Supporting Information

Synthesis of 3-Acyltetramates by Side Chain Manipulation and their Antibacterial Activity

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Experimetal procedures, spectroscopic and analytical data for new compounds **5a-d**, **7a-d**, **10a-h**, **11a-c**, **12**.

Experimental

General Procedures

^{1}H NMR spectra were recorded on Brucker DPX400 (400 MHz), DQX400 (400 MHz), AVC500 (500 MHz) spectrometers. Chemical shifts (δ_H) are reported in parts per million (ppm) and are referenced to the residual protonated solvent peak. The abbreviations used to describe multiplicity are as follows: s (singlet), br s (broad singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), q (quartet), m (multiplet), at (apparent triplet) and br (broad). Coupling constants (*J*) are given in Hertz (Hz). Two-dimensional COSY (correlation spectroscopy) spectra were obtained on a Brucker DQX400 (400 MHz), AVC500 (500 MHz) spectrometers.

^{13}C NMR spectra were recorded on a Bruker DQX400 spectrometer at 100.6 MHz or Bruker AVC500 spectrometer at 125.8 MHz with proton decoupling. Chemical shifts (δ_C) are reported in parts per million (ppm) and are referenced to the residual protonated solvent peak. Assignment was aided by the use of edited HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear multiple-bond correlation spectroscopy). HMBC experiments were performed on a Bruker AVC500 (500 MHz) spectrometer.

Infrared (IR) spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer. Absorption maxima (υ_{max}) are reported in wavenumbers (cm^{-1}) and only selected peaks are reported.

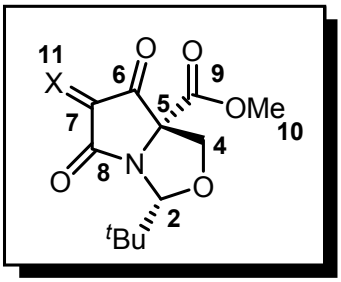
Low resolution mass spectra (*m/z*) were recorded on a Fisons Platform spectrometer using electrospray ionisation (ESI) or a Fisons AutoSpec-oaTof spectrometer using electron impact ionisation (EI) or field ionisation (FI). High resolution mass spectra (HRMS) were recorded on a Bruker microTOF (ESI). The *m/z* values of major peaks are reported in Daltons and their intensities given as percentages of the base peaks.

Optical rotations were recorded on a Perkin-Elmer 341 polarimeter at the stated temperature, with concentrations *c* given in g/100ml $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

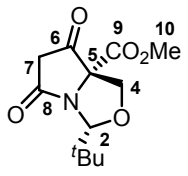
Thin layer chromatography (TLC) was performed using Merck aluminium foil backed sheets precoated with 0.2 mm Kieselgel 60 F_{254}. Product spots were visualised by quenching of UV fluorescence (λ_{max} 254 nm) or by staining with an aqueous solution of $KMnO_4$ followed by heating. Both dips were prepared according to J. Leonard, B. Lygo and G. Procter, "Advanced Practical Organic Chemistry", second edition, Blackie A & P, 1995. Retention factors (R_f) are quoted to the nearest 0.01. Flash column chromatography was carried out using Lancaster silica gel 60, 0.040-0.063 mm (230-400 mesh).

Reaction times are recorded in minutes (min) and hours (h). Temperatures below ambient room temperature were obtained using the following cold baths: 0°C ice/water, -10°C to -15°C ice/methanol. All reactions were carried out in oven-dried reaction flasks under inert (N_2) atmosphere unless otherwise stated. 'Petroleum ether' (PE) refers to that fraction of light petroleum ether boiling at 40-60°C and was used as received.

The tetramic acid system is numbered non-systematically as shown below:



Compound **3** (*J. Chem Soc, Perkin Trans 1*, **1998**, 223-236) is a known compound and was synthesised according to the procedures as described in the literature. The respective spectroscopic data was consistent with those reported in the literature.



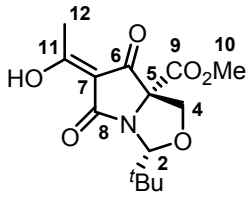
3

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-3-oxabicyclo[3.3.0]-octane, 3
δ_H(400 MHz, $CDCl_3$) 5.09 (s, 1H, H-2), 4.81 (d, *J* = 9.0 Hz, 1H, H-4), 3.83 (s, 3H, H-10), 3.74 (d, *J* = 21.2 Hz, 1H, H-7), 3.62 (d, *J* = 9.0 Hz, 1H, H-4'), 3.17 (d, *J* = 21.2 Hz, 1H, H-7'), 0.91 (-^{t}Bu);
δ_C (100 Mhz, $CDCl_3$) 198.29 (C-6), 172.30 (C-9), 166.84 (C-8), 98.27 (C-2), 80.51 (C-5), 67.89 (C-4), 53.72 (C-10), 44.79 (C-7), 35.59 (-<u>C</u>-^{t}Bu), 24.64 (-^{t}Bu);

<u>General Procedure for Synthesis of 3-acyl tetramic acids **5a-d**</u>

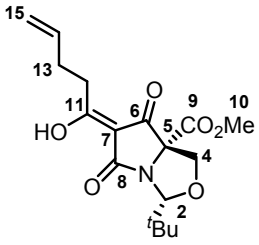
The respective acid (1.1 eq.) was added dropwise to a CH_2Cl_2 solution of tetramic acid **3** (1.0 eq.), DMAP (1.3 eq.) and DCC (1.1 eq.). The reaction mixture was then stirred at r.t. overnight (17-20 h). The suspension was filtered and the solvents evaporated to give the crude which was purified via flash column chromatography on silica gel. The product thus obtained was washed with a solution of sat. NH_4Cl and 10% aq. HCl, dried with anhydrous $MgSO_4$, filtered and the solvents removed to afford the 3-acyl tetramic acids **5a-d**.
The structures as drawn correspond to the major tautomeric form present.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-methylvinylidene]-3-oxabicyclo[3.3.0]-octane, 5a



R_f = 0.45 (10% MeOH/EtOAc);
$[\alpha]_D^{20}$ = +66.0 (c= 1.20 in $CHCl_3$);
Orange oil;
ν_{max} / cm^{-1} (film) 1756 (s, C=O), 1719 (s, C=O), 1660 (s, C=O);
δ_H(400 MHz, $CDCl_3$) 4.83 (s, 1H, H-2), 4.81 (d, *J* = 8.8 Hz, 1H, H-4), 3.77 (s, 3H, -CO_2<u>Me</u>), 3.48 (d, *J* = 8.8 Hz, 1H, H-4'), 2.47 (s, 3H, H-12), 0.91 (s, 9H, -^{t}Bu);
δ_C (125 Mhz, $CDCl_3$) 188.39 (C-11), 187.96 (C-6), 179.84 (C-8), 167.42 (C-9), 101.73 (C-7), 98.20 (C-2), 78.39 (C-5), 68.16 (C-4), 53.30 (-CO_2<u>Me</u>), 35.15 (-<u>C</u>-^{t}Bu), 24.60 (-^{t}Bu), 19.93 (C-12);
m/z (ESI-) 296.09 ([M-H]$^-$, 94%); HRMS (ESI-) calculated for $C_{14}H_{18}NO_6$ ([M-H]$^-$) 296.1140, found 296.1142.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(but-3-ene)vinylidene]-3-oxabicyclo[3.3.0]-octane, 5b



R_f = 0.26 (EtOAc);

$[\alpha]_D^{20}$ = +75.9 (c= 1.28 in $CHCl_3$);

Orange oil;

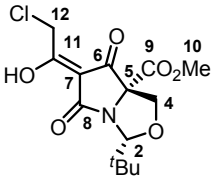
ν_{max} / cm^{-1} (film) 1756 (s, C=O), 1718 (s, C=O), 1659 (s, C=O);

δ_H(400 MHz, $CDCl_3$) 5.75-5.85 (m, 1H, H-14), 5.05 (dt, *J* = 8, 21 Hz, 2H, H-15), 4.85 (s, 1H, H-2), 4.82 (d, *J* = 8.8 Hz, 1H, H-4), 3.78 (s, 3H, -CO_2Me), 3.49 (d, *J* = 8.8 Hz, 1H, H-4'), 2.96 (apparent t, *J* = 8 Hz, 2H, H-12), 2.40-2.46 (m, 2H, H-13), 0.92 (s, 9H, -*t*Bu);

