Supporting Information for

Carborane clusters increase the potency of bis-substituted cyclam derivatives against Mycobacterium tuberculosis

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Contents

1. General Procedures  \hfill S2

2. Synthesis and Characterisation Data \hfill S3

3. \textsuperscript{1}H and \textsuperscript{11}B\textsuperscript{[1]H} NMR Spectra of Novel Compounds \hfill S6

4. High- and Low-Resolution Mass Spectra \hfill S14

5. References \hfill S29
1. General Procedures

Synthesis

Anhydrous solvents were collected from a PureSolv MD7 solvent purification system containing activated alumina and copper columns. 1,2-closo-carborane and nido-decaborane(14) were purchased from Boron Specialties LLC (US) and Katchem (Czech Republic), respectively, while all other commercially available reagents and solvents were purchased from Sigma Aldrich, Alfa Aesar, Combi-Blocks, and Merck. Automated flash column chromatography was performed on a Biotage Isolera Spektra One using Biotage SNAP KP-SI cartridges filled with silica gel 60 LR, 0.04-0.06 mm (230-400 mesh ASTM). $^1$H and $^{11}$B[$^1$H] NMR spectra were recorded on Bruker Advance DPX 300 and 500 MHz spectrometers. Chemical shifts are reported relative to tetramethylsilane (0 ppm) or residual solvent resonance as internal standards. All low resolution ESI-MS and APCI-MS data were recorded on a Finnigan LCQ mass spectrometer. High resolution ESI-MS data were recorded on a Thermo Velos Pro Orbitrap mass spectrometer via syringe infusion on the Ion-Max Electrospray ionisation source. Infrared spectra were recorded on a Bruker ALPHA FT-IR spectrometer. Syntheses of compounds 7, 11, 12 and 16 have been reported previously and characterization data collected during this study for each of these compounds were in accordance with the reported literature values.

Bacterial Growth Conditions

Mycobacterial strain (M. tuberculosis H37Rv) was grown at 37°C to log phase (OD$_{600}$ nm between 0.6-0.8) in 7H9 media containing albumin, dextrose and catalase, 20% Tween 80 and 50% glycerol.

Resazurin Assay of Growth Inhibition

The minimal inhibitory concentration (MIC) of each compound was determined using a modified resazurin viability assay. All compounds were initially prepared at 10 mM stock solutions in 100% DMSO and then adjusted to the required concentration in diluent (0.1% DMSO). Compounds (0.1-100 µM) were added to wells in 2-fold dilutions and incubated with bacteria previously diluted to OD$_{600}$ nm of approximately 0.001. Compounds and bacteria were incubated in complete 7H9 media at 37°C in a humidified 5% CO$_2$ incubator for 4 days. Resazurin and Tween 80 (1:1) was then added and incubated at 37°C for a further 24 hours. MICs were calculated by detection of fluorescence at 590 nm using a FLUOstar Omega microplate reader (BMG, Labtech).

Safety note: Decaborane(14), sodium azide and organic azides are hazardous and potentially explosive materials. Only small amounts of these materials should be prepared, and these should be handled with caution.
2. Synthesis and Characterisation Data

Di-tert-butyl 4,11-bis(closo-1,2-carboran-1-ylmethyl)-1,4,8,11-tetrazacyclopentadecane-1,8-dicarboxylate (8)

A solution of compound 7 (100 mg, 0.21 mmol) in toluene (5 mL) was added dropwise to a solution of nido-decaborane(14) (39 mg, 0.32 mmol) and CH₃CN (0.1 mL) in toluene (20 mL). The reaction mixture was heated to reflux and stirred for 24 h. The reaction was quenched with MeOH (10 mL) and allowed to stir for a further 24 h. The crude mixture was concentrated under reduced pressure and purified by column chromatography with 10% NH₃ in MeOH and DCM (1:9) to obtain product 8 as a yellow powder (90 mg, 60% yield).

1H NMR (300 MHz, CDCl₃): δ = 1.10-3.25 (br m, 20H), 1.47 (s, 18H), 1.73 (t, 4H, J 7.3), 2.57 (t, 4H, J 6.6), 2.65 (t, 4H, J 6.1), 3.04-3.20 (m, 12H), 3.80 (s, 2H).

11B{¹H} NMR (500 MHz, CDCl₃): δ = -2.52 (s, 2B), -5.04 (s, 2B), -9.18 (s, 4B), -11.54 (s, 4B), -13.00 (s, 8B). LRMS (ESI+) [M+Na]⁺ m/z = 736.76.

