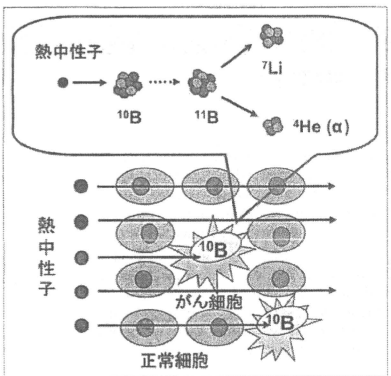

図1　BNCTの原理

表1　さまざまな核種の中性子捕捉断面積

nuclide	cross section capture value*
^{7}Li	942
^{10}B	3,838
^{113}Cd	20,000
^{135}Xe	2,720,000
^{149}Sm	41,500
^{151}Eu	59,002
^{157}Gd	240,000
^{174}Hf	400
H	0.332
C	0.0037
N	1.75
O	<0.0002
P	0.19
S	0.52
Na	0.536
K	2.07

*Cross section capture values in barns

子がホウ素の同位体であるホウ素10と核反応を起こし，その際に生成するα線によりがん細胞を死滅させる治療法である．BNCTの特徴として，α線の飛程が細胞一つの大きさ(5～9 μm)とほぼ等しく，細胞一つを殺傷するのに充分なエネルギー(2.4 MeV)であること，熱中性子による正常細胞(人体)への影響が少ないことが挙げられる(式)．このことから予めホウ素分子をがん細胞選択的に取り込ませ，そこに中性子照射を行えばがん細胞のみを選択的に破壊することができる(図1)．このように，BNCTは副作用の非常に低い次世代型細胞選択的放射線療法として注目されている．

$$^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He + 2.4\ MeV$$

では，なぜホウ素分子なのか？中性子を原子核に照射した際に，中性子を捕捉する大きさ“中性子捕捉断面積”を主な元素について比較した(表1)[4]．中性子捕捉断面積はバーン(1 barn=10^{-24} cm^2)という単位で表される．^{135}Xe，^{149}Sm，^{151}Eu，^{157}Gdなどがきわめて大きい値を示している．^{10}Bの中性子捕捉断面積は3,838バーンとそれほど大きな値は示していないのに，中性子捕捉療法に有望であるのは主につぎに挙げる四つの理由からである．① ^{10}Bは非放射性で天然のホウ素に約20%含まれるため入手容易である．② 上で述べたように核反応の際のα線の飛程が1個の細胞内に限られる．③ ホウ素の広範な化学反応性と安定性により種々の生物活性分子や生体関連物質への導入が可能である．④ 重金属のような高い毒性を示さない．

一方，生体中の元素も中性子を捕捉して放射線を生じるが，その中性子捕捉断面積は^{10}Bよりも数桁小さな値なので，通常は無視できる．しかしながら水素と窒素は生体中に高濃度に存在するため，中性子の照射線量に大きく影響する．したがってこれらの影響を最小限にするためにも，腫瘍組織内の^{10}B濃度が20～35 μg/gであれば，放射線量のおよそ85%が^{10}Bの中性子捕捉反応から生じると計算されている[5]．最終的には照射できる中性子線量の上限は，水素と窒素が中性子を捕捉して出す放射線に周囲の正常組織がどれほど耐えられるかに依存する．このためにも^{10}Bががん細胞に選択的に集積することが必要であり，実際に臨床上の立場から腫瘍組織内^{10}B濃度が30 μg/g以上，^{10}B濃度の腫瘍組織/血液および腫瘍組織/正常組織の比がいずれも5以上が望ましいとされている．

現在までBNCTは，原子炉から得られる熱中性子を用いて行われている．わが国には，医療用原子炉として京都大学原子炉実験所にあるKURと日本原子力研究機構東海研究開発センターにあるJRR4の2基がある．図2には，JRR4におけるBNCT用

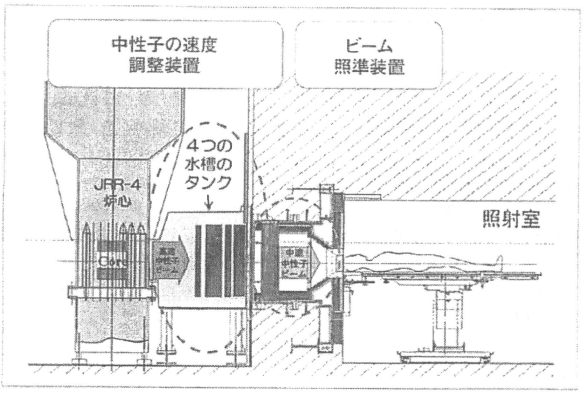

図2　日本原子力研究機構東海研究開発センター内にある原子炉(JRR4)医療照射設備
病状に応じて低速中性子から中速中性子までの32通りのビームを発生できる"医療照射設備"を開発

医療照射設備を示したが，これらの設備を用いてBNCTが行われているが，小型加速器から充分な熱中性子が得られるようになれば，都市型病院への併設が可能となる．したがって，BNCTが放射線療法の一つとして一般に普及するためは，病院に併設可能な小型加速器の開発が急務である．現在京都大学では，医療機器としての治験に向けたBNCT用小型加速器開発研究が進んでいる．

BNCT用ホウ素薬剤

BNCTではホウ素薬剤をいかにしてがん細胞にのみ選択的に高濃度で集積させるかが課題である．現在，わが国ではアミノ酸誘導体であるBPA(*p*-boronophenylalanine)と，分子内にホウ素原子を12個含む20面体の特異的な化学構造を有する水溶性ホウ素イオンクラスターであるBSH(mercaptoundecahydrododecaborate)を用いてそれぞれ悪性黒色腫[6,7]および脳腫瘍[8,9]のBNCTが行われてきた(図3)．

1994年には，京都府医大の今堀・上田らにより^{18}F-BPAを用いたPET(positron emission tomography)診断法が開発され，あらかじめ腫瘍部位のホウ素蓄積量を見積もることができるようになった[10]と同時に，BPAが多くの悪性腫瘍に集積することもわかってきた[11~13]．2001年には，大阪大の加藤・由良らと京都大の小野らのグループは共同して世界に先駆けて頭頸部悪性腫瘍のBNCTに成功した．頭頸部悪性腫瘍は現在でも手術が中心であり，審美障害，嚥下・咀嚼障害などの機能障害が後遺することがある．彼らは，再発耳下腺がん患者に対し，BSHとBPAの併用BNCTを行ったところ，

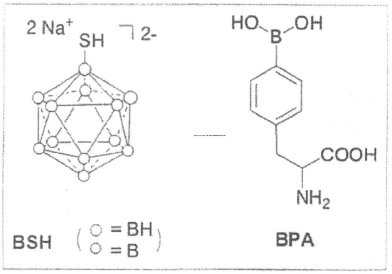

図3　現在臨床に用いられているホウ素薬剤の化学構造

9カ月後にはがんが完全に消失し，皮膚への放射線障害もほとんどみられなかった．この成功をきっかけにBNCTの適応拡大が進められ，頭頸部がん以外にも，肝臓がん，肺がん，胸壁腫瘍などの適応疾患の拡大とともにBNCTの症例数が増加している．

その一方で，ホウ素薬剤である^{10}B濃縮したBSHとL体BPAは，海外からの輸入に頼っていたため，臨床に必要なホウ素薬剤の確保がしばしば困難であった．大阪府立大の切畑らはステラケミファ社と共同で，^{10}B濃縮したBSHとBPAの国産化に成功し，この二つのホウ素薬剤のGMPレベルでの供給体制が整った．現在，BPAを用いた小型加速器BNCTの臨床治験に向けた研究が進んでいる．

次世代型ホウ素デリバリーシステム

さて，効果的にBNCTを行うためには選択的かつ高濃度でがん細胞にホウ素10を送り込むことが必要である．実際の臨床では，BSHとL体BPAを併用して，腫瘍内ホウ素濃度が25～100 ppm，腫瘍/血液のホウ素濃度比ならびに腫瘍/正常組織のホウ素濃度比が2～3で行われている．はじめに述べたように，BNCTの望まれる条件(腫瘍内ホウ素濃度が30 ppm以上でなおかつ，腫瘍/血液ならびに腫瘍/正常組織のホウ素濃度比が5以上)を達成するために，さまざまなホウ素キャリアーの開発研究が行われてきた．ホウ素キャリアーに望まれることは，① 500 mg/kg程度の濃度で投与が可能なくらい毒性が低いこと，② 充分に水溶性であること，③ 腫瘍細胞への蓄積が選択的であること，が挙げられる．これらの条件を満たすためには，従来の抗がん剤開発とはまったく異なるアプローチが必要であり，リポソームを用いたホウ素デリバリーシステムが有望である注目されている．その利点として① 一度に大量のホウ素10をがん細胞に送り込むことが可能であること，② ホウ素薬剤自体に薬理活性を持たせる必要がないこと，③ 受容体選択性を高めるためにリポソーム表面に対応するリガンドを導入することにより能動的な送達(アクティブターゲティング)が可能であること，が挙げられる．

リポソームを用いたホウ素デリバリー法として，大きく二つの方法に分けられる．一つは，リポソーム内にホウ素薬剤を封入する方法である．この方法は，一般的なリポソームを用いたDDSを応用するものであり，BSHなどのホウ素化合物を封入する．もう一つの方法は，リポソーム膜にホウ素を埋め込む方法である．この方法では，リポソーム内にさらに抗がん剤などの薬剤を封入することができるため，化学療法との複合治療が期待できる．いずれの場合も，さまざまなリガンドをリポソーム膜に結合させることにより，能動的に標的細胞に取り込ませるような機能を持たせることが可能となってきた．

1. ホウ素薬剤内封型リポソーム

1991年に柳衛らによってホウ素薬剤を内封したリポソームははじめて報告された[14]．エッグPC(phosphatidylcholine)，コレステロール，DTP-DPPE(3-(2-pyridyldithio)propionyl-dipalmitoylphosphatidylethanolamine))からリポソームを調製し，BSHを封入した後，anti-human CEA(carcinoembryonic antigen)モノクローナル抗体を結合させたBSH内封イムノリポソームであった．AsPC-1(ヒト膵臓がん)細胞を移植したヌードマウスにBSH封入イムノリポソームを投与し中性子照射を行ったところ，腫瘍増殖が50%以下に抑えられることを見いだしている[15]．Hawthorneらは，DSPC(distearoylphosphatidylcholine)とコレステロールを用いて，さまざまなホウ素イオンクラスターを封入したリポソームを報告している[16,17]．

ホウ素封入リポソームを細胞選択的かつ能動的に取り込ませるために葉酸[18]，細胞増殖因子の一つであるEGF(上皮細胞増殖因子)[19]，トランスフェリン[20,21]，EGFRモノクローナル抗体[22]などのリガンドを表面に修飾したリポソームの研究が行われてきた．

2. ホウ素脂質型リポソーム

前述のようにホウ素イオンクラスターを封入した内封型リポソームは高い治療効果が得られる可能性がある．しかしながら，このホウ素イオンクラスターが内封されているリポソームは非常に高いイオン濃度であり高浸透圧的な溶液であることから，内

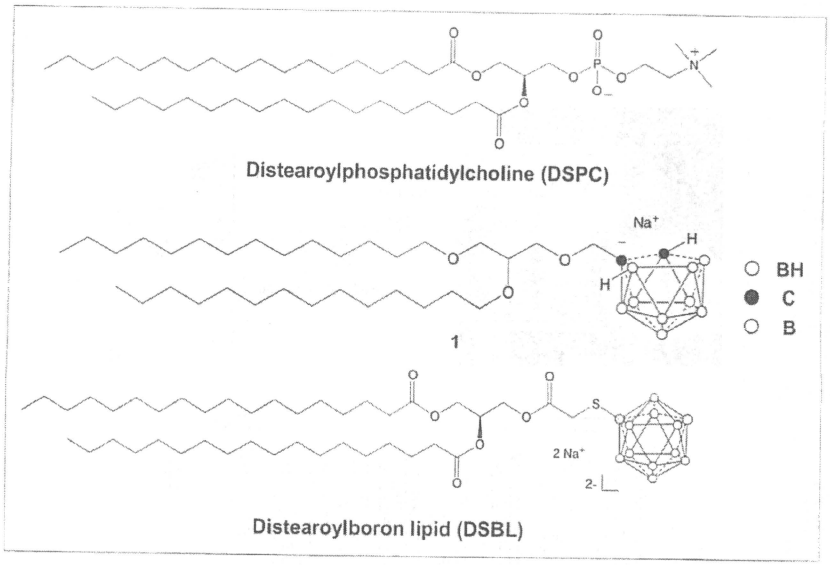

図4　ホウ素脂質の化学構造

封されているホウ素薬剤がリポソーム内部から容易に漏れ出てしまう問題が生じている．分子間相互作用により自己集合化しているリポソームの脂質二分子膜は非常に密度が高いため，この二分子膜へホウ素分子を導入できれば，非常に高濃度でホウ素をデリバリーできると考えられる．このようにリポソーム膜内にホウ素を導入させることで，リポソーム内には抗がん剤などさまざまな薬剤が封入できることから，BNCT と化学療法の複合治療も可能となる．

