Targeting *Staphylococcus aureus* Quorum Sensing with Non-Peptidic Small Molecule Inhibitors

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Scheme S1. Synthesis of 3-decanoyl-5-methyltetramic acid analogues

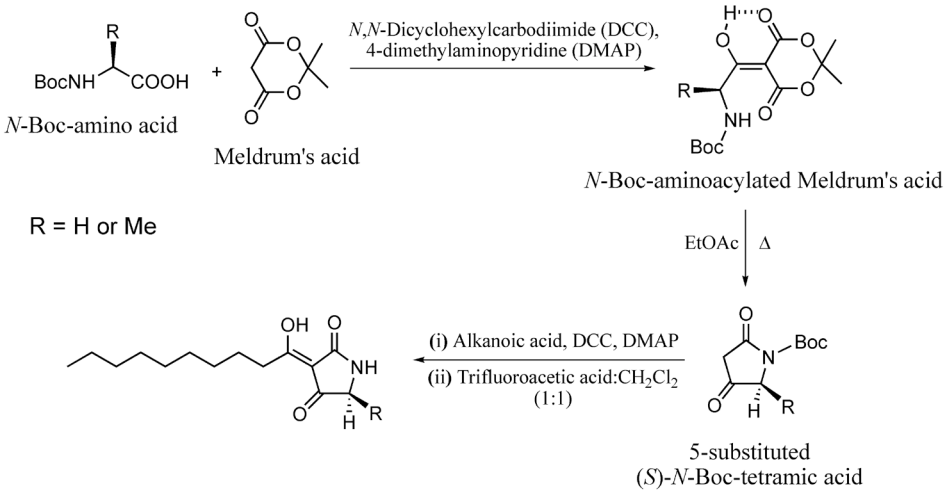


Figure S1. Chemical structure of 3-oxo-C_{12}-HSL **1** highlighting the structural units targeted for the SAR study.

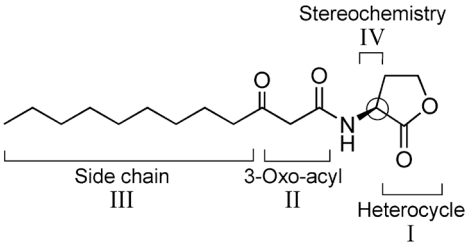
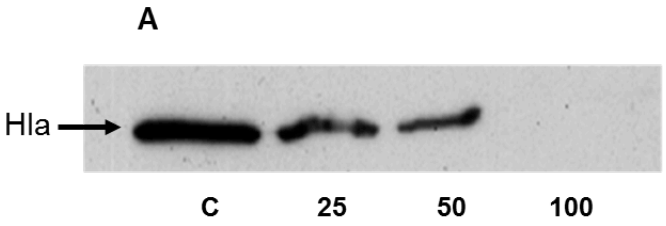


Figure S2. 5-HE-C8-TMA **4** abolishes α-hemolysin (Hla) production without inhibiting the growth of *S. aureus* USA300. (**A**) Western blot showing the effect of 5-HE-C8-TMA **4** added at concentrations of 0, 25, 50 and 100 μM. (**B**) Growth curves of *S. aureus* USA300 in the absence (control) and presence of 5-HE-C8-TMA **4** at 25, 50 and 100 μM.



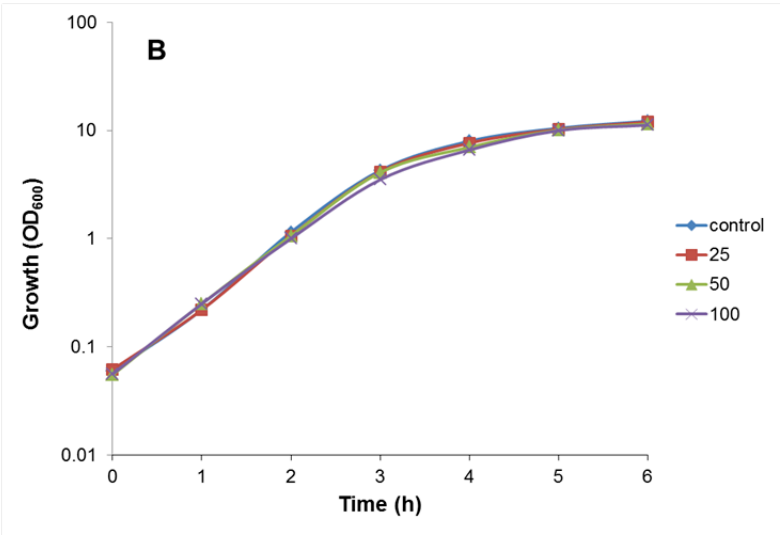


Figure S3. C14-TOA **17** substantially reduces α-hemolysin (Hla) production without inhibiting the growth of *S. aureus* USA300. (**A**) Western blot showing the effect of C14-TOA **17** added at concentrations of 0, 5 and 10 μM. (**B**) Growth curves of *S. aureus* USA300 in the absence (–◆–) and presence of C14-TOA **17** at 5 (–■–) and 10 μM (–▲–).