4,11-Bis(closo-1,2-carboran-1-ylmethyl)-1,4,8,11-tetrazacyclopentadecane-1,8-diium (9)

Hydrochloric acid (4.0 M in dioxane, 2.0 mL, 2.0 mmol) was added to a solution of compound 8 (35 mg, 0.049 mmol) in dioxane (5 mL) and stirred at room temperature for 4 hours. The solvent was evaporated off and the crude material was triturated with ice-cold ethyl acetate (2 x 10 mL) to obtain product 9 as a colourless powder (28 mg, 97% yield).

11B{¹H} NMR (500 MHz, DMSO): δ = -2.70 (s, 2B), -4.92 (S, 2B), -9.37 (S, 4B), -12.07 (S, 4B), -12.74 (S, 8B). FTIR: ν max cm⁻¹ 2985, 2749, 2583, 2342, 1727, 1436, 1140, 1068, 898, 721. LRMS (ESI+) [M+H]⁺ m/z = 514.63. HRMS (ESI+): m/z calcd for C₁₆H₄₉B₂₂N₄⁺ 514.59222 found 514.59206.

Cesium 4,11-bis(nido-7,8-carboran-1-ylmethyl)-1,4,8,11-tetrazacyclopentadecane-1,8-diium (Cs₂₁₀)

A mixture of CsF (42 mg, 0.28 mmol) and 9 (32 mg, 0.06 mmol) in EtOH (10 mL) was heated to reflux under nitrogen and stirred for 24 h. The solvent was evaporated off and the crude material was dissolved in acetone (30 mL). Insoluble borate impurities were removed by centrifugation and the supernatant was concentrated under reduced pressure to obtain compound Cs₂₁₀ as a colourless powder (39 mg, 95% yield).

1H NMR (500 MHz, acetone-d₆): δ = -3.12 (br s, 2H), -0.23-3.76 (br m, 16H), 1.16 (s, 2H), 1.65-1.75 (m, 4H), 2.13-2.30 (m, 2H), 2.42-2.53 (m, 2H), 2.59 (s, 2H), 2.68 (d, 2H, J 12.2), 2.76 (d, 2H, J 13.0), 2.83-2.98 (m, 6H), 3.02-3.07 (m, 2H), 3.14-3.26 (m, 2H). 11B{¹H} NMR (500 MHz, acetone-d₆): δ = -10.16 (s, 2B), -12.35 (S, 2B), -13.39 (S, 2B), -15.32 (S, 2B), -19.74 (S, 4B), -20.62 (S, 2B), -32.27 (S, 2B), -36.67 (S, 2B). FTIR: ν max cm⁻¹ 2920, 2835, 2502, 1587, 1451, 1364, 1117, 1023, 974. LRMS (ESI-) [M-2Cs+H]⁻ m/z = 492.56. HRMS (ESI-) [M-2Cs+H]⁻: m/z calcd for C₁₆H₄₉B₁₈N₄⁻ 492.57468 found 492.57480.

Di-tert-butyl 4,11-bis(2-(closo-1,2-carboran-1-ylmethyl)-1,2,3-triazol-1-yl)ethyl)-1,4,8,11-tetrazacyclopentadecane-1,8-dicarboxylate (13)

A mixture of CuSO₄·5H₂O (3.5 mg, 0.014 mmol) and sodium ascorbate (3.5 mg, 0.018 mmol) in H₂O (5 mL) was added to a mixture of 11 (80 mg, 0.440 mmol) and 12 (29 mg, 0.055 mmol) in THF (10 mL) and stirred at reflux under nitrogen for 16 h. The reaction mixture was quenched with the addition of NH₄Cl (sat, 5 mL) and the crude product was extracted with DCM (2 x 10 mL). Organic phases were combined, dried over MgSO₄.
and concentrated under reduced pressure. The product was isolated by column chromatography with 10% NH$_3$ in MeOH and DCM (1:9) to obtain product 13 as a yellow oil (39 mg, 81% yield). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 1.36-3.45 (m, 20H), 1.45 (s, 18H), 1.59 (qn, 4H, J 7.7), 2.47 (t, 4H, 6.3), 2.54-2.63 (m, 4H), 2.91 (t, 4H, J 6.1), 3.09-3.23 (m, 8H), 3.66 (s, 4H), 3.92 (s, 2H), 4.36 (t, 4H, J 6.0), 7.52 (s, 2H). $^{13}$B($^1$H) NMR (500 MHz, CDCl$_3$): $\delta$ = -1.95 (s, 3B), -5.43 (s, 2B), -9.34 (s, 5B), -10.95 (s, 4B), -12.60 (s, 6B). FTIR: $\nu_{\max}$ cm$^{-1}$ 2924, 2586, 1684, 1367, 1246, 1156, 1089, 1020, 913, 774, 532. LRMS (ESI+) [M+H]$^+$ m/z = 904.85, [M+Na]$^+$ m/z = 926.81.