δ_C (100 Mhz, $CDCl_3$) 191.12 (C-11), 187.72 (C-6), 180.11 (C-8), 167.40 (C-9), 135.59 (C-4), 116.32 (C-15), 101.33 (C-7), 98.23 (C-2), 78.31 (C-5), 68.18 (C-4), 53.29 (-CO_2Me), 35.14 (-C-*t*Bu), 32.29 (C-12), 29.47 (C-13), 24.61 (-*t*Bu);

m/z (ESI-) 336.13 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{17}H_{22}NO_6$ ([M-H]$^-$) 336.1453, found 336.1453.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(chloromethyl)vinylidene]-3-oxabicyclo[3.3.0]-octane, 5c



R_f = 0.56 (5% MeOH/EtOAc);

$[\alpha]_D^{20}$ = +49.8 (c= 1.20 in $CHCl_3$);

Pale orange oil;

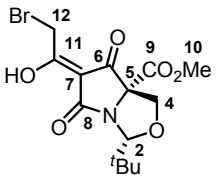
ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1720 (s, C=O), 1662 (s, C=O);

δ_H(500 MHz, $CDCl_3$) 12.05 (br, s, enol –OH), 4.86 (s, 1H, H-2), 4.83 (d, *J* = 9.1 Hz, 1H, H-4), 4.63 (d, *J* = 13.6 Hz, 1H, H-12), 4.53 (d, *J* = 13.6 Hz, 1H, H-12'), 3.80 (s, 3H, -CO_2Me), 3.53 (d, *J* = 9.1 Hz, 1H, H-4'), 0.93 (s, 9H, -*t*Bu);

δ_C (125 Mhz, $CDCl_3$) 186.69 (C-6), 181.91 (C-11), 179.40 (C-8), 166.74 (C-9), 101.53 (C-7), 98.42 (C-2), 78.43 (C-5), 68.01 (C-4), 53.48 (-CO_2Me), 38.84 (C-12), 35.16 (-C-*t*Bu), 24.57 (-*t*Bu);

m/z (ESI-) 330.08 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{14}H_{17}ClNO_6$ ([M-H]$^-$) 330.0750, found 330.0750.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(bromomethyl)vinylidene]-3-oxabicyclo[3.3.0]-octane, 5d



R_f = 0.65 (5% MeOH/EtOAc);

$[\alpha]_D^{20}$ = +39.9 (c= 1.0 in $CHCl_3$);

Yellow oil;

ν_{max} / cm^{-1} (film) 1753 (s, C=O), 1720 (s, C=O), 1661 (s, C=O);

δ_H(500 MHz, $CDCl_3$) 4.88 (s, 1H, H-2), 4.84 (d, *J* = 9.1 Hz, 1H, H-4), 4.41 (d, *J* = 10.7 Hz, 1H, H-12), 4.35 (d, *J* = 10.7 Hz, 1H, H-12'), 3.80 (s, 3H, -CO_2Me), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.93 (s, 9H, -*t*Bu);

δ_C (125 Mhz, $CDCl_3$) 186.95 (C-6), 181.70 (C-11), 179.11 (C-8), 166.84 (C-9), 101.43 (C-7), 98.40 (C-2), 78.47 (C-5), 68.06 (C-4), 53.48 (-CO_2Me), 35.19 (-C-*t*Bu), 24.60 (-*t*Bu), 23.21 (C-12);

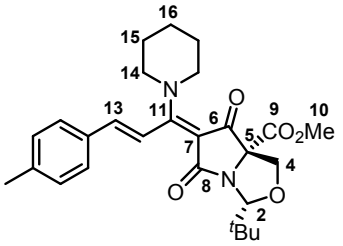
m/z (ESI-) 374.00 ([M-H]$^-$, 68%); HRMS (ESI-) calculated for $C_{14}H_{17}BrNO_6$ ([M-H]$^-$) 374.0245, found 374.0242.

General Procedure for Synthesis of Piperidyl Enamines **7a-c**

A solution of **5a**, the corresponding aromatic aldehyde (0.9 eq.) and piperidine (1.5 eq.) in toluene was heated at reflux (120°C) overnight. The reaction mixture was then cooled to r.t., the solvents evaporated and

the crude was purified via flash column chromatography on silica gel. The product thus obtained was washed with a solution of saturated aqueous NH_4Cl and 10% aq. HCl, dried with anhydrous $MgSO_4$, filtered and the solvents removed to afford the pure product.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-3-(*E*)-(4'-methylphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 7a



R_f = 0.31 (EtOAc);

$[\alpha]_D^{20}$ = +321.2 (c=1.05 in $CHCl_3$);

Dark yellow oil;

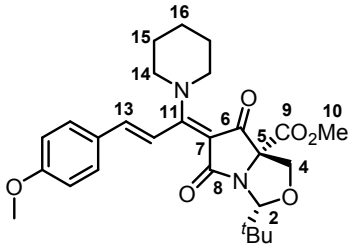
ν_{max} / cm^{-1} (film) 1745 (s, C=O), 1693 (s, C=O), 1634 (s, C=O);

δ_H(500 MHz, Acetone-d_6) 7.58 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.34 (d, *J* = 15.8 Hz, 1H, H-13), 7.27 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.02 (d, *J* = 15.8 Hz, 1H, H-12), 4.79 (s, 1H, H-2), 4.69 (d, *J* = 8.2 Hz, 1H, H-4), 3.92 (broad unresolved signal, 1H, one of H-14), 3.74 (s, 6H, -CO_2Me and one of H-14 and H-14'; base of singlet is broad), 3.57 (d, *J* = 8.5 Hz, 1H, H-4'), 2.38 (s, 3H, Ar-Me), 1.96 (broad unresolved signal, 2H, one of H-15 and one of H-15'), 1.80 (broad unresolved signal, 4H, H-16, one of H-15 and one of H-15'), 0.89 (s, 9H, -*t*Bu);

δ_C (125 Mhz, Acetone-d_6) 187.27 (C-6), 177.58 (C-8), 170.89 (C-9), 168.09 (C-11), 148.12 (C-13), 141.64, 133.62, 130.42, 129.41 (ArC), 119.73 (C-12), 98.64 (C-2), 95.91 (C-7), 78.29 (C-5), 69.44 (C-4), 56.41 (C-14 or C-14'), 52.81 (-CO_2Me), 52.66 (C-14 or C-14'), 35.97 (-C-*t*Bu), 27.35 (C-15), 25.38 (-*t*Bu), 24.22 (C-16), 21.46 (Ar-Me);

m/z (ESI+) 467.28 ([M+H]$^+$, 100%); HRMS (ESI+) calculated for $C_{27}H_{34}N_2NaO_5$ 489.2360, found 489.2367.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-3-(*E*)-(4'-methoxyphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 7b



R_f = 0.32 (EtOAc);

$[\alpha]_D^{20}$ = +400.9 (c= 0.96 in $CHCl_3$);

Orange oil;

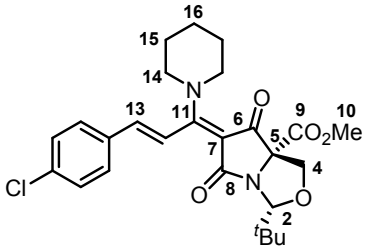
ν_{max} / cm^{-1} (film) 1745 (s, C=O), 1693 (s, C=O), 1634 (s, C=O);

δ_H(400 MHz, Acetone-d_6) 7.64 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.34 (d, *J* = 15.7 Hz, 1H, H-13), 6.98 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.93 (d, *J* = 15.7 Hz, 1H, H-12), 4.78 (s, 1H, H-2), 4.69 (d, *J* = 8.6 Hz, 1H, H-4), 3.85 (s, 3H, Ar-OMe), 3.79 (s, 3H, -CO_2Me), 3.78-3.80 (broad unresolved signals, 2H, H), 3.57 (d, *J* = 8.6Hz, 1H, H-4'), 1.93 (broad unresolved signals, 2H), 1.78 (broad unresolved signals, 2H), 0.88 (s, 9H, -*t*Bu);