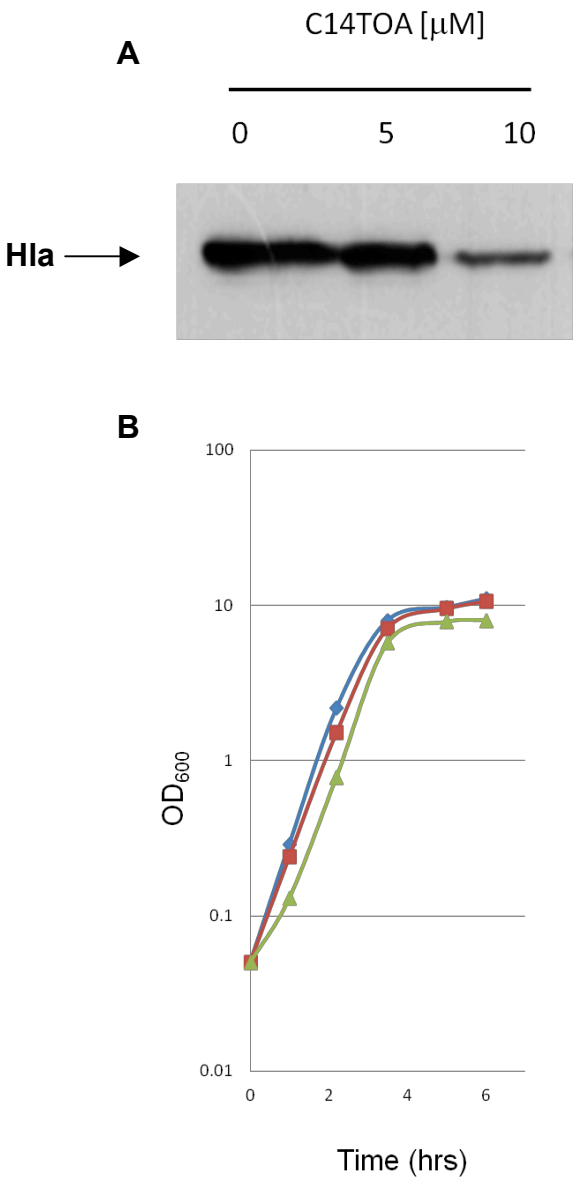


Figure S4. Impact of C14-TOA **17** (dark grey symbols and bars) in the staphylococcal mouse arthritis infection model compared with control (PBS; white symbols and bars) with respect to (**A**) Changes in mouse weight change (**B**) synovitis and mouse joint destruction and (**C**) bacterial viable counts in the kidneys.

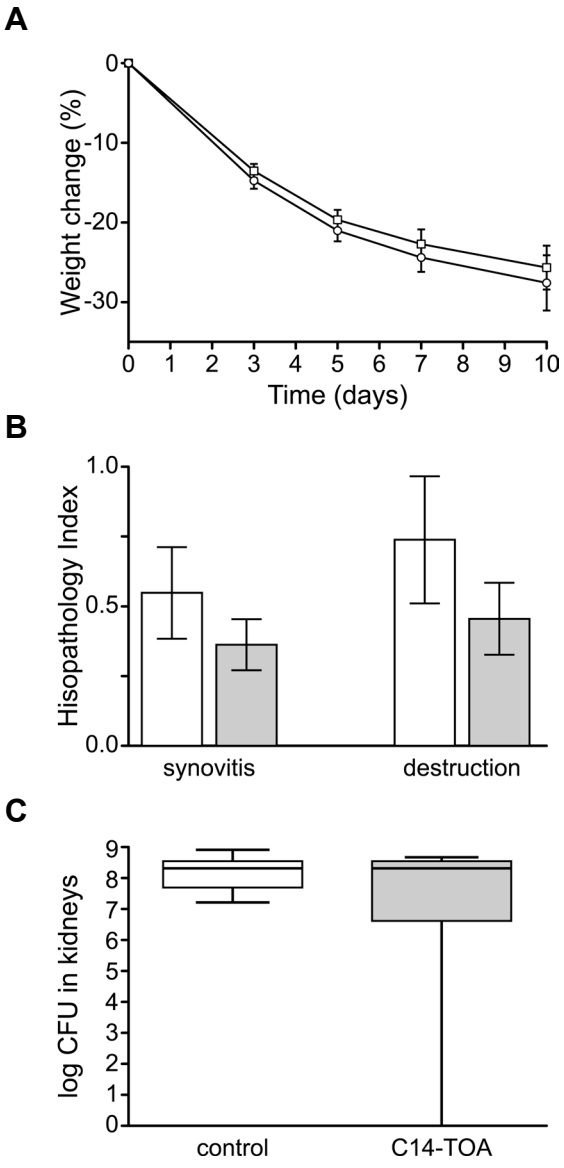
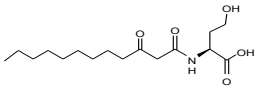
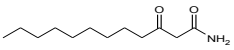
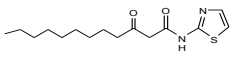
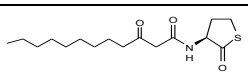
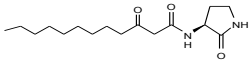
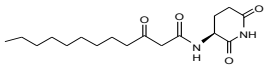
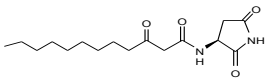
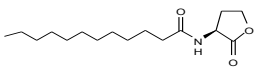
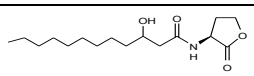
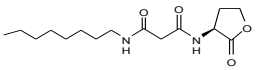
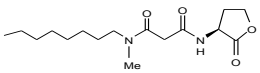
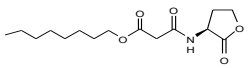
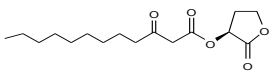
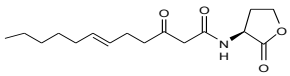
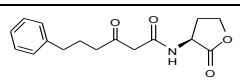
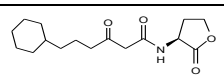
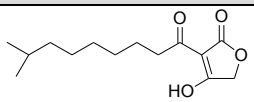
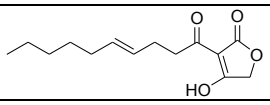
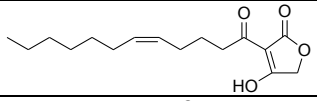
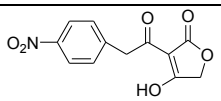
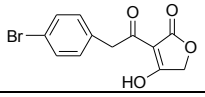
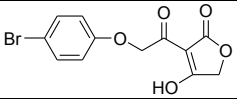
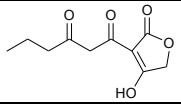
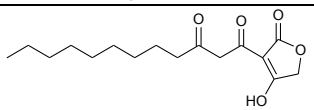


Table S1. QS and growth inhibitory activities of other 3-oxo-C_{12}-HSL analogues

Compound	Structure	Abbreviation	MIC (μM)	*agr* IC_{50} (μM)
	***Changes to*: Ring**			
S1		3-oxo-C_{12}-HS	-*	- **
S2		3-oxo-C_{12}-NH_2	-	-
S3		3-oxo-C_{12}-AT	-	-
S4		3-oxo-C_{12}-HTL	-	-
S5		3-oxo-C_{12}-AP	-	-
S6		3-oxo-C_{12}-GM	-	-
S7		3-oxo-C_{12}-Am	-	-
	3-Position			
S8		C_{12}-HSL	-	-
S9		3-OH-C_{12}-HSL	-	-
	4-Position			
S10		4-aza-3-oxo-C_{12}-HSL	-	-
S11		4-Me-4-aza-3-oxo-C_{12}-HSL	-	-
S12		4-oxa-3-oxo-C_{12}-HSL	-	-
	Amide			
S13		3-oxo-C_{12}-HBL	-	-
	Acyl chain			
S14		Δ^{6E}-3-oxo-C_{12}-HSL	-	-
S15		6-Ph-3-oxo-C_6-HSL	-	-
S16		6-Cy-3-oxo-C_6-HSL	-	-