4-11-bis(2-(closo-1,2-carboran-1-ylmethyl-1,2,3-triazol-1-yl)ethyl)-1,4,8,11-tetraazacyclotetradecane-1,8-diium (14)

HCl (4.0 M in dioxane, 2.0 mL, 2.0 mmol) was added to a solution of 13 (35 mg, 0.049 mmol) in dioxane (5 mL) and stirred at room temperature for 4 h. The solvent was evaporated off and the crude material was triturated with ice-cold ethyl acetate (2 x 10 mL) to obtain product 14 as a white powder (24 mg, 82% yield). $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ = 1.37-3.67 (br m, 20H), 1.91 (s, 6H), 2.65 (s, 4H), 2.87 (s, 4H), 3.03 (s, 8H), 3.17 (s, 6H), 4.57 (s, 4H), 5.17 (s, 2H), 8.20 (s, 2H), 8.74 (s, 4H). $^{11}$B($^1$H) NMR (500 MHz, DMSO): $\delta$ = -3.21 (s, 2B), -6.03 (s, 2B), -9.71 (s, 4B), -11.81 (s, 6B), -12.80 (s, 6B). FTIR: $\nu_{\max}$ cm$^{-1}$ 2934, 2580, 1435, 1062, 778, 721. LRMS (ESI+) [M+H]$^+$ m/z = 704.76. HRMS (ESI+) [M+H]$^+$: m/z calcd for C$_{24}$H$_{59}$B$_{20}$N$_{10}$+ 703.69252 found 703.69320.

Cesium 1,8-bis((E)-5-nido-7,8-carboran-1-ylpent-2-en-1-yl)-1,4,8,11-tetraazatricyclo[9.3.1.1$^{4,8}$]hexadecane-1,8-diium (Cs$_2$15)

A mixture of CsF (20 mg, 0.126 mmol) and 14 (30 mg, 0.039 mmol) in EtOH (10 mL) was heated to reflux under nitrogen and stirred for 24 h. The solvent was evaporated off and the crude material was dissolved in acetone (30 mL). Insoluble borate impurities were removed by centrifugation and the supernatant was concentrated under reduced pressure to obtain compound Cs$_2$15 as a colourless powder (33 mg, 89% yield). $^1$H NMR (500 MHz, acetone-d$_6$): $\delta$ = -2.87 (br s, 2H), -0.38-3.43 (br m, 16H), 1.74 (s, 6H), 2.09 (s, 2H), 2.15 (s, 2H), 2.64-2.68 (m, 4H), 2.85 (s, 4H), 2.92-2.97 (m, 8H), 3.03 (d, 6H, J15.4), 4.61-4.64 (m, 4H), 7.86 (s, 2H). $^{11}$B($^1$H) NMR (500 MHz, acetone-d$_6$): $\delta$ = -10.34 (s, 2B), -10.95 (s, 2B), -13.54 (s, 2B), -15.99 (s, 2B), -18.93 (s, 4B), -21.83 (s, 2B), -33.24 (s, 2B), -36.85 (s, 2B). LRMS (ESI-) [M-2Cs+H]$^-$ m/z = 682.85. HRMS (ESI-) [M-2Cs+H]$^-$: m/z calcd for C$_{24}$H$_{59}$B$_{18}$N$_{10}$- 682.67138 found 682.67148.

(E)-2-(5-Bromopent-3-en-1-yl)-1,2-closo-carborane (17)

A solution of 16 (219 mg, 1.10 mmol) in anhydrous DCM (5 mL) was added dropwise to a mixture of Grubbs 2nd generation catalyst (36 mg, 4 mol%, 0.04 mmol) and allyl bromide (0.2 mL, 280 mg, 2.31 mmol) in anhydrous DCM (10 mL). The reaction mixture was stirred at room temperature under nitrogen for 4 h. The crude mixture was concentrated under reduced pressure and the product was isolated by column chromatography with hexane (100%) to obtain product 17 as a clear oil (119 mg, 37% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 1.04-3.28 (m, 10H), 2.17-2.36 (m, 4H), 3.58 (s, 1H), 3.87-3.93 (m, 2H), 5.61-5.85 (m, 2H).
FTIR: $\nu_{\text{max}}$ cm$^{-1}$ 3061, 2959, 2570, 1449, 1202, 962, 721, 589. LRMS (APCI+) [M-Br+H]$^+$ m/z = 211.23 (debrromination). HRMS (APCI+) [M-Br+H]$^+$: m/z calcd for C$_7$H$_{19}$B$_{10}$+ 212.24844 found 212.24848.

