Design and Synthesis of a Candidate α-Human Thrombin Irreversible Inhibitor Containing a Hydrophobic Carborane Pharmacophore

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Abstract: α-Human thrombin is a potent platelet agonist involved in the blood coagulation cascade and is an attractive target for an anticoagulant agent due to its involvement in several debilitating diseases. In this contribution we present attempts to develop a new architecture for size-selective serine protease inhibitors that utilize a fully methylated icosahedral p-carborane as a dominating hydrophobic pharmacophore. Using a computational docking program, flexX, a carborane-containing inhibitor was designed and synthesized. Computationally, this compound displayed the ability to provide ligand–protein binding interactions throughout the thrombin’s main active site (S1–S3), while positioning an acylating group for facile irreversible attack at the Ser195 hydroxyl group.

Key words: thrombin, inhibitor, carborane, docking

α-Human thrombin, a serine protease, is a potent platelet agonist involved in the final sequence of the blood coagulation cascade that cleaves fibrinogen to fibrin, forming a haemostatic mesh over wounds and preventing the loss of blood. The coagulation cascade is regulated by natural anticoagulants such as antithrombin. Thrombin’s main recognition site (Figure 1) is comprised of three subsites:1 1) active site S1 consists of a catalytic triad and an oxyanion hole formed by Ser195, His57 and Asp102; 2) active site S2 is a hydrophobic pocket that contains a thrombin-specific Tyr-Pro-Trp insertion loop, which includes both Tyr60A and Trp60D; 3) active site S3 is a distal hydrophobic pocket created by Leu99, Ile174 and Trp215.

In recent years, development of synthetic small-molecule direct thrombin and prothrombin activating factor Xa inhibitors has led to a number of highly potent anticoagulant compounds.1,2 Bicyclic trans-lactone 1 (Figure 2) is a thrombin inhibitor (IC50 = 4 nM) isolated from the leaves of Lantana camara (wild sage).3 The inhibitory mechanism of 1 in the active site of thrombin involves cleavage of the strained lactone ring system and a concerted acylation of the nucleophilic Ser195 hydroxyl group. However, the lactone moiety is susceptible to hydrolysis in plasma, and of little utility as a therapeutic agent.4 The synthetic analogues 2 and 3 are more stable to hydrolysis and retained the reactivity of the strained ring system by acylation of the Ser195 located in the enzyme active site.4,5

This contribution presents an approach to a new class of potentially effective and selective irreversible serine protease inhibitors by substituting a known [5,5]-trans-lactam thrombin inhibitor with a novel icosahedral carborane cage-linker construct that has the potential ability to extend favorable ligand–protein binding interactions throughout the three main regions of the thrombin’s primary active site (see Figure 1).

Icosahedral carboranes and polyhedral boranes have been the focus of an immense amount of biologically relevant...
research due to their continued use in the development of boron neutron capture therapy (BNCT) and in molecular imaging. They can be considered as three-dimensional counterparts of the phenyl ring system. Endo et al. employed carborane derivatives as hydrophobic pharmacophores in the design of synthetic analogues of estradiol and retinoic acid. The activity of these analogues is comparable to their endogenous counterparts. More recently, Konvalinka et al. investigated cobaltacarborane derivatives as very effective HIV protease inhibitors and showed that the metallacarboranes are versatile building blocks capable of strong interactions with hydrophobic regions of the enzyme binding site. In this laboratory, the chemistry of carboranes and polyhedral boranes has been well established since 1959. Recently we have shown that the carborane pharmacophore, when substituted for a phenyl ring in non-steroidal anti-inflammatory drugs (NAIDS) with known transthyretin amyloidosis inhibition, forms derivatives which are equally potent in transthyretin binding and show no detrimental COX inhibition, a common side effect resulting from the use of NAIDS. The suggested use of a substituted icosahedral carborane as a hydrophobic pharmacophore in an irreversible thrombin inhibitor is novel and unprecedented.

**Inhibitor Design**

Using a computational docking program, FlexX, we evaluated the docking of several rationally designed ligands in the primary active site of \( \alpha \)-human thrombin. Lead structure 4 (Figure 3) was identified as a synthetically feasible candidate that demonstrated favorable interactions with the protein crystal structure. Ligand 4 contains three structural components; a permethylated \( p \)-carborane cage, a piperazine linker and a \([5,5]\)-trans-lactam ring (see Figure 3). In designing this molecule, we hypothesized that a ligand containing a hydrophobic substituted \( p \)-carborane cage would have an increased affinity for binding the protein, based on the ability of a boron cluster to lodge itself in the hydrophobic S3 pocket of the enzyme.

