Novel Carboranes with a DNA Binding Unit for the Treatment of Cancer by Boron Neutron Capture Therapy

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The synthesis and biological evaluation of two ortho*-carborane derivatives which contain a 5,6,7-trimethoxyindole (TMI) unit for use in boron neutron capture therapy is described. The TMI moiety is known to be the DNA-binding part of the highly potent anticancer agent duocarmycin A. The* ortho*-carborane derivatives were prepared from amino alkynes which were bound to a protected TMI carboxylic acid. Addition of decaborane(14) to the alkyne triple bond with subsequent removal of the* tert*-butoxycarbonyl (Boc) and benzyl protecting groups gave the desired product. Boron uptake from the* ortho*-carborane derivatives into B-16 melanoma cells was higher and faster than that observed with* L*-*p*-boronophenylalanine (BPA), which is in use in the clinic.*

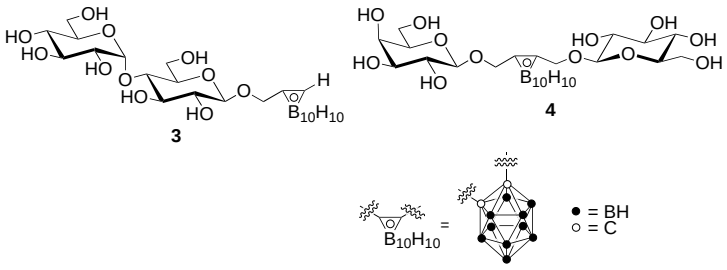
KEYWORDS:

antitumor agents · boron neutron capture therapy · carboranes · DNA · indoles

Introduction

Boron neutron capture therapy (BNCT) for the treatment of cancer has received intense attention over the last few years.[1] This method makes use of the cytotoxic neutron capture reaction $^{10}B(^{1}n,^{4}He)^{7}Li$, in which an alpha particle and a lithium ion are produced and release enough energy to kill the tumor cells. Boron is accumulated in cancer cells when suitable boron compounds are administered and subsequent radiotherapy with slow neutrons results in the $^{10}B(^{1}n,^{4}He)^{7}Li$ reaction. The effect of this binary system depends on the amount of boron in the malignant cells and the distance of the boron atoms from the cell nucleus. Boron levels should be greater than 20 μg per gram of tumor tissue[2] to be effective.

Substituted carborane derivatives are attractive boron sources for BNCT because of their stability in aqueous media and their high boron content, but the cytotoxicity and low water solubility of carboranes hampers their use in BNCT. However, we have recently shown that *ortho*-carboranes (1,2-dicarba-*closo*-dodecaboranes) connected to sugar moieties such as maltoside (**3**, Scheme 1) possess good water solubility, show no cytotoxicity towards human bronchial carcinoma cells of the line A549 up to *ortho*-carborane concentrations of 0.4 mM,[3] and are accumulated very well by tumor cells.[4] Moreover, carboranediyl bisglycosides such as **4** show nearly no uptake into C6 glioma cells due to the enhanced hydrophilicity of these carborane derivatives;[5] they may therefore be used for a selective delivery into malignant cells by employing glycohydrolases connected to monoclonal antibodies, which bind to tumor-associated antigens. The glycohydrolases can transform the bisglycosides into lipophilic compounds.[6]



Scheme 1. *Carboranyl maltoside* ***3*** *and carboranyl bisglycoside* ***4****, which may be used for delivered selectively into malignant cells.*

The cytotoxic effect of the boron neutron capture reaction is enhanced by a factor of between two and five when the boron-containing compound is accumulated at the DNA of malignant cells compared to when the boron is distributed equally throughout the cytoplasm.[7] Consequently, there is great interest in the synthesis of DNA-binding boron compounds.[1e, 8] Some substances in this category which contain alkylators[9] and interactors/intercalators[10–12] have been prepared and preliminary in vitro/in vivo investigations have been carried out.

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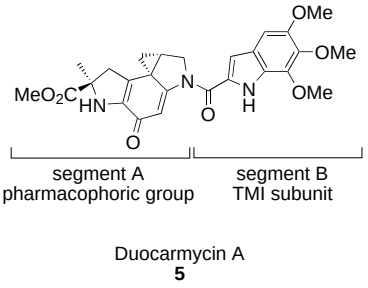
However, disadvantages such as high general cytotoxicity or accumulation outside of the tumor tissue have so far prevented the therapeutic application of most of these compounds.

Herein we report the synthesis and analysis of the new *ortho*-carboranes **1**, **2**, **16**, and **17** (Scheme 4), which carry a trimethoxy indole (TMI) unit. We have estimated the cytoxicity of these compounds on different cell lines and the level of boron uptake they provide. The TMI unit is a naturally occurring DNA binding segment found in the highly potent cytotoxic antibiotic duocarmycine A (**5**, Scheme 2). This antibiotic contains a spirocyclopropyl cyclohexadienyl moiety responsible for its alkylating effect and the TMI unit responsible for sequence-specific intercalation into DNA by noncovalent interactions.[13, 14]

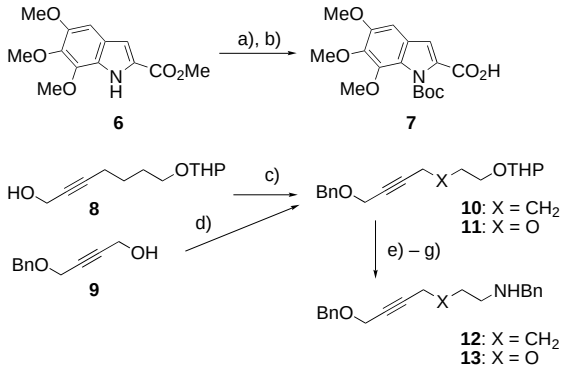


Scheme 2. *Duocarmycin A (**5**), a highly potent anticancer agent.*

Results and Discussion

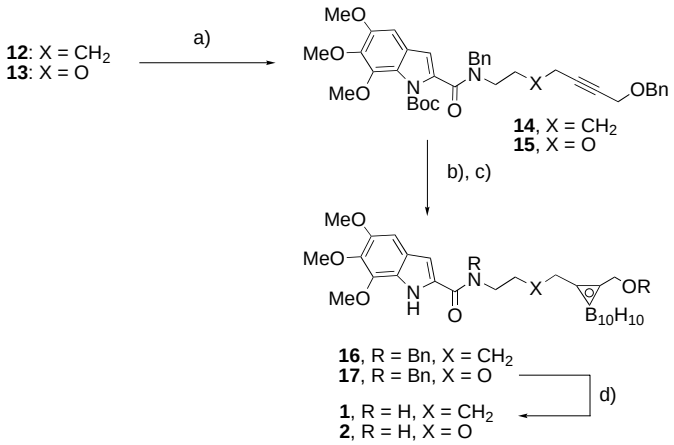
Syntheses

N-tert-Butoxycarbonyl-protected (*N*-Boc-protected) TMI carboxylic acid **7** (Scheme 3) was prepared in two steps and 60% overall yield from the known *N*-unprotected indole acid methyl ester **6**.[15] *N*,*O*-dibenzylaminoalkyne **12** was accessible in four steps and 76% overall yield from the known heptynediol derivative **8**[16] by benzylation to give **10**, acidic cleavage of the tetrahydropyranyl (THP) protecting group, Dess–Martin oxidation, and subsequent reductive amination of the generated carbonyl group with $BnNH_2 \cdot HCl$ (Bn = benzyl). The ether **13** was prepared from **9** in a similar way (Scheme 3), in an overall yield of 44%; monobenzylated butynediol **9**[17] was subjected to alkylation with THP-protected bromoethanol by using KOH in dimethyl sulfoxide (DMSO).