*no growth inhibition up to 100 μM; **no inhibition of *agr* observed at concentrations up to 100 μM

Table S2. Growth and QS inhibitory activities of other 3-acyltetronic acids

Compound	Structure	Abbreviation	MIC (µM)	*agr* IC_{50} (µM)
S17		(8Me-C9)-TOA	-*	-**
S18		Δ^{4E}-C10-TOA	50	22 ± 5
S19		Δ^{5Z}-C12-TOA	-	88 ± 8
S20		pNPC2-TOA	-	-
S21		pBrPC2-TOA	-	-
S22		pBrPOC2-TOA	-	-
S23		3O-C6-TOA	-	-
S24		3O-C12-TOA	-	-

*no growth inhibition up to 100 µM; **no inhibition of *agr* observed at concentrations up to 100 µM.

Chemical synthesis and characterization of *N*-acylhomoserine lactone analogues

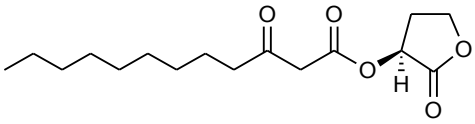
General information

The starting materials were purchased from Aldrich Chemical Co. and Alfa Aesar. The solvents used were of HPLC grade. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Avance-400 operating at 400 MHz. ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Avance-400 or Bruker Avance(III)-500 operating at 100 or 125 MHz, respectively. Chemical shifts were referenced to an internal standard $SiMe_4$.

The optical rotation was measured at 22 ºC using a polarimeter ADP220 (Bellingham & Stanley Ltd). High resolution ES-MS (in either positive or negative mode) were recorded using Waters Micromass LCT spectrometer. TLC was performed using Merck silica gel 60 GF_{254} pre-coated (0.2 mm) aluminium plates. Preparative layer chromatography (PLC) was performed using Merck silica gel 60 GF_{254} pre-coated (1.0 mm) glass plates. For column purification a Biotage Flashmaster Personal system was used with Biotage Isolute Flash Series II cartridges (10 g, 20 g and 70 g). All products during chromatography were visualized using either UV light (λ = 254 nm) or by staining with dilute potassium permanganate solutions.

Analytical RP-HPLC was used to establish purity, and was performed using a Waters setup, comprised of two 510 pumps, a 2487 Dual λ Absorbance Detector and Millennium software. The separation was performed using a Phenomenex Onyx Monolithic C18 column (4.6 x 100 mm) and a linear gradient of 30–70% solvent B in 16.0 min, then 70–100% solvent B in 1.0 min at a flow rate of 3.0 mL/min. Solvent A was 0.06% TFA in water and solvent B was 0.06% TFA in MeCN:H_2O (90:10), and UV detection at 214 and 254 nm.

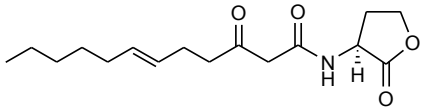
(*S*)-Tetrahydro-2-oxofuran-3-yl 3-oxododecanoate (3-Oxo-C_{12}-HBL) S13



3-Oxododecanoic acid (prepared from methyl 3-oxododecanoate by saponification at room temperature) (0.348 g, 1.63 mmol), dry DCM (40 ml), DMAP (0.298 g, 2.45 mmol), and α-hydroxy-γ-butyrolactone (0.166 g, 1.63 mmol) were stirred at room temperature under nitrogen. DCCI (0.386 g, 1.87 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was filtered to remove precipitated DCU and the filtrate was washed with 1 M HCl (2 x 10 ml), saturated sodium bicarbonate (2 x 10 ml), and brine (10 ml). The organic layer was dried over magnesium sulphate and concentrated to dryness to give an oily white solid. The oily solid was triturated with hexane and purified using Flash chromatography (30% ethyl acetate in hexane followed by 100% ethyl acetate) to deliver the title compound **S13** as a white solid (0.103 g, 21%).

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28 (12H, m, (CH_2)$_6$Me), 1.60 (2H, m, CH_2(CH_2)$_6$Me), 2.40 (1H, m, 4α-H), 2.57 (2H, m, CH_2(CH_2)$_7$Me), 2.78 (1H, m, 4β-H), 3.59 (2H, ABq, $COCH_2CO$), 4.35 (1H, m, 5α-H), 4.51 (1H, m, 5β-H), 5.47 (1H, m, 3-H). ES-MS *m/z* 299.1845 [M+H], $C_{16}H_{27}O_5^+$ requires 299.1858.

***N*-[(*E*)-3-Oxo-6-dodecenoyl]-L-homoserine lactone (Δ^{6E}-3-oxo-C_{12}-HSL) S14**



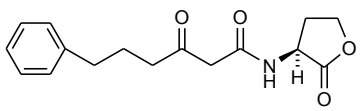
Ethyl *trans*-4-decenoate (1.0 g, 5.0 mmol) in methanol (2 ml) was treated with a solution of NaOH (0.5 g, 12.5 mmol) in water (5 ml) and stirred overnight at room temperature. The methanol was removed under reduced pressure. The aqueous solution was washed with ethyl acetate and then acidified with 1 M HCl (10 ml). The product was extracted with DCM (2 x 10 ml) and the pooled extract dried over magnesium sulphate and concentrated to dryness to give *trans*-4-decenoic acid as clear oil (0.743 g, 87%).

Using *trans*-4-decenoic acid, *trans*-4-decenoyl Meldrum's acid was prepared by a procedure similar to that described for acylated Meldrum's acid by Chhabra *et al.*[1]

trans-4-Decenoyl Meldrum's acid (0.528 g, 1.78 mmol), L-homoserine lactone HCl (0.245 g, 1.78 mmol), triethylamine (0.180 g, 1.78 mmol) and acetonitrile (20 ml) were stirred for 72 h at room temperature. The mixture was heated at reflux for 2 h and then the solvent was removed under reduced pressure. The residue was taken up in ethyl acetate (20 ml) and insoluble inorganics were removed by filtration. The filtrate was washed with saturated sodium bicarbonate (2 x 10 ml), 1 M HCl (2 x 10 ml) and brine (10 ml). The solution was dried over magnesium sulphate and concentrated to dryness to give a cream/white solid which was recrystallised from ethyl acetate/hexane to give the title compound **S14** as an off white solid (0.196 g, 37%).

