foster S.; Fairchild R. G. *Radiat. Res*. **1987**, *111*, 14.

26. Welton, T. *Chem. Rev.* **1999**, *99*, 2071.

27. Walden, P. *Bull. Acad. Imp. Sci. St. -Petersbourg* **1914**, 405.

28. Sugden, S.; Wilkins, H. *J. Chem. Soc.* **1929**, 1291.

29. Hakkou, H.; Carrie, D.; Paquin, L.; Bazureau, J. P. *Russ. J. Org. Chem.* **2011**, *47*, 371.

30. Sawant, A. D.; Raut, D. G.; Darvatkar, N. B.; Salunkhe, M. M. *Green Chem. Lett. Rev.* **2011**, *4*, 41.

31. Ni, B.; Headley, A. D. *Chem. Eur. J.* **2010**, *16*, 4426.

32. Poole, C. F.; Poole, S. K. *J. Chromatogr., A.* **2010**, *1217*, 2268.

33. Marciniak, A. I. *Fluid Phase Equilib.* **2010**, *294*, 213.

34. Han, D.; Row, K. H. *Molecules.* **2010**, *15*, 2405.

35. Olivier-Bourbigou, H.; Magna, L.; Morvan, D. *Appl. Catal. A.* **2010**, *373*, 1.

36. Lee, J. W.; Shin, J. Y.; Chun, Y. S.; Jang, H. B.; Song, C. E.; Lee, S. G. *Acc. Chem. Res.* **2010**, *43*, 985.

37. Zhang, L. F.; Fu, X. L.; Gao, G. H. *ChemCatChem* **2011**, *3*, 1359.

38. Gordon, C. M. *Appl. Catal. , A.* **2001**, *222*, 101.

39. Hurley, F. H.; Wier, T. P., Jr.. *J. Electrochem. Soc.* **1951**, *98*, 207.

40. Endres, F.; Zein El Abedin, S. *Phys. Chem. Chem. Phys.* **2006**, *8*, 2101.

41. Chum, H. L.; Koch, V. R.; Miller, L. L.; Osteryoung, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 3264.

42. Robinson, J.; Osteryoung, R. A. *J. Am. Chem. Soc.* **1979**, *101*, 323.

43. Wilkes, J. S.; Levisky, J. A.; Wilson, R. A.; Hussey, C. L. *Inorg. Chem.* **1982**, *21*, 1263－.

44. Appleby, D.; Hussey, C. L.; Seddon, K. R.; Turp, J. E.. *Nature.* **1986**, *323*, 614.

45. Scheffler, T. B.; Hussey, C. L.; Seddon, K. R.; Kear, C. M.; Armitage, P. D. *Inorg. Chem.* **1983**, *22*, 2099.

46. Wilkes, J. S. *Green Chem.* **2002**, *4*, 73.

47. Hussey, C. L. *Adv. Molten Salt Chem.* **1983**, *5*, 185.

48. Fry, S. E.; Pienta, N. J. *J. Am. Chem. Soc.* **1985**, *107* , 6399.

49. Boon, J. A.; Levisky, J. A.; Pflug, J. L.; Wilkes, J. S. *J. Org. Chem.* **1986**, *51*, 480.

50. Dupont, J. *J. Braz. Chem. Soc.* **2004**, *15*, 341.

51. Wilkes, J. S.; Zaworotko, M. J. *J. Chem. Soc. , Chem. Commun.* **1992**, 965.

52. Davis, J. H., Jr.; Forrester, K. J.; Merrigan, T. *Tetrahedron Lett.* **1998**, *39*, 8955.

53. Plechkova, N. V.; Seddon, K. R. *Chem. Soc. Rev.* **2008**, *37*, 123.

54. Rogers, R. D.; Seddon, K. R. *Science* **2003**, *302*, 792.

55. Seddon, K. R. *Kinet. Catal.* **1996**, *37*, 69.

Table 1. Yields and ^{1}H NMR for 1-alkyl-3-methylimidazolium ionic liquids

Entry	NO.	Yields (%)	^{1}H-NMR			
			N-CH_3	N-CH_2-N	N-CH_2	C-CH_3
1	**4**	80.0	3.83	9.07	4.15	0.89
2	**5**	57.0	3.92	9.06	4.25	0.83
3	**6**	96.0	3.87	9.09	4.18	0.86
4	**7**	12.3	3.84	9.50	4.15	0.89
5	**8**	55.0	3.84	9.53	4.14	0.85
6	**9**	66.6	3.93	9.48	4.25	0.83
7	**10**	49.8	3.86	9.37	4.18	0.84
8	**11**	51.4	3.84	9.32	4.14	0.86
9	**12**	34.5	3.89	9.32	4.14	0.85
10	**25**	54.0	3.92	9.13	4.23	0.87
11	**26**	48.0	3.86	9.09	4.19	0.84
12	**27**	52.0	3.84	9.12	4.14	0.85

Table 2. Yields and ^{1}H NMR for 4-dimethylaminopyridinium ionic liquids

Entry	NO.	Yields (%)	^{1}H-NMR	
			N-CH_2	C-CH_3
1	**19**	50.6	4.19	0.84
2	**20**	45.4	4.17	0.90
3	**21**	54.4	4.15	0.89
4	**22**	7.2	4.13	0.84
5	**23**	0.6	4.14	0.85
6	**24**	4.2	4.19	0.85
7	**28**	54.0	4.14	0.84
8	**29**	47.0	4.15	0.84
9	**30**	60.0	4.15	0.84

Table 3. Cytotoxicity (IC_{50}) for HCT116 Cancer Cells

Entry	Compd	IC50 (μM)
		HCT116
1	**4**	>1000
2	**5**	445.7 (± 6.7)
3	**6**	>1000
4	**7**	>1000
5	**8**	203.1 (± 1.7)
6	**9**	44.9 (± 0.3)
7	**10**	450.4 (± 1.3)
8	**11**	110.5 (± 3.9)
9	**12**	39.8 (± 1.0)
10	**19**	60.9 (± 0.5)
11	**20**	22.8 (± 1.3)
12	**21**	13.7 (± 0.8)
13	**22**	9.4 (± 0.2)
14	**23**	9.0 (± 0.3)
15	**24**	3.9 (± 0.6)
16	**25**	70.8 (± 0.8)
17	**26**	48.5 (± 0.5)
18	**27**	23.7 (± 0.6)
19	**28**	20.8 (± 0.3)
20	**29**	14.7 (± 0.5)
21	**30**	7.8 (± 0.2)
22	**BPA**	44.95 (± 0.3)