δ_C (125 Mhz, Acetone-d_6) 187.26 (C-6), 177.73 (C-8), 170.95 (C-9), 168.28 (C-11), 162.74 (ArC), 148.47 (C-13), 131.26, 128.96 (ArC), 117.95 (C-12), 115.22 (ArC), 98.61 (C-2), 95.57 (C-7), 78.28 (C-5), 69.44 (C-4), 55.84 (Ar-OMe), 55.63 (C-14 or C-14'), 52.79 (-CO_2Me), 52.60 (C-14 or C-14'), 35.97 (-C-*t*Bu), 27.35 (C-15), 25.36 (-*t*Bu), 24.25 (C-16);

HRMS (FI+) calculated for $C_{27}H_{34}N_2O_6$ 482.2417, found 482.2437.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-3-(*E*)-(4'-chlorophenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 7c



R_f = 0.30 (EtOAc);

$[\alpha]_D^{20}$ = +344.2 (c= 0.28 in $CHCl_3$);

Orange oil;

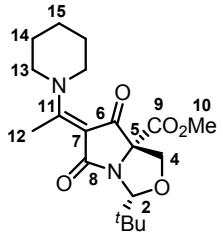
ν_{max} / cm^{-1} (film) 1747 (s, C=O), 1693 (s, C=O), 1637 (s, C=O);

δ_H(400 MHz, $CDCl_3$) 7.46 (d, *J* = 8.6Hz, 2H, Ar-H), 7.36 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.22 (d, *J* = 15.9 Hz, 1H, H-13), 6.74 (d, *J* = 15.9 Hz, 1H, H-12), 4.87 (s, 1H, H-2), 4.84 (d, *J* = 8.6 Hz, 1H, H-4), 3.79 (s, 3H, -CO_2Me), 3.78-3.80 (broad unresolved signals, 2H, H-14 or H-14'), 3.64 (broad unresolved signals, 2H, H-14 or H-14'), 3.54 (d, *J* = 8.6 Hz, 1H, H-4'), 1.80 (broad unresolved signals, 6H, H-15, H-15', H-16), 0.91 (s, 9H, -^{t}Bu);

δ_C (100 Mhz, $CDCl_3$) 187.58 (C-6), 177.06 (C-8), 169.63 (C-9), 166.57 (C-11), 146.03 (C-13), 136.63, 133.11, 129.52, 129.22 (Ar<u>C</u>), 119.78 (C-12), 98.16 (C-2), 96.39 (C-7), 77.57 (C-5), 68.91 (C-4), 56.10 (C-14 or C-14'), 52.89 (-CO_2<u>Me</u>), 52.15 (C-14 or C-14'), 35.34 (-<u>C</u>-^{t}Bu), 26.61 (C-15), 24.86 (-^{t}Bu), 23.46 (C-16);

m/z (ESI+) 487.23 ($[M+H]^+$, 100%); HRMS (FI+) calculated for $C_{26}H_{31}ClN_2O_5$ 486.1921, found 486.2147.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-piperidyl-1'-methyl-vinylidene]-3-oxabicyclo[3.3.0]-octane, 6c



A solution of **5a** (140 mg, 0.471 mmol, 1.0 eq.) and piperidine (68.9 µl, 0.706 mmol, 1.5 eq.) in toluene was heated at reflux (120°C) overnight. The reaction mixture was then cooled to r.t., the solvents evaporated and the crude material was purified via flash column chromatography (Eluent: EtOAC/PE 10% to 70%) on silica gel to afford **6c** (140 mg, 82%).

R_f = 0.19 (EtOAc);

$[\alpha]_D^{20}$ = +272.7 (c= 0.91 in $CHCl_3$);

Orange oil;

ν_{max} / cm^{-1} (film) 1745 (s, C=O), 1693 (s, C=O), 1634 (s, C=O);

δ_H(500 MHz, $CDCl_3$) 4.84 (s, 1H, H-2), 4.79 (d, *J* = 8.5 Hz, 1H, H-4), 3.79 (s, 3H, -CO_2Me), 3.69-3.73 (m, 2H, one of H-13 and one of H-13'), 3.62-3.67 (m, 1H, one of H-13), 3.52-3.57 (m, 1H, one of H-13'), 3.45 (d, *J* = 8.6 Hz, 1H, H-4'), 2.59 (s, 3H, H-12), 1.90-1.94 (m, 1H, one of H-14), 1.68-1.83 (m, 5H, one of H-14, H-14' and H-15), 0.92 (s, 9H, -^{t}Bu);

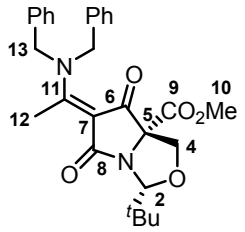
δ_C (125 MHz, $CDCl_3$) 186.89 (C-6), 177.42 (C-8), 170.38 (C-11), 169.58 (C-9), 98.18 (C-2), 97.11 (C-7), 77.12 (C-5; in the middle of $CDCl_3$ signals but correlation observed in HMBC), 68.85 (C-4), 56.43 (C-13), 52.82 (-CO_2<u>Me</u>), 50.48 (C-13'), 35.28 (-<u>C</u>-^{t}Bu), 26.77 (C-14), 26.23 (C-14'), 24.82 (-^{t}Bu), 23.40 (C-15), 18.85 (C-12);

m/z (ESI+) 365.23 ($[M+H]^+$, 100%); HRMS (ESI+) calculated for $C_{19}H_{28}N_2NaO_5$ ($[M+Na]^+$) 387.1890, found 387.1883.

Synthesis of *N*, *N'*-dibenzyl Enamines **7d** and **6d**

A solution of **5a** (90 mg, 0.303 mmol, 1.0 eq.), *p*-anisaldehyde (36.6 µl, 0.303 mmol, 1.0 eq.), and dibenzylamine (87.3 µl, 0.454 mmol, 1.5 mmol) in toluene (5 ml) was heated to 120°C for 22 h. The solution was cooled to r.t., diluted with EtOAc and washed with aq. 5% HCl. The organic layer was then dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, 5% to 35%) gave **6d** (25.5 mg,18%) and **7d** (32 mg, 18%).

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-(*N*,*N'*-dibenzyl)-1'-methyl-vinylidene]-3-oxabicyclo[3.3.0]-octane, 8b



R_f = 0.45 (1:1 PE:EtOAc);

Bright yellow oil;

$[\alpha]_D^{20}$ = +348.7 (*c*= 1.28 in $CHCl_3$);

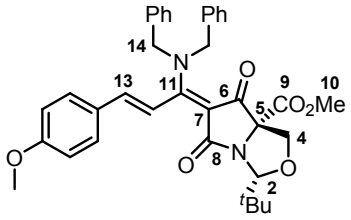
ν_{max} / cm^{-1} (film) 1746 (s, C=O), 1695 (s, C=O), 1639 (s, C=O);

δ_H(500 MHz, $CDCl_3$) 7.30-7.39 (m, 6H, ArH), 7.12-7.18 (m, 4H, ArH), 5.15 (d, *J* = 14.8 Hz, 1H, H-13), 4.93 (s, 1H, H-2), 4.82 (d, *J* = 8.4 Hz, 1H, H-4), 4.77 (d, *J* = 16.1 Hz, 1H, H-13a), 4.73 (d, *J* = 14.8 Hz, 1H, H-13'), 4.50 (d, *J* = 16.4 Hz, 1H, H-13a'), 3.77 (s, 3H, -CO_2Me), 3.47 (d, *J* = 8.8 Hz, 1H, H-4'), 2.68 (s, 3H, H-12), 0.94 (-*t*Bu);

δ_C (125 Mhz, $CDCl_3$) 187.76 (C-6), 176.39 (C-8), 173.39 (C-11), 169.37 (C-9), 134.38, 133.47, 129.33, 129.10, 128.69, 128.61, 128.22 126.39 (ArC), 98.81 (C-7), 98.08 (C-2), 77.06 (C-5, obscured by $CDCl_3$ signals but correlation seen on HSQC), 68.85 (C-4), 61.00 (C-13), 53.71 (C-13a), 52.89 (C-10), 35.34 (-C-*t*Bu), 24.84 (-*t*Bu), 19.77 (C-12) ;

m/z (ESI+) 499.23 ([M+Na]$^+$, 100%); HRMS (ESI+) calculated for $C_{28}H_{33}N_2O_5$ ([M+H]$^+$) 477.2384, found 477.2380.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[1-(*N*,*N'*-dibenzyl)-3-(*E*)-(4'-methoxyphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 7d