1,8-Bis((E)-5-closo-1,2-carboran-1-ylpent-2-en-1-yl)-1,4,8,11-tetraazatricyclo[9.3.1.1$^4$8]hexadecane-1,8-diium (19)

A solution of 17 (119 mg, 0.41 mmol) in anhydrous CH$_3$CN (5 mL) was added to a solution of bridged cyclam 18 (34 mg, 0.15 mmol) in anhydrous CH$_3$CN (10 mL). The reaction mixture was stirred at room temperature for 24 h, monitored by TLC and ESI-MS. The precipitate was filtered and washed with ice cold CH$_3$CN (2 x 20 mL). The crude product was then dissolved in MeOH (20 mL) and stirred with NaOH (2.5 M, 1 mL, 1 mmol) at room temperature for an additional 1 h. The crude mixture was concentrated under reduced pressure and the product was isolated by column chromatography with 10% NH$_3$ in MeOH and DCM (1:9) to obtain product 19 as a colourless powder (70 mg, 74% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 1.20-3.40 (br m, 20H), 1.33 (s, 2H), 1.61 (p, 2H, J 3.0), 1.86-2.00 (m, 4H), 2.21-2.29 (m, 4H), 2.41 (t, 2H, J 4.2), 2.58 (t, 4H, J 5.2), 2.66-2.77 (m, 4H), 2.88-2.98 (m, 6H), 3.15-3.21 (m, 2H), 3.41 (t, 2H, J 6.0), 3.99 (s, 2H), 5.47-5.66 (m, 4H). FTIR: $\nu_{\text{max}}$ cm$^{-1}$ 2926, 2588, 1672, 1456, 1183, 1138, 1020, 918, 722. LRMS (ESI+) [M]$^+$ m/z = 323.85 (bridged). LRMS (ESI+) [M+H]$^+$ m/z = 622.76 (free base). HRMS (ESI+) [M+H]$^+$: m/z calcd for C$_{24}$H$_{61}$B$_{20}$N$_4$+ 621.68993 found 621.68993.

Cesium 1,8-bis((E)-5-nido-7,8-carboran-1-ylpent-2-en-1-yl)-1,4,8,11-tetraazatricyclo[9.3.1.1$^4$8]hexadecane-1,8-diium (Cs$_2$20)

A mixture of CsF (20 mg, 0.126 mmol) and 19 (30 mg, 0.048 mmol) in EtOH (10 mL) was heated to reflux under nitrogen and stirred for 24 h. The solvent was evaporated off and the crude material was dissolved in acetone (30 mL). Insoluble borate impurities were removed by centrifugation and the supernatant was concentrated under reduced pressure to obtain compound Cs$_2$20 as a colourless powder (40 mg, 96% yield). $^1$H NMR (500 MHz, Acetone-$d_6$): $\delta$ = -2.65 (br s, 2H), -0.34-2.75 (br m, 16H), 1.19 (s, 2H), 1.49-1.56 (m, 2H), 1.65 (s, 2H), 1.67-1.75 (m, 6H), 2.14-2.23 (m, 4H), 2.49-2.56 (m, 8H), 2.64 (dt, 6H, J 15.5, 5.3), 3.07 (d, 4H, J 6.6), 5.48 (dt, 2H, J 15.3, 6.7), 5.61 (dt, 2H, J 15.3, 6.7). $^{11}$B[$^1$H] NMR (500 MHz, Acetone-D6): $\delta$ = -11.15 (s, 5B), -14.20 (s, 2B), -17.33 (s, 2B), -18.48 (s, 4B), -22.40 (s, 1B), -33.54 (s, 2B), -37.45 (s, 2B). FTIR: $\nu_{\text{max}}$ cm$^{-1}$ 2914, 2830, 2506, 1615, 1448, 1354, 1025, 972. LRMS (ESI-) [M-2Cs+H]$^-$ m/z = 600.74. HRMS (ESI-) [M-2Cs+H]$^-$: m/z calcd for C$_{24}$H$_{61}$B$_{18}$N$_4$- 600.66857 found 600.66881.
3. $^1$H and $^{11}$B($^1$H) NMR Spectra of Novel Compounds

Figure S1. $^1$H NMR spectrum of compound 8 in CDCl$_3$.