筆者らはリポソームを構成する脂質二分子膜中のリン脂質の骨格に着目した．リン脂質 DSPC の水溶性部位であるホスファチジルコリン部位(図4)にかご状のホウ素イオンクラスター(*nido* 型カルボラン)を導入した二本鎖ホウ素イオンクラスター脂質1を設計し合成にはじめて成功した[23]．合成したイオン性ホウ素クラスター脂質1は安定なリポソームを形成することが電子顕微鏡などで確認された．しかし *in vivo* 実験においてこのリポソームを腫瘍移植マウスに投与したところ，7 mgB/kg 投与において中性子照射後に顕著な延命効果がみられたものの，14 mgB/kg 投与した場合に重篤な急性毒性がみられた[21,23]．筆者らはこの *nido* 型カルボランが毒性に深く関わっていると考え，その代わりにより低毒性で非常に代謝が早く，実際の臨床に用いられ

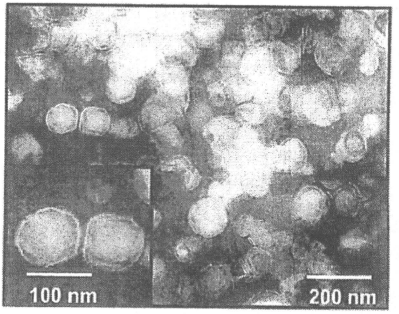

図5　DSBL リポソームの二分子膜構造形成
(電子顕微鏡写真)

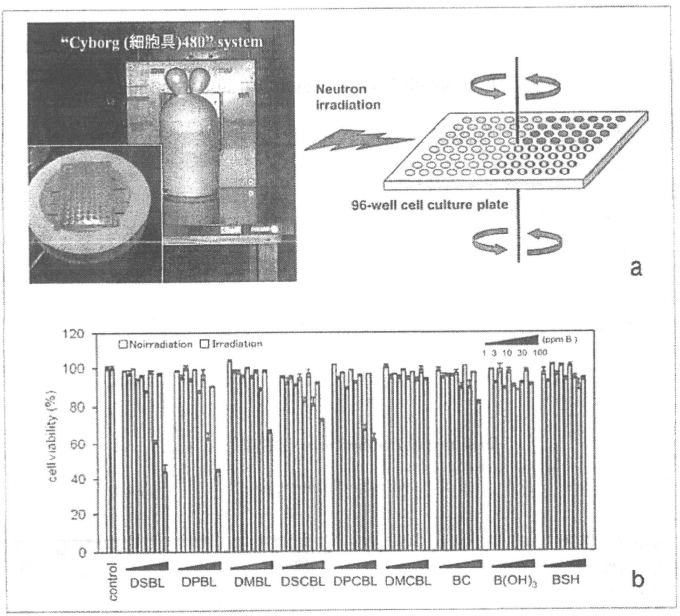

図6　ハイスループット細胞レベル BNCT 効果アッセイ法(a)と BNCT 致死効果(b)

ている BSH を導入した二本鎖ホウ素イオンクラスター脂質 DSBL(distearoylboron lipid)を再設計しその開発に成功した[26,27]．

ホウ素脂質 DSBL のリポソーム化

合成したホウ素クラスター脂質 DSBL，DSPC，コレステロール(X：1-X：1，X=0～0.5)を用いて逆相蒸発(REV)法によりリポソームを調製した後，100 nm のフィルターを用いてサイジングした．ICP-AES 法を用いて得られたリポソームのリン脂質とホウ素の濃度を定量し，各々モル比に換算した．その結果，形成したリポソーム膜内の DSBL/DSPC 比は，調整した混合比に比例していることがわかった．さらに，得られたリポソームの粒子径ならびにゼータ電位を測定した結果，DSBL の混合比に関わらず粒子径は 100 nm 前後に分布しており，ゼータ電位は DSBL により負に大きく帯電していることがわかった．また，DSBL(25%)のリポソームに関して，透過型電子顕微鏡測定を行ったところ，図5に示すように，直径 100 nm の二分子膜構造を形成していることがわかった．

細胞レベルでの BNCT 効果

BNCT のためのホウ素薬剤としての有用性を評価する方法として，筆者らは，図6aに示すように 96 ウェルプレートを用いたハイスループットスクリーニング評価法を開発した．この方法により，1 度の中性子照射で 480 サンプル(5 プレート)の評価が可能となる．colon 26 細胞を 96 ウェルプレートで 24 時間培養し，その後ホウ素リポソームを 1～100 ppm ホウ素濃度(ホウ素 10 濃縮化合物を使用)に 30 分間接触させた後，培地を新しく交換し，中

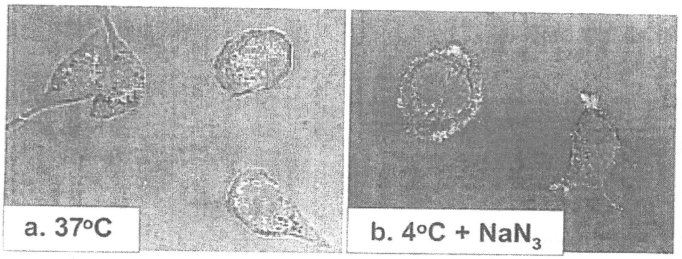

図7　エンドサイトーシスによる蛍光標識ホウ素リポソームの細胞内取り込み

性子照射を行った．その結果，**図6b**に示すように，BSHおよびホウ酸ともに細胞増殖抑制効果はみられないにも関わらず，DPBLやDSBLでは，細胞増殖抑制効果がみられた．このことから，BSHおよびホウ酸は細胞内から，新しい培地中にwash outされてしまうが，ホウ素リポソーム(DPBL，DSBL)は細胞内に長時間留まることが明らかとなった．

そこで，蛍光脂質(PKH67)を用いてホウ素リポソームを蛍光標識し，マウス大腸がん細胞(colon 26)に37度で3時間暴露したところ，**図7a**に示すように細胞質に取り込まれていることがわかった．一方，この蛍光標識ホウ素リポソームを細胞のエンドサイトーシスが起こらない条件であるアジ化ナトリウム存在下，4度で同様に3時間暴露したところ，**図7b**に示すように細胞膜表面に存在しているものの細胞質には取り込まれていないことから，ホウ素リポソームはエンドサイトーシスを経由して細胞内に取り込まれていることが明らかとなった[20]．

腫瘍移植マウスを用いたBNCT効果

1. 急性毒性試験と生体内残存濃度

*nido*型カルボランを導入した二本鎖ホウ素イオンクラスター脂質1を用いた場合，急性毒性が問題であったため，まず健康マウスを用いてホウ素脂質DSBLを25%および50%含むホウ素リポソームの限界投与量および，肝臓，脾臓，腎臓といった主要臓器での代謝を確認した．ホウ素リポソームにはRES(細網内皮系組織)を回避するため，PEG 2000を結合したDSPE(distearoyl phosphatidylethanolamine)を脂質に対して10%用いた．その結果，DSBL-50%リポソームをホウ素濃度で30 mgB/kg投与した場合に，急性毒性がみられたもののそれ以下の濃度では急性毒性はみられなかった．30 mgB/kgの濃度では，投与脂質濃度が非常に高いために静脈注射によって血管が詰まったためであると考えられる．さらに，投与3週間後の各臓器におけるホウ素残存量を調べたところ，いずれの臓器にもほとんど蓄積していなかった．このことから，dodecaborate型ホウ素脂質は生体内から速やかに排出されることがわかった．

2. 腫瘍移植マウスを用いたホウ素リポソームのBNCT効果

Colon 26細胞を移植したBALB/cマウス(生後6週間，16～18 g)にPEG化DSBL-25%ホウ素リポソーム(20 mg B/kg)を尾静脈投与してホウ素の体内挙動を調べた．投与24～72時間後に各臓器を分画し，ホウ素濃度をICP-AESにて測定した．**図8**に示すように，脾臓・肝臓では非常に高いホウ素蓄積がみられ，腫瘍内ホウ素蓄積量は，投与24時間後にホウ素濃度で23 ppmで，時間とともに蓄積濃度は低下した．BNCTは，標的部位へのホウ素デリバリーと中性子照射のダブルターゲティング治療法であるため，脾臓・肝臓のようにホウ素が高濃度で集積しても，中性子照射をしない限り細胞毒性を示さないことが特徴である．

そこで，ホウ素リポソーム(20 mg^{10}B/kg)をColon 26細胞を左太腿部に移植したBALB/cマウス

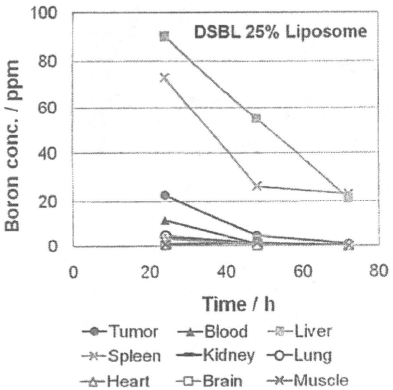

図8 Colon 26 マウス大腸がん移植マウスへのホウ素リポソームの各臓器分布

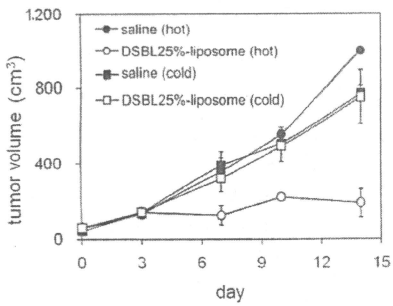

図9 Colon 26 マウス大腸がん移植マウスを用いた BNCT 効果

に尾静脈投与し，24 時間後にマウスの左太腿部に 30 分中性子照射(1.8×10^{12} neutrons/cm^2)を行った．中性子照射は独立行政法人・日本原子力研究開発機構東海研究開発センターの原子炉 JRR4 において行い，中性子照射後は経過を 2 週間観察した．その結果，図 9 に示すように，中性子照射を行ったのみのマウスおよび中性子照射をしていないマウス群では，腫瘍が成長したのに対し，ホウ素リポソームを投与し中性子照射したマウス群では，照射後 1 週間で腫瘍の萎縮がみられ，2 週間後も腫瘍の成長を顕著に抑制した[28]．このことから，リポソームを用いるホウ素デリバリーシステムは BNCT に対して非常に有効な手法となる可能性が示唆された．

おわりに

現在 BNCT に必須な熱中性子源として世界的に小型加速器の開発が行われており，日本でも現在京都大学で開発されている BNCT 用小型加速器は，すでに動物実験による BNCT 効果の検証が終わり，いよいよ医療機器としての認可を目指し，臨床治験実施に向けた研究が進んでいる．したがって，近い将来都市部病院に併設型加速器の設置が実現すれば，BNCT が放射線療法の一般的治療法の一つになるであろう．

その一方で，BNCT では 1950 年代に開発された BSH，BPA という 2 剤以外には，まだ臨床応用されたホウ素薬剤は登場してない．高い治療効果と適応疾患拡大のためにも，腫瘍部位への高効率ホウ素デリバリーシステム開発が急務となっており，本稿で紹介したリポソームホウ素デリバリーは，次世代 BNCT のための有望な手段となると期待される．

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Undecahydro-*closo*-dodecaborates as good leaving groups in organic synthesis: generation of substituted styrenes *via* elimination of arylethyl dodecaborates

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New functionalized arylethyl undecahydro-*closo*-dodecaborates (*S*,*S*-disubstituted $B_{12}H_{11}SH^{2-}$, *N*,*N*-disubstituted $B_{12}H_{11}NH_3^-$ and *O*-substituted $B_{12}H_{11}OH^{2-}$) are prepared by a simple one-step reaction. Moderate to good yields are obtained in the presence of various functional aryl groups. The synthesis of functionalized styrene derivatives can be readily achieved by treating arylethyl undecahydro-*closo*-dodecaborates with various bases. The scope and limitations of this procedure are demonstrated by investigating an array of alkylated dodecaborates. Based on an E2 elimination reaction, we identify the mechanistic pathway for dealkylation of arylethyl dodecaborates. Mechanistic studies indicate the following essential requirements to promote the elimination reaction: (i) the presence of α-CH acidity of the phenethyl group; (ii) steric hindrance; (iii) a substituted heteroatom on the *closo*-$B_{12}H_{11}^{2-}$ cage and (iv) the presence of an electron-withdrawing group on the aromatic ring.