R_f = 0.36 (1:1 PE:EtOAc);

Bright yellow oil;

$[\alpha]_D^{20}$ = +312.7 (*c*= 1.07 in $CHCl_3$);

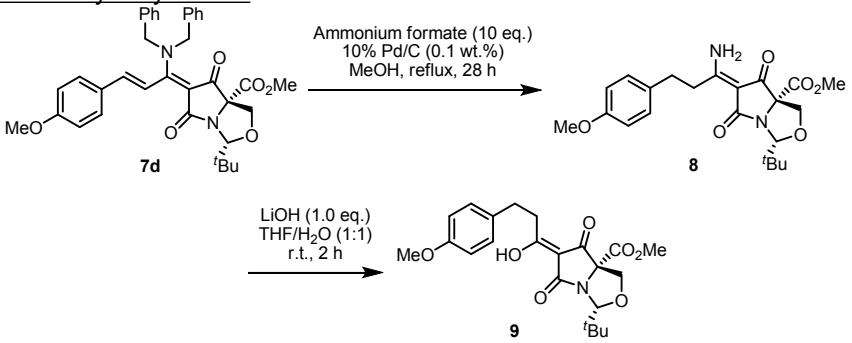
ν_{max} / cm^{-1} (film) 1746 (s, C=O), 1695 (s, C=O), 1638 (s, C=O);

δ_H(500 MHz, $CDCl_3$) 7.47 (d, *J* = 8.8 Hz, 2H, ArH), 7.31-7.36 (m, 7H, ArH and H-13), 7.15 (d, *J* = 6.9 Hz, 4H, ArH), 6.89 (d, *J* = 8.8 Hz, 2H, ArH), 6.84 (d, *J* = 15.4 Hz, 1H, H-12), 4.96 (s, 1H, H-2), 4.93 (d, *J* = 14.8 Hz, 2H, H-14), 4.86 (d, *J* = 8.5 Hz, 1H, H-4), 4.58-5.60 (broad signal, 2H, H-14'), 3.84 (s, 3H, Ar-OMe), 3.78 (s, 3H, -CO_2Me), 3.56 (d, *J* = 8.5 Hz, 1H, H-4'), 0.94 (-*t*Bu);

δ_C (125 Mhz, $CDCl_3$) 187.86 (C-6), 176.82 (C-8), 169.66 (C-9), 162.10 (Ar<u>C</u>-OMe), 149.44 (C-13), 134.12 (broad), 130.52, 128.97, 128.39 (broad), 127.41 (ArC), 116.92 (C-12), 114.42 (ArC), 98.05 (C-2), 97.14 (C-7), 77.62 (C-5), 69.01 (C-4), 55.43 (Ar-O<u>Me</u>), 52.86 (-CO_2<u>Me</u>), 35.40 (-<u>C</u>-^{t}Bu), 24.88 (-^{t}Bu).
m/z (ESI+) 617.29 ([M+Na]$^+$, 100%), 595.32 ([M+H]$^+$, 65%); HRMS (ESI+) calculated for $C_{36}H_{39}N_2O_6$ ([M+H]$^+$) 595.2803, found 595.2797.

In a separate experiment, **8b** (26 mg, 0.055 mmol, 1.0 eq.), *p*-anisaldehyde (6.6 µl, 0.055 mmol, 1.0 eq.), dibenzylamine (5.2 µl, 0.027 mmol, 0.5 eq.) was heated in toluene (4 ml) at 120°C for 42 h. The solution was cooled to r.t., diluted with EtOAc and washed with aq. 5% HCl. The organic layer was then dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. ^{1}H NMR shows **8b**: **7d** = 1:2.5, based on H-4 signals of the product.

<u>Debenzylation of **7d** and hydrolysis of **8**</u>



Debenzylation:
To a solution of **7d** (30 mg, 0.0504 mmol, 1.0 eq.) in MeOH (5 ml), was added 10% Pd/C (10% of **7d** wt.) and ammonium formate (31.8 mg, 0.504 mmol, 10.0 eq.). The reaction mixture was stirred at r.t. for 19 h. As there is no conversion based on TLC, the reaction mixture was then heated at 60°C for another 28 h, cooled to r.t. and filtered over Celite. The filtrate was evaporated and CH_2Cl_2 was added to the residue, followed by filtration to remove the precipitated solids. The filtrate was evaporated to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/petroleum ether, 20% to 100%, then 5% MeOH/EtOAc) gave 15 mg of the presumed –NH_2 enamine. Analysis via low resolution mass spectrometry gave the desired mass peak.

Hydrolysis:
LiOH (1 mg) was added to the presumed enamine (15 mg, 0.036 mmol) in THF/H_2O (1:1) (3 ml). The reaction mixture was stirred at r.t. for 2 h. The reaction mixture was then acidified with 10% aq. HCl, saturated with brine and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude which was then purified via flash column chromatography on silica gel (Eluent: EtOAc/petroleum ether, 20% to 100%, then 5% MeOH/EtOAc), to give the product (<5 mg). Analysis via low resolution mass spectrometry gave the desired mass peak; ^{1}H NMR suggests enamine hydrolysed due to absence of resonance signals ~10 ppm.

<u>Procedure for Hydrolysis of Piperidine Enamines **7a-c** to give 3-enoyl tetramic acids **10a-c**</u>

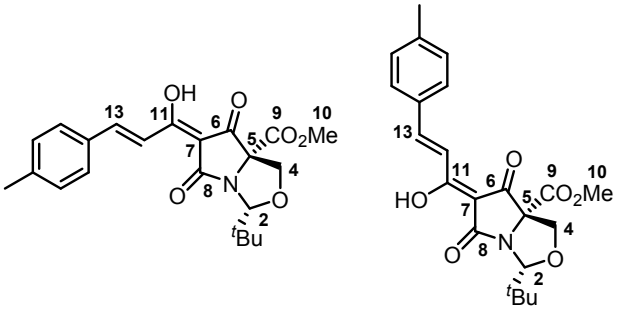
Solid LiOH (1.0 eq.) was added to a solution of **7a-c** (1.0 eq.) in THF/H_2O (1:1) (approx. concentration 0.02-0.05 M) and the deep yellow solution was stirred at r.t for 1.0-1.5 h. (The solution begins as a deep yellow solution and becomes pale yellow in colour when the reaction is completed). The solution was then acidified with 10% aq. HCl and saturated with aq. sat. NH_4Cl, followed by extraction with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents evaporated to give the desired 3-enoyl tetramic acids **10a-c** with sufficiently good purity as determined by ^{1}H NMR.
(See below for characterisation of **10a-c**).

<u>General Procedure for Aldol Condensation of Tetramic Acid **5a** with Aldehydes</u>

A solution of **5a** (1.0 eq.) in anhydrous THF was added to 1.8 M LDA solution (2.1 eq.), at -10°C and the solution was stirred at -10°C for 1 h. A solution of aldehyde (1.0 eq.) in anhydrous THF was then added dropwise to the reaction mixture at -10°C (Overall approx. concentration 0.07-0.1 M, based on **5a**). The

reaction mixture was allowed to warm to r.t. and left to stir at r.t. for 20 h. The reaction mixture was then quenched with aq. sat. NH_4Cl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, 10% to 100%) gave the product as a yellow or orange oil. The product was then washed with 10% aq. HCl to remove chelated metals from the 3-acyltetramic acid moiety to give a well resolved ^{1}H NMR spectrum.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-(4'-methylphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 10a



major isomer (*Z*)

R_f = 0.70 (5% MeOH/EtOAc);

$[\alpha]_D^{20}$ = +66.8 (*c*= 1.00 in $CHCl_3$) (*E*:*Z* = 0.48:1);

Bright yellow oil;

ν_{max} / cm^{-1} (film) 1753 (s, C=O), 1707 (s, C=O), 1652 (s, C=O);