The description of docking computations is presented in the experimental section. As shown in Figure 4, the spherically permethylated \( p \)-carborane cage could be positioned in the distal lipophilic S3 region and is able to correctly orient the inhibitor towards the catalytic cleft of the active site. After rationally substituting the cage with linkers of varying sizes and lengths, a piperazine ring was chosen as a synthetically acceptable moiety with which to interact with the smaller S2 region. Lastly, a \([5,5]\)-trans lactam ring was chosen as a template to be attached to the cage-linker system because of its known ability to interact with the S1 pocket of the protease and acylate Ser. This known synthetic moiety emerged as an optimal target with which to demonstrate the utility of the carborane cage as a hydrophobic pharmacophore, transforming a known inhibitor into a potent biologically active species, based upon the propensity of carborane to bind in the S3 site of the protein.

**Precursor Synthesis**

Starting from commercially available \( p \)-carborane, the ten available B-H vertices of the carborane structure were selectively methylated in iodomethane solvent using catalytic aluminum(III) chloride. As shown in Scheme 1, this reaction proceeded smoothly yielding \( B \)-decamethyl-\( p \)-carborane (5) in 95% yield. Having completely sheathed the boron atoms with methyl groups, one of the cage vertex carbon atoms was converted into the alcohol to give 6 in 70% yield through the use of butyl lithium followed by ethylene oxide. Control in this reaction was dictated by the precise addition of exactly one equivalent of base in order to ensure that only a limited amount of the disubstituted product was formed. Alcohol 6 was protected with tert-butyldimethylchlorosilane using 95% sodium hydride as base. This reaction proceeded with 72% conversion and the starting material was recovered and converted into the desired protected compound in subsequent reactions.

The remaining C-H vertex of 7 was lithiated using methyl lithium and methylated with an excess of iodomethane in 99% yield; the progression of this reaction was monitored by \(^{11} \)B NMR spectroscopy. If the reaction was quenched prior to completion, the resulting mixture of decamethyl borane...
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**Scheme 1** Reagents and conditions: a) MeI, AlCl₃, 55 °C, 9 d; b) BuLi, ethylene oxide, THF, −78 °C → r.t., overnight; c) 95% NaH, TBDMSCl, THF, 0 °C, 2.5 h; d) MeLi, MeI, THF, −78 °C → r.t., 48 h; e) TBAF (1 M in THF), 2.5 h.

(7) and undecamethyl-p-carborane (8) was quite difficult to separate. Upon completion, removal of the silyl protecting group was accomplished using four equivalents of tetra-n-butyrammonium fluoride (TBAF) in tetrahydrofuran at room temperature for 2.5 hours, to provide 9 as a colorless crystalline solid in 90% yield.

Initially, oxidation of 9 was attempted using pyridinium dichromate in N,N-dimethylformamide; however, these conditions led to exclusive isolation of the corresponding aldehyde derivative. As shown in Scheme 2, obtaining the desired carboxylic acid derivative 10 necessitated the use of four equivalents of periodic acid and a catalytic amount of chromium oxide. Following successful oxidation, 10 was easily converted into the acid chloride 11 using thionyl chloride. Although this reaction is normally rapid, due to apparent stereochemical effects, the reaction required heating overnight. Evidence of conversion into 11 was apparent by monitoring the shift of the carbonyl resonance in the 13C NMR spectra. Without further purification, 11 was subjected to nucleophilic substitution using the commercially available tert-butyl 1-piperazinecarboxylate (1-Boc-piperazine) to form 12 in 71% yield. After quantitatively removing the protecting group (TFA), the crude product 13 was converted into the bisamido derivative 14 using bromo acetylbromide in N,N-diisopropylethylamine (DIEA). The successful synthesis of 14 signified the synthetic completion of a versatile linker-cage system that could be attached to a 5,5-trans-lactam template that is known to acylate Ser195 resulting in irreversible inhibition of α-human thrombin.

Compound 18 was synthesized in seven steps from commercially available cyclopentene oxide as shown in Scheme 3. Using sodium azide, cyclopentene oxide was opened in ammonium chloride and aqueous ethanol. The azide was reduced using triphenyl phosphine and gradual heating in toluene; rapid heating caused effervescence and the release of N₂ gas. The resulting amine was protected in situ in a biphasic mixture of di-tert-butyl dicarbonate, ethyl acetate and a saturated aqueous solution of sodium bicarbonate, to afford 15 in 22% yield over three steps. Using Mitsunobu conditions, 15 was closed to the protected aziridine 16 in 75% yield. The enolate of dimethyl malonate was used to open the aziridine system to yield 17 in.

