Norbornene-based anion receptors as D-alanine binders

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(Received 28 May 2015; accepted 26 August 2015)

Vancomycin is currently used as last-line therapy against many Gram-positive bacterial pathogens. Herein, we report a series of peptidomimetic norbornene-based anion receptors that were designed as simple vancomycin mimics. New hosts were evaluated for their affinity to both acetate and acetyl D-Ala by \textsuperscript{1}H NMR titration. Modest binding to both anions was observed in DMSO-\textsubscript{d}6 (Log $K_a$ 1–2 for TBA Acetyl D-Alanine) in the anticipated 1:1 mode of binding.

**Keywords**: anion binding; NMR titration; antibacterial; vancomycin

Introduction

An increasing incidence of bacterial resistance to clinically used antibiotic agents (such as methicillin-resistant \textit{Staphylococcus} and vancomycin-resistant \textit{enterococci}) combined with decreased investment in antibacterial agents by the pharmaceutical industry has led to an antibiotic crisis (1).

Vancomycin I is a naturally occurring antibiotic and, in a supramolecular chemistry context, is an elegant example of anion recognition by means of both a preorganised (achieved largely by the linked aromatic amino acid side chains) and complementary (matched H-bond donors/acceptors) host (Figure 1) (2). The ‘guest’ is the terminal D-alanine-D-alaninate (D-Ala-D-Ala, 2) portion of Lipid II, a key substrate in the late-stage enzymatic cross-linking to form the bacterial peptidoglycan outer membrane (2, 3).

Vancomycin binds D-Ala-D-Ala through five hydrogen bonds (2, 3) with three of these combining to target the terminal carboxylate. The strength of binding (Log $K_a$ of 5.6 in an aqueous environment) of this interaction has been measured using a segment of the peptide portion of Lipid II, namely diacetyl-L-Lys-D-Ala-D-Ala (4). In the presence of vancomycin, the transpeptidase enzyme responsible for cross-linking the cell wall is unable to access the D-Ala-D-Ala, thus, construction of the cell wall is incomplete and the bacteria are rendered susceptible to lysis and cell death (5).

As vancomycin is an excellent example of anion recognition, supramolecular chemists have sought to mimic the binding, and in turn, the antibacterial properties of vancomycin by designing preorganised hosts specific for D-Ala-D-Ala (6, 7). Examples include that of Schumuck who produced functionalised pyrroles that bind D-Ala-D-Ala (2, 3, 8). Bremner and co-workers synthesised functionalised binols with cationic amino acid sequences and the resultant peptidomimetics were active against vancomycin-susceptible \textit{S. aureus} and vancomycin-resistant \textit{S. aureus}. (9) Cohen \textit{et al.} developed a vancomycin mimetic based on the calix[4]arene scaffold and studied its binding to D-alanine using diffusion NMR (10).

The structure of vancomycin can be viewed as a rigidified aryl ether backbone supporting, and preorganising, the peptide chain responsible for the hydrogen bonding interactions. Our work in the field of alicyclic frameworks (11) led us to believe that a similarly preorganised peptide (such as 2, Figure 1) might be constructed through the use of a rigid \textit{[n]polynorbornane} backbone. Herein, we report our initial efforts to replicate the carboxylate-binding portion of Vancomycin in which a series of peptide-functionalised norbornenes were designed and synthesised as carboxylate receptors (Figures 1, 3–9).

The design of these anion receptors allows for rapid synthesis with potential to incorporate a broad range of structural modifications. It is envisioned that the fragments discussed here will be elaborated in future through extension of both the peptide chain (using standard peptide coupling reagents) and also the norbornane scaffold (using 1,3-dipolar cycloaddition chemistry) to provide a macrocyclic peptidoframework such as \textit{[3]polynorbornane} 2. (11, 12) If the complete peptidoframework was to be successful it was envisioned that sections of this molecule, such as receptors 3–9, could bind to the carboxylate of D-Ala-D-Ala as vancomycin does (2, 3, 8).

The design of this fragment library (Figure 1) includes functionalisation for variation of (i) urea/thiourea moiety (X) to mediate the pK$_a$; (ii) electron-withdrawing group (Y), for further tuning of the urea/...
Figure 1. (Colour online) Vancomycin 1 bound to Ac-D-Ala-D-Ala (the hydrogen bond donors/acceptors of vancomycin are highlighted in red); (top right) design of [n]polynorbornane-supported peptide and (bottom right) receptors 3–9 with intended binding to Ac-D-Ala.