Major Tautomer

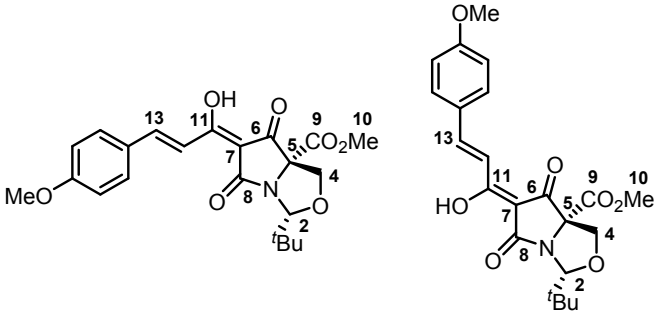
δ_H(500 MHz, $CDCl_3$) 7.98 (d, *J* = 16.1 Hz, 1H, H-13), 7.68 (d, *J* = 16.1 Hz, 1H, H-12), 7.57 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.24 (d, *J* = 7.9 Hz, 2H, Ar-H), 4.90 (s, 1H, H-2), 4.87 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.55 (d, *J* = 8.8 Hz, 1H, H-4'), 2.41 (s, 3H, Ar-Me), 0.96 (s, 9H, -*t*Bu);

Major Tautomer

δ_C (125 Mhz, $CDCl_3$) 187.99 (C-6), 180.72 (C-8), 176.99 (C-11), 167.71 (C-9), 147.34 (C-13), 142.92, 131.42, 129.92, 129.64, 129.47 (ArC), 116.12 (C-12), 99.76 (C-7), 98.27 (C-2), 78.31 (C-5), 68.34 (C-4), 53.26 (C-10), 35.20 (-C-*t*Bu), 24.66 (-*t*Bu), 21.69 (Ar-Me);

m/z (ESI-) 398.17 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{22}H_{24}NO_6$ ([M-H]$^-$) 398.1609, found 398.1603.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-(4'-methoxyphenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 10b



major isomer (*Z*)

R_f = 0.58 (5% MeOH/EtOAc);

$[\alpha]_D^{20}$ = +74.3 (*c*= 1.04 in $CHCl_3$) (*E*:*Z* = 0.28:1);

Bright orange oil;

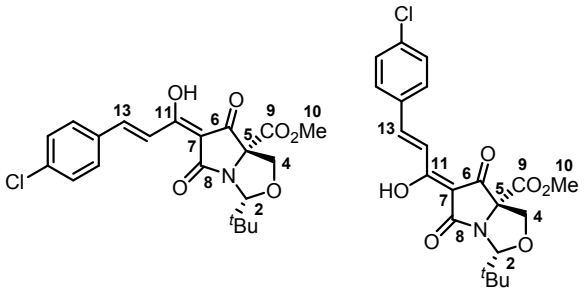
ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1705 (s, C=O), 1651 (s, C=O);

Major Tautomer

δ_H(500 MHz, $CDCl_3$) 7.97 (d, *J* = 15.8 Hz, 1H, H-13), 7.63 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.60 (d, *J* = 15.8 Hz, 1H, H-12), 6.94 (d, *J* = 8.8 Hz, 2H, Ar-H), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 8.8 Hz, 1H, H-4), 3.87 (s, 3H, Ar-OMe), 3.81 (s, 3H, -CO_2Me), 3.55 (d, *J* = 8.8 Hz, 1H, H-4'), 0.96 (s, 9H, -*t*Bu);

Major Tautomer
δ_C (125 Mhz, $CDCl_3$) 187.99 (C-6), 180.92 (C-8), 177.06 (C-11), 167.81 (C-9), 162.96 (Ar<u>C</u>-OMe), 147.19 (C-13), 131.74, 131.52, 126.92, 114.69 (ArC), 114.59 (C-12), 99.31 (C-7), 98.24 (C-2), 78.30 (C-5), 68.37 (C-4), 55.51 (Ar-O<u>Me</u>), 53.23 (C-10), 35.19 (-<u>C</u>-^{t}Bu), 24.66 (-^{t}Bu);
m/z (ESI-) 414.16 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{22}H_{24}NO_7$ ([M-H]$^-$) 414.1558, found 414.1560.

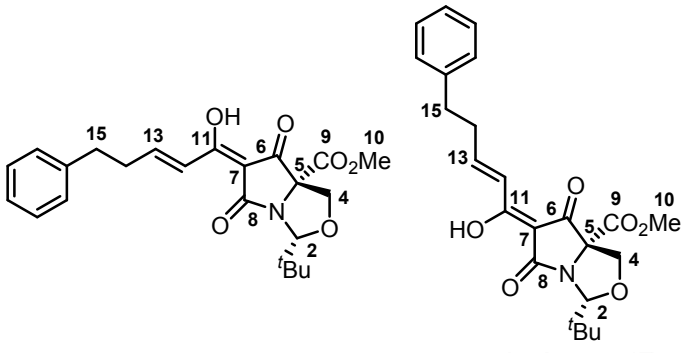
(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-(4'-chlorophenyl)allylidene]-3-oxabicyclo[3.3.0]-octane, 10c



R_f = 0.49 (5% MeOH/EtOAc);
$[\alpha]_D^{20}$ = +63.6 (*c*= 0.5 in $CHCl_3$) (*E*:*Z* = 0.26:1);
Bright yellow oil;
ν_{max} / cm^{-1} (film) 1755 (s, C=O), 1711 (s, C=O), 1655 (s, C=O);
Major Tautomer
δ_H(500 MHz, $CDCl_3$) 7.92 (d, *J* = 16.0 Hz, 1H, H-13), 7.70 (d, *J* = 16.0 Hz, 1H, H-12), 7.59 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.41 (d, *J* = 8.5 Hz, 2H, Ar-H), 4.90 (s, 1H, H-2), 4.87 (d, *J* = 8.9 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.55 (d, *J* = 8.9 Hz, 1H, H-4'), 0.96 (s, 9H, -^{t}Bu);
δ_C (125 Mhz, $CDCl_3$) 188.01 (C-6), 180.38 (C-8), 176.30 (C-11), 167.56 (C-9), 145.31 (C-13), 137.93, 132.55, 130.36, 129.48 (ArC), 117.70 (C-12), 100.35 (C-7), 98.32 (C-2), 78.34 (C-5), 68.29 (C-4), 53.31 (C-10), 35.21 (-<u>C</u>-^{t}Bu), 24.64 (-^{t}Bu);
Minor Tautomer
δ_H(500 MHz, $CDCl_3$) 4.92 (s, 1H, H-2), 3.82 (s, 3H, -CO_2Me), 0.94 (s, 9H, -^{t}Bu); (the other signals from the minor isomer were not resolved)

δ_C (125 Mhz, $CDCl_3$) 196.78 (C-6), 176.77 (C-11), 172.57 (C-8), 167.46 (C-9), 145.97 (C-13), 116.98 (C-12), 102.56 (C-7), 98.45 (C-2), 68.22 (C-4), 53.42 (C-10), 35.35 (-<u>C</u>-^{t}Bu), 24.76 (-^{t}Bu);
m/z (ESI-) 418.21 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{21}H_{21}ClNO_6$ ([M-H]$^-$) 418.1062, found 418.1063.

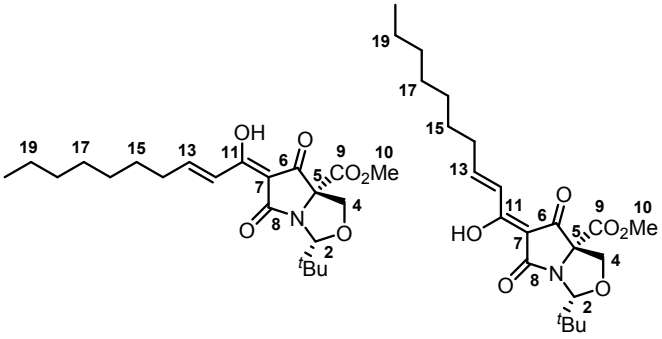
(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-ethylphenylallylidene]-3-oxabicyclo[3.3.0]-octane, 10d



R_f = 0.39 (EtOAc);
$[\alpha]_D^{20}$ = +72.5 (*c*= 0.91 in $CHCl_3$) (*E*:*Z* = 0.23:1, based on H-2 signal);