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Introduction

Functionalized derivatives of the undecahydro-*closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ are promising candidates for boron neutron capture therapy (BNCT).[1,2] For example, the thiol-substituted derivative, mercaptoundecahydro-*closo*-dodecaborate(2–) [HS–$B_{12}H_{11}]^{2-}$ **1a**, is utilized in the treatment of gliomas.[3,4] To link the boron cluster $[B_{12}H_{12}]^{2-}$ to organic moieties known to be tumor-seeking,[5–10] the icosahedron itself is not suitable. There are two approaches to functionalize the $[B_{12}H_{12}]^{2-}$ anion: (i) the introduction of a reactive centre such as an amino, mercapto or hydroxy group into the boron cage, followed by the attachment of a side chain containing a functional group;[11–14] (ii) direct synthesis of $[B_{12}H_{12}]^{2-}$ oxonium and its use in the syntheses of various functional derivatives of the $[B_{12}H_{12}]^{2-}$ anion for BNCT.[15]

Alkylation of **1a** with excess halide generally results in the formation of *S*,*S*-disubstituted sulfonium derivatives.[12] Moreover, stable thioesters were obtained from the acylation of **1a** with acid halides.[12] The alkylation of amino-undecahydro-*closo*-dodecaborate(1–) $[H_3N–B_{12}H_{11}]^-$ **1c**, which was first described by Hertler and Raasch,[16] leads to mixtures of mono-, di- and trialkylated products.[11] The degree of alkylation is governed by the steric demand of the alkyl chain. The formation of a Schiff base and its subsequent reduction to a primary amine has been reported for **1c** using aromatic and α,β-unsaturated aldehydes.[17] Alkylation of hydroxyundecahydro-*closo*-dodecaborate(2–) [HO–$B_{12}H_{11}]^{2-}$ **1d**[18] was also studied.[13] It was demonstrated that **1d** is a very weak nucleophile, and that its alkylation under strong basic conditions results in monoalkylated derivatives. Furthermore, monoalkylated derivatives of **1d** were prepared *via* the ring-opening reaction of the tetramethylene oxonium derivative with different nucleophiles.[14] Recently, there was a demonstration of the possibility of alkylation of undecahydro-*closo*-dodecaborates with alkyne halides to provide dodecaborate terminal alkyne groups for click chemistry.[19,20]

In contrast, under basic conditions in certain cases, a boron cluster acts as a good leaving group. For example, *ortho*-carboranyl carbinols undergo a retroaddition reaction in the presence of NaOH, revealing that *ortho*-carborane can be used as a protective group for carbonyl groups.[21] Furthermore, a similar retroaddition can also be observed in [3 + 2] annulations between *o*-carboranyltrimethylsilane and conjugated carbonyl compounds.[22] In this example, in the protection of the sulfur atom, both *ortho*-carborane and thioether derivatives of **1a** behaved as good leaving groups.[12] In this reaction, a cyanoethyl group was dealkylated from unsymmetric *S*,*S*-dialkylated sulfoniums $[B_{12}H_{11}SR^1R^2]^-$ in the presence of tetramethylammonium hydroxide (TMAOH) at room temperature.

In this paper, we report that under basic conditions, mercapto-, amino- and hydroxyl-undecahydro-*closo*-dodecaborane anions act as good leaving groups in the formation of substituted styrenes from the corresponding arylethyl-substituted dodecaborate derivatives. In this transformation, dianion formation is more favorable as compared to the formation of monoanion molecules.

Results and discussion

Alkylation reactions

The preparation of symmetrical *S*,*S*-di-alkylated sulfonium salts $[B_{12}H_{11}SR_2]^-$ (**2–13**) is illustrated in Scheme 1. Alkylation of **1a** with phenethyl bromide proceeded in acetonitrile–water (4 : 1) for 24 h at room temperature to provide *S*,*S*-bisphenethyl sulfonium **2** in 81% yield. Similarly, *S*,*S*-disubstituted derivatives **3** and **7–12** were obtained from **1a** in 72–90% yields. However, alkylation of **1a** with methoxyphenethyl bromides and 2-bromo-1-phenylpropane required 48 h to provide the desired products **4–6** and **13** in 45–53% yields. The longer reaction times and lower product yields were the result of either the electron-donating effect of the methoxy group

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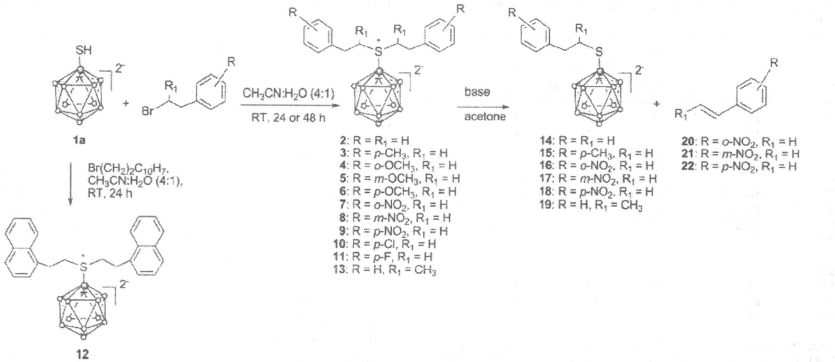
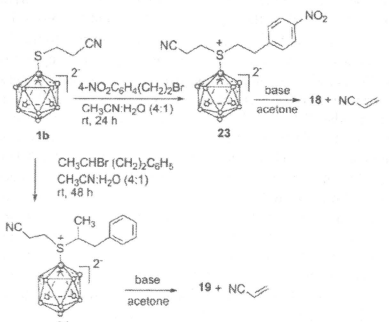


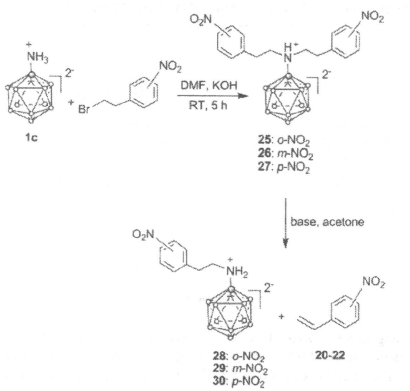
Scheme 1

of the methoxyphenethyl bromides or the steric effect of the methyl group of 2-bromo-1-phenylpropane.

Unsymmetrical sulfonium salts (**23** and **24**) were prepared from the reaction of **1b** with *p*-nitrophenethyl bromide and 2-bromo-1-phenylpropane in 87% and 53% yields, respectively, (Scheme 2).



Scheme 2



Scheme 3

Gabel *et al.* have reported a method for preparing mono-, di- and tri-alkylated amino-undecahydro-*closo*-dodecaborates $[B_{12}H_{11}NH_2R]^-$, $[B_{12}H_{11}NHR_2]^-$ and $[B_{12}H_{11}NR_3]^-$, by the reaction of **1c** with various alkyl halides in dimethylsulfoxide (DMSO), under strong basic conditions at room temperature.[11] We applied this protocol for preparing *N*,*N*-dialkylated derivatives (**25–27**). Using *N*,*N*-dimethylformamide (DMF) instead of DMSO as a solvent was effective for the dialkylation of **1c** with *o*-nitro-, *m*-nitro- and *p*-nitrophenethyl bromides to produce **25–27** in 20–30% yields (Scheme 3). Low yields of **25–27** can be explained by the initial attack of a base (hydroxyl anion) on nitrophenethyl bromide to produce nitrostyrene. We were able to separate nitrostyrene from the reaction medium as a byproduct in 28–79% yield, with a structure in accord with that described in the literature.[23]

An extension of a similar alkylation strategy to $[HO{-}B_{12}H_{11}]^{2-}$ was also examined. In this case, *O*-alkylated derivatives **31–33** were obtained by the reaction of **1d** with *o*-nitro-, *m*-nitro- and *p*-nitrophenethyl bromides in 22%, 27% and 18% yields, respectively, as shown in Scheme 4.

Elimination of aryl dodecaborates to form styrenes

We investigated the reactivity of the resulting compounds (**2–13**, **23–27** and **31–33**) in elimination reactions. The results are shown in Schemes 1 and 2, and Table 1. Conversion of symmetrical (**2–13**) and unsymmetrical (**23** and **24**) sulfonium salts into the desired thioethers **14–19** was carried out in acetone at room temperature,

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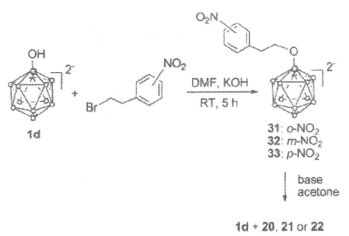
Table 1 Dealkylation of *S,S*-disubstituted derivatives **2–13**, **23** and **24** with different bases

Substrate	Thioether (nitrostyrene)	Yield (%) KOH	TMAOH	DMAP	TEA
2[a]	**14**	15	77	67	52
3[a]	**15**	5	72	58	43
4[a]	—	—	—	—	—
5	—	—	—	—	—
6[b]	—	—	—	—	—
7[c]	**16 (20)**	62 (39)	93 (74)	80 (61)	71 (52)
8[c]	**17 (21)**	45 (28)	82 (66)	69 (47)	57 (35)
9[d]	**18 (22)**	63 (42)	97 (79)	80 (63)	69 (56)
10[b]	—	—	—	—	—
11[b]	—	—	—	—	—
12[b]	—	—	—	—	—
13[e]	**19**	40	90	69	55
23[e]	**18**	67	98	90	82
24[e]	**19**	70	97	88	80

[a] Reaction time: 24 h in KOH, 10 min in TMAOH, 6 h in DMAP and 6 h in TEA. [b] Reaction time: 24 h in KOH, 20 min in TMAOH, 24 h in DMAP and 24 h in TEA. [c] Reaction time: 1 h in KOH, 10 min in TMAOH, 1 h in DMAP and 1 h in TEA. [d] Reaction time: 30 min. in KOH, 10 min in TMAOH, 30 min in DMAP and 30 min in TEA. [e] Reaction time: 2 h in KOH, 10 min in TMAOH, 30 min in DMAP and 30 min in TEA.



Scheme 4

using KOH, TMAOH, *N,N*-dimethylaminopyridine (DMAP) or triethylamine (TEA) as the base (Schemes 1 and 2, and Table 1).

This method was also applied to the dealkylation of the *N,N*-disubstituted derivatives **25–27** and *O*-alkylated derivatives **31–33** (Schemes 3 and 4, and Table 2). Unlike the volatile styrenes, which are derived by base treatment of compounds **2**, **3**, **10**, **11** and **13** *in situ*, nitrostyrenes (**20–22**) are sufficiently stable to be bottled and stored.

Gabel *et al.* reported that bis-cyanoethylmercaptoundecahydro-*closo*-dodecaborate loses one substituent upon treatment with TMAOH to yield the corresponding thioether.[12] However, studies on the mechanistic pathways of dealkylation reactions of the aryl dodecaborates have not yet been conducted. Therefore, in this study, we have attempted the dealkylation of aryl dodecaborates using different bases. As predicted, the products formed in the reaction were the elimination products, monoalkylated derivatives and styrenes.

To understand the effect of the base strength on the present elimination reaction, the reaction of **2** with different bases was carried out in acetone. TMAOH showed the highest activity, giving **14** in 77% yield. DMAP and TEA were also effective for

Table 2 Dealkylation of *N,N*-disubstituted derivatives of **1c** and *O*-substituted derivatives of **1d** with different bases

Substrate	Product	Yield (%) KOH	TMAOH	DMAP	TEA
25[a]	**28**	51	82	70	65
26[a]	**29**	33	70	65	49
27[a]	**30**	64	87	72	50
31[b]	**1d**	39	62	55	39
32[b]	**1d**	21	56	48	34
33[b]	**1d**	52	70	60	47

[a] Reaction time: 3 h in KOH, 30 min in TMAOH, 1 h in DMAP and 1 h in TEA. [b] Reaction time: 24 h in KOH, 120 min in TMAOH, 8 h in DMAP and 8 h in TEA.

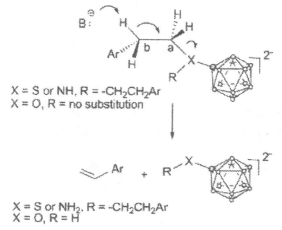
the reaction but **14** was obtained in lower yields of 67 and 52%, respectively (Table 1). When KOH was used, the reaction rates and yields were low. This can be explained by the incomplete solubility of KOH in acetone. Equimolar reactions of base and aryl dodecaborates (**2–13** and **23–27**) led to the loss of one aryl group. However, the excessive amount of base led to the formation of **1a** or **1c**.

The reaction of dodecaborates containing a cyanobenzyl group with base was unsuccessful under similar reaction conditions. For this reason the presence of β-hydrogen on the boron-cluster-substituted ethylene group is an important factor in the elimination reaction.

On the other hand the elimination of these aryl dodecaborates is strongly affected by the aryl moiety attached at the β-position of the ethyl group. Aryl groups constituting electron-withdrawing substituents will increase the acidity of the β-hydrogen. In this case, the overall reaction rate was greater, with satisfactory yields occurring even in the presence of a weak base. Better results were obtained when **7–9** or **23–27** were used as substrates. However, aryl groups with an electron-donating substituent will increase electron density at the β-CH_2 on the boron-cluster-substituted ethylene group, thus presumably decreasing the acidity of the β-hydrogen atom. Although **10** and **11** have electron-withdrawing substituents on the aromatic group, the resonance effect of the halogen groups may affect the β-hydrogen absorption by bases. Therefore, we were unable to convert *S,S*-disubstituted derivatives (**4–6** and **10–12**) into the desired thioethers. Based on these results, we note the following trend of aryl group influence on the reaction rate: *p*-$NO_2C_6H_5$ > *o*-$NO_2C_6H_5$ > *m*-$NO_2C_6H_5$ > C_6H_5 > *p*-$CH_3C_6H_5$.

Furthermore, the effect of steric hindrance on the elimination reaction was also studied using compound **13** as a substrate. The observed reactivity of the branched derivative **13** towards elimination, as compared to that of compound **2**, can be attributed to the steric effect of the methyl group and the higher stability of the resulting (1*E*)-1-propenylbenzene as compared to styrene.