Results and discussion

A four-step protocol including imide formation, amide coupling, deprotection and anionophore formation was used to synthesise the norbornene receptors 3–9. As an example, synthesis of receptor 3 (Scheme 1) began with imide formation using norbornene anhydride 10 and Boc-protected dianimopropanoic acid to generate imide 11 in 95% yield. Imide 11 was coupled with glycine methyl ester using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and hydroxybenzotriazole (HOBt) to form amide 12 in 80% yield. Amide 12 was deprotected (20% trifluoroacetic acid in CH$_2$Cl$_2$) and treated with 4-fluorophenyl isothiocyanate to form thiourea receptor 3 in 67% yield. The synthesis of the norbornene receptors 3–9 all followed a similar synthetic route (Table 1).

Next, receptors 3–9 were evaluated for their ability to bind both acetate and acetyl D-alanine as model anions representing the carboxyl portion of the bacterial cell wall precursor. Using $^1$H NMR titration, all receptors were tested against both anions and the results are presented in Table 1. Anions were used as their tetrabutylammonium salts and the titrations were conducted in DMSO-d$_6$ using an initial host concentration of 2.5 mM. The strength of binding (Log $K_a$) was calculated using WinEQNMR2 (13) (see ESI for titration isotherms, modified Job plots and fit plots).

All receptors (3–9) were found to bind to both acetate and acetyl D-alanine in a 1:1 Host:Guest (H:G) stoichiometry. Although the modified Job plots used in this study indicated that the H:G stoichiometry was 1:2, we (14) and others (15) have shown previously that Job Plots can provide misleading results (in particular the modified methodology) and a sensible 1:2 H:G stoichiometry could not be envisioned.$^1$ Nevertheless, both 1:1 and 1:2 fitting protocols were used to analyse the data. The results of the 1:2 fitting provided large asymptotic errors (20–500%) and nonsensical $K_a$ values; the errors for the 1:1 fitting were less than 15%. Given, the quality of fit of the data to the 1:1 model, all receptors were deemed to bind acetate in a 1:1 stoichiometry with an average Log $K_a$ of 2.6 which is typical for (thio)ureas in DMSO (16).

The R=H receptor 9 provided an example where the receptor amide N–H was also able to contribute to binding of the anion (as indicated by the small downfield $^1$H NMR chemical shift change, $\Delta \delta = 0.16$), but for receptors such as 5, with an isopropyl side chain, the bulky group likely restricted the ability of the receptor to adopt the desired tridentate binding conformation. Unfortunately, only modest binding to acetyl-D-alanine was observed across this series (average Log $K_a = 1.9$), with no hydrogen bonding identified from the acetylamide. Nevertheless, receptor 8, the most abundantly available, was submitted for a disc diffusion assay against S. aureus, and unfortunately no activity was noted.

It was pleasing to see that in this initial investigation into norbornene receptors for D-alanine, the binding stoichiometry was 1:1 as predicted. While the strength of association was modest, the receptors lack the same...
number of H–bond donors and the same level of preorganisation present in vancomycin. Future efforts will involve using a larger, more preorganised, fused polynorbornane framework (such as 2) that will be functionalised with a more complete array of H–bond donors and acceptors.

Conclusion
A series of low-molecular weight norbornane-based vancomycin mimics were synthesised and evaluated for binding of acetyl-D-alaninate. Consistent 1:1 binding of these substrates with the desired target was noted and the compounds serve as an entry point for a larger study involving highly functionalised fused [n]polynorbornane-based vancomycin mimics.