**Scheme 2** Reagents and conditions: a) H₂IO₆, CrO₃, wet MeCN, 0 °C → r.t., overnight; b) SOCl₂, CH₂Cl₂, reflux 24 h; c) tert-butyl 1-piperazinecarboxylate, DIPEA, CH₂Cl₂, 0 °C → r.t., 2 h; d) i) TFA, CH₂Cl₂, 0 °C, 100 min, ii) bromo acetyl bromide, DIPEA, CH₂Cl₂, 0 °C → r.t., overnight.

**Scheme 3** Reagents and conditions: a) NH₄Cl, NaN₃, EtOH–H₂O (50:50), reflux, overnight; b) PPh₃, toluene, reflux, 1.5 h; c) aq NaHCO₃, EtOAc, Boc₂O, 0 °C → r.t., overnight; d) PPh₃, DIAD, THF, −78 °C → r.t.; e) 95% NaH, dimethyl malonate, DME, reflux, 24 h; f) 1 M KOH, MeOH, KH₂PO₄; g) xylenes, reflux, 4 h; h) 4 M HCl, r.t., 14 h; i) 2-chloro-1-methylpyridinium iodine, Et,N, CH₂Cl₂, 48 h; j) Boc₂O, HMDS, BuLi, −78 °C → r.t., 15 h; k) MeSO₂Cl, HMDS, BuLi, −78 °C → r.t., 15 h.
a stereoselective fashion that set the required trans-stereochemistry of the required ring junction. This reaction had previously been reported as proceeding in N,N-dimethylformamide in 73% yield. During our optimization, it was discovered that 17 was produced more efficiently (91% yield) and with fewer byproducts if the reaction was carried out in freshly distilled glyme. The lower boiling point and facile removal in vacuo made this solvent a preferred medium. Diester 17 was subjected to selective hydrolysis in 4.8 equivalents of aqueous potassium hydroxide in order to convert one of the methyl esters into the corresponding acid derivative that then underwent decarboxylation upon heating in either N,N-dimethylformamide or xylene. The lower boiling point and facile removal of xylene also made this solvent more desirable. This two-step protocol provided monoester 18 in 68% yield as a colorless crystalline solid following flash column chromatography. To our knowledge, this is the first time the 1H NMR (compound 18) and the 13C NMR (compounds 15–18) data have been reported.

Additionally, using hydrochloric acid and vigorous stirring overnight, 19 was easily obtained in 80% yield. Without further purification, this was cyclized to the desired lactam 20 in 73% yield, using Mukaiyama’s reagent,\(^4\) eight equivalents of triethylamine, and dichloromethane (1 mg/mL).\(^5\) Lactam 20 was protected with either di-tert-butyl dicarbonate or methanesulfonyl chloride in lithium hexamethyldisilazide (LHMDs), to yield compounds 21 and 22, respectively. The deliberate use of the mesyl protecting group was a direct result of the enhanced reactivity of the lactam towards attack by Ser\(^{195}\) in the active site of thrombin when the ring nitrogen is substituted by a highly electron-withdrawing group.\(^4\)

Alkylation of lactams 21 and 22 with 14 was accomplished in moderate yields using lithium hexamethyldisilazide in anhydrous tetrahydrofuran at –78 °C, to give 23 and 4 in 55% and 49% yield respectively (see Scheme 4). NMR analysis confirmed that the substitution was diastereoselective. It has been reported that when lithium hexamethyldisilazide is used in the presence of tetrahydrofuran at or below –78 °C, the β-conformation is the major isomer produced because the electrophile can approach the enolate from the molecular face opposite the major isomer produced because the electrophile can approach the enolate from the molecular face opposite the stereocenter. This approach was the result of the enhanced reactivity of the lactam towards attack by Ser\(^{195}\) in the active site of thrombin when the ring nitrogen is substituted by a highly electron-withdrawing group.\(^4\)

Scheme 4 Synthesis of 4

In conclusion, this novel approach to the synthesis of a rationally designed irreversible inhibitor of α-human thrombin using a substituted carborane as a hydrophobic pharmacophore, is innovative and unprecedented. Efforts are ongoing to evaluate the potency of this inhibitor and the results will be published elsewhere. The strategic design of 4 can probably be improved by including an amidine-containing side chain that can form an ionic interaction with Asp\(^{189}\) in the S1\(^*\) recognition site. This plays a critical role in anchoring substrates in the correct orientation towards acylation in the active site.\(^4\) As previously mentioned, 14 is a versatile compound that can be substituted with numerous other templates known to interact in the active site of thrombin to extend favorable interactions with future lead compounds throughout the active site of the enzyme. These prospects are currently being investigated and will be communicated elsewhere.