Based on these data, a plausible reaction pathway is depicted in Scheme 5. This implies that there is an interaction between the base used and the aryl dodecaborate. This pathway is a concerted process with the following characteristics: (i) simultaneous removal of the proton by the base; (ii) loss of X–dodecaborate (where X = S, N or O) as a leaving group and (iii) formation of the π-bond. Therefore, highly substituted systems undergo E2 elimination more rapidly due to the stability of the resulting products. The C–$XB_{12}H_{11}^{2-}$ bond (where X = S, N or O) is broken



Scheme 5

during the rate-determining step. Therefore, the rate does depend on the nature of the leaving group. Since the base is involved in the rate-determining step, the nature of the base is a very important factor in an E2 reaction. More reactive bases will favour an E2 reaction, as indicated from the results in Tables 1 and 2.

Conclusions

We succeeded in preparing a series of structurally interesting arylethyl undecahydrododecaborates in good yields. The *S,S*-dialkylated sulfonium salts $[B_{12}H_{11}SR_2]^-$, *N,N*-dialkyalted derivatives $[B_{12}H_{11}NHR_2]^-$ and *O*-alkylated derivatives $[B_{12}H_{11}OR]^{2-}$ underwent elimination reactions in the presence of various bases such as KOH, TMAOH, DMAP and TEA. The presence of electron-withdrawing groups substituted at the aromatic ring as well as a substituent (R^1) at the α-position of the symmetric and unsymmetric *S,S*-dialkylated sulfonium salts of the undecahydrododecaborate accelerated the rate of elimination. Although undecahydrododecaborate cluster derivatives have attracted attention as potential water-soluble boron carriers in neutron capture therapy, the behaviour of undecahydrododecaborate derivatives has not been thoroughly studied earlier. In this regard, the current investigation is important for the development of undecahydrododecaborate-conjugated molecules in organic synthesis.

Experimental section

Materials and instruments

^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL JNM-AL 300 (300 MHz) and VARIAN UNITY-INOVA 400 (400 MHz) spectrometers. Chemical shifts of ^{1}H NMR and ^{13}C NMR were expressed in parts per million (ppm, δ units), and coupling constant (J) values were expressed in units of hertz (Hz). ^{11}B NMR spectra were recorded on a JEOL JNM-AL 300 spectrometer (96.3 MHz) and the chemical shifts were reported in δ units relative to external $BF_3{\cdot}Et_2O$ in $CDCl_3$. IR (cm^{-1}) spectra were determined as KBr discs on a Shimadzu FTIR-8600PC spectrometer. Electron spray ionization (ESI) mass spectra were recorded on a Shimadzu LCMS-2010 eV spectrometer. Elemental analyses were performed by a Perkin-Elmer 2400 automatic elemental analyzer. All compounds gave elemental analysis within ± 0.4% of the theoretical values. Analytical thin layer chromatography (TLC) was performed on glass plates of silica gel 60 GF_{254} (Merck). Visualization was accompanied by UV light (254 nm), I_2, $KMnO_4$, or $PdCl_2$. Melting points (mp) were determined on an Asone ATM-01 melting point apparatus. Preparative TLC was carried out using 0.75 mm layers of silica gel 60 GF_{254} (Merck) made from water slurries on glass plates of dimensions 20 × 20 cm^2, followed by drying in air at 100 °C. Column chromatography was conducted on silica gel (Merck Kieselgel 70–230 mesh). Analytical grade aryl bromides were purchased from Aldrich Chemical Co. and were not generally purified prior to use. Starting materials (**1a–1d**) were prepared as described in the literature.[11,12,16,18]

S,S-Bis(phenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (2)

A solution of phenethyl bromide (1.57 g, 8.5 mmol) in 10 ml CH_3CN was added dropwise over 20 min to a stirring solution of **1a** (500 mg, 1.55 mmol) in acetonitrile–water (4 : 1, 125 ml). The stirring continued for 24 h (48 h for compounds **4–6** and **13**) at room temperature. The solvent was removed on a rotary evaporator and the obtained solid was redissolved in acetonitrile (10 ml). The insoluble material was removed by filtration and the product was precipitated with ether (300 ml). A white precipitate was collected by filtration and recrystallized from water to yield (576 mg, 81%) white needle crystals of the desired product: mp 278–280 °C; IR (KBr, cm^{-1}) 3024, 2954 (ν_{CH}), 2495 (ν_{BH}), 1481, 1415 (ν_{CH}), 1049 ($\nu_{B\text{-}B}$), 948, 837, 718 (ν_{CH}). ^{1}H NMR (300 MHz, CD_3CN) δ = 7.17–7.3 (m, 10H, CH-phenyl), 3.27 (t, 4H, J_{CH} = 14.4 Hz, S-CH_2), 3.09 (s, 12H, $N(CH_3)_4$), 3.05 (t, 4H, J_{CH} = 14.4 Hz, phenyl-CH_2), 1.85–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN) δ = 129.9, 129.3, 128.9 (12C, CH and C-phenyl), 56.2 (4C, $N(CH_3)_4$), 42.2 (2C, phenyl-CH_2), 37.4 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ −15.59 (bs, 1B, B1), −18.98 (d, J_{BH} = 163.9 Hz, 11B, B2-12). MS (ESI) m/z 382.4 [100, M^-]. Anal. Calc. for $C_{20}H_{41}B_{12}NS$: C, 52.52; H, 9.04; N, 3.06%. found: C, 52.37; H, 8.76; N, 2.89%.

S,S-Bis(4-methylphenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (3)

This compound was prepared from **1a** (500 mg, 1.55 mmol) and *p*-methylphenethyl bromide (1.69 g, 8.5 mmol) using the procedure described for **2** to give **3** (544 mg, 72%) as a white solid. mp 276–278 °C. IR (KBr, cm^{-1}) 3024, 2954 (ν_{CH}), 2496 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 ($\nu_{B\text{-}B}$), 948, 837, 721 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.12 (d, J_{CH} = 8.4 Hz, 4H, CH-phenyl), 7.05 (d, J_{CH} = 8.0 Hz, 4H, CH-phenyl), 3.25 (m, 4H, S-CH_2), 3.09 (s, 12H, $N(CH_3)_4$), 3.06 (m, 4H, phenyl-CH_2), 1.82–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 130.2, 128.7, 125.9 (12C, CH and C-phenyl), 56.2 (4C, $N(CH_3)_4$), 41.8 (2C, phenyl-CH_2), 37.3 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ −15.52 (bs, 1B, B1), −18.79 (d, J_{BH} = 161.87 Hz, 11B, B2-12). MS (ESI) m/z 411.4 [100, M^-]. Anal. Calc. for $C_{22}H_{45}B_{12}NS$: C, 54.44; H, 9.34; N, 2.89%. found: C, 54.37; H, 8.99; N, 2.73%.

S,S-Bis(2-methoxyphenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (4)

This compound was prepared from **1a** (500 mg, 1.55 mmol) and *o*-methoxyphenethyl bromide (1.83 g, 8.5 mmol) using the procedure described for **2** to give **4** (379 mg, 47%) as a white solid: mp > 300 °C. IR (KBr, cm^{-1}) 3026, 2955 (ν_{CH}), 2487 (ν_{BH}), 1485,

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1415 (ν_{CH}), 1049 ($\nu_{B\text{-}B}$), 949, 839, 725 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.19–6.87 (m, 8H, CH-phenyl), 3.81 (s, 6H, -OMe), 3.24 (m, 4H, S-CH_2), 3.09 (s, 12H, N(CH_3)$_4$), 3.05 (m, 4H, phenyl-CH_2), 1.75–0.51 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 158.3 (2C, O-Cphenyl), 129.8, 119.8, 113.8, 113.3 (10C, CH and C-phenyl), 56.2 (4C, N(CH_3)$_4$), 55.1 (2C, O-CH_3), 42.1 (2C, phenyl-CH_2), 36.8 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.56 (bs, 1B, B1), –19.02 (d, J_{BH} = 161.88 Hz, 11B, B2-12). MS (ESI) m/z 444.4 [100, M$^-$]. Anal. Calc. for $C_{22}H_{45}B_{12}NO_2S$: C, 51.07; H, 8.77; N, 2.71%. found: C, 50.88; H, 8.51; N, 2.59%.

S,S-Bis(3-methoxyphenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (5)

This compound was prepared analogously to **4**, using *m*-methoxyphenethyl bromide as the halide source to give **5** (411 mg, 51%) as a white solid: mp 265–267 °C; IR (KBr, cm^{-1}) 3025, 2954 (ν_{CH}), 2489 (ν_{BH}), 1481, 1415 (ν_{CH}), 1045 ($\nu_{B\text{-}B}$), 948, 838, 721 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.2–6.75 (m, 8H, CH-phenyl), 3.77 (s, 6H, -OMe), 3.25 (m, 4H, S-CH_2), 3.09 (s, 12H, N(CH_3)$_4$), 3.05 (m, 4H, phenyl-CH_2), 1.81–0.54 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 159.5 (2C, O-Cphenyl), 130.1, 119.4, 113.6, 113.5 (10C, CH and C-phenyl), 56.2 (4C, N(CH_3)$_4$), 55.1 (2C, O-CH_3), 42.1 (2C, phenyl-CH_2), 37.0 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.49 (bs, 1B, B1), –19.06 (d, J_{BH} = 162.52 Hz, 11B, B2-12). MS (ESI) m/z 444.4 [100, M$^-$]. Anal. Calc. for $C_{22}H_{45}B_{12}NO_2S$: C, 51.07; H, 8.77; N, 2.71%. found: C, 50.75; H, 8.49; N, 2.51%.

S,S-Bis(4-methoxyphenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (6)

This compound was prepared analogously to **4**, using *p*-methoxyphenethyl bromide as the halide source to give **6** (363 mg, 45%) as a white solid: mp 235–237 °C; IR (KBr, cm^{-1}) 3025, 2954 (ν_{CH}), 2489 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 ($\nu_{B\text{-}B}$), 945, 837, 722 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.22 (d, J_{CH} = 7.85 Hz, 4H, CH-phenyl), 6.77 (d, J_{CH} = 7.85 Hz, 4H, CH-phenyl), 3.75 (s, 6H, -OMe), 3.25 (m, 4H, S-CH_2), 3.09 (s, 12H, N(CH_3)$_4$), 3.03 (m, 4H, phenyl-CH_2), 1.82–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 159.7 (2C, O-Cphenyl), 131.0, 122.7, 113.5 (10C, CH and C-phenyl), 56.3 (4C, N(CH_3)$_4$), 55.4 (2C, O-CH_3), 42.2 (2C, phenyl-CH_2), 37.4 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.62 (bs, 1B, B1), –19.02 (d, J_{BH} = 161.26 Hz, 11B, B2-12). MS (ESI) m/z 444.4 [100, M$^-$]. Anal. Calc. for $C_{22}H_{45}B_{12}NO_2S$: C, 51.07; H, 8.77; N, 2.71%. found: C, 50.92; H, 8.63; N, 2.55%.

S,S-Bis(2-nitrophenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (7)

This compound was prepared from **1a** (500 mg, 1.55 mmol) and *o*-nitrophenethyl bromide (1.83 g, 8.5 mmol) using the procedure described for **2** to give **7** (724 mg, 85%) as a white solid: mp 285–287 °C; IR (KBr, cm^{-1}) 3028, 2956 (ν_{CH}), 2480 (ν_{BH}), 1527, 1353 (ν_{NO_2}), 1485, 1415 (ν_{CH}), 1049 ($\nu_{B\text{-}B}$), 949, 837, 725 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.99, 7.63–6.47 (m, 8H, CH-phenyl), 3.34 (m, 4H, S-CH_2), 3.07 (s, 12H, N(CH_3)$_4$), 3.02 (m, 4H, phenyl-CH_2), 1.69–0.32 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149.2 (2C, NO_2-Cphenyl), 130.1, 126.2, 122.5, 113.2 (10C, CH and C-phenyl), 56.3 (4C, N(CH_3)$_4$), 41.8 (2C, phenyl-CH_2), 37.0 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.57 (bs, 1B, B1), –18.93 (d, J_{BH} = 162.41 Hz, 11B, B2-12). MS (ESI) m/z 473.4 [100, M$^-$]. Anal. Calc. for $C_{20}H_{39}B_{12}N_3O_4S$: C, 43.89; H, 7.18; N, 7.68%. found: C, 43.66; H, 6.91; N, 7.42%.

S,S-Bis(3-nitrophenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (8)

This compound was prepared analogously to **7**, using *m*-nitrophenethyl bromide as the halide source to give **8** (673 mg, 79%) as a white solid: mp > 300 °C; IR (KBr, cm^{-1}) 3025, 2955 (ν_{CH}), 2495 (ν_{BH}), 1523, 1350 (ν_{NO_2}), 1485, 1415 (ν_{CH}), 1045 ($\nu_{B\text{-}B}$), 945, 837, 721 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 8.06, 7.67–7.52 (m, 8H, CH-phenyl), 3.35 (m, 4H, S-CH_2), 3.07 (s, 12H, N(CH_3)$_4$), 3.02 (m, 4H, phenyl-CH_2), 1.75–0.52 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 148.2 (2C, NO_2-Cphenyl), 130.1, 128.4, 119.6, 114.4 (10C, CH and C-phenyl), 56.2 (4C, N(CH_3)$_4$), 42.1 (2C, phenyl-CH_2), 37.1 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.55 (bs, 1B, B1), –18.96 (d, J_{BH} = 163.73 Hz, 11B, B2-12). MS (ESI) m/z 473.3 [100, M$^-$]. Anal. Calc. for $C_{20}H_{39}B_{12}N_3O_4S$: C, 43.89; H, 7.18; N, 7.68%. found: C, 43.52; H, 6.85; N, 7.39%.