Scheme 3. *Synthesis of TMI carboxylic acid **7** and amino alkynes **12** and **13**. a) Boc_2O, DMAP, CH_3CN, RT, 60% yield of **19**; b) LiOH, MeOH, H_2O, quant.; c) NaH, BnBr, THF, RT, 95% yield; d) 2-(2-bromo-ethoxy) tetrahydropyran, KOH, DMSO, RT, 72% yield; e) PTS, MeOH, RT, 92% yield of **20** from **10** or 85% yield of **21** from **11**; f) Dess-Martin periodinane, CH_2Cl_2, 0 °C, 87% yield of **22** from **20** or 80% yield of **23** from **21**; g) $BnNH_2 \cdot HCl$, $NaBH_3CN$, MeOH, 80% yield of **12** from **10**, 61% yield of **13** from **11**. For definitions of abbreviations and compounds **19** and **20**–**23**, see the text and Experimental Section.*

Connection of amino alkynes **12** and **13** to the TMI carboxylic acid **7** was achieved by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) in dimethylformamide (DMF) to give amides **14** and **15** in 75% and 86% yield, respectively (Scheme 4). It is important for the formation of carboranes from alkynes that the



Scheme 4. *Synthesis of carboranes **1** and **2**. a) **7**, EDC·HCl, HOBt·H_2O, DMF, RT, 75% yield from **12**, 86% yield from **13**; b) $B_{10}H_{14}$, CH_3CN, reflux, then **14** or **15** in toluene, reflux; c) TFA, CH_2Cl_2 RT, 26% yield from **14**, 43% yield from **15**; d) H_2 (3 bar), Pd/C, EtOAc, MeOH, RT, 83% yield from **16**, 64% yield from **17**. For definitions of abbreviations, see the text and Experimental Section.*

substrates do not contain any acidic hydrogens. Thus, preliminary experiments have shown that indole and amide NH groups must be protected to obtain reasonable yields and suppress side-reactions. Compounds **14** and **15** (Scheme 4) were treated with a $B_{10}H_{12} \cdot 2CH_3CN$ adduct[18] which was formed in situ by heating decaborane(14) in acetonitrile for 30 minutes under reflux ($B_{10}H_{14}$ = decaborane(14)). Subsequent removal of the Boc protecting group led to carboranes **16** and **17** in 26% and 43% overall yield, respectively. In these transformations, butyne diethers such as **15** gave consistantly higher yields than monoethers such as **14**.[19] A clear explanation for this phenomenon can not yet be given, however, it is possible that coordination of the decaborane complex to the diethers may facilate the addition. Simple electronic explanations can also be assumed.

Carboranes **16** and **17** were debenzylated by hydrogenolysis to give hydroxymethylcarboranes **1** and **2** in good yields, by using Pd on charcoal as the catalyst with a MeOH/EtOAc mixture as solvent; the *N*-debenzylation occurred as fast as the *O*-debenzylation.

Structure determination

The structures of the new compounds were mainly determined by ^{1}H and ^{13}C NMR spectroscopy. A broad signal for the 10 protons attached to boron is found at $\delta = 0.5 - 3.5$ in the ^{1}H NMR spectra, as is typical for carboranes. In addition, the IR spectra of these compounds displayed a strong B–H stretch signal at approximately 2590 cm^{-1}. The prepared boron compounds contain the natural isotopic distribution of boron. In the mass spectra of the new compounds, a broad family of peaks is therefore detected together with the peak of highest intensity which correlates to the most abundant ^{10}B/^{11}B ratio. Both ^{1}H and ^{13}C NMR spectra of amides **14**, **15**, **16**, and **17** showed strong line broadenings at ambient temperature and were therefore recorded at 100 °C in $C_2D_2Cl_4$ or [D_6]DMSO.

In Vitro Studies

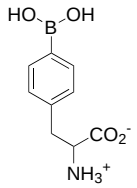
Toxicities: The cytotoxicity of the carboranes **1**, **2**, **16**, and **17** was determined in cloning efficiency tests on human bronchial carcinoma cells of line A549[20] and human melanoma cells of line B-16, with 1.0% (A549) or 0.5% (B-16) DMSO, respectively, to enhance water solubility of the carboranes. The debenzylated carboranes **1** and **2** display modest cytotoxicities with ED_{50} values in the range of 7.5 – 42.5 μM (see Table 1; ED_{50} = drug concentration required for 50% effect on target cells). Amazingly, the more lipophilic carboranes **16** and **17**, which were also investigated for comparison, displayed almost no cytotoxicity, with ED_{50} values of up to 0.1 mM (Table 1). Usually, cytotoxicity increases with the lipophilicity of the compounds in question. The ED_{50} values found for **1** and **2** are in the same range as established for the hydroxymethylcarboranes investigated in our previous work.[3] Thus, the cytotoxicity of hydroxymethylcarborane is not significantly enhanced by conjugation to the TMI unit.

Table 1. *Cytotoxicities of indole carboranes **1**, **2**, **16**, and **17**.*

	ED_{50} values [μM] human bronchial carcinoma cell line A549[a]	human melanoma cell line B-16[b]
16	> 137[c]	> 137[c]
17	> 131.5[c]	92.5[c]
1	32	7.5
2	42.5	10

[a] Incubation time = 1 day. [b] Incubation time = 3 days. [c] Compound partially insoluble under these conditions.