$[\alpha]^D$ = +5.9 (c = 1.53, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.25-1.37 (6H, m, $(CH_2)_3$Me), 1.99 (2H, m, CHCH_2CH$_2$CO), 2.22-2.35 (3H, m, 4α-H & CH_2CHCH), 2.62 (2H, t, CH$_2$CH_2COCH$_2$) 2.79 (1H, m, 4β-H), 3.49 (2H, s, COCH$_2$CO), 4.31 (1H, m, 5α-H), 4.51 (1H, m, 5β-H), 4.61 (1H, m, 3-H), 5.37 (1H, m, CH$_2$C*H*CHCH$_2$), 5.45 (1H, m, CH$_2$CH*CH*CH$_2$). 7.70 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 14.05, 22.50, 26.37, 29.08, 29.78, 31.36, 32.45, 43.74, 48.24, 49.04, 65.88, 127.37, 132.28, 166.31, 174.83, 205.87. ES-MS *m/z* 296.1851 [M+H], $C_{16}H_{26}NO_4^+$ requires 296.1862.

***N*-(3-Oxo-6-phenylhexanoyl)-L-homoserine lactone (6-Ph-3-oxo-C_6-HSL) S15**

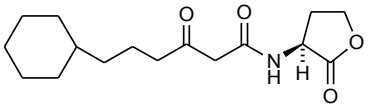


The title compound was prepared using 4-phenylbutyric acid and following the procedure described for **S14**. The crude oily solid was purified using silica plate chromatography (50% toluene in acetone) and then by trituration with ether to give **S15** as a white solid (0.155 g, 29%).

$[\alpha]^D$ = +15.1 (c = 1.72, $CHCl_3$). ^{1}H NMR ($CDCl_3$) 1.96 (2H, m, CH$_2$CH_2CH$_2$), 2.23 (1H, m, 4α-H), 2.56 (2H, t, CH_2(CH$_2$)$_2$), 2.66 (2H, t, (CH$_2$)$_2$CH_2), 2.78 (1H, m, 4β-H), 3.45 (2H, s, COCH$_2$CO), 4.30 (1H, m, 5α-H), 4.50 (1H, m, 5β-H), 4.60 (1H, m, 3-H), 7.18 (5H, m, Ph), 7.60

(1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 24.73, 24.94, 29.75, 33.92, 34.80, 42.92, 48.38, 49.05, 65.90, 126.12, 128.46, 128.48, 141.17, 166.33, 174.89, 205.99. ES-MS *m/z* 290.1382 [M+H], $C_{16}H_{20}NO_4^+$ requires 290.1392.

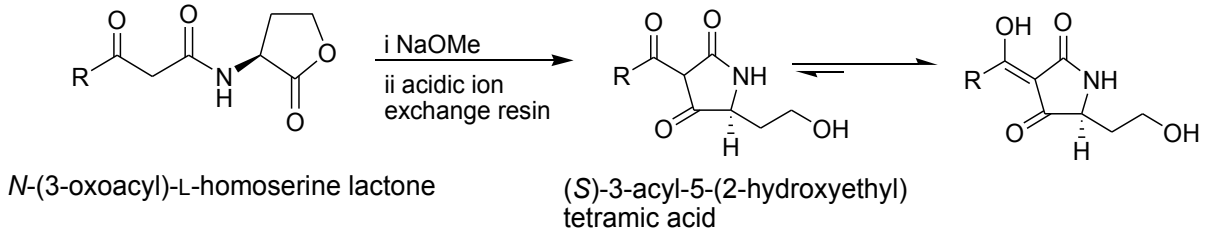
_N_-(3-oxo-6-cyclohexylhexanoyl)-L-homoserine lactone (6-Cy-3-oxo-C_6-HSL) S16



The title compound was prepared using 4-cyclohexylbutyric acid and following the procedure described for **S14**. The crude off white solid was purified using flash chromatography (90% ethyl acetate in hexane) and trituration with ether to give **S16** as a white solid (0.226 g, 40%).

^{1}H NMR ($CDCl_3$) 0.90 (2H, m, C*H*$_2$(CH_2)$_2$CO), 1.15-1.25 & 1.59-1.72 (13H, 2 x m, CH_2C*H*$_2$$CH_2$ & CyH_{11}), 2.25 (1H, m, 4α-H), 2.53 (2H, t, (CH_2)$_2$C*H*$_2$CO), 2.78 (1H, m, 4β-H), 3.49 (2H, s, $COCH_2CO$), 4.30 (1H, m, 5α-H), 4.50 (1H, m, 5β-H), 4.61 (1H, m, 3-H), 7.68 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 20.73, 26.30, 26.61, 29.72, 33.20, 36.71, 37.42, 44.16, 48.20, 49.03, 65.90, 166.47, 174.89, 206.56. ES-MS *m/z* 296.1857[M+H], $C_{16}H_{26}NO_4^+$ requires 296.1862.

Chemical synthesis and characterization of 3-acyltetramic acid (TMA) analogues



N-(3-oxoacyl)-L-homoserine lactone

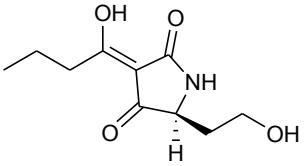
(*S*)-3-acyl-5-(2-hydroxyethyl) tetramic acid

Scheme 1: Synthesis of (*S*)-3-acyl-5-(2-hydroxyethyl)tetramic acids

General Procedure 1 (GP1)

A solution of sodium methoxide in methanol (0.5 M, 2.0 mL, 1.0 mmol) was added to a stirred solution of *N*-(3-oxoacyl)-L-homoserine lactone (1.0 mmol) in methanol (3 mL) under nitrogen. The reaction mixture was stirred for 3 h at 55 °C and then overnight at 50 °C. The mixture was cooled to room temperature and then passed through an acidic ion exchange resin (Dowex 50 WX2-200). The resin was eluted with MeOH (30 mL), the eluants were combined and concentrated *in vacuo* to afford the tetramic acids as a colourless solid.

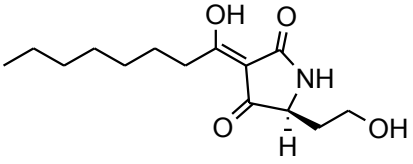
(*S*)-3-Butyryl-5-(2-hydroxyethyl)tetramic acid (5-HE-C4-TMA) 3



Use of *N*-(3-oxohexanoyl)-L-homoserine lactone in **GP1** gave an oily orange solid which was triturated with ether to give **3** as a cream/white solid (55%).