Experimental

General experimental
All reagents were obtained commercially and used without purification; all solvents used were analytical reagent grade. Petroleum spirits refers to the fraction boiling between 40 and 60 °C. NMR spectra were collected on either a JEOL JNM-Ex 270 MHz, a Varian Unity Plus 300 MHz, an Eclipse JNM-ECP 400 MHz FT-NMR or a Bruker AVANCE III 500 MHz FT-NMR spectrometer as indicated. Samples were dissolved in CDCl3, DMSO-d6 or CD3CN (~0.5 mL) and reported relative to TMS = 0.00 ppm. 1H NMR spectra are reported as chemical shift (ppm) (integral, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, sept = septet, m = multiplet), J coupling (Hz), assignment). High-resolution mass spectral data were collected on either an Agilent Technologies LC/MSD TOF mass spectrometer or an LC Agilent 1200 MS 6520 QTOF with dual-electrospray ionisation source. Samples were dissolved in acetonitrile or MeOH at a concentration of less than 0.1 mg/mL. Thin-layer chromatography was performed on Merck TLC silica gel 60 F plates, and visualised using UV light (λ = 254 and 365 nm) and/or potassium permanganate (KMnO4, H2O, K2CO3) as an oxidising dip. Column chromatography was performed with silica gel 60 (230–400 mesh). All microwave reactions were performed using a CEM Discover S-class microwave reactor. IR spectra were collected on a Bruker AlphaP ATR–FTIR spectrometer. Compounds were named according to the IUPAC guidelines with the V on Baeyer system for polycyclic compounds and the carbohydrate α/β system for ring substituents (17).

NMR titration
A stock solution of each host was made up to 2.5 × 10−3 M in DMSO-d6, and then 600 μL of this solution was transferred to a 5-mm NMR tube and the 1H NMR spectrum acquired. The chemical shifts (ppm) of the resonances corresponding to the amide and both thiourea H-bond donors were recorded. An aliquot of the stock guest solution (5.0 μL of a 3.0 × 10−2 M in DMSO-d6 solution, 0.1 equiv of guest) was then added to the host
solution in the NMR tube by autopipette. The NMR tube was recapped, the solution was shaken by hand and then the $^1$H NMR spectrum was collected. Again, the chemical shifts of the resonances corresponding to the amide and both thiourea H-bond donors were recorded; this process was repeated until 2.0 equiv. of guest had been added. The aliquot was then increased (10 μL, 0.2 equiv of guest), and the procedure was repeated until a total of 4.0 equiv of guest had been added. The final additions were made using larger aliquots (20 μL, 0.4 equiv of guest) until a total of 6.0 equiv. of guest had been added. At this point, an additional 150 μL of aliquot (3.0 equiv.) was added. The data were corrected for dilution then plotted as a titration isotherm and Jobs plot.

(2'S) (1a, 2β, 6β, 7α)-N-[2'-
(tert-butoxy carbonylamino)carboxyethyl] 4-azatricyclo
[5.2.1.02,6]dec-8-en-3,6 dione (11)

A microwave vessel was charged with norbornene anhydride (82 mg, 0.5 mmol) and N’(tert-butoxycarbonyl) di-1,2-aminopropinoic acid (Boc-Dap-OH) (102 mg, 0.5 mmol) and EtOAc (0.5 mL). The resulting mixture was heated to 120 °C under microwave irradiation for 45 min. The solution was cooled, diluted with EtOAc (20 mL), washed with 1 M HCl (3 × 15 mL), dried with MgSO$_4$, filtered and concentrated in vacuo to afford the title compound (146 mg, 83%) as a white solid Mp 165 °C; $\delta$ 7.05 (1H, s, Thiourea Ar); 3.91 (2H, m, H1″); 3.89 (2H, m, H2″); 3.27 (2H, m, H8, H9); 3.25 (3H, s, OMe); 3.16 (2H, m, H1, H7); 3.01 (2H, m, H2, H6); 2.98 (2H, m, H4); 1.62 (3H, s, H3′); 1.54 (1H, d, J 7.7, H10b); 1.46 (1H, d, J 7.7, H10b); 1.30 (2H, m, H1, H7), 3.38 (2H, m, H2, H6), 3.77 (2H, m, H1″), 4.39 (1H, m, H2″), 5.26 (1H, d, J 5.8, NH), 6.12 (2H, m, H8, H9); $\delta$C (75 MHz, CDCl$_3$) 178.2, 177.6, 169.8, 155.6, 134.7, 134.0, 80.3, 77.2, 52.2, 52.0, 45.9, 45.0, 44.9, 38.8, 28.3; HRMS m/z (+ESI) 464.2356 ([C$_{20}$H$_{22}$N$_2$O$_6$-H] requires 464.2391).