In vitro boron incorporation into B-16 melanoma cells: Boron incorporation into B-16 cells was determined by using inductively coupled plasma atomic emission spectrometry (ICP-AES). The cells were cultured in Falcon dishes and grown until they filled the dishes. The cells were incubated for 3 – 24 h in Eagle's Minimum Essential Medium (Eagle-MEM) with the experimental compounds and 0.5% DMSO to enhance water solubility. At 3, 12, and 24 h, the cells were washed three times with calcium-and magnesium-free phosphate-buffered saline (PBS(-)) and processed for the determination of boron concentration by ICP-AES. The results are shown in Table 2. Carboranes **1** and **2** already display a high boron uptake into B-16 cells after the remarkably short time of three hours. Incubation with 10 μM **1** in the medium (a boron concentration of 1.1 ppm) led to a cellular boron concentration of 2.3 ppm per 10^7 cells after three hours. This represents a 24% consumption of the total boron content of the medium, as determined by ICP-AES experiments with the remaining medium. Under the same conditions, the carborane **2** gave an even higher boron concentration of 3.7 ppm per 10^7 cells after three hours. The same boron uptake was achieved for B-16 cells with the clinically used *p*-boronophenylalanine **18** (BPA; Scheme 5) only after administration of a tenfold greater boron concentration (or hundredfold greater molar boron concentration in the medium) than described above and incubation for 24 hours.



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Scheme 5. p-*Boronophenylalanine, currently in use in the clinic for boron neutron capture therapy.*

The boron content after incubation of the cells with **1** and **2** was highest after 3 hours and then decreased slowly. Astoundingly, the more lipophilic dibenzyl compounds **16** and **17** displayed a lower uptake into B-16 cells than **1** and **2** even though boron uptake usually increases with lipophilicity. This phenomenon may be explained by precipitation of carboranes **16** and **17** from the medium due to their low water solubility, which was observed in some cases. Uptake into the cells is thus prohibited. Consequently, this precipitation could also be the reason for the low cytotoxicity of **16** and **17**.

Table 2. *Boron uptake by B-16 melanoma cells.[a]*

	Concentration of boron in the medium [ppm] (μM)	Concentration of boron in the cells[b] After 3 h	After 12 h	After 24 h
16	0.81 (7.5)	–[c]	0.01 ± 0.01	–[c]
16	8.1 (75)	0.79 ± 0.12[d]	0.56 ± 0.07	0.71 ± 0.02
17	8.1 (75)	0.85 ± 0.08	0.69 ± 0.03	1.3 ± 0.11
1	1.1 (10)	2.3 ± 0.03	1.6 ± 0.07	1.1 ± 0.08
2	1.1 (10)	3.7 ± 0.07[d]	2.3 ± 0.03	2.3 ± 0.08
BPA **18**[5]	11 (1000)	1.4 ± 0.21	1.9 ± 0.06	3.1 ± 0.31

[a] The cells were incubated for 3 – 24 h with Eagle MEM containing the boron compounds and the administered boron concentration indicated. [b] The boron concentration is given as multiples of 10^{-6} g boron per 10^7 cells. Each concentration represents the mean value ± the standard error in the duplicate experiments. [c] The boron concentration was too low to be determined by ICP-AES. [d] Experiment carried out in triplicate.

Conclusions

For the treatment of cancer by using boron neutron capture therapy, a high boron content in the malignant cells is required. Present compounds used for BNCT such as BPA **18** display both low and slow uptake into cells; therefore, high concentrations in the medium and long incubation times prior to neutron irradiation are required.

The killing effect of boron compounds is enhanced two- to fivefold if boron is accumulated near the DNA of the cell. The new compounds **1** and **2**, accesible in a short and convenient synthesis, show high boron uptakes into B-16 cells after 3 hours of administration and allow lower incubation concentrations and shorter incubation times than conventional BPA **18**.

Experimental Section

Synthesis of the carboranyl indoles

^{1}H and ^{13}C NMR spectroscopies were carried out on Varian XL-500, XL-300, and VXR-200, Bruker AM-300 spectrometers; multiplicities were determined with an APT (attached proton test) pulse sequence. Mass spectrometry was performed on a Finnigan MAT 95 spectrometer. IR spectroscopy was done on a Bruker Vector 22. Elemental analyses were carried out in the analytical laboratory of the University of Göttingen. All solvents were distilled prior to use. Reagents and materials were obtained from commercial suppliers and were used without further purification. All reactions were carried out under a positive pressure of argon and monitored by thin-layer chromatography (TLC; Macherey-Nagel & Co., Polygram SIL G/UV$_{254}$). Products were isolated by column chromatography on silica gel (Merck).

5,6,7-Trimethoxy-indole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (19): Boc_2O (2.17 g, 9.94, 2.0 equiv) in CH_3CN (10 mL) followed by 4-dimethylaminopyridine (DMAP; 29 mg, 0.23 mmol, 5 mol%), was added to a solution of indole **6** (1.32 g, 4.98 mmol) in CH_3CN (10 mL). The solution was stirred at RT for 9 h then poured into HCl (0.5 M, 80 mL) and extracted with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated. The residue was purified by gradient column chromatography (*n*-pentane/diethyl ether (5:2 $\rightarrow$2:1)) to afford **19** (1.09 g, 2.98 mmol, 60%) as a white solid. $R_f = 0.50$ (*n*-pentane/diethyl ether (1:1)); IR (KBr): $\tilde{\nu} =$ 2939 (C–H), 1759, 1715 (C=O), 1538, 1371, 1233, 1004, 833, 755; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.68$ (s, 9H, $C(CH_3)_3$), 3.88, 3.90, 3.95, 3.98 (4 s, 4 $\times$ 3H, 4 OCH_3), 6.80 (s, 1H, 4-H), 7.10 (s, 1H, 3-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 27.38$ ($C(CH_3)_3$), 51.93 (CO_2CH_3), 56.19, 61.23, 61.28 (3 OCH_3), 85.00 ($C(CH_3)_3$), 98.06 (C-4), 111.2 (C-3), 122.2, 126.6, 127.3 (C-3a, C-6, C-7a), 139.9, 142.0, 150.6 (C-2, C-5, C-7), 153.6, 161.0 (2 C=O); MS (EI): *m/z* (%): 365 (20) $[M]^+$, 265 (100) $[M - CO_2 - C(CH_3)_3]^+$, 57 (80) $[C(CH_3)_3]^+$; elemental analysis (%) calcd for $C_{18}H_{23}NO_7$ (365.4): C 59.17, H 6.34; found: C 58.87, H 6.41.