^{1}H NMR ($CDCl_3$) δ 1.04 (3H, t, Me), 1.71-1.83 (3H, m, C*H*$_2$Me & C*H*HCH$_2$OH), 2.15 (1H, m, CH*H*CH$_2$OH), 2.85 (2H, m, C*H*$_2$CH$_2$Me), 3.85-3.99 (3H, m, ring CH & CH$_2$C*H*$_2$OH), 6.41 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 13.73, 19.43, 34.09, 34.72, 60.91, 61.81, 100.66, 175.08, 189.66, 195.51. ES-MS *m/z* 214.1060 [M+H], $C_{10}H_{16}NO_4^+$ requires 214.1079. RP-HPLC R_T 0.93 min.

(*S*)-3-Octanoyl-5-(2-hydroxyethyl)tetramic acid (5-HE-C8-TMA) 4

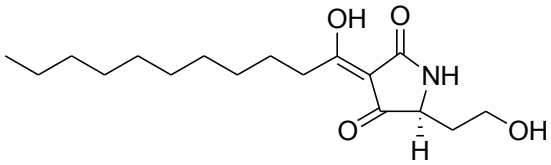


Use of *N*-(3-oxodecanoyl)-L-homoserine lactone in **GP1** gave **4** as a white solid (21%).

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.33-1.42 (8H, m, (C*H*$_2$)$_4$Me), 1.69 (2H, m, C*H*$_2$(CH$_2$)$_4$Me), 1.82 & 2.15 (2H, 2 x m, C*H*$_2$CH$_2$OH), 2.87 (2H, m, C*H*$_2$(CH$_2$)$_5$Me), 3.87-.3.99 (3H, m, ring CH & CH$_2$C*H*$_2$OH), 6.38 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 14.07, 22.59, 25.88,

28.92, 29.22, 31.63, 33.00, 34.11, 60.82, 61.77, 100.55, 175.13, 189.93, 195.54. ES-MS *m/z* 270.1689 [M+H], $C_{14}H_{24}NO_4^+$ requires 270.1705. RP-HPLC R_T 5.55 min.

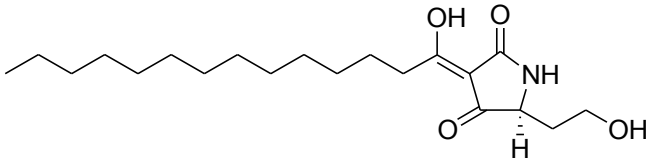
(*S*)-3-Undecanoyl-5-(2-hydroxyethyl)tetramic acid (5-HE-C11-TMA) 6



Use of *N*-(3-oxotridecanoyl)-L-homoserine lactone in **GP1** gave **6** as an off white solid (67%).

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28-1.40 (14H, m, (C*H*$_2$)$_7$Me), 1.68 (2H, m, C*H*$_2$(CH$_2$)$_7$Me), 1.82 & 2.12 (2H, 2 x m, C*H*$_2$CH$_2$OH), 2.86 (2H, m, C*H*$_2$(CH$_2$)$_8$Me), 3.87-3.99 (3H, m, ring CH & CH$_2$C*H*$_2$OH), 6.38 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 14.10, 22.67, 25.91, 29.26, 29.29, 29.43, 29.54, 31.87, 32.94, 34.11, 60.82, 61.76, 100.53, 175.14, 189.94, 195.54. ES-MS *m/z* 312.2167 [M+H], $C_{17}H_{30}NO_4^+$ requires 312.2175. RP-HPLC R_T 11.24 min.

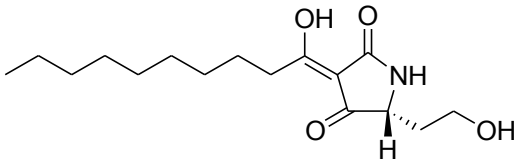
(*S*)-3-Tetradecanoyl-5-(2-hydroxyethyl)tetramic acid (5-HE-C14-TMA) 8



Use of *N*-(3-oxohexadecanoyl)-L-homoserine lactone in **GP1** gave **8** as a white solid (95%).

$[\alpha]^D$ = –9.1 (c = 1.70, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28-1.40 (20H, m, (C*H*$_2$)$_{10}$Me), 1.69 (2H, m, C*H*$_2$(CH$_2$)$_{10}$Me), 1.82 & 2.15 (2H, 2 x m, C*H*$_2$CH$_2$OH), 2.86 (2H, m, C*H*$_2$(CH$_2$)$_{11}$Me), 3.84-4.00 (3H, m, ring CH & CH$_2$C*H*$_2$OH), 6.34 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 14.11, 22.68, 25.91, 29.27, 29.35, 29.44, 29.59, 29.64, 29.66, 31.92, 32.94, 34.09, 60.89, 61.80, 100.52, 175.12, 189.91, 195.48. ES-MS *m/z* 354.2624 [M+H], $C_{20}H_{36}NO_4^+$ requires 354.2644. RP-HPLC R_T 17.17 min.

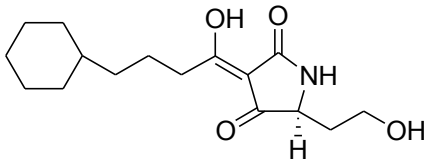
(*R*)-3-Decanoyl-5-(2-hydroxyethyl)tetramic acid (*R*-5-HE-C10-TMA) 9



Use of *N*-(3-oxododecanoyl)-D-homoserine lactone in **GP1** gave **9** as a cream/white solid (53%).

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28-1.41 (12H, m, (C*H*$_2$)$_6$Me), 1.68 (2H, m, C*H*$_2$(CH$_2$)$_6$Me), 1.81 & 2.14 (2H, 2 x m, C*H*$_2$CH$_2$OH), 2.87 (2H, m, C*H*$_2$(CH$_2$)$_7$Me), 3.84-4.01 (3H, m, ring CH & CH$_2$C*H*$_2$OH), 6.33 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 14.11, 22.66, 25.91, 29.25, 29.27, 29.33, 29.40, 31.85, 33.00, 34.11, 60.91, 61.81, 100.53, 175.13, 189.98, 195.51. ES-MS *m/z* 298.2025 [M+H], $C_{16}H_{28}NO_4^+$ requires 298.2018. RP-HPLC R_T 9.28 min.