Orange oil;
ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1711 (s, C=O), 1641 (s, C=O);
Major Tautomer
δ_H(500 MHz, $CDCl_3$) 7.36 (d, *J* = 15.8 Hz, 1H, H-13), 7.28-7.33 (m, 2H, Ar-H), 7.19-7.24 (m, 3H, Ar-H), 7.12 (d, *J* = 15.8 Hz, 1H, H-12), 4.87 (s, 1H, H-2), 4.84 (d, *J* = 8.8 Hz, 1H, H-4), 3.80 (s, 3H, -CO_2Me), 3.51 (d, *J* = 8.8 Hz, 1H, H-4'), 2.83-2.86 (m, 2H, H-15), 2.67-2.71 (m, 2H, H-14), 0.94 (s, 9H, -*t*Bu);
Major Tautomer
δ_C (125 Mhz, $CDCl_3$) 187.85 (C-6), 180.62 (C-8), 176.77 (C-11), 167.58 (C-9), 152.46 (C-13), 140.29, 128.59, 128.57, 128.29, 126.32 (ArC), 121.43 (C-12), 99.43 (C-7), 98.27 (C-2), 78.25 (C-5), 68.25 (C-4), 53.27 (C-10), 35.30 (-C-*t*Bu), 35.08 (C-14), 34.17 (C-15), 24.63 (-*t*Bu);
m/z (ESI-) 412.20 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{23}H_{27}NNaO_6$ ([M+Na]$^+$) 436.1731, found 436.1727.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-3-(*E*)-heptylallylidene]-3-oxabicyclo[3.3.0]-octane, 10e

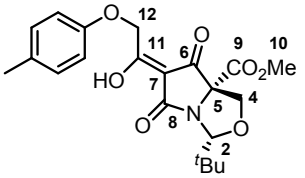


R_f = 0.42 (EtOAc);
$[\alpha]_D^{20}$ = +64.9 (*c*= 1.23 in $CHCl_3$) (*E*:*Z* = 0.24:1, based on H-2 signal);
Orange oil;
ν_{max} / cm^{-1} (film) 1755 (s, C=O), 1714 (s, C=O), 1640 (s, C=O);
Major Tautomer
δ_H(500 MHz, $CDCl_3$) 7.36 (d, *J* = 15.4 Hz, 1H, H-13), 7.07 (d, *J* = 15.4 Hz, 1H, H-12), 4.87 (s, 1H, H-2), 4.84 (d, *J* = 8.8 Hz, 1H, H-4), 3.79 (s, 3H, -CO_2Me), 3.51 (d, *J* = 8.8 Hz, 1H, H-4'), 2.36 (apparent quartet, *J* = 6.9 Hz, 2H, H-14), 1.49-1.53 (m, 2H, H-15), 1.25-1.36 (m, 8H, H-16, H-17, H-18, H-19), 0.94 (s, 9H, -*t*Bu), 0.89 (t, *J* = 6.9 Hz, 3H, H-20);
Major Tautomer
δ_C (125 Mhz, $CDCl_3$) 187.85 (C-6), 180.77 (C-8), 177.08 (C-11), 167.63 (C-9), 154.33 (C-13), 120.92 (C-12), 101.54 (C-7), 98.25 (C-2), 78.25 (C-5), 68.28 (C-4), 53.24 (C-10), 35.18 (-C-*t*Bu), 33.53 (C-14), 31.65, 29.21, 29.00 (C-16, C-17, C-18), 27.95 (C-15), 24.63 (-*t*Bu), 22.57 (C-19), 14.04 (C-20);
m/z (ESI-) 406.24 ([M-H]$^-$, 100%); HRMS (ESI+) calculated for $C_{22}H_{33}NNaO_6$ ([M+Na]$^+$) 430.2200, found 430.2196.

General Procedure for Synthesis of *O*-arylether tetramic acids **11a-c**

Cs_2CO_3 (3.0 eq.) and the corresponding phenol (1.1 eq.) was added to **5c** (1.0 eq.) dissolved in acetonitrile. The solution was stirred at r.t. for 17 h, afterwhich the solvents were evaporated and water and EtOAc were added to the residue. The aqueous layer was acidified with dilute aq. 10% aq. HCl and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and evaporated to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/Petroleum Ether) gave the product, which was then washed with a solution of aq. sat. NH_4Cl and 10% aq. HCl, dried with anhydrous $MgSO_4$, filtered and the solvents evaporated.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(4''-methylphenoxy)methyl-allylidene]-3-oxabicyclo[3.3.0]-octane, 11a



R_f = 0.37 (EtOAc);

$[\alpha]_D^{20}$ = +61.3 (c= 1.10 in $CHCl_3$);

Pale orange oil;

ν_{max} / cm^{-1} (film) 1755 (s, C=O), 1717 (s, C=O), 1661 (s, C=O), 1286, 1176, 1047 (s, C-O-C);

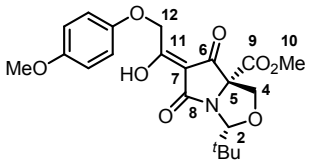
Major Isomer

δ_H(500 MHz, $CDCl_3$) 7.10 (d, *J* = 8.2 Hz, 2H, ArH), 6.85 (d, *J* = 8.2 Hz, 2H, ArH), 5.26 (d, *J* = 17.0 Hz, 1H, H-12), 5.18 (d, *J* = 17.0 Hz, 1H, H-12'), 4.87 (s, 1H, H-2), 4.86 (d, *J* = 9.1 Hz, 1H, H-4), 3.82 (s, 3H, -CO_2Me), 3.55 (d, *J* = 9.1 Hz, 1H, H-4'), 2.30 (s, 3H, Ar-Me), 0.94 (s, 9H, -^{t}Bu);

δ_C (125 Mhz, $CDCl_3$) 187.27 (C-6), 185.92 (C-11), 179.83 (C-8), 166.95 (C-9), 155.55, 131.47, 130.07, 114.60 (Ar-C), 100.80 (C-7), 98.35 (C-2), 78.28 (C-5), 68.06 (C-4), 65.21 (C-12), 53.43 (C-10), 35.16 (-C-^{t}Bu), 24.59 (-^{t}Bu), 20.47 (Ar-Me);

m/z (ESI-) 402.14 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{21}H_{24}NO_7$ ([M-H]$^-$) 402.1558, found 402.1561.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(4''-methoxyphenoxy)methyl-allylidene]-3-oxabicyclo[3.3.0]-octane, 11b



R_f = 0.33 (EtOAc);

$[\alpha]_D^{20}$ = +24.6 (c= 0.97 in $CHCl_3$);

Yellow oil;

ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1716 (s, C=O), 1661 (s, C=O), 1285, 1178 (br), 1045 (br) (s, C-O-C);

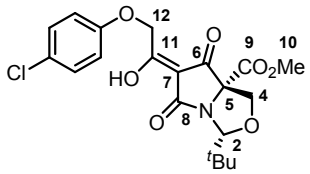
Major Isomer

δ_H(500 MHz, $CDCl_3$) 6.90 (d, *J* = 9.1 Hz, 2H, ArH), 6.83 (d, *J* = 9.1 Hz, 2H, ArH), 5.23 (d, *J* = 17.3 Hz, 1H, H-12), 5.14 (d, *J* = 17.0 Hz, 1H, H-12'), 4.87 (s, 1H, H-2), 4.85 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.77 (s, 3H, Ar-OMe), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.94 (s, 9h, -^{t}Bu);

δ_C (125 Mhz, $CDCl_3$) 187.26 (C-6), 185.94 (C-11), 179.81 (C-8), 166.94 (C-9), 154.79, 151.78, 116.03, 114.70 (Ar-C), 100.81 (C-7), 98.35 (C-2), 78.28 (C-5), 68.06 (C-4), 65.96 (C-12), 55.65 (Ar-OMe), 53.43 (C-10), 35.15 (-C-^{t}Bu), 24.58 (-^{t}Bu);

m/z (ESI-) 418.13 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{21}H_{24}NO_7$ ([M-H]$^-$) 418.1507, found 418.1513.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(4''-chlorophenoxy)methyl-allylidene]-3-oxabicyclo[3.3.0]-octane, 11c



R_f = 0.38 (EtOAc);

$[\alpha]_D^{20}$ = +20.4 (c= 0.95 in $CHCl_3$);

Yellow oil;

ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1716 (s, C=O), 1662 (s, C=O), 1284, 1173, 1045 (s, C-O-C);