5,6,7-Trimethoxy-indole-1,2-dicarboxylic acid 1-*tert*-butyl ester (7): $LiOH \cdot H_2O$ (3.00 g, 71.5 mmol, 4.5 equiv) in H_2O (50 mL) at 0 °C was added to a solution of **19** (5.83 g, 16.0 mmol) in MeOH (125 mL). The solution was allowed to warm slowly to RT, stirred for 40 h, then cooled to 0 °C and neutralized with HCl (3 M). The MeOH was evaporated, the residue poured into HCl (0.5 M) and the mixture extracted with CH_2Cl_2 (monitored with TLC). The combined organic layers were washed with brine and dried over Na_2SO_4. CH_2Cl_2 was evaporated to afford pure **7** (5.62 g, 16.0 mmol) as a white solid. $R_f = 0.20$ (*n*-pentane/diethyl ether (1:1)); IR (KBr): $\tilde{\nu} = 3425$ (OH), 2939 (C–H), 1765, 1681 (C=O), 1246; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta =$ 1.67 (s, 9H, $C(CH_3)_3$), 3.91, 3.94, 3.98 (3 s, 3 $\times$ 3H, 3 OCH_3), 6.80 (s, 1H, 4-H), 7.10 (s, 1H, 3-H), 12.6 (br s, 1H, CO_2H); ^{13}C NMR (50 MHz, [D$_6$]DMSO): $\delta = 26.90$ ($C(CH_3)_3$), 55.96, 60.76, 61.02 (3 OCH_3), 84.27 ($C(CH_3)_3$) 98.66 (C-4), 109.8 (C-3), 122.0, 125.2, 129.1 (C-3a, C-6, C-7a), 139.0, 141.1, 150.1 (C-2, C-5, C-7), 150.3, 161.6 (2 C=O); MS (EI): *m/z* (%): 351 (20) $[M]^+$, 251 (100) $[M - CO_2 - C(CH_3)_3]^+$, 236 (40) $[M - CO_2 - C(CH_3)_3 - CH_3]^+$; elemental analysis (%) calcd for $C_{17}H_{21}NO_7$ (351.4): C 58.11, H 6.02; found: C 57.86, H 6.07.

2-(7-Benzyloxy-hept-5-ynyloxy)-tetrahydropyran (10): A solution of **8** (9.33 g, 43.9 mmol) in tetrahydrofuran (THF; 15 mL) at 0 °C was added to a suspension of NaH (2.25 g, 93.8 mmol, 2.1 equiv) in dry THF (300 mL). The reaction mixture was allowed to warm to RT, benzyl bromide (10.0 mL, 84.2 mmol, 1.9 equiv) was added, and the mixture was stirred for 20 h. To work up the reaction, dry MeOH (10 mL) was added, followed by addition of H_2O (100 mL) after 30 min. The mixture was concentrated in vacuo and extracted with diethyl ether. The combined organic layers were dried over Na_2SO_4 and evaporated. Purification of the remaining oily residue by gradient column chromatography (*n*-pentane/diethyl ether (50:1 $\rightarrow$1:1)) afforded **10** (12.6 g, 41.7 mmol, 95%) as a pale yellow oil. $R_f = 0.69$ (*n*-pentane/diethyl ether (10:1)); IR (film): $\tilde{\nu} = 3031$, 2942 (C–H), 1073 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.44 - 1.93$ (m, 10H, 3-H, 4-H, 5-H, 2′-H, 3′-H), 2.30 (tt, $J = 6.6$, 1.7 Hz, 2H, 4′-H), 3.32 – 3.58 (m, 2H, 1′-H), 3.68 – 3.94 (m, 2H, 6-H), 4.15 (t, $J = 1.7$ Hz, 2H, 7′-H), 4.56 (t, $J = 1.7$ Hz, 1H, 2-H), 4.57 (s, 2H, PhCH_2), 7.26 – 7.41 (m, 5H, Ph–H); ^{13}C NMR (50 MHz, C_6D_6): $\delta = 18.85$, 19.63 (C-4, C-4′), 25.91, 25.97 (C-5, C-3′), 29.30 (C-3), 31.01 (C-2′), 57.84 (C-7′), 61.57 (C-1′), 66.83 (C-6), 71.25 (PhCH_2), 77.05 (C-6′), 88.85 (C-5′), 98.94 (C-2), 127.7, 128.1, 128.3 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 138.6 (*i*-Ph–C); $C_{19}H_{26}O_3$ (302.4).

2-[2-(4-Benzyloxy-but-2-ynyloxy)-ethoxy]-tetrahydropyran (11): A mixture of KOH (16.8 g, 299 mmol, 3.4 equiv) and DMSO (140 mL) was stirred at 0 °C. Compound **9** (15.5 g, 88.0 mmol) was added followed by 2-(2-bromo-ethoxy)-tetrahydropyran (19.6 g, 93.7 mmol, 1.1 equiv). The mixture was allowed to warm to RT, (upon which it became darker), stirred for 20 h, and cooled to 0 °C for work-up. H_2O (200 mL) was added and the mixture was extracted with diethyl ether. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, and evaporated. The residual oil was purified by gradient column chromatography (*n*-pentane/ethyl acetate (13:1 $\rightarrow$8:1)) to afford **11** (19.3 g, 63.4 mmol, 72%) as a colourless liquid. $R_f = 0.58$ (*n*-pentane/ethyl acetate (2:1)); IR (film): $\tilde{\nu} = 2942$ (C–H), 1074 (C–O); ^{1}H NMR (200 MHz, C_6D_6): $\delta = 1.10 - 1.90$ (m, 6H, 3-H, 4-H, 5-H), 3.38 – 4.05 (m, 6H, 6-H, 2′-H, 1′-H,), 4.02, 4.09 (2 $\times$ t, $J =$ 1.7 Hz, 2 $\times$ 2H, 1′′-H, 4′′-H), 4.49 (s, 2H, PhCH_2), 4.66 (t, $J = 3.3$ Hz, 1H, 2-H), 7.05 – 7.40 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, C_6D_6): $\delta = 19.40$ (C-4), 25.78 (C-5), 30.81 (C-3), 57.39, 58.61 (C-1′′, C-4′′), 61.52 (C-1′), 66.70 (C-6), 69.38 (C-2′), 71.73 (PhCH_2), 82.74, 83.18 (C-2′′, C-3′′), 98.94 (C-2), 127.8, 128.1, 128.5 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 138.3 (*i*-Ph–C); MS (DCI): *m/z* (%): 322 (100) $[M+NH_4]^+$; elemental analysis (%) calcd for $C_{18}H_{24}O_4$ (304.4): C 71.03, H 7.95; found: C 70.80, H 7.69.