Standard Schlenk- and vacuum-line techniques were employed for all manipulations of air- and moisture-sensitive compounds. Reaction solvents were distilled from appropriate drying agents under argon before use. Deca-β-methyl-p-carborane (5),\(^15\) trans-2-(2-tert-butoxycarbonylamino)cyclopentyl)malonic acid dimethyl ester (17)\(^21\) and racemic (3aR,6aS)-2-oxocyclopentyl)pyrrol-2-one (20),\(^22\) were prepared according to literature methods. Other reagents were used as purchased commercially. NMR spectra were obtained on a Bruker ARX 500 at 500 MHz (\(^1H\)), 125 MHz (\(^13C\)) and 160 MHz (\(^11B\)). Chemical shifts for \(^1H\) and \(^13C\) NMR spectra were referenced to TMS and measured with respect to residual proton spectra in deuterated solvents. Chemical shift values for \(^11B\) NMR spectra were referenced relative to external BF\(_3\)OEt\(_2\). Mass spectra were obtained on a VG Autospec.

Computational Docking Procedure
Various docking programs are available to study the protein–ligand interactions and, while various force fields are available to deal with
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1-(2-Hydroxyethyl)-closo-2,3,4,5,6,7,8,9,10,11-decamethyl-1,12-dicarbadodecaborane (6)

A solution of 5 (2.80 g, 9.84 mmol) in anhydrous THF (80 mL) was cooled to −78 °C and a solution of n-ButLi (4.13 mL, 10.33 mmol, 2.5 M in hexanes) was added dropwise. The reaction and stirred for 20 min at −78 °C then at r.t. for an additional 2 h. The reaction was again cooled to −78 °C and ethylene oxide (0.53 mL, 11.71 mmol) was condensed and added via syringe in one portion. After 20 min, the reaction was warmed to r.t. and stirred overnight. EtOAc (100 mL) was added to the reaction flask and the organic layer was washed with a sat. aq NH4Cl (2 × 75 mL) and brine (2 × 75 mL). The organic phase was dried over MgSO4, filtered and the solvent removed in vacuo. The resulting residue was purified by flash chromatography (silica gel; pentane–EtOAc, 9:9.0:1→9:1) to give 6.

Yield: 2.25 g (70%); colorless crystalline solid.

1H NMR (CDCl3): δ = 3.44 (t, J = 8 Hz, 2 H, CH2), 2.01 (br s, 1 H, CH), 1.67 (t, J = 8 Hz, 2 H, CH3), 1.25 (br s, 1 H, OH), 0.03 (br s, 30 H, B(CH3)3).

11B{1H} NMR (CDCl3): δ = 74.69 (CH), 53.42 (OCH3), 34.45 (CH3), –3.27 (vbr s, B(CH3)3).

1B{1H} NMR (Et2O): δ = –8.24 (s, B(CH3)3), –9.59 (s, B(CH3)3).

HRMS (FAB+): m/z calc. for C20H50B10O3Si: 438.26; found: 439.27 (M+ + H+).

1-(2-tert-Butylimidethylsiloxoyethyl)-closo-2,3,4,5,6,7,8,9,10,11-decamethyl-1,12-dicarbadodecaborane (7)

Alcohol 6 (1.6 g, 4.88 mmol) and NaH (95% dispersion in oil, 0.70 g, 29.3 mmol) were stirred in anhydrous THF (50 mL) at 0 °C for 30 min then allowed to warm to r.t. TBDMSiCl (0.81 g, 5.37 mmol) was slowly added to the reaction mixture at 0 °C and the reaction was warmed to r.t. After 2.5 h, the reaction mixture was partitioned between Et2O (3 × 60 mL) and aq K2CO3 (10%, 60 mL). The combined organic layers were washed with brine (2 × 75 mL), dried over MgSO4 and evaporated under vacuum. The resulting white foam residue was purified by flash chromatography (silica gel; pentane–EtOAc, 9:9.0:1→7) as a colorless oil which solidified overnight. The unreacted starting material was also recovered from the column (pentane–EtOAc, 9:1).

1H NMR (CDCl3): δ = 3.40 (t, J = 8 Hz, 2 H, CH2), 2.01 (br s, 1 H, CH), 1.63 (t, J = 8 Hz, 2 H, CH3), 0.99 (s, 9 H, r-Bu), 0.04 (s, 30 H, B(CH3)3), 0.005 (s, 6 H, 2 × CH3).

13C{1H} NMR (CDCl3): δ = 74.69 (CH), 59.98 (OCH3), 34.64 (CH3), 25.86 (r-Bu), 18.21 (C), –3.27 (vbr s, B(CH3)3), –5.38 (2 × CH3).

1B{1H} NMR (Et2O): δ = –8.33 (s, B(CH3)3), –9.56 (s, B(CH3)3).

1B{1H} NMR (Et2O): δ = –8.33 (s, B(CH3)3), –9.56 (s, B(CH3)3).