**General procedures**

**General procedure for amino acid amide coupling**

To a suspension of norbornene carboxylic acid, C-protected amino acid salt (1.2–1.3 equiv.), EDCI (1.2–1.3 equiv) and HOBt (0.5–0.1 equiv) in dry CHCl$_3$ was added NEt$_3$ (3 equiv.) The resulting solution was stirred at room temperature for 24 h. The reaction mixture was then transferred to a separatory funnel, diluted with CHCl$_3$ (20 mL), washed with 1 M HCl (3 × 20 mL), sat. sodium bicarbonate (3 × 20 mL). The organic fraction was separated, dried with MgSO$_4$, filtered and concentrated in vacuo to afford the norbornene amide.

**General procedure for Boc deprotection and (thio)urea formation**

A solution of Boc-protected norbornene peptide in 20% TFA in CH$_2$Cl$_2$ (5 mL) was stirred at room temperature until complete Boc deprotection by TLC analysis. The resultant mixture was concentrated in vacuo, added ca. 2 mL of CH$_2$Cl$_2$, concentrated in vacuo, added ca. 2 mL PhMe and concentrated in vacuo. The resultant TFA salt was used directly in the next step. To the trifluoroacetic acid salt, 15 mL of solvent was added. The solution was basified using NEt$_3$ (3 eq.) and stirred at room temperature for 10 min. To the resultant amine, an iso(thio)cyanate (2 eq.) was added, and stirred for at room temperature for 16 h. The resultant mixture was concentrated in vacuo and purified by flash column chromatography.

(2'S) (1a, 2β, 6β, 7α)-N-[2'-
(tert-butoxy carbonylamino) N’-(5’methoxycarbonyl)methyl propylamide] 4-azatricyclo
[5.2.1.02,6]dec-8-en-3,6 dione (12)

Following general procedure A, a solution of acid 11 (150 mg, 0.38 mmol), glycine methyl ester hydrochloride (62 mg, 0.49 mmol), EDCI (94 mg, 0.49 mmol), HOBt (10 mg, 0.08 mmol) and NEt$_3$ (157 μL, 1.14 mmol) were reacted to afford the title compound (85 mg, 48%) as a pale brown oil. $\delta$H (270 MHz, CDCl$_3$) 1.34 (9H, s, t-Bu), 1.46 (1H, d, J 7.7, H10b), 1.63 (1H, d, J 7.7, H10a), 1.38 (2H, m, H2, H6), 3.28 (2H, bs, H1, H7), 3.61 (2H, m, H5″), 3.65 (3H, s, H8′), 3.95 (2H, m, H5″), 4.26 (1H, m, H2′), 5.34 (1H, d, J 8.0, NH), 6.00 (2H, m, H8, H9), 7.10 (1H, bs, H4′), $\delta$C (75 MHz, CDCl$_3$) 178.3, 177.4, 169.8, 155.6, 134.7, 134.0, 80.3, 77.3, 52.2, 52.1, 45.8, 45.8, 45.4, 44.7, 41.1, 39.0, 29.2, 28.1, 16.6; HRMS m/z (+ESI) 464.2356 ([C$_{20}$H$_{22}$N$_2$O$_6$+H] requires 464.2391).

(2'S) (1a, 2β, 6β, 7α)-N-[2'-
(4'-phenylthio redo) N’-(5’methoxycarbonyl)methyl propylamide] 4-azatricyclo
[5.2.1.02,6]dec-8-en-5,6 dione (3)