7-Benzyloxy-hept-5-yn-1-ol (20): A solution of **10** (12.6 g, 41.7 mmol) in dry MeOH (300 mL) was stirred with p-toluenesulfonic acid (PTS; 87 mg, 0.456 mmol, 1 mol%) for 28 h. NEt_3 (2 mL) was added and the solvent evaporated. The residual oil was purified by gradient column chromatography (*n*-pentane/ethyl acetate (5:2 $\rightarrow$100% ethyl acetate)) to afford **20** (8.40 g, 38.5 mmol, 92%) as a pale yellow oil. $R_f = 0.16$ (*n*-pentane/ethyl acetate (3:1)); IR (film): $\tilde{\nu} = 3405$ (OH), 3031, 2939 (C–H), 1070 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.30$ (br s, 1H, OH), 1.65 (m, 4H, 1-H, 2-H), 2.30 (tt, $J = 6.0$, 1.7 Hz, 2H, 4-H), 3.67 (t, $J = 6.0$ Hz, 2H, 1-H), 4.15 (t, $J = 1.7$ Hz, 2H, 7-H), 4.57 (s, 2H, PhCH_2), 7.26 – 7.44 (m, 5H, Ph–H); ^{13}C NMR (50 MHz,

$CDCl_3$): $\delta = 18.56$ (C-4), 24.87 (C-5), 31.77 (C-6), 57.69 (C-1), 62.24 (C-7), 71.39 (PhCH$_2$), 76.20 (C-2), 86.84 (C-3), 127.8, 128.1, 128.4 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 137.6 (*i*-Ph–C); MS (DCI): *m/z* (%): 236 (100) $[M+NH_4]^+$; elemental analysis (%) calcd for $C_{14}H_{18}O_2$ (219.3): C 77.03, H 8.31; found: C 77.77, H 8.24.

2-(4-Benzyloxy-but-2-ynyloxy)-ethanol (21): **21** (a colourless liquid) was prepared in 85% yield from **11** by using a method similar to that described for **20**. $R_f = 0.50$ (*n*-pentane/ethyl acetate (1:1)); IR (film): $\tilde{\nu} = 3406$ (OH), 3032, 2861 (C–H), 1070 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.41$ (br s, 1H, OH), 3.65, 3.78 (2 × m, 2 × 2H, 1-H, 2-H), 4.22, 4.28 (2 × t, $J = 1.7$ Hz, 2 × 2H, 1′-H, 4′-H), 4.60 (s, 2H, PhCH_2), 7.26 – 7.37 (m, 5H, Ph–H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 57.28$ (C-4′), 58.60 (C-1′), 61.58 (C-1), 71.09, 71.60 (C-2, PhCH_2), 82.20, 82.50 (C-2′, C-3′), 127.8, 128.0, 128.4 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 137.2 (*i*-Ph–C); MS (DCI): *m/z* (%): 238 (100) $[M+NH_4]^+$; elemental analysis (%) calcd for $C_{13}H_{16}O_3$ (220.3): C 70.89, H 7.32; found: C 70.71, H 7.18.

7-Benzyloxy-hept-5-ynal (22): Dess – Martin periodinane (2.08 g, 4.90 mmol, 1.1 equiv) was added to an ice-cold solution of **20** (972 mg, 4.45 mmol) in CH_2Cl_2 (50 mL). The solution was stirred for 2 h at 0 °C then diethyl ether (100 mL) was added and the mixture was washed with NaOH (1 M, 50 mL). The layers were separated, the organic layer washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (*n*-pentane/ethyl acetate (1:1)) to afford **22** (837 mg, 3.87 mmol, 87%) as a colourless liquid containing traces of impurities. $R_f = 0.50$ (*n*-pentane/ethyl acetate (2:1)); IR (film): $\tilde{\nu} = 3031$, 2939 (C–H), 2773 (C(O)–H), 1773 (C=O), 1071 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta =$ 1.83 (m, 2H, H-3), 2.30 (tt, $J = 6.7$, 2.1 Hz, 2H, 4-H), 2.58 (td, $J = 7.3$, 1.4 Hz, 2H, 2-H), 4.13 (t, $J = 2.1$ Hz, 2H, 7-H), 4.56 (s, 2H, PhCH_2), 7.26 – 7.38 (m, 5H, Ph–H), 9.78 (t, $J = 1.4$ Hz, 1H, CHO); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 18.18$ (C-4), 20.98 (C-3), 42.69 (C-2), 57.62 (C-7), 71.51 (PhCH_2), 77.05 (C-6), 85.72 (C-5), 127.8, 128.1, 128.4 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 137.5 (*i*-Ph–C), 201.8 (CHO); MS (EI): *m/z* (%): 216 (100) $[M]^+$, 107, $[M - C_7H_7 - H_2O]^+$, 91 (100) $[C_7H_7]^+$; $C_{14}H_{16}O_2$ (216.3).

(4-Benzyloxy-but-2-ynyloxy)-acetaldehyde (23): **23** (a colourless liquid) was prepared from **21** in 80% yield at 64% conversion in a similar way to that described for **22**. $R_f = 0.54$ (*n*-pentane/ethyl acetate (1:1)); IR (film): $\tilde{\nu} = 3031$ (C–H), 2857 (C(O)–H), 1735 (C=O), 1072 (C–O); ^{1}H NMR (200 MHz, C_6D_6): $\delta = 3.58$ (t, $J = 1.0$ Hz 2H, 2-H), 3.88, 3.91 (2 × t, $J = 2.2$ Hz, 2 × 2H, 1′-H, 4′-H), 4.39 (s, 2H, PhCH_2), 7.00 – 7.20 (m, 3H, *m*-Ph–H, *p*-Ph–H), 7.25 – 7.30 (m, 2H, *o*-Ph–H), 9.26 (t, $J = 1.0$ Hz, 1H, CHO); ^{13}C NMR (50 MHz, C_6D_6): $\delta = 57.24$, 58.60 (C-1′, C-4′), 71.62 (PhCH_2), 74.51 (C-2), 81.79, 84.05 (C-2′, C-3′), 127.9, 128.1, 128.6 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 138.1 (*i*-Ph–C), 199.1 (CHO); MS (DCI): *m/z* (%): 236 (100) $[M+NH_4]^+$; $C_{13}H_{14}O_3$ (218.3).

Benzyl-(7-benzyloxy-hept-5-ynyl)-amine (12): $BnNH_2 \cdot HCl$ (14.0 g, 95.5 mmol, 4.2 equiv) was added to a solution of **22** (4.87 g, 22.5 mmol) in dry MeOH (200 mL) and the solution stirred at RT for 30 min before $NaBH_3CN$ (1.62 g, 25.0 mmol, 1.11 equiv) was added. The mixture was stirred for a further 19 h, then saturated K_2CO_3 solution was added and the mixture was concentrated in vacuo and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over Na_2SO_4. The solvents were evaporated and excess $BnNH_2$ removed by distillation. The residue was filtered through a short silica plug to yield **12** (7.10 g, quantitative) as a yellow oil which was used for the next step without further purification. $R_f = 0.20$ (*n*-pentane/ethyl acetate (1:1)); IR (film): $\tilde{\nu} = 3303$ (NH), 3029, 2930 (C–H), 1454, 1072 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.55$ (m, 5H, 2-H, 3-H, NH), 2.26 (m, 2H, 4-H), 2.65 (t, $J = 7.5$ Hz, 2H, 1-H), 3.78 (s, 2H, PhCH_2N), 4.14 (t, $J = 1.7$ Hz, 2H, 7-H), 4.58 (s, 2H, PhCH_2O), 7.20 – 7.40 (m, 10H, Ph–H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 18.70$ (C-4), 26.37 (C-3), 29.19 (C-2), 48.81 (C-1), 53.95 (PhCH_2N) 57.72 (C-7), 71.36 (PhCH_2O), 76.13 (C-6), 86.87 (C-5), 126.9, 127.7, 128.0, 128.1, 128.3, 128.4 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 137.63, 140.3 (*i*-Ph–C); MS (EI): *m/z* (%): 307 (2) $[M]^+$, 306 (12) $[M-H]^+$, 91 (100) $[C_7H_7]^+$; elemental analysis (%) calcd for $C_{21}H_{25}NO$ (307.4): C 82.04, H 8.20; found C 81.92, H 8.11.