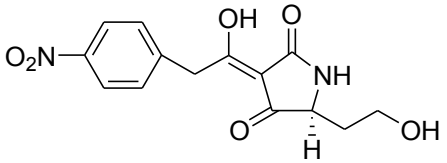
(*S*)-3-(4-Cyclohexylbutyryl)-5-(2-hydroxyethyl)tetramic acid (5-HE-CyC4-TMA) 10



Use of *N*-(6-cyclohexyl-3-oxohexanoyl)-L-homoserine lactone **S16** in **GP1** gave **10** as a cream/white solid (89%).

^{1}H NMR ($CDCl_3$) δ 0.88-1.72 (15H, m, CyHs & $Cy(CH_2)_2$), 1.82 & 2.15 (2H, 2 x m, CH_2CH_2OH), 2.85 (2H, m, $Cy(CH_2)_2CH_2$), 3.87-3.99 (3H, m, NHC*H* & CH_2OH), 6.29 (1H, br s, NH). ES-MS *m/z* 296.1891 [M+H], $C_{16}H_{26}NO_4^+$ requires 296.1862. RP-HPLC R_T 7.19 min.

(*S*)-5-(2-Hydroxyethyl)-3-(*p*-nitrophenylacetyl)tetramic acid (5-HE-pNPC2-TMA) 11

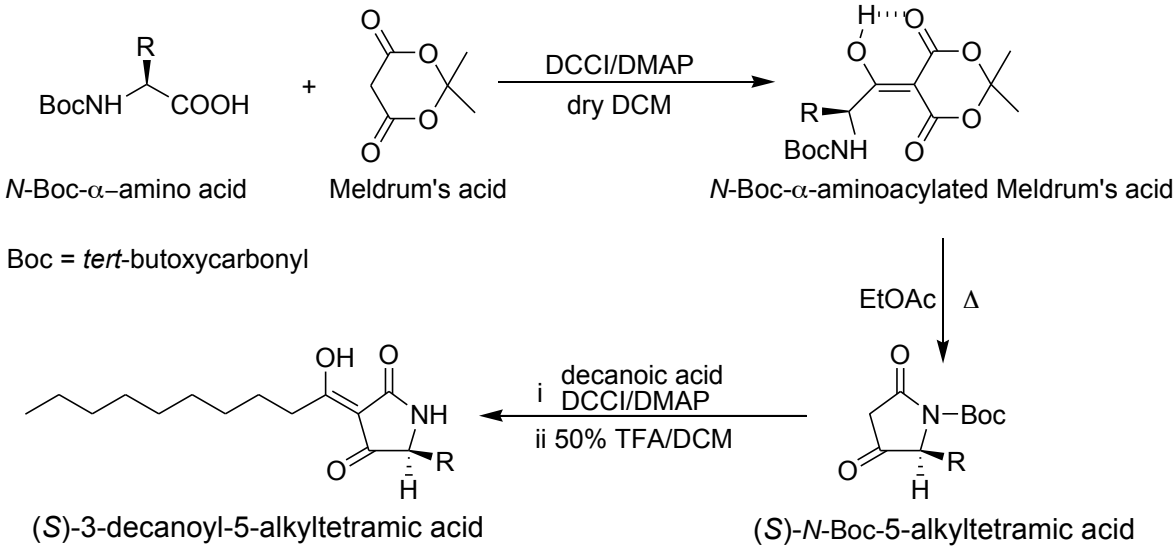


p-Nitrophenylacetyl Meldrum's acid was prepared as a yellow oil in 86.6% yield by a procedure similar to that described for acylated Meldrum's acid by Chhabra *et al*.[1] and condensed with L-homoserine lactone HCl as described for **S14** to furnish *N*-[4-(*p*-nitrophenyl)-3-oxobutyryl]-L-homoserine lactone as a pale yellow solid in 18% yield.
^{1}H NMR ($CDCl_3$) δ 2.26 (1H, m, 4α-H), 2.80 (1H, m, 4β-H), 3.60 (2H, s, $COCH_2CO$) 4.01 (2H, s, CH_2CO), 4.30 (1H, m, 5α-H), 4.48-4.62 (2H, m, NHC*H* & 5β-H), 7.25 (1H, br s, NH), 7.41 (2H, ABq, Ar 2H & 6H), 8.23 (2H, ABq, Ar 3H & 5H).

Use of *N*-[4-(*p*-nitrophenyl)-3-oxobutyryl]-L-homoserine lactone in **GP1** gave **11** as a cream solid (95%).

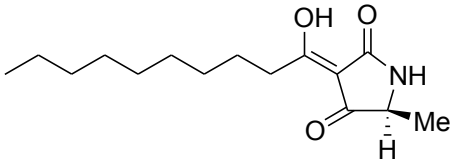
^{1}H NMR ($CDCl_3$) δ 1.23 & 1.57 (2H, m, CH_2CH_2OH), 3.22 (2H, m, CH_2CH_2OH), 3.6 (1H, br s, ring CH), 3.73 (2H, s, CH_2COH), 7.08 (2H, ABq, Ar 2H & 6H), 7.67 (2H, ABq, Ar 3H & 5H), 8.28 (1H, br s, NH). ^{13}C NMR (DMSO-d_6) δ 35.01, 57.45, 63.56, 100.81, 123.84, 124.02, 130.85, 131.09, 143.64, 147.03, 175.25, 185.81, 195.40. ES-MS *m/z* 307.0933 [M+H], $C_{14}H_{15}N_2O_6^+$ requires 307.0930. RP-HPLC R_T 1.53 min.

The 3-acyl-5-alkyltetramic acids **12** and **13** were prepared as shown in the Scheme 2.[2]



Scheme 2: Synthesis of (*S*)-3-decanoyl-5-alkyltetramic acids

(*S*)-3-Decanoyl-5-methyltetramic acid (5-Me-C10-TMA) 12



5-(N-tert-Butoxycarbonylalanyl) Meldrum's acid

N-tert-Butoxycarbonyl-L-alanine *(*Boc-Ala-OH) (0.378 g, 2 mmol), dry DCM (20 ml), DMAP (0.366 g, 3 mmol), and Meldrum's Acid (0.302 g, 2.1 mmol) were stirred at room temperature under nitrogen. DCCI (0.473 g, 2.3 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was filtered to remove the precipitated DCU and the filtrate was washed with 1 M $KHSO_4$ (2 x 15 ml). The organic layer was dried over magnesium sulphate and concentrated to dryness. The resulting yellow oily solid was suspended in cold acetone (2 ml) and filtered to remove the insoluble solid. The filtrate was concentrated to dryness to give 5-(*N*-tert-butoxycarbonylalanyl) Meldrum's acid as a yellow oil (0.50 g, 79%).