Following general procedure B, N-Boc norbornene 12 (185 mg, 0.44 mmol) was deprotected then reacted with phenylisothiocyanate (92 μL, 0.0.48 mmol) and NEt$_3$ (122 μL, 0.42 mmol) in CH$_2$Cl$_2$. The reaction mixture was diluted with CH$_2$Cl$_2$, washed with 0.1 M HCl (3 × 10 mL) and H$_2$O (10 mL), concentrated in vacuo and subjected to flash column chromatography (50:49:1 Petroleum spirits/EtOAc/EtOH) to afford the title compound (150 mg, 75%) as an off-white solid mp 103.6 °C; $\delta$H (270 MHz, CDCl$_3$) 1.47 (1H, d, J 8.7, H10a), 1.66 (1H, d, J 8.5, H10b), 3.24 (2H, s, H1′), 3.29 (2H, m, H2′), 3.69 (3H, s, OMe), 3.73 (2H, m, H1′), 3.97 (2H, d, J 5.5, H5′), 5.32 (1H, m, H2′), 6.04 (2H, m, H8, H9), 6.92 (1H, d, thiourea NH), 7.27 (3H, m, H1″ and H3″), 7.40 (3H, m, H2″ and Amide NH), 8.41 (1H, s, Thiourea Ar–NH); $\delta$C (100 MHz, CDCl$_3$) 180.7, 178.6, 177.8, 170.0, 169.5, 136.6, 134.7, 134.6, 129.9, 127.1, 125.0, 56.3, 52.5, 52.3, 46.2, 46.1, 44.9, 44.8, 42.6.
Following general procedure for B, N-Boc norbornene 12 (102 mg, 0.24 mmol) was deprotected then reacted with 4-fluoro phenylisothiocyanate (47 mg, 0.31 mmol) and NEt3 (0.1 mL, 0.72 mmol) in THF. The mixture was subjected to flash column chromatography (15% petroleum spirits in EtOAc) to afford the title compound (79 mg, 67%) as an off-white solid. mp 84.7–97.0 °C; δH (270 MHz, DMSO-d6) 1.51 (1H, d, J 8.8, H10a) 1.69 (1H, d, J 8.8, H10b), 3.27 (2H, m, H1,7), 3.22 (2H, m, H2,6), 3.71 (5H, m, OMe and H1), 3.98 (2H, d, J 5.5, H5′), 5.29 (1H, td, J 8.4, 4.5, H2′), 6.06 (2H, m, H8,9), 6.82 (1H, d, J 8.0, Thioiurea NH), 7.10 (2H, dd, J 8.5, 3.6, H2′, H3′), 7.26 (3H, m, H1′′ and amidne NH), 8.12 (1H, s, Thioiurea Ar–NH); δC (100 MHz, CDCl3) 181.2, 178.6, 177.8, 169.9, 169.5, 161.36 (d, J′′ 248, C4′), 134.6 (J′′F 19.5, C′′3), 132.4, 127.6 (J′′F 8.5, C′′2), 116.7 (J′′F 22.7, C′′4′), 56.4, 52.5, 52.3, 46.2, 46.1, 44.9, 44.8, 41.3, 39.6, 29.8; HRMS m/z (+ESI) 475.14460 ([C23H23FN3O4S + H]+ requires 475.14560).

Following general procedure B, 11a (145 mg, 0.31 mmol) was deprotected then reacted with 4-fluorophenylisocyanate (76 μL, 0.40 mmol) and NEt3 (87 μL, 0.62 mmol) in CH2Cl2. The reaction mixture was diluted with CH2Cl2, washed with 0.1 M HCl (3 × 15 mL) and sat. NaCl (3 × 15 mL), concentrated in vacuo and subjected to flash column chromatography (16:3 Petroleum spirits/EtOAc/EtOH, Rp = 0.19) to afford the title compound (115 mg, 86%) as an pale brown solid. mp 86.9–91.2 °C; [α]D25 = −29.55° (c 1.14 in CHCl3; vmax/cm−1 1743 s, 1628 vs. and 1598 (C=O), 1634 (NH), 1152 (C=S), 723 and 689 (Aryl CH); δH (400 MHz, DMSO-d6) 0.86 (3H, d, J 6.5, H2′ or H3′) 0.88 (3H, d, J 6.5, H2′ or H3′) 1.54 (2H, bs, H10), 2.04 (1H, qdd, J 6.2, 6.2, 6.2, H1′), 3.22 (2H, bs, H1, H7), 3.31 (2H, m, H2, H6), 3.57 (2H, d, J 5.8, H1′), 3.66 (3H, s, H8′), 4.17 (1H, dd, J 7.7, 7.7, H5′), 5.25 (1H, m, H2′) 6.02 (2H, m, H8, H9), 7.14 (1H, dd, J 7.0, 7.0, H7′′), 7.34 (2H, dd, J 7.3, 7.3, H6′, H7′), 7.44 (2H, d, J 7.7, H5′′, H9′), 7.54 (H1, d, J 8.4, H1′), 8.50 (1H, d, J 6.9, H4′), 9.93 (1H, s, H3′′); δC (100 MHz, DMSO-d6) 180.2, 177.3, 177.2, 171.5, 169.2, 138.9, 134.5, 134.2, 128.7, 124.5, 123.2, 57.6, 54.0, 51.8, 51.8, 45.4, 45.3, 44.2, 44.1, 39.5, 29.9, 18.9, 18.3; HRMS m/z (+ESI) 499.2002 ([C23H30N4O5S + H]+ requires 499.2010).