HRMS (FAB+): m/z calc. for C20H50B10O3Si: 442.24; found: 443.35 (M+ + H+).

1-(2-tert-Butylimidethylsiloxoyethyl)-closo-2,3,4,5,6,7,8,9,10,11,12-undecamethyl-1,12-dicarbadodecaborane (8)

Compound 7 (1.0 g, 2.26 mmol) was dissolved in anhydrous THF (30mL) and the solution was cooled to −78 °C. A solution of MeLi (2.82 mL, 4.52 mmol, 1.6 M in Et2O) was added dropwise and the mixture was stirred for 20 min then warmed to r.t. and stirred for an additional 2 h. MeI (0.28 mL, 4.52 mmol) was added at −78 °C and the reaction mixture was slowly warmed to r.t. and stirring was continued for 48 h. The progress of the reaction was monitored by 11B NMR spectroscopy until a single peak at δ = 9.92 ppm (decoupled) was the only detected resonance. The solvent was removed in vacuo and the resulting residue was dissolved in Et2O (80 mL). The organ-
ic layer was washed H₂O (2 × 60 mL), brine (2 × 60 mL), dried over MgSO₄ and evaporated. The crude colorless residue was purified by flash chromatography (silica gel; pentane–EtOAc, 9:9:0.1) to give 8.

Yield: 1.02 g (99%); colorless crystalline solid.

1H NMR (CDCl₃): δ = 3.41 (t, J₁₂ = 8 Hz, 2 H, CH₂), 1.63 (t, J₁₂ = 8 Hz, 2 H, CH₂), 0.89 (s, 9 H, CH₃), 0.07 (s, 15 H, B(CH₃)₂), 0.03 (s, 6 H, 2 × CH₂), –0.05 (s, 15 H, B(CH₃)₂).

11B NMR (Et₂O): δ = –7.81 (s, B(CH₃)).

1-(2-Hydroxyethyl)-closo-2,3,4,5,6,7,8,9,10,11,12-undecamethyl-1,12-dicarbaborane (9)

Compound 9 (1.20 g, 2.63 mmol) in anhydrous THF (30 mL) was treated with a solution of TBAF (5.94 mL, 6.00 mmol, 1 M in THF) at r.t. for 2.5 h. After addition of sat. aq NaHCO₃ (20 mL), the mixture was extracted with Et₂O (3 × 60 mL). The combined organic phase was washed with H₂O (3 × 50 mL) and brine (2 × 60 mL), dried over MgSO₄ and evaporated. The residue was purified by flash chromatography (silica gel; pentane–EtOAc, 1:1) to give 9.

Yield: 0.81 g (90%); colorless crystalline solid.

1H NMR (CDCl₃): δ = 3.50 (t, J₁₂ = 8 Hz, 2 H, CH₂), 1.73 (t, J₁₂ = 8 Hz, 2 H, CH₂), 1.34 (s, 1 H, OH), 0.81 (s, 3 H, CH₃), 0.07 (s, 15 H, B(CH₃)₂), –0.05 (s, 15 H, B(CH₃)₂).

11B NMR (CDCl₃): δ = 3.46 (br m, 4 H, CH₂ piperazine), 3.43 (br m, 4 H, CH₂ piperazine), 2.33 (s, 2 H, CH₂), 1.45 (s, 9 H, t-Boc), 0.76 (s, 3 H, CH₃), 0.04 (s, 15 H, B(CH₃)₂), –0.01 (s, 15 H, B(CH₃)₂).

11B NMR (Et₂O): δ = –7.81 (s, B(CH₃)).

HRMS (EI+): m/z calc’d for C₁₅H₃₈B₁₀O_{11}: 535.22; found: 534.2 [M + H].

1-(2-Carboxymethyl)-closo-2,3,4,5,6,7,8,9,10,11,12-undecamethyl-1,12-dicarbaborane (10)

A stock solution of H₅IO₆ (1.14 g) and CrO₃ (2.42 mg) was stirred in MeCN (11 mL) and H₂O (8.25 mL) at 0 °C for 2 h. 2.3 mL of this stock solution was added to a stirred mixture of 2,3,4,5,6,7,8,9,10,11,12-undecamethyl-1,12-dicarbadodecaborane (12) tert-Butyl 1-piperazinecarboxylate (0.55 g, 2.97 mmol) was dissolved in anhydrous CH₂Cl₂ (10 mL) and deprotonated with DIPEA (0.52 mL, 2.98 mmol) at r.t. for 20 min. This anionic solution was added to 11 (1.0 g, 2.67 mmol) in CH₂Cl₂ (50 mL) at 0 °C and the reaction mixture was stirred at r.t. for 2 h. The solvent was removed and the residue was purified by flash chromatography (silica gel; pentane–EtOAc, 7.5:2.5) to yield 12.