Benzyl-[2-(4-benzyloxy-but-2-ynyloxy)-ethyl]-amine (13): **13** (a pale yellow oil) was prepared in 89% yield from **23** in a similar way to that described for **12** and used without further purification. $R_f = 0.28$ (ethyl acetate); IR (film): $\tilde{\nu} = 3364$ (NH), 3028, 2924 (C–H), 1454, 1072 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.02$ (br s, 1H, NH), 2.82 (t, $J = 7.0$ Hz, 2H, 1-H), 3.66 (t, $J = 7.0$ Hz, 2H, 2-H), 3.86 (s, 2H, PhCH_2N), 4.20 – 4.22 (m, 4H, 1′-H, 4′-H), 4.57 (s, 2H, PhCH_2O), 7.23 – 7.36 (m, 10H, Ph–H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 48.55$ (C-1), 53.81 (PhCH_2N), 57.39, 58.57 (C-1′, C-4′), 69.35 (C-2), 71.64 (PhCH_2O), 82.33, 86.84 (C-2′, C-3′), 126.9, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 137.6, 140 1 (*i*-Ph–C); MS (DCI): *m/z* (%): 310 (100) $[M+H]^+$; $C_{20}H_{23}NO_2$ (309.4).

2-[Benzyl-(7-benzyloxy-hept-5-ynyl)-carbamoyl]-5,6,7-trimethoxy-indole-1-carboxylic acid *tert*-butyl ester (14): A solution of **7** (1.86 g, 5.29 mmol), EDC · HCl (1.15 g, 5.82 mmol, 1.1 equiv) and HOBt · H_2O (912 mg, 5.96 mmol, 1.1 equiv) in DMF (70 mL) was stirred at RT for 40 min before a solution of **12** (1.79 g, 5.82 mmol, 1.1 equiv) in DMF (10 mL) was added. Stirring was continued for 24 h, the mixture poured into HCl (1 M, 200 mL), and extracted with ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by gradient column chromatography (*n*-pentane/ethyl acetate (3:1 → 2:1)) to give **14** (2.54 g, 3.96 mmol, 75%) as a yellow oil. $R_f = 0.50$ (*n*-pentane/ethyl acetate (1:1)); IR (film): $\tilde{\nu} = 2937$ (C–H), 1761, 1637 (C=O), 1254; ^{1}H NMR (300 MHz, $C_2D_2C_{l4}$, 100 °C): $\delta = 1.52$ (m, 2H, 3′-H), 1.74 (s, 9H, C(CH_3)$_3$), 1.75 (m, 2H, 2′-H), 2.23 (m_c, 2H, 4′-H), 3.48 (dd, $J = 8.0$, 7.5 Hz, 2H, 1′-H), 3.89, 3.94, 4.00 (3 s, 3 × 3H, OCH_3), 4.17 (t, $J = 1.7$ Hz, 2H, 7′-H), 4.58, 4.74 (2 s, 2 × 2H, PhCH_2), 6.50 (s, 1H, 4-H), 6.76 (s, 1H, 3-H), 7.25 – 7.40 (m, 10H, Ph–H); ^{13}C NMR (75 MHz, $C_2D_2Cl_4$, 100 °C): $\delta = 18.24$ (C-4′), 25.79, 26.77 (C-2′, C-3′), 27.50 (C(CH_3)$_3$), 44.63 (C-1′), 56.40 (OCH_3), 57.66 (C-7′, PhCH_2N), 60.64, 60.85 (2 × OCH_3), 71.20 (PhCH_2O), 76.59 (C-6′), 84.04 (*C*(CH_3)$_3$), 86.31 (C-5′), 98.84 (C-4), 106.4 (C-3), 124.1, 124.2 (C-6, C-7 a), 127.3, 127.4, 127.6, 128.0, 128.3, 128.4 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 133.16 (C-3 a), 136.9, 137.8 (*i*-Ph–C), 141.4, 141.7 (C-2, C-7), 149.0 (C-5), 151.0, 163.7 (2 × C=O); MS (EI): *m/z* (%): 640 (8) $[M]^+$, 540 (100) $[M - CO_2 - C(CH_3)_3]^+$, 340 (38) $[C_{19}H_{20}N_2O_4]^+$, 234 (44), 91 (44) $[C_7H_7]^+$; high-resolution mass spectrometry (HRMS): calcd for $C_{38}H_{44}N_2O_7$: 640.3148; found: 640.3148.

2-{Benzyl-[2-(4-benzyloxy-but-2-ynyloxy)-ethyl]-carbamoyl}-5,6,7-trimethoxy-indole-1-carboxylic acid *tert*-butyl ester (15): **15** (a white wax-like solid) was prepared in 86% yield from **13** using the method described for **14**. $R_f = 0.71$ (*n*-pentane/ethyl acetate (1:1)); IR (film): $\tilde{\nu} = 2938$ (C–H), 1763, 1638 (C=O), 1131; ^{1}H NMR (300 MHz, [D_6]DMSO, 100 °C): $\delta = 1.55$ (s, 9H, C(CH_3)$_3$), 3.62 (m, 4H, 1′-H, 2′-H), 3.80, 3.81, 3.88 (3 s, 3 × 3H, OCH_3), 4.15, 4.20 (2 t, $J = 1.7$ Hz, 2 × 2H, 1″-H, 4″-H), 4.52, 4.77 (2 s, 2 × 2H, PhCH_2), 6.67 (s, 1H, 4-H), 6.90 (s, 1H, 4-H), 7.20 – 7.40 (m, 10H, Ph–H); ^{13}C NMR (75 MHz, [D_6]DMSO, 100 °C): $\delta = 26.74$ (C(CH_3)$_3$), 55.92 (OCH_3), 57.35, 57.66 (C-1″, C-4″), 60.07, 60.15 (2 × OCH_3), 66.58 (C-2′), 70.52 (PhCH_2O), 82.07, 82.26 (C-2′, C-3′), 83.59 (*C*(CH_3)$_3$), 98.88 (C-4), 106.2 (C-3), 122.9, 123.4 (C-6, C-7 a), 126.7, 126.9, 127.1 (4 C), 127.6, 127.8 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 132.12 (C-3 a), 136.6, 137.3 (*i*-Ph–C), 140.0, 140.9 (C-2, C-7), 148.3 (C-5), 150.3, 162.9 (2 × C=O), C-1′ and PhCH_2N are not visible under these conditions because of line broadening; MS (EI): *m/z* (%): 642 (2) $[M]^+$, 542 (4) $[M - CO_2 - C(CH_3)_3]^+$, 340 (100) $[C_{19}H_{20}N_2O_4]^+$, 234 (40) $[C_{12}H_{12}NO_4]^+$, 91 (30) $[C_7H_7]^+$; HRMS: calcd for $C_{37}H_{42}N_2O_8$: 642.2941; found: 642.2941.