A microwave vessel was charged with norbornene anhydride 10 (82 mg, 0.55 mmol) and α-Boc-Lys-OH (123 mg, 0.55 mmol) and EtOAc (0.25 mL). The resulting mixture was heated to 120 °C under microwave irradiation for 45 min. The solution was cooled, diluted with EtOAc (20 mL), washed with 1 M HCl (3 × 15 mL), dried with MgSO4, filtered and concentrated in vacuo to afford imide 11a (146 mg, 77%) as a pale yellow viscous oil. δH (270 MHz, CDCl3) 1.16–1.91 (6H, m, H2′,3′,4′), 1.43 (9H, s, -Bu), 1.52 (1H, d, J 8.7, H10a), 1.71 (1H,
aza-tricyclo[5.2.1.02,6]deca-8-ene-3,5-dione (8)

1.50 (1H, d, \( \delta \)) requires 499.20097).

151.1 \( J \)

amide

J 1.53 (1H, d, \( \delta \)) 7.41 (2H, m, H2, H6), 3.47 (2H, m, H1, H7), 3.38 (2H, bs, H2, H6), 3.61 (2H, bs, H1'), 3.71 (3H, s, OMe), 4.25 (1H, m, H2'), 4.47 (1H, dd, \( J = 4.5, 8.9 \)), 5.17 (H1, d, \( J = 7.9, \) NHboc), 6.09 (2H, m, H8, H9), 6.93 (1H, d, \( J = 7.4, \) NH4)); \( \delta _C \) (75 MHz, CDCl3) 178.45, 177.33, 171.74, 169.54, 155.85, 134.85, 133.90, 80.49, 77.29, 57.02, 52.42, 52.08, 45.91, 45.70, 45.24, 44.96, 44.75, 38.29, 31.01, 28.18, 18.87, 17.43; HRMS m/z (+ESI) 464.2356 ([C\(_2\)H\(_3\)N\(_3\)O\(_2\) + H\(^+\)] requires 464.2391).

Following general procedure for B, N-Boc norbornene 12a (185 mg, 1.04 mmol) was deprotected then reacted with 4-fluorophenylisocyanate (128 \( \mu \)L, 1.14 mmol) and NEt\(_3\) (122 \( \mu \)L, 0.42 mmol) in CH\(_2\)Cl\(_2\). The reaction mixture was diluted with CH\(_2\)Cl\(_2\), washed with 0.1 M HCl (3 × 15 mL) and sat. NaCl (3 × 15 mL), concentrated in vacuo and subjected to flash column chromatography (16:3:1 Petroleum spirits/EtOAc/EtOH, \( R_f = 0.19 \)) to afford the title compound (328 mg, 63%) as an off-white solid. mp 94.4–98.7 °C; \( [\alpha]_D^{25} = -3.43 \pm 0.15 \) (c 1.11 in CHCl\(_3\)).

Following general procedure for B, amide 12c (65 mg, 0.14 mmol) was deprotected then reacted with phenylisothiocyanate (34 \( \mu \)L, 0.18 mmol) and NEt\(_3\) (58 \( \mu \)L, 0.42 mmol) in THF. The mixture was subjected to flash column chromatography (gradient elution: 20% petroleum spirits in EtOAc to 15% petroleum spirits in EtOAc) to afford the title compound (94%, 11 mg) as an off-white solid. mp 70–81.8 °C; \( \delta _H \) (270 MHz, CDCl3) 1.20 (4H, m, Alkyl), 1.44 (2H, m, alky), 1.50 (1H, d, \( J = 8.8 \)), 1.70 (1H, d, \( J = 8.05 \)), 1.94 (2H, m, Alkyl), 3.21 (2H, m, H1, H7), 3.29 (4H, m, H1’ and H2’), 3.71 (3H, s, OMe), 4.01 (2H, m, H8’), 5.06 (1H, m, H5’), 6.06 (2H, m, H8, H9), 6.77 (1H, d, \( J = 8.1 \)), Thiourea NH), 6.92 (1H, t, \( J = 6.9 \), Amide NH), 7.27 (3H, m, H1” and H3”) 7.41 (2H, m, H2”), 8.01 (1H, s, Thiourea Ar-NH); \( \delta _C \) (100 MHz, CDCl3) 180.3, 178.1, 172.0, 170.0, 136.5, 134.6, 134.5, 130.0, 127.0, 124.9, 57.9, 52.5, 52.3, 45.8, 45.0, 41.3, 37.8, 31.6, 29.8, 27.3, 22.3; HRMS m/z (+ESI) 499.20013 ([C\(_{2}\)H\(_{3}\)N\(_{4}\)O\(_{2}\)S + H\(^+\)] requires 499.20079).