^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s, CMe_3), 1.76, (6H, s, CMe_2), 2.20 (3H, d, $HCCH_3$) 5.10 (1H, br s, NH), 5.57 (1H, m, $HCCH_3$), 15.56 (1H, s, enolic OH).

N-Boc-5-methyltetramic acid

A solution of 5-(*N-tert*-butoxycarbonylalanyl) Meldrum's acid (0.490 g) in ethyl acetate (100 ml) was heated at reflux for 30 min. The solution was cooled to room temperature, washed with 1 M $KHSO_4$ (2 x 10 ml) and brine (10 ml) before being dried over magnesium sulphate and concentrated to dryness to give *N*-Boc-5-methyltetramic acid as a pale yellow solid (0.280 g, 85%).

^{1}H NMR ($CDCl_3$) δ 1.54 (3H, d, ring Me), 1.59 (9H, s, CMe_3), 3.26 (2H, m, ring CH_2), 4.45 (1H, q, ring CH).

5-N-tert-Butoxycarbonyl-3-decanoyl-5-methyltetramic acid

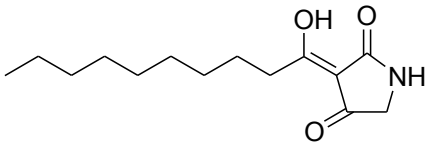
A solution of *N*-Boc-5-methyltetramic acid (0.173 g, 0.811 mmol), DMAP (0.148 g, 1.217 mmol), and decanoic acid (0.139 g, 0.811 mmol) in dry DCM (20 ml) was stirred at room temperature under nitrogen. Triethylamine (0.164 g, 1.62 mmol) and DCCI (0.192 g, 0.933 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was filtered to remove insoluble DCU and the filtrate was washed with 1 M $KHSO_4$ (2 x 15 ml). The organic layer was dried over magnesium sulphate and concentrated to dryness. The resulting yellow oily solid was suspended in cold acetone (1 ml) and filtered to remove the remaining insoluble DCU. The filtrate was concentrated to dryness to give 5-*N*-tert-butoxycarbonyl-3-decanoyl-5-methyltetramic acid as a yellow oil (0.228 g, 77%).

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28-1.41 (12H, m, (CH_2)$_6$Me), 1.52 (3H, m, ring Me), 1.58 (9H, s, CMe_3), 1.69 (2H, m, $CH_2(CH_2)_6Me$), 2.93 (2H, m, $CH_2(CH_2)_7Me$), 4.21 & 4.46 (1H, dq, ring CH).

A solution of *N-tert*-butoxycarbonyl-3-decanoyl-5-methyltetramic acid (0.114 g) in DCM (5 ml) and TFA (5 ml) was stirred at room temperature for 2 h. The solvent was removed under reduced pressure with the aid of acetonitrile to give an impure beige solid (0.095 g) which was purified by trituration with ether and hexane to give the title (*S*)-3-decanoyl-5-methyltetramic acid **12** as a cream solid (0.015 g, 18%).

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28-1.43 (15H, m, ring Me & (CH_2)$_6$Me), 1.71 (2H, m, $CH_2(CH_2)_6Me$), 2.85 (2H, m, $CH_2(CH_2)_7Me$), 3.91 & 4.08 (1H, dq, ring CH), 5.76 (1H, s, NH). ES-MS *m/z* 266.1785 [M-H], $C_{15}H_{24}NO_3^-$ requires 266.1756. RP-HPLC R_T 12.25 min.

3-Decanoylltetramic acid (C10-TMA) 13



5-(N-tert-Butoxycarbonyglycyl) Meldrum's acid

A solution of *N-tert*-butoxycarbonylglycine (Boc-Gly-OH) (0.35 g, 2 mmol), DMAP (0.366 g, 3 mmol), and Meldrum's acid (0.302 g, 2.1 mmol) in dry DCM (20 ml) was stirred at room temperature under nitrogen. DCCI (0.473 g, 2.3 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was filtered to remove insoluble DCU and the filtrate was washed with 1 M $KHSO_4$ (2 x 15 ml). The organic layer was dried over magnesium sulphate and concentrated to dryness. The resulting yellow oily solid was purified by dissolving in cold acetone (2 ml) and filtered to remove any remaining insoluble DCU. The filtrate was concentrated to dryness to give 5-(*N*-tert-butoxycarbonyglycyl) Meldrum's acid *as* yellow oil (0.463 g, 78%).

^{1}H NMR ($CDCl_3$) δ 1.39 (9H, s, CMe_3), 1.77 (6H, s, CMe_2), 4.69 (2H, d, NHCH_2), 5.14 (1H, br s, NH), 15.69 (1H, br s, enolic OH).

N-Boc-tetramic acid

A solution of 5-(*N-tert*-butoxycarbonyglycyl) Meldrum's acid (0.461 g) in ethyl acetate (100 ml) was heated at reflux for 30 min. The solution was cooled to room temperature, washed with 1 M $KHSO_4$ (2 x 10 ml) and brine (10 ml) before being dried over magnesium sulphate and concentrated to dryness to give *N*-Boc-tetramic acid as a white solid (0.285 g, 93%).

^{1}H NMR ($CDCl_3$) δ 1.58 (9H, s, CMe_3), 3.27 (2H, s, $COCH_2CO$), 4.27 (2H, s, $COCH_2N$).

N-tert-Butoxycarbonyl-3-decanoyltetramic acid

A solution of *N*-Boc-tetramic acid (0.280 g, 1.41 mmol), DMAP (0.256 g, 2.1 mmol), and decanoic acid (0.241 g, 1.41 mmol) in dry DCM (20 ml) was stirred at room temperature under nitrogen. Triethylamine (0.28 g, 2.8 mmol) and DCCI (0.33 g, 1.62 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was filtered to remove insoluble DCU and the filtrate was washed with 1 M $KHSO_4$ (2 x 15 ml). The organic layer was dried over magnesium sulphate and concentrated to dryness. The resulting yellow oily solid was suspended in cold acetone (1 ml) and filtered to remove any remaining insoluble DCU. The filtrate was concentrated to dryness to give an orange oil which was further purified using Flash chromatography (50% ethyl acetate in hexane) to give *N*-tert-butoxycarbonyl-3-decanoyltetramic acid as a glassy solid (0.138 g, 28%).