S,S-Bis(4-nitrophenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (9)

This compound was prepared analogously to **7**, using *p*-nitrophenethyl bromide as the halide source to give **9** (766 mg, 90%) as a white solid: mp 293–295 °C; IR (KBr, cm^{-1}) 3022, 2955 (ν_{CH}), 2491 (ν_{BH}), 1525, 1352 (ν_{NO_2}), 1489, 1416 (ν_{CH}), 1049 ($\nu_{B\text{-}B}$), 947, 838, 725 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 8.08 (d, J_{CH} = 8.4 Hz, 4H, CH-phenyl), 7.45 (d, J_{CH} = 9.2 Hz, 4H, CH-phenyl), 3.41 (m, 4H, S-CH_2), 3.09 (s, 12H, N(CH_3)$_4$), 3.01 (m, 4H, phenyl-CH_2), 1.81–0.54 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149.1 (2C, NO_2-Cphenyl), 131.7, 130.9, 123.5 (10C, CH and C-phenyl), 56.2 (4C, N(CH_3)$_4$), 42.0 (2C, phenyl-CH_2), 37.2 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.57 (bs, 1B, B1), –18.87 (d, J_{BH} = 163.59 Hz, 11B, B2-12). MS (ESI) m/z 473.5 [100, M$^-$]. Anal. Calc. for $C_{20}H_{39}B_{12}N_3O_4S$: C, 43.89; H, 7.18; N, 7.68%. found: C, 43.71; H, 7.02; N, 7.48%.

S,S-Bis(4-chlorophenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (10)

This compound was prepared from **1a** (500 mg, 1.55 mmol) and *p*-chlorophenethyl bromide (1.87 g, 8.5 mmol) using the procedure described for **2** to give **10** (622 mg, 76%) as a white solid: mp > 300 °C; IR (KBr, cm^{-1}) 3025, 2956 (ν_{CH}), 2495 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 ($\nu_{B\text{-}B}$), 948, 837, 721 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.3 (d, J_{CH} = 18.0 Hz, 4H, CH-phenyl), 7.17 (d, J_{CH} = 17.2 Hz, 4H, CH-phenyl), 3.28 (m, 4H, S-CH_2), 3.11 (s, 12H, N(CH_3)$_4$), 3.02 (m, 4H, phenyl-CH_2), 1.68–0.33 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 132.8 (2C, Cl-Cphenyl), 130.9, 129.6, 122.7 (10C, CH and C-phenyl), 56.2 (4C, N(CH_3)$_4$), 42.0 (2C, phenyl-CH_2), 37.3 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.53 (bs, 1B, B1), –18.99 (d, J_{BH} = 162.66 Hz, 11B, B2-12). MS (ESI) m/z 450.4 [100, M$^-$]. Anal. Calc. for $C_{20}H_{39}B_{12}Cl_2NS$: C, 45.65; H, 7.47; N, 2.66%. found: C, 45.29; H, 7.19; N, 2.34%.

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_S,S_-Bis(4-fluorophenethyl)sulfonio-undecahydro-_closo_-dodecaborate (1–) tetramethylammonium salt (11)

This compound was prepared from **1a** (500 mg, 1.55 mmol) and _p_-fluorophenethyl bromide (1.73 g, 8.5 mmol) using the procedure described for **2** to give **11** (614 mg, 80%) as a white solid: mp > 300 °C; IR (KBr, cm^{-1}) 3020, 2952 (ν_{CH}), 2480 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 949, 837, 721 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 7.2 (d, J_{CH} = 8.8 Hz, 4H, CH-phenyl), 7.05 (d, J_{CH} = 8.8 Hz, 4H, CH-phenyl), 3.32 (m, 4H, S-CH_2), 3.07 (s, 12H, N(CH_3)$_4$), 3.01 (m, 4H, phenyl-CH_2), 1.75–0.5 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 162.1 (2C, Cl-Cphenyl), 131.3, 130.3, 123.7 (10C, CH and C-phenyl), 56.3 (4C, N(CH_3)$_4$), 41.9 (2C, phenyl-CH_2), 37.3 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.55 (bs, 1B, B1), –18.98 (d, J_{BH} = 163.52 Hz, 11B, B2-12). MS (ESI) _m/z_ 419.3 [100, M^-]. Anal. Calc. for $C_{20}H_{39}B_{12}F_2NS$: C, 48.69; H, 7.97; N, 2.84%. found: C, 48.46; H, 7.68; N, 2.73%.

_S,S_-Bis(naphthethyl)sulfonio-undecahydro-_closo_-dodecaborate (1–) tetramethylammonium salt (12)

This compound was prepared from **1a** (500 mg, 1.55 mmol) and 1-(2-bromoethyl)naphthalene (2.0 g, 8.5 mmol) using the procedure described for **2** to give **12** (649 mg, 75%) as a white solid: mp 295–297 °C; IR (KBr, cm^{-1}) 3020, 2954 (ν_{CH}), 2491 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 948, 837, 718 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 7.46–7.07 (m, 14H, CH-naphthalene), 3.39 (m, 4H, S-CH_2), 3.11 (s, 12H, N(CH_3)$_4$), 3.07 (m, 4H, naphthalene-CH_2), 1.75–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 134.3, 133.1 132.2, 129.9, 129.2, 128.2, 127.3, 126.3, 125.6, 124.4 (20C, CH and C-naphthalene), 56.2 (4C, N(CH_3)$_4$), 42.0 (2C, phenyl-CH_2), 37.2 (2C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.57 (bs, 1B, B1), –18.97 (d, J_{BH} = 161.92 Hz, 11B, B2-12). MS (ESI) _m/z_ 482.4 [100, M^-]. Anal. Calc. for $C_{28}H_{45}B_{12}NS$: C, 60.33; H, 8.14; N, 2.51%. found: C, 59.98; H, 7.82; N, 2.28%.

_S,S_-Bis(1-phenypropan-2-yl)sulfonioundecahydro-_closo_-dodecaborate (1–) tetramethylammonium salt (13)

This compound was prepared from **1a** (500 mg, 1.55 mmol) and 2-bromo-1-phenylpropane (1.69 g, 8.5 mmol) using the procedure described for **2** to give **12** (576 mg, 53%) as a white solid: mp 255–257 °C; IR (KBr, cm^{-1}) 3028, 2958 (ν_{CH}), 2487 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 948, 840, 721 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 7.35–7.07 (m, 10H, CH-phenyl), 3.89 (m, 4H, S-CH), 3.08 (s, 12H, N(CH_3)$_4$), 3.02 (m, 4H, phenyl-CH_2), 1.35 (d, 6H, J_{CH} = 8.0 Hz, CH_3), 1.85–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 129.5, 129.1, 127.8 (12C, CH and C-phenyl), 56.2 (4C, N(CH_3)$_4$), 42.4 (2C, phenyl-CH_2), 40.8 (2C, S-CH), 22.7 (2C, CH_3). ^{11}B NMR (96.3 MHz; CD_3CN): δ –15.54 (bs, 1B, B1), –18.96 (d, J_{BH} = 163.21 Hz, 11B, B2-12). MS (ESI) _m/z_ 411.4 [100, M^-]. Anal. Calc. for $C_{22}H_{45}B_{12}NS$: C, 54.44; H, 9.34; N, 2.89%. found: C, 54.19; H, 9.07; N, 2.74%.

_S_-Phenethyl-thioundecahydro-_closo_-dodecaborate (2–) ditetramethylammonium salt (14)

To a solution of **2** (228 mg, 0.5 mmol) in acetone (15 ml), 1 equiv. of a 25% solution of TMAOH in methanol was added dropwise. A white precipitate of the product formed immediately. The precipitate was filtered off and dried to give **14** (163 mg, 77%) as a white solid: mp > 300 °C;. IR (KBr, cm^{-1}) 3025, 2955 (ν_{CH}), 2492 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 947, 838, 722 (ν_{CH}). ^1H NMR (300 MHz, CD_3CN) δ = 7.15–7.29 (m, 5H, CH-phenyl), 3.25 (m, 2H, S-CH_2), 3.11 (s, 24H, N(CH_3)$_4$), 3.02 (m, 2H, phenyl-CH_2), 1.79–0.52 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (75 MHz, CD_3CN) δ = 129.1, 128.6, 128.0 (6C, CH and C-phenyl), 54.5 (8C, N(CH_3)$_4$), 41.0 (1C, phenyl-CH_2), 37.2 (1C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.87 (bs, 1B, B1), –19.98 (d, J_{BH} = 161.7 Hz, 11B, B2-11), –21.59 (bs, 1B, B12). MS (ESI) _m/z_ 138.8 [100, M^-/2]. Anal. Calc. for $C_{16}H_{44}B_{12}N_2S$: C, 45.08; H, 10.4; N, 6.57%. found: C, 44.87; H, 10.11; N, 6.22%.

_S_-4-Methylphenethyl-thioundecahydro-_closo_-dodecaborate (2–) ditetramethylammonium salt (15)

This compound was prepared from **3** (242 mg, 0.5 mmol) using the procedure described for **14** to give **15** (158 mg, 72%) as a white solid: mp > 300 °C; IR (KBr, cm^{-1}) 3020, 2951 (ν_{CH}), 2495 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 947, 838, 725 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 7.15 (d, J_{CH} = 7.52 Hz, 2H, CH-phenyl), 7.07 (d, J_{CH} = 7.52 Hz, 2H, CH-phenyl), 3.29 (m, 2H, S-CH_2), 3.12 (s, 24H, N(CH_3)$_4$), 3.02 (m, 2H, phenyl-CH_2), 1.82–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 130.3, 129.1, 126.1 (6C, CH and C-phenyl), 54.3 (6C, N(CH_3)$_4$), 42.5 (1C, phenyl-CH_2), 36.9 (1C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.85 (bs, 1B, B1), –19.97 (d, J_{BH} = 161.97 Hz, 11B, B2-11), –21.56 (bs, 1B, B12). MS (ESI) _m/z_ 145.8 [100, M^-/2]. Anal. Calc. for $C_{17}H_{47}B_{12}N_2S$: C, 46.26; H, 10.73; N, 6.35%. found: C, 46.01; H, 10.69; N, 6.17%.

_S_-2-Nitrophenethylthio-undecahydro-_closo_-dodecaborate (2–) ditetramethylammonium salt (16)

This compound was prepared from **7** (236 mg, 0.5 mmol) using the procedure described for **14** to give **16** (189 mg, 93%) as a white solid: mp > 300 °C; IR (KBr, cm^{-1}) 3025, 2955 (ν_{CH}), 2487 (ν_{BH}), 1525, 1355 (ν_{NO_2}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 949, 837, 725 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 7.95, 7.61–6.43 (m, 4H, CH-phenyl), 3.35 (m, 2H, S-CH_2), 3.12 (s, 24H, N(CH_3)$_4$), 2.99 (m, 2H, phenyl-CH_2), 1.79–0.51 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 150.0 (1C, NO_2-Cphenyl), 130.3, 127.5, 123.1, 114.7 (5C, CH and C-phenyl), 54.3 (8C, N(CH_3)$_4$), 41.5 (1C, phenyl-CH_2), 37.2 (1C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.86 (bs, 1B, B1), –19.98 (d, J_{BH} = 162.19 Hz, 11B, B2-11), –21.55 (bs, 1B, B12). MS (ESI) _m/z_ 161.5 [100, M^-/2]. Anal. Calc. for $C_{16}H_{43}B_{12}N_3O_2S$: C, 40.77; H, 9.20; N, 8.92%. found: C, 40.55; H, 9.08; N, 8.68%.

_S_-3-Nitrophenethylthio-undecahydro-_closo_-dodecaborate (2–) ditetramethylammonium salt (17)

This compound was prepared from **8** (236 mg, 0.5 mmol) using the procedure described for **14** to give **17** (166 mg, 82%) as a white solid: mp > 300 °C; IR (KBr, cm^{-1}) 30204, 2956 (ν_{CH}), 2494 (ν_{BH}), 1526, 1351 (ν_{NO_2}), 1485, 1415 (ν_{CH}), 1045 (ν_{B-B}), 947, 838, 725 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 8.02, 7.63–7.5 (m, 4H, CH-phenyl), 3.37 (m, 2H, S-CH_2), 3.11 (s, 24H, N(CH_3)$_4$), 3.05 (m, 2H, phenyl-CH_2), 1.8–0.54 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz,

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CD_3CN) δ = 149.4 (1C, NO_2-Cphenyl), 13.8, 128.6, 120.3, 115.1 (5C, CH and C-phenyl), 56.2 (8C, $N(CH_3)_4$), 42.1 (1C, phenyl-CH_2), 37.1 (1C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.85 (bs, 1B, B1), –19.95 (d, J_{BH} = 162.24 Hz, 11B, B2-11), –21.61 (bs, 1B, B12). MS (ESI) m/z 161.5 [100, $M^-/2$]. Anal. Calc. for $C_{16}H_{43}B_{12}N_3O_2S$: C, 40.77; H, 9.20; N, 8.92%. found: C, 40.49; H, 9.11; N, 8.73%.