5,6,7-Trimethoxyindole-2-carboxylic acid benzyl-(7-benzyloxy-5*C*,6*C*-dicarba-*closo*-dodecaboranylheptyl)-amide (16): Boron hydride (317 mg, 2.60 mmol, 1.3 equiv) was heated in CH_3CN (12 mL) under reflux. After 30 min the solution turned yellow, which indicated the formation of the adduct $B_{10}H_{12}\cdot 2CH_3CN$. A solution of **14** (1.28 g, 2.00 mmol) in a toluene/CH_3CN mixture (15 mL, 5:1) was added and heating continued for 16 h. For work-up MeOH (1 mL) was added, the mixture heated to reflux for 30 min, cooled to RT, and concentrated in vacuo. Baseline impurities were removed by filtration through a short silica plug with ethyl acetate as eluent. The crude product (1.07 g) was dissolved in CH_2Cl_2 (300 mL), trifluoroacetic acid (TFA) (2.50 mL, 32.5 mmol, 16.2 equiv) was added and the solution stirred for 14 h at RT. For work-up, the solution was treated with saturated K_2CO_3 solution and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (100 mL) and the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated in vacuo and the residue purified by column chromatography (toluene/acetone (10:1)) to give **16** (339 mg, 515 μmol, 26%) as a white foam. $R_f = 0.30$ (toluene/acetone (10:1)); IR (KBr): $\tilde{\nu} = 2935$ (C–H), 2581 (B–H), 1606 (C=O), 1427, 1232, 1109; ^{1}H NMR (300 MHz, $C_2D_2Cl_4$, 100 °C): $\delta = 0.50 - 3.50$ (br s, 10H, BH), 1.51 – 1.65 (m, 4H, 2′-H, 3′-H), 2.20 (m_c, 2H, 4′-H), 3.54 (t, $J = 7.0$ Hz, 2H, 1′-H), 3.88, 3.93 (2s, 2×3H, OCH_3), 3.99 (s, 2H, 7′-H), 4.09 (s, 3H, OCH_3), 4.57, 4.91 (2s, 2×2H, PhCH_2), 6.61 (d, $J = 2.0$ Hz, 1H, 3-H), 6.77 (s, 1H, 4-H), 7.22 – 7.46 (m, 10H, Ph–H), 9.16 (br s 1H, NH); ^{13}C NMR (75 MHz, $C_2D_2Cl_4$, 100 °C): $\delta = 26.62$, 27.17 (C-2′, C-3′), 34.43 (C-4′), 46.83 (C-1′), 51.64 (PhCH_2N), 56.50, 60.64, 60.85 ($3 \times OCH_3$), 70.03, 73.48 (C-7′, PhCH_2O), 78.13, 78.21 (C-5′, C-6′), 98.47 (C-4), 104.9 (C-3), 123.2, 125.1 (C-6, C-7a), 126.8, 127.3, 127.4, 127.9, 128.3, 128.7 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 129.0 (C-3a), 136.4, 136.7 (*i*-Ph–C), 138.6, 140.2 (C-2, C-7), 150.2 (C-5), 163.7 (C=O); MS (EI): *m/z* (%): 659 (76) $[M]^+$, 234 (100) $[C_{12}H_{12}NO_4]^+$, 91 (100) $[C_7H_7]^+$; elemental analysis (%) calcd for $C_{33}H_{46}B_{10}N_2O_5$ (658.8): C 60.16, H 7.04; found: C 59.88, H 6.89.

5,6,7-Trimethoxyindole-2-carboxylic acid benzyl-[2-(4-benzyloxy-2*C*,3*C*-dicarba-*closo*-dodecaboranylbutyloxy)-ethyl]-amide (17): **17** (white foam) was prepared from **15** in 43% yield in a preparation similar to that described for **16**. $R_f = 0.56$ (*n*-pentane/ethyl acetate (1:1)); IR (KBr): $\tilde{\nu} = 2936$ (C–H), 2586 (B–H), 1607 (C=O), 1461, 1113; ^{1}H NMR (300 MHz, $C_2D_2Cl_4$, 100 °C): $\delta = 0.50 - 3.50$ (br s, 10H, BH), 3.73 (m_c, 4H, 1′-H, 2′-H), 3.86, 3.91 (2s, 2×3H, OCH_3), 3.95, 3.99 (2s, 2×2H, 1″-H, 4″-H), 4.09 (s, 3H, OCH_3), 4.55, 5.00 (2s, 2×2H, PhCH_2), 6.67 (d, $J = 2.0$ Hz, 1H, 3-H), 6.77 (s, 1H, 4-H), 7.22 – 7.44 (m, 10H, Ph–H), 9.17 (br s 1H, NH); ^{13}C NMR (75 MHz, $C_2D_2Cl_4$, 100 °C): $\delta = 46.83$ (C-1′), 52.43 (PhCH_2N), 56.49, 60.61, 61.04 ($3 \times OCH_3$), 70.06, 70.13, 71.28, 73.48 (C-2′, C-1″, C-4″, PhCH_2O), 76.42, 76.76 (C-2″, C-3″), 98.47 (C-4), 105.4 (C-3), 123.2, 125.1 (C-6, C-7a), 126.9, 127.3, 127.4, 127.9, 128.3, 128.7 (*o*-Ph–C, *m*-Ph–C, *p*-Ph–C), 128.8 (C-3a), 136.4, 136.7 (*i*-Ph–C), 138.6, 140.2 (C-2, C-7), 150.2 (C-5), 163.1 (C=O); MS (EI): *m/z* (%): 661 (100) $[M]^+$, 234 (50) $[C_{12}H_{12}NO_4]^+$, 91 (36) $[C_7H_7]^+$; HRMS: calcd for $C_{32}H_{44}B_{10}N_2O_6$: 661.4193; found: 661.4193.