Yield: 0.99 g (71%); colorless foamy solid.

HRMS (EI+): m/z calc’d for C₁₅H₃₈B₁₀O_{11}Na₂O₃: 524.49; found: 525.50 [M + H].

1-[2-(4-Tert-Butylcarboxy-1-piperazine)carbonylmethyl]-closo-2,3,4,5,6,7,8,9,10,11,12-undecamethyl-1,12-dicarbaborane (12)

A solution of acid 10 (1.15 g, 3.23 mmol) in anhydrous CH₂Cl₂ (150 mL) was cooled to 0 °C and SOCl₂ (1.18 mL, 16.15 mmol) was added. The reaction was allowed to warm to r.t. over 30 min then the slightly yellow solution was refluxed for 24 h. The solvent and excess of SOCl₂ were removed under vacuum to give 11, which was analyzed and used without further purification.

Yield: 1.15 g (98%); pale-yellow solid.

1H NMR (CDCl₃): δ = 2.92 (s, 2 H, CH₃), 0.79 (s, 3 H, CH₃), 0.08 (s, 15 H, B(CH₃)₂), –0.07 (s, 15 H, B(CH₃)₂).

13C{¹H} NMR (CDCl₃): δ = 167.91 (CO), 46.94 (CH₃), 13.28 (CH₂), –4.58 (vbr s, B(CH₃)).

11B NMR (Et₂O): δ = –8.29 (s, B(CH₃)).

1B NMR (Et₂O): δ = –7.57 (s, B(CH₃)).

HRMS (EI+): m/z calc’d for C₁₅H₂₀B₁₀O₃N₂O₃: 524.49; found: 525.50 [M + H].

1-[2-(1-Piperazine)carbonylmethyl]-closo-2,3,4,5,6,7,8,9,10,11,12-undecamethyl-1,12-dicarbaborane (13)

To a solution of 12 (2.2 g, 4.20 mmol) in anhydrous CH₂Cl₂ (50 mL), TFA (4 mL) was added at 0 °C. The reaction was stirred for 100 min at 0 °C then warmed to r.t. and stirred for an additional 40 min. The reaction mixture was concentrated and placed under high vacuum to remove excess TFA to give 13, which was used without any further purification.

Yield: 1.71 g (quant); beige solid.

1H NMR (CDCl₃): δ = 8.78 (br s, 1 H, NH), 3.82 (br m, 4 H, CH₂ piperazine), 3.23 (br m, 4 H, CH₂ piperazine), 2.37 (br s, 2 H, CH₂), 0.76 (s, 3 H, CH₃), 0.05 (s, 15 H, B(CH₃)₂), –0.08 (s, 15 H, B(CH₃)₂).

13C{¹H} NMR (CDCl₃): δ = 167.13 (CO), 47.25 (CH₃), 43.59 (CH₂ piperazine), 30.95 (CH₂ piperazine), 13.26 (CH₃), –4.29 (vbr s, B(CH₃)).

11B NMR (Et₂O): δ = –7.76 (s, B(CH₃)).

1B NMR (Et₂O): δ = –7.76 (s, B(CH₃)).

HRMS (EI+): m/z calc’d for C₁₅H₂₀B₁₀O₃N₂O₃: 524.49; found: 525.50 [M + H].

1-[2-(4-Bromomethylcarbonyl-1-piperazine)carbonylmethyl]-closo-2,3,4,5,6,7,8,9,10,11,12-undecamethyl-1,12-dicarbaborane (14)

A solution of acid 10 (1.15 g, 3.23 mmol) in anhydrous CH₂Cl₂ (7 mL) was added DIPEA (0.62 mL, 3.55 mmol) and the solution was stirred for 20 min at 0 °C. Bromo acetyl bromide (0.31 mL, 3.55 mmol) was slowly added to the cooled reaction mixture,
which was then warmed to r.t. and allowed to continue stirring overnight. An aqueous extraction was performed and the organic layers were washed with H2O (2 × 10 mL) and brine (2 × 10 mL), dried over MgSO4, filtered and concentrated to yield a light-brown residue. The crude material was purified by flash chromatography (silica gel; pentane–Et2O, 6:4→1:1) to give 14.

Yield: 1.55 g (81%); colorless solid.

1H NMR (CDCl3): δ = 3.85 (s, 2 H, BrCH2), 3.60 (br m, 4 H, CH2 piperazine), 3.45 (br m, 4 H, CH2 piperazine), 2.36 (s, 2 H, CH2), 0.77 (s, 3 H, CH3), 0.06 (s, 15 H, B(CH3)3), –0.09 (s, 15 H, BCH3).