S-4-Nitrophenethylthio-undecahydro-*closo*-dodecaborate (2–) ditetramethylammonium salt (18)

This compound was prepared from **9** (236 mg, 0.5 mmol) using the procedure described for **14** to give **18** (197 mg, 97%) as a white solid: mp 275–277 °C; IR (KBr, cm^{-1}) 3024, 2954 (ν_{CH}), 2493 (ν_{BH}), 1522, 1355 (ν_{NO_2}), 1489, 1416 (ν_{CH}), 1049 (ν_{B-B}), 948, 837, 722 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 8.05 (d, J_{CH} = 6.75 Hz, 2H, CH-phenyl), 7.41 (d, J_{CH} = 6.75 Hz, 2H, CH-phenyl), 3.49 (m, 2H, S-CH_2), 3.11 (s, 24H, $N(CH_3)_4$), 3.03 (m, 2H, phenyl-CH_2), 1.79–0.52 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149. 9 (1C, NO_2-Cphenyl), 130.5, 130.0, 125.5 (5C, CH and C-phenyl), 54.2 (8C, $N(CH_3)_4$), 42.0 (1C, phenyl-CH_2), 37.6 (1C, S-CH_2). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.88 (bs, 1B, B1), –19.98 (d, J_{BH} = 162.34 Hz, 11B, B2-11), –21.59 (bs, 1B, B12). MS (ESI) m/z 161.5 [100, $M^-/2$]. Anal. Calc. for $C_{16}H_{43}B_{12}N_3O_2S$: C, 40.77; H, 9.20; N, 8.92%. found: C, 40.59; H, 9.15; N, 8.74%.

S-(1-Phenylpropan-2-yl)thioundecahydro-*closo*-dodecaborate (2–) ditetramethylammonium salt (19)

This compound was prepared from **13** (220 mg, 0.5 mmol) using the procedure described for **14** to give **19** (179 mg, 90%) as a white solid: mp 284–286 °C; IR (KBr, cm^{-1}) 3026, 2954 (ν_{CH}), 2489 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 947, 838, 722 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 7.33–7.04 (m, 5H, CH-phenyl), 3.77 (m, 2H, S-CH), 3.10 (s, 24H, $N(CH_3)_4$), 3.03 (m, 2H, phenyl-CH_2), 1.38 (d, 3H, J_{CH} = 9.1 Hz, CH_3), 1.82–0.53 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 130.0, 129.9, 127.0 (6C, CH and C-phenyl), 54.2 (8C, $N(CH_3)_4$), 42.6 (1C, phenyl-CH_2), 42.0 (1C, S-CH), 23.1 (1C, CH_3). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.87 (bs, 1B, B1), –19.98 (d, J_{BH} = 161.83 Hz, 11B, B2-11), –21.59 (bs, 1B, B12). MS (ESI) m/z 145.8 [100, $M^-/2$]. Anal. Calc. for $C_{17}H_{46}B_{12}N_2S$: C, 46.37; H, 10.53; N, 6.36%. found: C, 46.02; H, 10.23; N, 6.21%.

S-(2-Cyanoethyl)-*S*-(4-nitrophenethyl)sulfonio-undecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (23)

This compound was prepared from **1b** (187 mg, 0.5 mmol) and 4-nitrophenethyl bromide (630 mg, 8.5 mmol), using the procedure described for **2** to give **23** (195 mg, 87%) as a white solid: mp 239–241 °C; IR (KBr, cm^{-1}) 3024, 2952 (ν_{CH}), 2491 (ν_{BH}), 1523, 1355 (ν_{NO_2}), 1489, 1416 (ν_{CH}), 1049 (ν_{B-B}), 946, 837, 722 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 8.06 (d, J_{CH} = 7.3 Hz, 2H, CH-phenyl), 7.42 (d, J_{CH} = 7.3 Hz, 2H, CH-phenyl), 3.56 (m, 2H, S-CH_2), 3.29 (m, 2H, S-CH_2), 3.12 (s, 12H, $N(CH_3)_4$), 3.03 (m, 2H, phenyl-CH_2), 2.98 (m, 2H, CH_2-CN), 1.82–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 148.8 (1C, NO_2-Cphenyl), 131.2, 130.7, 123.5 (5C, CH and C-phenyl), 119.3 (C, CN), 56.3 (4C, $N(CH_3)_4$), 42.0 (1C, phenyl-CH_2), 37.2 (1C, S-CH_2), 31.2 (C, S-CH_2), 16.3 (C, CH_2-CN). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.82 (bs, 1B, B1), –19.96 (d, J_{BH} = 161.51 Hz, 11B, B2-11), –21.51 (bs, 1B, B12). MS (ESI) m/z 376.6 [100, M^-]. Anal. Calc. for $C_{15}H_{35}B_{12}N_3O_2S$: C, 39.92; H, 7.82; N, 9.31%. found: C, 39.59; H, 7.61; N, 8.99%.

S-(2-Cyanoethyl)-*S*-(1-phenypropan-2-yl)sulfonioundecahydro-*closo*-dodecaborate (1–) tetramethylammonium salt (24)

This compound was prepared from **1b** (187 mg, 0.5 mmol) and 2-bromo-1-phenylpropane (545 mg, 8.5 mmol), using the procedure described for **2** to give **24** (576 mg, 53%) as a white solid: mp 271–273 °C; IR (KBr, cm^{-1}) 3024, 2955 (ν_{CH}), 2487 (ν_{BH}), 1485, 1415 (ν_{CH}), 1049 (ν_{B-B}), 946, 840, 722 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 7.33–7.05 (m, 5H, CH-phenyl), 3.82 (m, 2H, S-CH), 3.25 (m, 2H, S-CH_2), 3.11 (s, 12H, $N(CH_3)_4$), 3.01 (m, 2H, phenyl-CH_2), 2.95 (m, 2H, CH_2-CN), 1.39 (d, 3H, J_{CH} = 5.9 Hz, CH_3), 1.78–0.52 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 130.0, 129.4, 127.0 (6C, CH and C-phenyl), 119.8 (C, CN), 56.2 (4C, $N(CH_3)_4$), 42.6 (2C, phenyl-CH_2), 40.9 (2C, S-CH), 32.1 (C, S-CH_2), 22.4 (2C, CH_3), 16.6 (C, CH_2-CN). ^{11}B NMR (96.3 MHz; CD_3CN): δ –9.86 (bs, 1B, B1), –19.93 (d, J_{BH} = 161.42 Hz, 11B, B2-11), –21.52 (bs, 1B, B12). MS (ESI) m/z 345.6 [100, M^-]. Anal. Calc. for $C_{16}H_{38}B_{12}N_2S$: C, 45.72; H, 9.11; N, 6.67%. found: C, 45.53; H, 8.87; N, 6.43%.

General synthesis for compounds 25–27

A solution of **1c** (125 mg, 0.54 mmol) and KOH (152 mg, 2.72 mmol) in dry DMF (15 ml) was stirred at room temperature under argon atmosphere. Nitrophenethyl bromide (2.59 g, 11.27 mmol) was added. After stirring for 5 h, the solvent was evaporated *in vacuo* and the orange residue dissolved in 10 ml of acetonitrile. The insoluble material was filtered off to give the *N*,*N*-bisalkylated derivative. This was recrystallized from water and dissolved in water–acetonitrile. A solid was precipitated by addition of 175 mg of tetrabutylammonium bromide (0.54 mmol) and chromatographed on TLC using MeOH–CH_2Cl_2 (1 : 4) as a mobile phase to give the *N*,*N*-bisalkylated derivative as white needle crystals.

***N*,*N*-Bis(2-nitrophenethyl)amino-undecahydro-*closo*-dodecaborate (1–) tetrabutylammonium salt (25).** (141 mg, 25%, R_f 0.29), mp 177–179 °C. IR (KBr, cm^{-1}) 3500 (ν_{NH}), 3030, 2939 (ν_{CH}), 2500 (ν_{BH}), 1525, 1351 (ν_{NO_2}), 1510 (ν_{BN}), 1485, 1407, 1350 (ν_{CH}), 1145 (ν_{CN}), 1055 (ν_{B-B}), 998, 952, 856, 725, 675 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 5.45 (bs, 1H, NH), 7.79, 7.59–7.23 (m, 8H, CH-phenyl), 3.43 (m, 4H, NH-CH_2), 3.05 (m, 8H, $N(CH_2\text{-})_4$), 3.02 (m, 4H, phenyl-CH_2), 1.55 (m, 8H, $N(CH_2\mathbf{CH_2}\text{-})_4$), 1.35 (m, 8H, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 0.95 (t, J = 7.56 Hz, 12H, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$), 1.79–0.51 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 148.5 (2C, NO_2-Cphenyl), 130.2, 127.2, 123.6, 114.2 (10C, CH and C-phenyl), 59.3 (4C, $N(CH_2\text{-})_4$), 52.4 (2C, NH-CH_2), 41.5 (2C, CH_2-phenyl), 24.3 (4C, $N(CH_2\mathbf{CH_2}\text{-})_4$), 20.3 (4C, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 13.8 (4C, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –3.12 (s, 1B, B1), –20.89 (bs, 11B, B2-12). MS (ESI) m/z 454.4 [100, M^-]. Anal. Calc. for $C_{32}H_{64}B_{12}N_4O_4$: C, 55.02; H, 9.23; N, 8.02%. found: C, 54.83; H, 8.92; N, 7.79%.

***N*,*N*-Bis(3-nitrophenethyl)amino-undecahydro-*closo*-dodecaborate (1–) tetrabutylammonium salt (26).** (170 mg, 30%, R_f 0.26),

mp 199–201 °C. IR (KBr, cm^{-1}) 3489 (ν_{NH}), 3035, 2951 (ν_{CH}), 2494 (ν_{BH}), 1523, 1352 (ν_{NO_2}), 1489 (ν_{BN}), 1485, 1405, 1350 (ν_{CH}), 1149 (ν_{CN}), 1057 ($\nu_{B\text{-}B}$), 995, 955, 857, 722, 677 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 5.52 (bs, 1H, NH), 7.73, 7.53–7.37 (m, 8H, CH-phenyl), 3.45 (m, 4H, NH-CH_2), 3.12 (m, 8H, $N(CH_2\text{-})_4$), 3.01 (m, 4H, phenyl-CH_2), 1.56 (m, 8H, $N(CH_2\mathbf{CH_2}\text{-})_4$), 1.35 (m, 8H, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 0.96 (t, J = 6.75 Hz, 12H, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$), 1.82–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149.6 (2C, NO_2-Cphenyl), 130.1, 129.0, 126.0, 114.8 (10C, CH and C-phenyl), 59.3 (4C, $N(CH_2\text{-})_4$), 52.5 (2C, NH-CH_2), 41.7 (2C, CH_2-phenyl), 24.4 (4C, $N(CH_2\mathbf{CH_2}\text{-})_4$), 20.3 (4C, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 13.8 (4C, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –3.07 (s, 1B, B1), –20.85 (bs, 11B, B2-12). MS (ESI) m/z 455.4 [100, M^-]. Anal. Calc. for $C_{32}H_{64}B_{12}N_4O_4$: C, 55.02; H, 9.23; N, 8.02%. found: C, 54.91; H, 8.95; N, 7.86%.

***N*,*N*-Bis(4-nitrophenethyl)amino-undecahydro-*closo*-dodecaborate (1–) tetrabutylammonium salt (27).** (113 mg, 20%, R_f 0.32), mp 169–171 °C. IR (KBr, cm^{-1}) 3505 (ν_{NH}), 3032, 2950 (ν_{CH}), 2499 (ν_{BH}), 1522, 1352 (ν_{NO_2}), 1499 (ν_{BN}), 1485, 1407, 1350 (ν_{CH}), 1145 (ν_{CN}), 1055 ($\nu_{B\text{-}B}$), 998, 952, 856, 725, 675 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 5.49 (bs, 1H, NH), 8.1 (d, J_{CH} = 8.4 Hz, 4H, CH-phenyl), 7.45 (d, J_{CH} = 9.2 Hz, 4H, CH-phenyl), 3.45 (m, 4H, NH-CH_2), 3.15 (m, 8H, $N(CH_2\text{-})_4$), 3.06 (m, 4H, phenyl-CH_2), 1.57 (m, 8H, $N(CH_2\mathbf{CH_2}\text{-})_4$), 1.36 (m, 8H, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 0.98 (t, J = 7.25 Hz, 12H, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$), 1.83–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 148.9 (2C, NO_2-Cphenyl), 130.2, 127.2, 125.9, 114.3 (10C, CH and C-phenyl), 59.3 (4C, $N(CH_2\text{-})_4$), 52.5 (2C, NH-CH_2), 41.6 (2C, CH_2-phenyl), 24.4 (4C, $N(CH_2\mathbf{CH_2}\text{-})_4$), 20.24 (4C, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 13.8 (4C, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –3.11 (s, 1B, B1), –20.91 (bs, 11B, B2-12). MS (ESI) m/z 455.4 [100, M^-]. Anal. Calc. for $C_{32}H_{64}B_{12}N_4O_4$: C, 55.02; H, 9.23; N, 8.02%. found: C, 54.77; H, 8.81; N, 7.69%.