5,6,7-Trimethoxyindole-2-carboxylic acid (7-hydroxy-5*C*,6*C*-dicarba-*closo*-dodecaboranaylheptyl)-amide (1): A mixture of **16** (49.6 mg, 75.3 μmol) and Pd/C (10%, 70 mg) in MeOH/ethyl acetate (1:1, 2 mL) was shaken in an H_2 atmosphere (3 bar) for 8 h at RT. Filtration, evaporation of the solvents, and column chromatography (*n*-pentane/ethyl acetate (1:1)) gave **1** (29.8 mg, 62.3 μmol, 83%) as a white foam. $R_f = 0.14$ (*n*-pentane/ethyl acetate (1:1)); IR (KBr): $\tilde{\nu} =$ 3344 (OH, NH), 2938 (C–H), 2583 (B–H), 1606 (C=O), 1561, 1465, 1262, 1105; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.50 - 3.50$ (br s, 10H, BH), 1.62 (m, 4H, 2′-H, 3′-H), 2.30 (m, 2H, 4′-H), 3.45 (m, 2H, 1′-H), 3.87, 3.92, 4.05 (3 s, 3×3H, OCH_3), 4.17 (s, 2H, 7′-H), 5.05 (br s, 1H, OH), 6.42 (t, $J = 6.0$ Hz, 1H, amide-NH), 6.72 (s, 1H, 4-H), 6.78 (d, $J = 2.0$ Hz, 1H, 3-H), 9.37 (s_{br} 1H, indole-NH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 25.87$, 29.28 (C-2′, C-3′), 33.62 (C-4′), 37.69 (C-1′), 61.12, 61.47, 61.48 ($3 \times OCH_3$), 63.50 (C-7′), 78.12, 81.38 (C-5′, C-6′), 97.32 (C-4), 103.1 (C-3), 123.2, 125.9 (C-6, C-7a), 129.5 (C-3a), 139.0, 140.1 (C-2, C-7), 150.2 (C-5), 162.7 (C=O); MS (EI): *m/z* (%): 478 (100) $[M]^+$, 233 (50) $[C_{12}H_{11}NO_4]^+$; HRMS: calcd for $C_{19}H_{34}B_{10}N_2O_5$: 478.3481; found: 478.3481.

5,6,7-Trimethoxyindole-2-carboxylic acid [2-(4-hydroxy-2*C*,3*C*-dicarba-*closo*-dodecaboranylbutyloxy)-ethyl]-amide (2): Compound **2** (a white foam) was prepared from **17** in 64% yield in a process similar to that described for **1**. $R_f = 0.17$ (*n*-pentane/ethyl acetate (1:1)); IR (KBr): $\tilde{\nu} = 3331$ (OH, NH) 2936 (C–H), 2583 (B–H), 1631 (C=O), 1556, 1465, 1106; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.50 - 3.50$ (br s, 10H, BH), 3.67 (m, 4H, 1′-H, 2′-H), 3.75 (br s, 1H, OH), 3.86, 3.91, 4.03 (3 s, 3×3H, OCH_3), 4.07, 4.14 (1 s, 1 br s, 2×2H, 1″-H, 4″-H), 6.66 (br tr, $J =$ 6.0 Hz, 1H, amide-NH), 6.74 (s, 1H, 4-H), 6.76 (d, $J = 2.0$ Hz, 1H, 3-H), 9.26 (br s 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 38.89$ (C-1′), 56.21, 61.13, 61.46 ($3 \times OCH_3$), 64.00, 71.00, 71.48, 73.48 (C-2′, C-1″, C-4″), 75.88, 78.34 (C-2″, C-3″), 97.36 (C-4), 102.9 (C-3), 123.2, 125.8 (C-6, C-7a), 129.9 (C-3a), 138.9, 140.0 (C-2, C-7), 150.1 (C-5), 161.9 (C=O); MS (EI): *m/z* (%): 480 (100) $[M]^+$, 336 (78) $[C_{17}H_{24}N_2O_5]^+$, 233 (80) $[C_{12}H_{11}NO_4]$; HRMS: calcd for $C_{18}H_{32}B_{10}N_2O_6$: 480.3273; found: 480.3273.

Biological Evaluation

Cytotoxicity tests: Adherent cells of the human bronchial carcinoma cell line A549 were sown in triplicate in six multiwell plates at concentrations of 10^2, 10^3 10^4, and 10^5 cells per cavity, and were incubated for 1 day with freshly prepared solutions of the compound to be tested at various concentrations. The medium was removed after cultivation for 12 days at 37 °C under an air atmosphere with CO_2 content enriched to 7.5%; the clones were dried, stained with Löffler's methylene blue, and counted under a microscope. The relative clone forming rate was determined according to Equation (1).

$$\text{relative clone forming rate [\%]} = (\text{number of clones counted after exposure}) \times 100 / (\text{number of clones counted in the control}) \quad (1)$$

Cells for the toxicity tests were cultivated at 37 °C under 7.5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; Biochrom) supplemented with L-glutamine (4 nM, Gibco), $NaHCO_3$ (44 nM, Biochrom), and 10% fetal calf serum (FCS; heat-inactivated for 30 min at 56 °C; Gibco).

Cytotoxicity tests with the adherent human melanoma cell line B-16 were performed as described above with the following alterations: Falcon dishes containing a similar cell concentration to that used for the human bronchial carcinoma cell line A549 (10^5 cells per dish) were incubated with the compounds at various concentrations and 0.5% DMSO for 3 days. The medium was removed and the remaining viable cells washed with PBS(-), trypsinized, and counted under a phase contrast microscope. B-16 cells were maintained in Eagle's MEM (Nissui Pure Industries, Osaka, Japan) supplemented with 10% fetal bovine serum (JRH biosciences) and 1% antibiotic/antimycotic solution (100X; Gibco BRL). C6 cells were maintained in Eagle's MEM supplemented with 10% fetal bovine serum (FBS, ICN biochemicals Japan Co., Ltd.), gentamicin sulfate (50 mg L^{-1}), and amphotericin B (250 mg L^{-1}). The results are shown in Table 1.

In vitro boron incorporation into B-16 melanoma cells: B-16 melanoma cells were cultured in Falcon 3025 dishes (150 mm diameter). When the cells had grown to fill up the dish, the cell number was counted ($4.0 - 5.0 \times 10^6$ cells/dish). The boron containing indoles **16** (7.5 and 75 μM, 0.81 and 8.1 ppm boron, respectively) and **17** (75 μM, 8.1 boron) as well as the indoles **1** and **2** (10 μM,

1.1 ppm boron) and BPA **18** (1 mM, 10.8 ppm boron), were added to separate dishes. The cells were incubated for 3 – 24 h at 37 °C in 20 mL of the medium (Eagle-MEM, 10% FBS). The cells were washed 3 times with PBS(-), collected, digested with 2 mL of 60% $HClO_4$/30% H_2O_2 (1:2) solution, and then decomposed for 3 h at 80 °C. The boron concentration was determined by ICP-AES (Shimadzu, ICPS-1000-III). Two to three repetitions of each experiment were carried out. The average boron concentration of each fraction is indicated in Table 2.

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