13C{1H} NMR (CDCl3): 51.60 (OCH3), 42.80 (NCH), 37.72 (CH), 33.03 (CH2), 29.96 (CH2), 19.35 (CH2), 17.09 (CH3) ppm.

HRMS (FAB+): m/z calcd for C15H25NO6: 315.27; found: 316.2 [M + H].

**trans-2-(2-Tert-Butyloxycarbonylamino)cyclopentylmalonic Acid tert-Butyl Ester (15)**

To a stirred solution of cyclopentene oxide (23.0 g, 0.27 mol) in a mixture of EtOH–H2O (50:50, 250 mL), NH4Cl (15.0 g, 0.28 mol) and NaN3 (18.0 g, 0.27 mol) were added and the resulting mixture was refluxed for 48 h. The mixture was concentrated to a small volume and partitioned between H2O (200 mL) and EtOAc (200 mL). The organic layer was dried over anhydrous MgSO4 and evaporated to give an orange semi-solid (30.8 g). The compound was analyzed by 1H NMR and was found to be sufficiently pure to be carried forward without further purification. The crude azide (30.8 g, 0.24 mol) was dissolved in toluene (500 mL) and Ph3P (95.42 g, 0.36 mol) was added. The resulting solution was slowly warmed to prevent the violent evolution of N2 gas. Once equilibrated, the vibrantly orange solution was refluxed for 1.5 h. The solution was cooled to 0 °C and diluted with EtOAc (50 mL) and sat. NaHCO3 (50 mL). An aqueous extraction was performed and the organic layers were washed with H2O (2 × 10 mL) and brine (2 × 10 mL), which was then warmed to r.t. and allowed to continue stirring overnight. The resulting biphasic mixture was vigorously stirred for 30 min then Boc2O (53.1 g, 0.24 mol) in EtOAc (420 mL) was slowly added over several minutes. The mixture was warmed to r.t. and stirred overnight. The organic phase was separated and concentrated to give a semi-solid, which was dissolved in EtOAc, dried over anhydrous MgSO4 and concentrated to a crude semi-solid. This material was treated with Ph3P (19.38 g, 0.074 mol) in THF (150 mL) at –60 °C and diluted with EtOAc (50 mL) was added. The resulting solution was slowly warmed to prevent the violent evolution of N2 gas. Once equilibrated, the vibrantly orange solution was refluxed for 24 h. The mixture was filtered and the filtrate was concentrated to give a beige foam. The crude product was dissolved in xylenes (150 mL) and stirred in the presence of air for 45 min. The mixture was then acidified with potassium hydrogen sulfate (1 M, 10 mL) and the mixture was dissolved in H2O (10 mL). The product was extracted into Et2O (3 × 60 mL) and the combined organic extracts were washed with brine (2 × 150 mL), dried over anhydrous MgSO4 and evaporated to give a beige foam. The crude product was dissolved in xylene (150 mL) and refluxed for 4 h. After cooling, the mixture was concentrated and the resulting solid was purified by flash chromatography (silica gel; petroleum ether–Et2O, 4:1) to give 16.

Yield: 6.75 g (75%); colorless oil.

1H NMR (CDCl3): δ = 2.9 (s, 2 H, H-1,5), 2.05 (dd, 2 H, H-2,4), 1.6 (m, 3 H, H-2,3,4, 1.4 (s, 9 H, t-Bu), 1.2 (m, 1 H, H-3).

13C{1H} NMR (CDCl3): δ = 161.40 (CO), 80.36 (C), 42.87 (NCH), 27.87 (CH, t-Bu), 26.34 (CH3), 19.35 (CH2).

HRMS (EI+): m/z calcd for C19H27NO2: 312.23; found: 312.2 [M + H].

Racemic (1R,2S)-2-tert-Butyloxycarbonylamino)cyclopentyl)-[S]-benzoylcarbonylaminocyclopentyl]acetic Acid Methyl Ester (18)

KOH (1 M, 8.85 mL, 8.85 mmol) was added to a solution of 17 (0.56 g, 1.76 mmol) in MeOH (20 mL) and the reaction mixture was stirred for 24 h. The mixture was filtered and the filtrate was concentrated to give a pale-yellow residue that was purified by flash chromatography (silica gel; petroleum ether–Et2O, 4:1) to give 16.

Yield: 6.75 g (75%); colorless oil.

1H NMR (CDCl3): δ = 4.55 (br s, 1 H, NH), 3.84 (s, 3 H, OCH3), 3.59 (m, 1 H, H-2), 2.63 (dd, J1,3 = 5 Hz, J2,3 = 15.0 Hz, H-1, H-2), 2.45–1.24 (m, 9 H, H-3,4,5), 1.47 (s, 9 H, t-Bu).