***N*-(2-Nitrophenethyl)amino-undecahydro-*closo*-dodecaborate (1–) tetrabutylammonium salt (28)**

This compound was prepared from **25** (175 mg, 0.25 mmol), using the procedure described for **14** to give **28** (112 mg, 82%) as a white solid: mp 225–227 °C; IR (KBr, cm^{-1}) 3520 (ν_{NH}), 3035, 2945 (ν_{CH}), 2493 (ν_{BH}), 1525, 1352 (ν_{NO_2}), 1510 (ν_{BN}), 1485, 1405, 1350 (ν_{CH}), 1145 (ν_{CN}), 1056 ($\nu_{B\text{-}B}$), 998, 955, 857, 721, 677 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 6.87 (bs, 2H, NH_2), 7.71, 7.55–7.31 (m, 4H, CH-phenyl), 3.42 (m, 2H, NH-CH_2), 3.14 (m, 4H, $N(CH_2\text{-})_4$), 3.02 (m, 2H, phenyl-CH_2), 1.59 (m, 8H, $N(CH_2\mathbf{CH_2}\text{-})_4$), 1.36 (m, 8H, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 0.95 (t, J = 6.85 Hz, 12H, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$), 1.73–0.46 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149.0 (1C, NO_2-Cphenyl), 130.3, 128.5, 124.5, 113.9 (5C, CH and C-phenyl), 59.2 (4C, $N(CH_2\text{-})_4$), 52.0 (1C, NH_2-CH_2), 41.2 (1C, CH_2-phenyl), 24.2 (4C, $N(CH_2\mathbf{CH_2}\text{-})_4$), 20.3 (4C, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 13.9 (4C, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –3.15 (s, 1B, B1), –20.05 (s, 10B, J = 86.27 Hz, B2-11), –16.18 (s, 1B, B12). MS (ESI) m/z 306.5 [100, M^-]. Anal. Calc. for $C_{24}H_{57}B_{12}N_3O_2$: C, 52.46; H, 10.46; N, 7.65%. found: C, 52.12; H, 10.24; N, 7.38%.

***N*-(3-Nitrophenethyl)amino-undecahydro-*closo*-dodecaborate (1–) tetrabutylammonium salt (29)**

This compound was prepared from **26** (175 mg, 0.25 mmol), using the procedure described for **14** to give **29** (96 mg, 70%) as a white solid: mp 188–190 °C; IR (KBr, cm^{-1}) 3515 (ν_{NH}), 3030, 2955 (ν_{CH}), 2494 (ν_{BH}), 1524, 1350 (ν_{NO_2}), 1495 (ν_{BN}), 1485, 1405, 1350 (ν_{CH}), 1149 (ν_{CN}), 1057 ($\nu_{B\text{-}B}$), 995, 955, 857, 725, 676 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 6.95 (bs, 2H, NH_2), 7.68, 7.55–7.35 (m, 4H, CH-phenyl), 3.42 (m, 2H, NH-CH_2), 3.11 (m, 8H, $N(CH_2\text{-})_4$), 3.02 (m, 2H, phenyl-CH_2), 1.55 (m, 8H, $N(CH_2\mathbf{CH_2}\text{-})_4$), 1.38 (m, 8H, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 0.97 (t, J = 7.5 Hz, 12H, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$), 1.82–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149.8 (1C, NO_2-Cphenyl), 130.2, 129.4, 125. 7, 114.1 (5C, CH and C-phenyl), 59.3 (4C, $N(CH_2\text{-})_4$), 52.5 (1C, NH_2-CH_2), 41.7 (1C, CH_2-phenyl), 24.3 (4C, $N(CH_2\mathbf{CH_2}\text{-})_4$), 20.3 (4C, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 13.8 (4C, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –3.08 (s, 1B, B1), –19.97 (s, 10B, J = 85.79 Hz, B2-11), –16.34 (s, 1B, B12). MS (ESI) m/z 306.6 [100, M^-]. Anal. Calc. for $C_{24}H_{57}B_{12}N_3O_2$: C, 52.46; H, 10.46; N, 7.65%. found: C, 52.19; H, 10.23; N, 7.41%.

***N*-(4-Nitrophenethyl)amino-undecahydro-*closo*-dodecaborate (1–) tetrabutylammonium salt (30)**

This compound was prepared from **27** (175 mg, 0.25 mmol), using the procedure described for **14** to give **30** (119 mg, 87%) as a white solid: mp 214–216 °C; IR (KBr, cm^{-1}) 3515 (ν_{NH}), 3035, 2952 (ν_{CH}), 2487 (ν_{BH}), 1523, 1351 (ν_{NO_2}), 1497 (ν_{BN}), 1485, 1407, 1350 (ν_{CH}), 1145 (ν_{CN}), 1055 ($\nu_{B\text{-}B}$), 998, 952, 856, 725, 675 (ν_{CH}). ^1H NMR (400 MHz, CD_3CN) δ = 6.79 (bs, 2H, NH_2), 8.05 (d, J_{CH} = 7.55 Hz, 2H, CH-phenyl), 7.42 (d, J_{CH} = 7.55 Hz, 2H, CH-phenyl), 3.42 (m, 2H, NH-CH_2), 3.15 (m, 8H, $N(CH_2\text{-})_4$), 3.05 (m, 4H, phenyl-CH_2), 1.55 (m, 8H, $N(CH_2\mathbf{CH_2}\text{-})_4$), 1.35 (m, 8H, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 0.96 (t, J = 7.2 Hz, 12H, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$), 1.83–0.55 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149.2 (1C, NO_2-Cphenyl), 130.2, 129.0, 125.7, 114.5 (5C, CH and C-phenyl), 59.3 (4C, $N(CH_2\text{-})_4$), 52.5 (1C, NH-CH_2), 41.5 (1C, CH_2-phenyl), 24.4 (4C, $N(CH_2\mathbf{CH_2}\text{-})_4$), 20.2 (4C, $N(CH_2CH_2\mathbf{CH_2}\text{-})_4$), 13.8 (4C, $N(CH_2CH_2CH_2\mathbf{CH_3})_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ –3.12 (s, 1B, B1), –20.06 (s, 10B, J = 87.01 Hz, B2-11), –16.23 (s, 1B, B12). MS (ESI) m/z 306.5 [100, M^-]. Anal. Calc. for $C_{24}H_{57}B_{12}N_3O_2$: C, 52.46; H, 10.46; N, 7.65%. found: C, 52.22; H, 10.19; N, 7.42%.

General synthesis for compounds 31–33

Nitrophenethyl bromide (1.61 g, 7 mmol) was added to a stirred solution of **1d** (0.5 g, 0.7 mmol) and KOH (198 mg, 3.55 mmol) in 20 ml dry DMF under argon atmosphere. The solution was stirred for 5 h at room temperature. The solvent was evaporated *in vacuo* and the residue was washed with diethyl ether and dissolved in 20 ml of water. Addition of 2.25 g (7 mmol) of tetrabutylammonium bromide gave a white precipitate of *O*-alkylated derivative. Crystallization from dichloromethane–ethanol yielded colorless needles.

***O*-(2-Nitrophenethyl)hydroxo-undecahydro-*closo*-dodecaborate (2–) ditetrabutylammonium salt (31).** (174 mg, 22%), mp 179–181 °C. IR (KBr, cm^{-1}) 3024, 2953 (ν_{CH}), 2492 (ν_{BH}), 1526, 1350 (ν_{NO_2}), 1705 (ν_{BO}), 1485, 1405, 1285 (ν_{CH}), 1045 ($\nu_{B\text{-}B}$), 995, 948,

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825, 722, 675 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.66, 7.52–7.33 (m, 2H, CH-phenyl), 3.89 (m, 2H, O-CH_2), 3.11 (m, 16H, N(CH_2-)$_4$), 3.06 (m, 2H, CH_2-phenyl), 1.57 (m, 16H, N(CH_2**CH_2**-)$_4$), 1.38 (m, 16H, N(CH_2CH_2**CH_2**-)$_4$), 0.95 (t, J = 14.41 Hz, 24H, N($CH_2CH_2CH_2$**CH_3**)$_4$), 1.87–0.35 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 148.2 (1C, NO_2-Cphenyl), 131.1, 129.3, 125.7, 114.8 (5C, CH and C-phenyl), 59.3 (8C, N(CH_2-)$_4$), 56.8 (C, O-CH_2), 41.3 (1C, CH_2-phenyl), 24.3 (8C, N(CH_2**CH_2**-)$_4$), 20.3 (8C, N(CH_2CH_2**CH_2**-)$_4$), 13.8 (8C, N($CH_2CH_2CH_2$**CH_3**)$_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ 8.51 (s, 1B, B1), –12.05 (d, J = 166.21 Hz, 10B, B2-11), –21.06 (s, 1B, B12). MS (ESI): m/z 153.5 [100, M$^-$/2]. Anal. Calcd. for $C_{35}H_{86}B_{12}N_2O$: C, 60.67; H, 11.58; N, 5.31%. found: C, 60.33; H, 11.29; N, 5.06%.

***O*-(3-Nitrophenethyl)hydroxo-undecahydro-*closo*-dodecaborate (2–) ditetrabutylammonium salt (32).** (187 mg, 27%), mp 168–170 °C. IR (KBr, cm^{-1}) 3025, 2955 (ν_{CH}), 2489 (ν_{BH}), 1525, 1352 (ν_{NO_2}), 1706 (ν_{BO}), 1485, 1405, 1285 (ν_{CH}), 1045 ($\nu_{B\ B}$), 995, 948, 825, 722, 675 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 7.72, 7.59–7.39 (m, 2H, CH-phenyl), 3.92 (m, 2H, O-CH_2), 3.11 (m, 16H, N(CH_2-)$_4$), 3.02 (m, 2H, CH_2-phenyl), 1.55 (m, 16H, N(CH_2**CH_2**-)$_4$), 1.35 (m, 16H, N(CH_2CH_2**CH_2**-)$_4$), 0.95 (t, J = 14.41 Hz, 24H, N($CH_2CH_2CH_2$**CH_3**)$_4$), 1.87–0.35 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 148.7 (1C, NO_2-Cphenyl), 131.4, 129.4, 125.5, 114.9 (5C, CH and C-phenyl), 59.4 (8C, N(CH_2-)$_4$), 56.8 (C, O-CH_2), 41.4 (1C, CH_2-phenyl), 24.4 (8C, N(CH_2**CH_2**-)$_4$), 20.3 (8C, N(CH_2CH_2**CH_2**-)$_4$), 13.8 (8C, N($CH_2CH_2CH_2$**CH_3**)$_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ 8.52 (s, 1B, B1), –12.11 (d, J = 165.96 Hz, 10B, B2-11), –20.99 (s, 1B, B12). MS (ESI): m/z 153.5 [100, M$^-$/2]. Anal. Calcd. for $C_{35}H_{86}B_{12}N_2O$: C, 60.67; H, 11.58; N, 5.31%. found: C, 60.30; H, 11.36; N, 5.12%.

***O*-(4-Nitrophenethyl)hydroxo-undecahydro-*closo*-dodecaborate (2–) ditetrabutylammonium salt (33).** (124 mg, 18%), mp 192–194 °C. IR (KBr, cm^{-1}) 3022, 2956 (ν_{CH}), 2492 (ν_{BH}), 1525, 1350 (ν_{NO_2}), 1705 (ν_{BO}), 1485, 1405, 1285 (ν_{CH}), 1152 (ν_{CN}), 1045 ($\nu_{B\ B}$), 995, 948, 825, 722, 675 (ν_{CH}). ^{1}H NMR (400 MHz, CD_3CN) δ = 6.79 (bs, 2H, NH_2), 8.06 (d, J_{CH} = 7.2 Hz, 2H, CH-phenyl), 7.42 (d, J_{CH} = 7.2 Hz, 2H, CH-phenyl), 3.95 (m, 2H, O-CH_2), 3.11 (m, 16H, N(CH_2-)$_4$), 3.05 (m, 2H, CH_2-phenyl), 1.55 (m, 16H, N(CH_2**CH_2**-)$_4$), 1.36 (m, 16H, N(CH_2CH_2**CH_2**-)$_4$), 0.95 (t, J = 14.41 Hz, 24H, N($CH_2CH_2CH_2$**CH_3**)$_4$), 1.82–0.42 (m, 11H, $B_{12}H_{11}$). ^{13}C NMR (100 MHz, CD_3CN) δ = 149.3 (1C, NO_2-Cphenyl), 131.6, 129.8, 125.9, 114.8 (5C, CH and C-phenyl), 59.3 (8C, N(CH_2-)$_4$), 56.6 (C, O-CH_2), 41.4 (1C, CH_2-phenyl), 24.4 (8C, N(CH_2**CH_2**-)$_4$), 20.3 (8C, N(CH_2CH_2**CH_2**-)$_4$), 13.8 (8C, N($CH_2CH_2CH_2$**CH_3**)$_4$). ^{11}B NMR (96.3 MHz; CD_3CN): δ 8.49 (s, 1B, B1), –12.07 (d, J = 165.76 Hz, 10B, B2-11), –21.02 (s, 1B, B12). MS (ESI): m/z 153.3 [100, M$^-$/2]. Anal. Calcd. for $C_{35}H_{86}B_{12}N_2O$: C, 60.67; H, 11.58; N, 5.31%. Found: C, 60.47; H, 11.33; N, 5.19%.