Major Isomer

δ_H(400 MHz, $CDCl_3$) 11.64 (s, br, enol –OH), 7.26 (d, *J* = 8.5 Hz, 2H, ArH), 6.89 (d, *J* = 9.0 Hz, 2H, ArH), 5.28 (d, *J* = 17.2 Hz, 1H, H-12), 5.20 (d, *J* = 13.5 Hz, 1H, H-12'), 4.87 (s, 1H, H-2), 4.86 (d, *J* = 9.0 Hz, 1H, H-4), 3.82 (s, 3H, -CO_2Me), 3.55 (d, *J* = 9.0 Hz, 1H, H-4'), 0.94 (s, 9h, -^{t}Bu);

δ_H(400 MHz, Acetone-d_6) 10.08 (s, br, enol –OH), 7.34 (d, *J* = 9.0 Hz, 2H, ArH), 7.01 (d, *J* = 9.0 Hz, 2H, ArH), 5.35 (s, 2H, H-12), 4.90 (s, 1H, H-2), 4.77 (d, *J* = 8.7 Hz, 1H, H-4), 3.82 (s, 3H, -CO_2Me), 3.77 (d, *J* = 8.7 Hz, 1H, H-4'), 0.93(s, 9h, -^{t}Bu); [broad signals in ^{13}C]

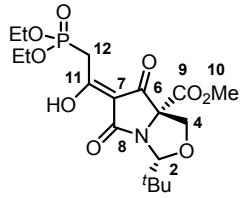
δ_H(700 MHz, CD_2Cl_2, 278K) 7.30 (d, *J* = 8.9 Hz, 2H, ArH), 6.92 (d , *J* = 8.5 Hz, 2H, ArH), 5.27 (d, *J* = 17.2 Hz, 1H, H-12), 5.22 (d, *J* = 17.2 Hz, 1H, H-12'), 4.90 (s, 1H, H-2), 4.83 (d, *J* = 9.1 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.58 (d, *J* = 9.0Hz, 1H, H-4'), 0.95 (s, 9H, -^{t}Bu);
δ_C(175 MHz, CD_2Cl_2, 278K) 187.20 (C-6), 184.88 (C-11), 179.78 (C-8), 167.04 (C-9), 156.32, 129.52, 126.73, 116.00 (Ar-C), 101.04 (C-7), 98.24 (C-2), 78.28 (C-5), 67.98 (C-4), 65.19 (C-12), 53.49 (C-10; obscured by CD_2Cl_2 signals but HMBC correlation with C-9 seen), 35.06 (-C-^{t}Bu), 24.35 (-^{t}Bu);

m/z (ESI-) 422.09 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{20}H_{21}ClNO_7$ ([M-H]$^-$) 422.1012, found 422.1010.

Synthesis of Phosphonate Ester **12**

NaH used was pre-washed with dry Et_2O.
A suspension of NaH (87.1 mg, 2.186 mmol, 3.5 eq.; 60% in mineral oil) in dry THF (3 ml) was cooled to 0°C. Diethyl phosphate (284 µl, 2.186 mmol, 3.5 eq.) was then added dropwise and the resulting suspension was stirred at 0°C for 30 min. **5d** (235 mg, 0.625 mmol, 1.0 eq.) in THF (5 ml) was then added slowly dropwise. The reaction mixture was stirred at 0°C for 1 h, then at r.t. for 16 h. Reaction was quenched with water and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, 10% to 100%, then 5% MeOH/EtOAc) gave **12** (228 mg, 84%) as an orange oil, which was then washed with sat. NH_4Cl and 10% aq. HCl to remove chelated metals from the 3-acyltetramic acid moiety.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-dioxo-7-[(*Z*)-1-hydroxy-1'-(diethyl-methylphosphonate)-allylidene]-3-oxabicyclo[3.3.0]-octane, 12

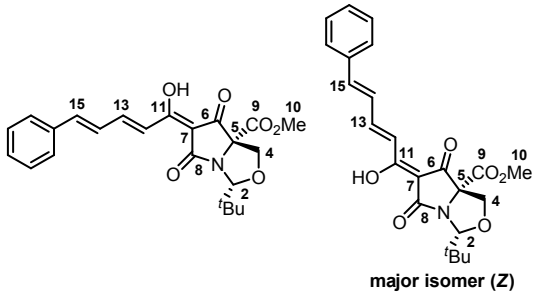


R_f = 0.11 (5% MeOH/EtOAc);
Orange oil;
$[\alpha]_D^{20}$ = +40.8 (*c*= 0.88 in $CHCl_3$);
ν_{max} / cm^{-1} (film) 1754 (s, C=O), 1718 (s, C=O), 1658 (s, C=O), 1265 (s, P=O), 1047, 1020, 956 (s, RO-P);
δ_H (500 MHz, $CDCl_3$) 4.86 (s, 1H, H-2), 4.83 (d, *J* = 8.8 Hz, 1H, H-4), 4.16 (apparent quintet, *J* = 7.3 Hz, 4H, -O$CH_2$$CH_3$), 3.78 (s, 3H, -$CO_2$Me), 3.55 (d, *J* = 24.0 Hz, 2H, H-12), 3.50 (d, *J* = 8.8 Hz, 1H, H-4'), 1.31 (dt, *J* = 6.9 Hz, 2.8 Hz, 6H, -O$CH_2$$CH_3$), 0.93 (s, 9H, -^{t}Bu);
δ_C (125 Mhz, $CDCl_3$) 187.38 (C-6), 181.25 (C-11, $^2J_{PC}$ = 9.5 Hz), 179.49 (C-8), 167.14 (C-9), 102.53 (C-7, $^3J_{PC}$ = 6.7 Hz), 98.32 (C-2), 78.46 (C-5), 68.09 (C-4), 63.05 (-O$CH_2$$CH_3$, $^2J_{PC}$ = 6.7 Hz), 53.30 (C-10), 35.13 (-C-^{t}Bu), 32.36 (C-12, $^1J_{PC}$ = 127.8 Hz), 24.58 (-^{t}Bu), 16.24 (-O$CH_2$$CH_3$, $^3J_{PC}$ = 6.7 Hz);
δ_P (200 Mhz, $CDCl_3$) 19.21;
m/z (ESI-) 432.11 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{18}H_{27}NO_9P$([M-H]$^-$) 432.1429, found 432.1431.

General Procedure for Horner-Wadsworth-Emmons Olefination Towards **10a-h**

Phosphonate **12** (1.0 eq.) in dry THF (1.5 ml), was added to solid ^{t}BuOK (2.1 eq.) at 0°C and the suspension was stirred at 0°C for 30 min. The corresponding aldehyde (1.1 eq.) in dry THF (1.5 ml) was then added. The reaction mixture was stirred at 0°C for 10 min, quenched with aq. sat. NH_4Cl, acidified with aq. 10% HCl and extracted with EtOAc. The combined organic layers was dried with anhydrous $MgSO_4$, filtered and the solvents removed in vacuo to give the crude. Purification via flash column chromatography on silica gel (Eluent: EtOAc/PE, then 5% MeOH/EtOAc) gave the product, which was then washed with aq. sat. NH_4Cl and 10% aq. HCl.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-7-[(*Z*)-1-hydroxy-1'-(*E*,*E*-phenyldienyl)vinylidene]-dioxo-3-oxabicyclo[3.3.0]-octane, 10f



R_f = 0.31 (EtOAc);

$[\alpha]_D^{20}$ = +51.8 (*c*= 0.88 in $CHCl_3$) (*E*:*Z* = 0.24:1, based on H-2 signal);

Orange oil;

ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1705 (s, C=O), 1651 (s, C=O);

Major Tautomer

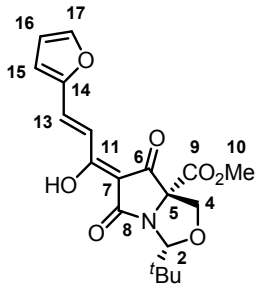
δ_H(500 MHz, $CDCl_3$) 7.78 (dd, *J* = 15.1 Hz, 10.1 Hz, 1H, H-13), 7.51-7.57 (m, 2H, ArH), 7.36-7.48 (m, 3H, ArH), 7.24 (d, *J* = 15.1 Hz, 1H, H-12), 6.95-7.03 (m, 2H, H-14 and H-15), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.96 (s, 9H, -^{t}Bu);