13C{1H} NMR (CDCl3): δ = 172.35 (CO), 155.26 (CO), 78.85 (C), 55.54 (NCH), 54.60 (CH), 52.40 (OCH3), 44.93 (CH3), 33.03 (CH2), 28.25 (CH, t-Bu), 21.47 (CH3).

HRMS (FAB+): m/z calcd for C19H27NO2: 312.23; found: 312.2 [M + H].

**Racemic (1R,2S)-2-tert-Butyloxycarbonylamino)cyclopentyl]-[S]-benzoylcarbonylaminocyclopentyl]acetic Acid (19)**

Ester 18 (0.27 g, 1.06 mmol) was stirred with HCl (4 M, 20 mL) at r.t. for 14 h. The reaction mixture was filtered and concentrated under vacuum and the resulting oil was triturated with acetonitrile to yield 19.

Yield: 0.15 g (80%).
1H NMR (D2O): δ = 11.97 (br s, 1 H, OH), 3.68 (s, NH2), 3.22 (m, 1 H, H-1), 2.43 (m, 2 H, 2'), 2.14 (s, 1 H, H-2), 1.97–1.82 (m, 2 H, H-4,5), 1.57 (m, 3 H, H-3,4,5), 1.21 (m, 1 H, H-3).

13C{1H} NMR (CDCl3): δ = 177.06 (CO), 67.53 (NCH), 48.91 (CH3), 45.70 (CH2), 41.98 (CH2 pipera-azine), 37.66 (CH3), 37.56 (CH3), 31.19 (CH2 piperazine), 27.77 (CH2), 26.63 (CH2), 23.67 (CH2), 22.95 (CH2), 13.21 (CH3), –4.43 (vbr s, BCH3).

HRMS (MALDI +ve): m/z calculated for C12H19NO3: 211.15; found: 211.13 [M+ + H].

1H NMR (CDCl3): δ = 3.35 (m, 1 H), 3.18 (s, 3 H, CH3), 2.57 (m, 1 H), 2.27–1.13 (m, 8 H).

13C{1H} NMR (CDCl3): δ = 178.05 (CO), 67.45 (NCH), 45.74 (CH3), 40.59 (CH3), 37.56 (CH3), 27.80 (CH3), 23.72 (CH2), 22.85 (CH3).

HRMS (EI+): m/z calculated for C21H39NO5S: 667.95; found: 668.57 [M+ + Na].

1H NMR (CDCl3): δ = 3.72 (m, 1 H), 3.60 (br m, 4 H, CH2 piperazine), 3.45 (br m, 4 H, CH2 piperazine), 3.18 (s, 3 H, CH3), 2.94 (m, 2 H, CH2), 2.57 (m, 1 H), 2.35 (s, 2 H, CH2), 2.24–1.26 (m, 8 H), 0.78 (s, 3 H, CH3), 0.07 (s, 15 H, BCH3a), –0.09 (s, 15 H, BCH3b).

13C{1H} NMR (CDCl3): δ = 177.75 (CO), 167.49 (CO), 166.35 (CO), 67.53 (NCH), 48.91 (CH3), 45.70 (CH2 piperazine), 37.66 (CH3), 37.56 (CH3), 31.19 (CH2 piperazine), 27.77 (CH2), 26.63 (CH2), 23.67 (CH2), 22.95 (CH2), 13.21 (CH3), –4.43 (vbr s, BCH3).

1B{1H} NMR (EtOAc): δ = –8.30 (s, CH3).

HRMS (MALDI +ve): m/z calculated for C21H39BrNO5S: 667.95; found: 668.57 [M+ + H].

1H NMR (CDCl3): δ = 3.72 (m, 1 H), 3.45–3.33 (br m, 8 H, CH2 piperazine), 2.69 (m, 2 H, CH2), 2.50 (s, 2 H, CH2), 2.03–1.26 (m, 8 H), 1.49 (s, 9 H, t-Bu), 0.78 (s, 3 H, CH3), 0.05 (s, 15 H, BCH3a), –0.10 (s, 15 H, BCH3b).

13C{1H} NMR (CDCl3): δ = 171.00 (CO), 167.49 (CO), 161.67 (CO), 67.53 (NCH), 48.91 (CH3), 45.70 (CH2 piperazine), 37.66 (CH3), 37.56 (CH3), 31.19 (CH2 piperazine), 27.77 (CH2), 26.63 (CH2), 23.67 (CH2), 22.95 (CH2), 13.21 (CH3), –4.43 (vbr s, BCH3).

1B{1H} NMR (EtOAc): δ = –8.41 (s, CH3).
HRMS (EI+): m/z calc for C_{15}H_{26}B_{2}N_{4}O_{5}: 690.57; found: 691.65 [M^+ + H].

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References