Following general procedure A, a solution of acid 11 (593 mg, 1.7 mmol), valine methyl ester hydrochloride (341 mg, 2.0 mmol), EDCI (504 mg, 2.0 mmol), HOBT (115 mg, 0.85 mmol) and NEt\(_3\) (0.5 mL, 3.4 mmol) were reacted and washed with sat. NaCl (3 × 15 mL) to afford amide 12a (595 mg, 75%) as a pale brown solid. mp 151.1–152.8 °C; \( \delta _H \) (270 MHz, CDCl3) 0.86 (3H, d, \( J = 6.9, \) H7’), 0.89 (3H, d, \( J = 6.9, \) H7’), 1.42 (9H, s, t-Bu), 1.53 (1H, d, \( J = 8.4, \) H10b), 1.72 (1H, d, \( J = 8.9, \) H10a), 2.15 (1H, m, H1”), 3.27 (2H, m, H1, H7), 3.38 (2H, bs, H2, H6), 3.61 (2H, bs, H1’), 3.71 (3H, s, OMe), 4.25 (1H, m, H2’), 4.47 (1H, dd, \( J = 4.5, 8.9 \)), 5.17 (H1, d, \( J = 7.9, \) NHboc), 6.09 (2H, m, H8, H9), 6.93 (1H, d, \( J = 7.4, \) NH4)); \( \delta _C \) (75 MHz, CDCl3) 178.45, 177.33, 171.74, 169.54, 155.85, 134.85, 133.90, 80.49, 77.29, 57.02, 52.42, 52.08, 45.91, 45.70, 45.24, 44.96, 44.75, 38.29, 31.01, 28.18, 18.87, 17.43; HRMS m/z (+ESI) 464.2356 ([C\(_2\)H\(_3\)N\(_3\)O\(_2\) + H\(^+\)] requires 464.2391).

Following general procedure for B, N-Boc norbornene 12 (154 mg, 0.31 mmol) was deprotected then reacted with 4-fluorophenylisocyanate (76 \( \mu \)L, 0.40 mmol) and triethylamine (87 \( \mu \)L, 0.62 mmol) in CH\(_2\)Cl\(_2\). The reaction mixture was diluted with CH\(_2\)Cl\(_2\), washed with 3 × 0.1 M HCl (15 mL) and 3 × sat. NaCl (15 mL), concentrated in vacuo and subjected to flash column chromatography (100% EtOAc, \( R_f = 0.32 \)) to afford the title compound (63.4 mg, 19%) as a pale brown solid. mp 94.4–98.7 °C; \( [\alpha]_D^{25} = -3.43 \pm 0.15 \) (c 1.11 in CHCl\(_3\)).

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Tetrabutylammonium acetyl D-alaninate
To a solution of acetyl D-alaninate methyl ester (55 mg, 0.30 mmol) in MeOH (5 mL) was added TBA OH (288 µL, 1.034 M in MeOH) and allowed to stir at room temperature overnight. The resulting solution was concentrated in vacuo to afford the title compound (112 mg, quantitative). δH (270 MHz, CDCl3) 0.98 (12H, m, Bu), 1.40 (12H, m, Bu and CH3), 1.63 (8H, m, Bu), 1.92 (3H, s, AcCH3), 3.31 (8H, m, Bu), 4.06 (1H, m, CH), 7.07 (1H, bs, AcNH); δc (100 MHz, CDCl3) 176.29, 168.92, 58.82, 51.17, 24.03, 23.75, 19.78, 13.69.

Acknowledgements
The authors would like to thank Professors Roger Nation and Jian Li for assistance with the disk diffusion assay and Dr Deni Taleski for his insight.

Supplemental data
Supplemental data for this article can be accessed here: http://dx.doi.org/10.1080/10610278.2015.1086764.

Note
1. While the modified Job Plots did not contribute reliable data for assessment of binding stoichiometry, in this study, they are included in the supplemental data for full disclosure of results.

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