Figure S2. $^1$H NMR spectrum of compound 10 in d$_6$-acetone.
Figure S3. $^1$H NMR spectrum of compound 13 in CDCl$_3$.

Figure S4. $^1$H NMR spectrum of compound 14 in d$_6$-DMSO.
Figure S5. $^1$H NMR spectrum of compound 15 in $d_6$-acetone.

Figure S6. $^1$H NMR spectrum of compound 17 in CDCl$_3$. 
Figure S7. $^1$H NMR spectrum of compound 19 in CDCl$_3$.

Figure S8. $^1$H NMR spectrum of compound 20 in d$_6$-acetone.
Figure S9. $^{11}$B$^{1}$H NMR spectrum of compound 8 in CDCl$_3$.

Figure S10. $^{13}$B$^{1}$H NMR spectrum of compound 9 in d$_6$-DMSO.
Figure S11. $^{11}$B($^1$H) NMR spectrum of compound 10 in d$_6$-acetone.

Figure S12. $^{11}$B($^1$H) NMR spectrum of compound 13 in CDCl$_3$. 
Figure S13. $^{11}$B($^1$H) NMR spectrum of compound 14 in d$_6$-DMSO.

Figure S14. $^{11}$B($^1$H) NMR spectrum of compound 15 in d$_6$-acetone.
Figure S15. $^{11}$B/$^1$H NMR spectrum of compound 20 in d$_6$-acetone.
4. High- and Low-Resolution Mass Spectra

Figure S16. HRMS spectrum of compound 9 m/z calcd for C_{16}H_{49}B_{20}N_{4}^+ 514.59222 found 514.59206.
Figure S17. HRMS spectrum of compound 10 m/z calcd for C_{16}H_{49}B_{18}N_{4} - 492.57468 found 492.57480.
Figure S18. HRMS spectrum of compound 14 m/z calcd for $C_{24}H_{59}B_{20}N_{10}^+$ 703.69252 found 703.69320.
Figure S19. HRMS spectrum of compound 15 m/z calcd for C_{24}H_{59}B_{19}N_{10}· 682.67138 found 682.67148.
Figure S20. HRMS spectrum of compound 17 m/z calcd for C$_7$H$_{19}$B$_{10}$ $^+$ 212.24844 found 212.24848.
Figure S21. HRMS spectrum of compound 19 m/z calcd for $C_{24}H_{61}B_{20}N_4^+$ 621.68985 found 621.68993.
Figure S22. HRMS spectrum of compound 20 $m/z$ calcd for $\text{C}_{24}\text{H}_{61}\text{B}_{18}\text{N}_{4}$ 600.66857 found 600.66881.
Figure S23. LRMS spectrum of compound 8 m/z calcd for C_{26}H_{65}B_{20}N_{4}O_{4} $^+$ 714.70 found 713.79 and calcd for C_{26}H_{64}B_{20}N_{4}O_{4}Na $^+$ 735.68 found 735.77.
Figure S24. LRMS spectrum of compound 9 $m/z$ calcd for $C_{16}H_{49}B_{20}N_4^+$ 514.59 found 514.62.
Figure S25. LRMS spectrum of compound 10 m/z calcd for C_{16}H_{49}B_{19}N_{4} 493.58 found 492.56.
Figure S26. LRMS spectrum of compound 13 m/z calcd for C$_{34}$H$_{75}$B$_{20}$N$_{10}$O$_4$+ 904.80 found 904.85 and calcd for C$_{34}$H$_{75}$B$_{20}$N$_{10}$O$_4$Na+ 926.78 found 926.81.
Figure S27. LRMS spectrum of compound 14 m/z calcd for C$_{24}$H$_{59}$B$_{20}$N$_{10}$ $^+$ 704.69 found 704.74.
Figure S28. LRMS spectrum of compound 15 m/z calcd for C_{24}H_{59}B_{18}N_{10} \cdot 682.67 found 682.75.
Figure S29. LRMS spectrum of compound 17 m/z calcd for C$_7$H$_{19}$B$_{10}^+$ 212.26 found 211.22.
Figure S30. LRMS spectrum of compound 19 $m/z$ calcd for $\text{C}_{24}\text{H}_{61}\text{B}_{20}\text{N}_4^+$ 622.69 found 622.76.
Figure S31. LRMS spectrum of compound 20 m/z calcd for C_{24}H_{61}B_{18}N_{4}^- 600.67 found 600.75.

5. References