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Reduction of *N*-Glycolylneuraminic Acid Xenoantigen on Human Adipose Tissue-Derived Stromal Cells/ Mesenchymal Stem Cells Leads to Safer and More Useful Cell Sources for Various Stem Cell Therapies

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Adipose tissue is an attractive source for somatic stem cell therapy. Currently, human adipose tissue-derived stromal cells/mesenchymal stem cells (hADSCs/MSCs) are cultured with fetal bovine serum (FBS). Recently, however, not only human embryonic stem cell lines cultured on mouse feeder cells but also bone marrow-derived human MSCs cultured with FBS were reported to express *N*-glycolylneuraminic acid (Neu5Gc) xenoantigen. Human serum contains high titers of natural preformed antibodies against Neu5Gc. We studied the presence of Neu5Gc on hADSCs/MSCs cultured with FBS and human immune response mediated by Neu5Gc. Our data indicated that hADSCs/MSCs cultured with FBS expressed Neu5Gc and that human natural preformed antibodies could bind to hADSCs/MSCs. However, hADSCs/MSCs express complement regulatory proteins such as CD46, CD55, and CD59 and are largely resistant to complement-mediated cytotoxicity. hADSCs/MSCs cultured with FBS could be injured by antibody-dependent cell-mediated cytotoxicity mechanism. Further, human monocyte-derived macrophages could phagocytose hADSCs/MSCs cultured with FBS and this phagocytic activity was increased in the presence of human serum. Culturing hADSCs/MSCs with heat-inactivated human serum for a week could markedly reduce Neu5Gc on hADSCs/MSCs and prevent immune responses mediated by Neu5Gc, such as binding of human natural preformed antibodies, antibody-dependent cell-mediated cytotoxicity, and phagocytosis. Adipogenic and osteogenic differentiation potentials of hADSCs/MSCs cultured with heat-inactivated human serum were not less than that of those cultured with FBS. For stem cell therapies based on hADSCs/MSCs, hADSCs/MSCs that presented Neu5Gc on their cell surfaces after exposure to FBS should be cleaned up to be rescued from xenogeneic rejection.

Introduction

ADIPOSE TISSUE is an attractive source for somatic cell therapy, because it is safe and abundant and many investigators have reported that the stromal cells derived from adipose tissue (adipose tissue-derived stromal cells [ADSCs]) could differentiate into various cell types.[1–4] ADSCs are also referred to as adipose tissue-derived mesenchymal stem cells (MSCs). Human ADSCs (hADSCs)/MSCs are very similar to bone marrow (BM)-derived human MSCs (hMSCs) and therefore reveal differentiation potential similar to BM-derived hMSCs.[5–7]

For stem cell therapies based on hMSCs including hADSCs/MSCs, it is essential that stem cells are handled and cultured in a manner that guarantees the efficacy and safety of the cellular therapy product. One such aspect is the choice

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of cell culture medium and supplements. In principle, most investigators agree that all animal materials should be avoided to maximize product safety. Currently, however, hADSCs/MSCs are cultured with fetal bovine serum (FBS), and the clinical efficacy of BM-derived hMSCs in human disease has been investigated using hMSCs cultured with FBS in a number of clinical trials.[8–12]

Recently, not only human embryonic stem cell (hESC) lines cultured on mouse feeder cells but also BM-derived hMSCs cultured with FBS were reported to express *N*-glycolylneuraminic acid (Neu5Gc) xenoantigen,[13,14] the so-called Hanganutziu–Deicher antigen.[15] Humans are incapable of synthesizing the common mammalian sialic acid, Neu5Gc, because of an *Alu* transposon-mediated inactivation of the cytidine monophosphate (CMP)-*N*-acetylneuraminic acid hydroxylase gene.[16,17] Despite this, both hESC lines and BM-derived hMSCs were reported to express the Neu5Gc, apparently originating from the mouse feeder layers, animal-derived components, and FBS.[13,14] The significant levels of Neu5Gc found on the surface of hESCs and hMSCs evidently originate from a Trojan Horse pathway involving endocytosis of extracellular glycoconjugates, delivery to the lysosome, release of Neu5Gc by lysosomal sialidase, active transport to the cytoplasm through the lysosomal sialic acid transporter, activation by CMP, and addition to nascent glycoproteins and glycolipids in the secretory pathway.[18] It is also possible that amphipathic molecules carrying Neu5Gc might be directly transferred into the hESC and hMSC plasma membranes.[19] Human serum contains high titers of natural preformed antibodies against Neu5Gc xenoantigen.[20–22] Thus, binding of these natural preformed antibodies may lead to immune responses such as complement-mediated cytotoxicity (CMC), antibody-dependent cell-mediated cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis. However, these immune responses mediated by natural preformed antibodies against human stem cells remain in controversary.[13,23] This study was therefore undertaken to study the presence of Neu5Gc on hADSCs/MSCs cultured with FBS and the human immune responses mediated by Neu5Gc on hADSCs/MSCs.

Materials and Methods

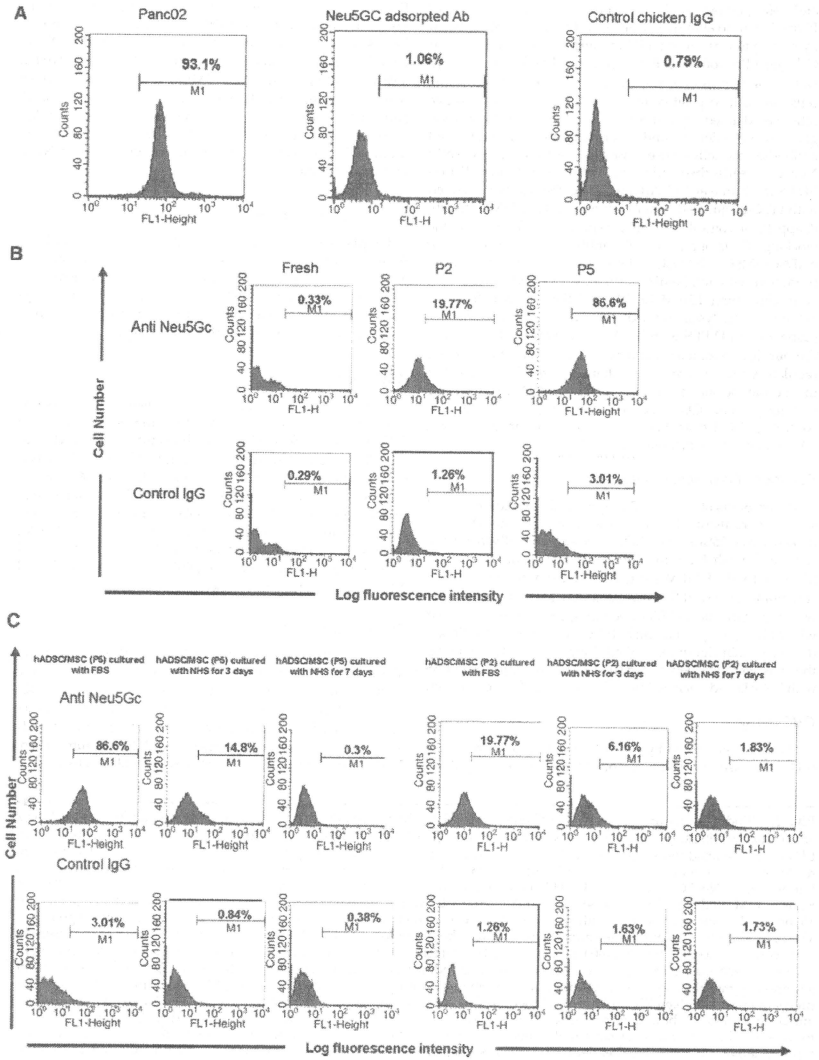
Cells

hADSCs/MSCs were prepared as described previously[1,2] with modifications.[3,4] Adipose tissue was resected during plastic surgery in five human subjects (four men and one woman; age, 20–60 years) as excess discards. Ten to 50 g of subcutaneous adipose tissue was collected from each subject. All subjects provided informed consent. The protocol was approved by the Review Board for Human Research of the Kobe University Graduate School of Medicine, Osaka University Graduate School of Medicine and Foundation for Biomedical Research and Innovation. All subjects fasted for at least 10 h before surgery and none was being treated with steroids. The resected excess adipose tissue was minced and then digested in Hank's balanced salt solution (Gibco Invitrogen, Grand Island, NY) containing 0.075% collagenase type II (Sigma Aldrich, St. Louis, MO) at 37°C for 1 h. Digests were filtered with a cell strainer (BD Bioscience, San Jose, CA) and centrifuged at 800 *g* for 10 min. Erythrocytes were excluded using density gradient centrifugation with Lymphoprep ($d = 1.077$; Nycomed, Oslo, Norway). The cells were then plated using Dulbecco's modified Eagle's medium (DMEM; Gibco Invitrogen) with 10% defined FBS (Hyclone, Northumberland, United Kingdom) and incubated for 24 h at 37°C. Following incubation, the adherent cells were washed extensively and treated with 0.2 g/L ethylenediaminetetraacetate (EDTA) solution (Nacalai Tesque, Kyoto, Japan), and the resulting suspended cells were replated at a density of 10,000 cells/cm^2 on human fibronectin-coated dishes (BD BioCoat, Franklin Lakes, NJ) in a medium containing 60% DMEM-low glucose, 40% MCDB-201 medium (Sigma Aldrich), 1×insulin–transferrin–selenium (Gibco Invitrogen), 1 nM dexamethasone (Sigma Aldrich), 100 μM ascorbic acid 2-phosphate (Sigma Aldrich), 10 ng/mL epidermal growth factor (PeproTec, Rocky Hill, NJ), and 5% FBS. For analysis of the effects of human serum on Neu5Gc expression on hADSCs/MSCs, the cells were cultured for 7 days, where FBS was replaced by 5% heat-inactivated normal human pooled serum (NHS) from type AB blood. As control cells, a murine pancreatic cell line, Panc02, was cultured with RPMI 1640 medium (Gibco Invitrogen) supplemented with 10% FBS and 1% antibiotic/antimycotic solution.

Flow cytometry

Cells were detached from culture dishes and suspended in Dulbecco's phosphate-buffered saline (D-PBS; Nacalai Tesque). Aliquots (5×10^5 cells) were incubated for 30 min at 4°C with a chicken anti-Neu5Gc polyclonal antibody (a gift from Prof. N. Wakamiya, Asahikawa Medical College, Hokkaido, Japan).[24] Cells incubated with D-PBS alone were used as negative control. After washing with D-PBS, cells were stained with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-chicken immunoglobulin G (IgG; Cappel, Cochranville, PA) as a second antibody. After staining, the cells were washed

FIG. 1. Expression of Neu5Gc on hADSCs/MSCs. (**A**) Specificity of anti-Neu5GC antibody. Panc02, a cell line derived from murine pancreatic carcinomas, expressed Neu5Gc. Flow cytometric analysis showed that chicken anti-Neu5Gc polyclonal antibody bound to the surfaces of Panc02, but Neu5Gc-preadsorbed anti-Neu5Gc polyclonal antibody could not react, showing specificity of the anti-Neu5Gc antibody. The percentage of cells that stained positive is indicated in the upper right corner of each panel. (**B**) Expression of Neu5Gc xenoantigen on hADSCs/MSCs. Fresh hADSCs/MSCs did not express Neu5Gc on their cell surface. In accordance with passage numbers, the population of Neu5Gc-positive cells increased by cultivation with FBS. The percentage of cells that stained positive is indicated in the upper right corner of each panel. (**C**) Reduction of Neu5Gc xenoantigen by chasing cultivation with human serum. After cultivation of hADSCs/MSCs with heat-inactivated NHS but not FBS, the percentages of Neu5Gc-positive cells have decreased in accordance with culture duration. The decrement manners of second passaged hADSCs/MSCs and fifth passaged ones have been in a similar fashion. The percentage of cells that stained positive is indicated in the upper right corner of each panel. Data are representative of four independent experiments. Neu5Gc, *N*-glycolylneuraminic acid; hADSCs/MSCs, human adipose tissue-stromal cells/mesenchymal stem cells; FBS, fetal bovine serum; NHS, normal human pooled serum; IgG, immunoglobulin G; M1, marked positive area 1; FL1, fluorescence1.


A
Panc02
93.1%
M1
Neu5GC adsorpted Ab
1.06%
M1
Control chicken IgG
0.79%
M1
Counts
FL1-Height
B
Fresh
P2
P5
Anti Neu5Gc
0.33%
19.77%
86.6%
Control IgG
0.29%
1.26%
3.01%
Cell Number
Log fluorescence intensity
C
hADSC/MSC (P5) cultured with FBS
hADSC/MSC (P5) cultured with NHS for 3 days
hADSC/MSC (P5) cultured with NHS for 7 days
hADSC/MSC (P2) cultured with FBS
hADSC/MSC (P2) cultured with NHS for 3 days
hADSC/MSC (P2) cultured with NHS for 7 days
Anti Neu5Gc
86.6%
14.8%
0.3%
19.77%
6.16%
1.83%
Control IgG
3.01%
0.84%
0.38%
1.26%
1.63%
1.73%
Cell Number
Log fluorescence intensity