Major Tautomer

δ_C (125 Mhz, $CDCl_3$) 186.64 (C-6), 179.68 (C-8), 175.45 (C-11), 166.74 (C-9), 146.41 (C-13), 143.86 (C-15), 129.10, 128.00, 127.09, 126.82 (ArC), 125.91 (C-14), 119.66 (C-12), 98.72 (C-7), 97.25 (C-2), 77.28 (C-5), 67.34 (C-4), 52.25 (C-10), 34.20 (-C-^{t}Bu), 23.66 (-^{t}Bu);

m/z (ESI-) 410.17 ([M-H]$^-$, 100%); HRMS (ESI-) calculated for $C_{23}H_{24}NO_6$ ([M-H]$^-$) 410.1609, found 410.1603.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-7-[(*Z*)-1-hydroxy-3-(*E*)-(2'-furan)allylidene]-dioxo-3-oxabicyclo[3.3.0]-octane, 10g



R_f = 0.32 (EtOAc);

Dark yellow oil;

$[\alpha]_D^{20}$ = +102.9 (*c*= 0.50 in $CHCl_3$);

ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1704 (s, C=O), 1651 (s, C=O), 1614, 1589, 1537, 1483, 1387, 1368 (s, furan C=C) ;

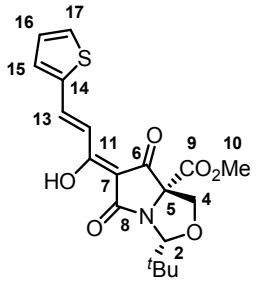
Major Tautomer

δ_H (500 MHz, $CDCl_3$) 7.72 (d, *J* = 15.4 Hz, 1H, H-13), 7.61 (d, *J* = 1.6 Hz, 1H, H-17), 7.54 (d, *J* = 15.4 Hz, 1H, H-12), 6.86 (d, *J* = 3.5 Hz, 1H, H-15), 6.56 (dd, *J* = 1.6, 3.5 Hz, 1H, H-16), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 9.1 Hz, 1H, H-4), 3.80 (s, 3H, -CO_2Me), 3.54 (d, *J* = 9.1 Hz, 1H, H-4'), 0.95 (s, 9H, -^{t}Bu);

δ_C (125 Mhz, $CDCl_3$) 187.71 (C-6), 180.62 (C-8), 176.26 (C-11), 167.71 (C-9), 151.28 (C-14), 147.06 (C-17), 132.11 (C-13), 118.81 (C-15), 114.81 (C-12), 113.38 (C-16), 99.84 (C-7), 98.24 (C-2), 78.28 (C-5), 68.33 (C-4), 53.24 (C-10), 35.20 (-C-^{t}Bu), 24.65 (-^{t}Bu);

m/z (ESI+) 398.13 ([M+Na]$^+$, 63%), 374.13 ([M-H]$^-$, 47%); HRMS (ESI+) calculated for $C_{19}H_{21}NNaO_7$ ([M+Na]$^+$) 398.1210, found 398.1215.

(2*R*,5*R*)-1-Aza-2-(tert-butyl)-5-methoxycarbonyl-6,8-7-[(*Z*)-1-hydroxy-3-(*E*)-(2'-thiophene)allylidene]-dioxo-3-oxabicyclo[3.3.0]-octane, 10h



R_f = 0.39 (EtOAc);
Dark yellow oil;
$[\alpha]_D^{20}$ = +148.2 (*c*= 0.48 in $CHCl_3$);
ν_{max} / cm^{-1} (film) 1752 (s, C=O), 1705 (s, C=O), 1651 (s, C=O), 1604, 1570, 1503, 1482, 1383, 1355 (s, thiophene C=C) ;
Major Tautomer
δ_H (500 MHz, $CDCl_3$) 8.09 (d, *J* = 15.8 Hz, 1H, H-13), 7.57 (d, *J* = 5.0 Hz, 1H, H-17), 7.48 (d, *J* = 15.4 Hz, 1H, H-12), 7.45 (d, *J* = 3.5 Hz, 1H, H-15), 7.13 (dd, *J* = 3.8, 5.0 Hz, 1H, H-16), 4.89 (s, 1H, H-2), 4.86 (d, *J* = 8.8 Hz, 1H, H-4), 3.81 (s, 3H, -CO_2Me), 3.54 (d, *J* = 8.8 Hz, 1H, H-4'), 0.95 (s, 9H, -^{t}Bu);
δ_C (125 Mhz, $CDCl_3$) 187.85 (C-6), 180.64 (C-8), 176.24 (C-11), 167.68 (C-9), 139.98 (C-14), 139.32 (C-13), 133.70 (C-15), 132.11 (C-17), 128.82 (C-16), 115.82 (C-12), 99.64 (C-7), 98.27 (C-2), 78.31 (C-5), 68.34 (C-4), 53.27 (C-10), 35.19(-C-^{t}Bu), 24.65 (-^{t}Bu);
m/z (ESI-) 390.11 ([M-H]$^-$, 55%); HRMS (ESI-) calculated for $C_{19}H_{20}NO_6S$ ([M-H]$^-$) 390.1017, found 390.1025.

Bioassay Procedures

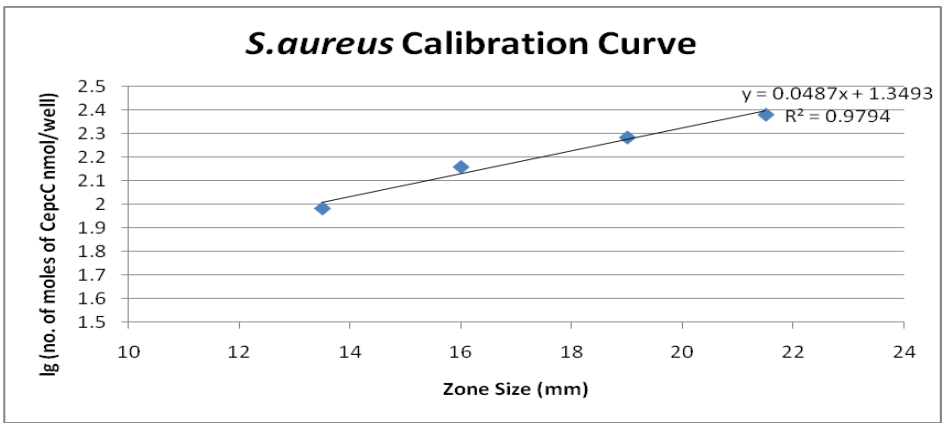
Antibacterial activity was assessed using hole-plate bioassay, with agar (Brain Heart Infusion Agar) plates inoculated with either *S. aureus* DS267 or *E. coli* X580. A calibration curve was obtained using Cephalosporin C (CepcC) for each of the bacteria species.

100 µl of each sample at the respective concentrations were loaded as 70% DMSO/H_2O solutions into 10 mm wells, and the agar plates incubated at 37°C for 18 h.

An Example of Bioassay Calibration
Each of the reference solutions were prepared by using the required amount of the respective stock solutions, and made up to a final volume of 100 µl with distilled water.

S.aureus (1 mg/ml stock solution)

Reference Solution	No. of moles of CepcC (nmol/well)	lg (no. of moles of CepcC nmol/well)	Zone size (mm)
40 µl + 60 µl H_2O (40 µg of CepcC)	96.29	1.984	13.5
60 µl + 40 µl H_2O (60 µg of CepcC)	144.44	2.160	16.0
80 µl + 20 µl H_2O (80 µg of CepcC)	192.59	2.285	19.0
100 µl + 0 µl H_2O (100 µg of CepcC)	240.73	2.382	21.5



E.coli (100 µg/ml and 10µg/ml stock solution)

Stock Solution used	Reference Solution	No. Of moles of CepcC (nmol/well)	lg (no. of moles of CepcC nmol/well)	Zone size (mm)
10 µg/ml	40 µl + 60 µl H_2O	0.9629	-0.0164	19
	60 µl + 40 µl H_2O	1.4444	0.1597	22
	80 µl + 20 µl H_2O	1.9259	0.2846	25
	100 µl + 0 µl H_2O	2.4073	0.3815	30
100 µg/ml	20 µl + 80 µl H_2O	4.8146	0.6826	31
	40 µl + 60 µl H_2O	9.6293	0.9836	36
	60 µl + 40 µl H_2O	14.4439	1.1597	38
	80 µl + 20 µl H_2O	19.2585	1.2846	42
	100 µl + 0 µl H_2O	24.07318	1.3815	44