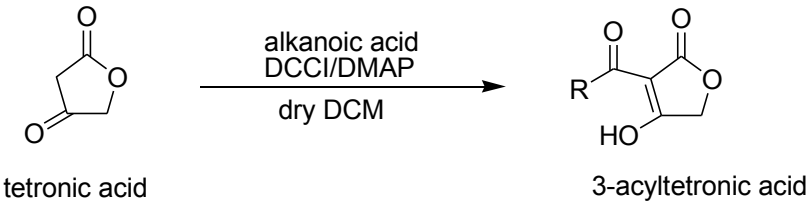
^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28-1.34 (12H, m, (CH_2)$_6$Me), 1.58 (9H, s, CMe_3), 1.69 (2H, m, CH_2(CH_2)$_6$Me), 2.94 (2H, m, CH_2(CH_2)$_7$Me), 4.08 & 4.26 (2H, 2 x s, ring CH_2).

A solution of *N*-tert-butoxycarbonyl-3-decanoyltetramic acid (0.135 g) in DCM (5 ml), water (0.1 ml) and TFA (5 ml) were stirred at room temperature for 2 h. The solvent was removed under reduced pressure with the aid of acetonitrile to give a brown oil (0.145 g), which was purified by trituration with ether and hexane to give the title 3-decanoylltetramic acid **13** as a cream solid (0.073 g, 75%).

^{1}H NMR (DMSO d_6) δ 0.86 (3H, t, Me), 1.24 (12H, m, (CH_2)$_6$Me), 1.52 (2H, m, CH_2(CH_2)$_6$Me), 2.73 (2H, m, CH_2(CH_2)$_7$Me), 3.65 (3H, m, ring CH_2 and NH masked by water peak). ^{13}C NMR (DMSO-d_6) δ 14.42, 22.57, 25.63, 29.12, 29.18, 29.39, 31.76, 34.13 101.88, 158.17, 174.95, 193.74. ES-MS *m/z* 252.1592 [M-H], $C_{14}H_{22}NO_3^-$ requires 252.1600.

Chemical synthesis and characterization of 3-acyltetronic acid (TOA) analogues

The 3-acyltetronic acids were prepared by the General Procedure 2 (**GP2**) as shown in **Scheme 3** by acylation of the commercially available tetronic acid [4-hydroxy-2(5*H*)-furanone] essentially by the procedure described by Nomura et al.[3]

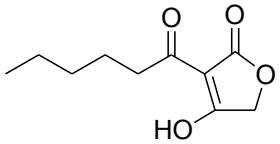


Scheme 3: Synthesis of 3-acyltetronic acids

General Procedure 2 (GP2)

N,*N*'-Dicyclohexylcarbodiimide (DCCI) (2.3 mmol) was added to a stirred solution of alkanoic acid (2 mmol) and 4-(dimethylamino)pyridine (DMAP) (3 mmol) in dry dichloromethane (20 ml) at room temperature under nitrogen. Tetronic acid (2.1 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was filtered to remove insoluble *N*,*N*'-dicyclohexylurea (DCU) and the filtrate was extracted with 1 M HCl (2 x 10 ml). The organic layer was dried over magnesium sulphate and concentrated to dryness to give 3-acyltetronic acids as solids.

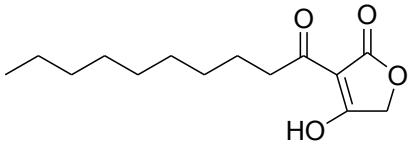
3-Hexanoyltetronic acid (C6-TOA) 14



Use of hexanoic acid in **GP2** gave **14** as a yellow solid in 80% yield.

^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t, Me), 1.35-1.44 (4H, m, (CH_2)$_2$Me), 1.74 (2H, m, CH_2(CH$_2$)$_2$Me), 2.95 (2H, m, CH_2(CH$_2$)$_3$Me), 4.58 & 4.70 (2H, 2 x s, ring CH$_2$). ^{13}C NMR ($CDCl_3$) δ 13.81, 22.28, 31.28, 32.79, 49.18, 68.81, 100.02, 168.16, 192.36, 197.78. ES-MS *m/z* 197.0816 [M-H], $C_{10}H_{13}O_4^-$ requires 197.0814. RP-HPLC R_T 0.99 min.

3-Decanoyltetronic acid (C10-TOA) 15

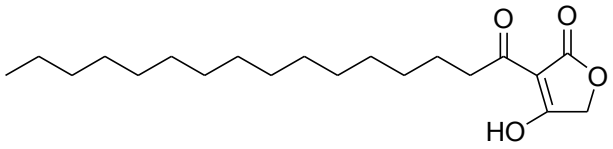


Use of decanoic acid in **GP2** gave **15** as a cream solid, after trituration with ether, in 31% yield.

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.29-1.41 (12H, m, (CH_2)$_6$Me), 1.74 (2H, m, CH_2(CH$_2$)$_6$Me), 2.95 (2H, m, CH_2(CH$_2$)$_7$Me), 4.58 & 4.70 (2H, 2 x s, ring CH$_2$). ^{13}C NMR

($CDCl_3$) δ 14.10, 22.65, 25.60, 29.19, 29.22, 29.34, 29.42, 31.84, 49.25, 68.83, 100.08, 168.34, 192.51, 197.74. ES-MS *m/z* 253.1493 [M-H], $C_{14}H_{21}O_4^-$ requires 253.1440.

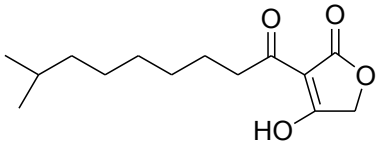
3-Hexadecanoyltetronic acid (C16-TOA) 18



Use of hexadecanoic acid in **GP2** gave **18** as a grey/white solid in 73% yield.

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.28-1.42 (24H, m, (C*H*$_2$)$_{12}$Me), 1.72 (2H, m, C*H*$_2$(CH$_2$)$_{12}$Me), 2.95 (2H, m, C*H*$_2$(CH$_2$)$_{13}$Me), 4.58 & 4.70 (2H, 2 x s, ring CH$_2$). ^{13}C NMR ($CDCl_3$) δ 14.12, 22.69, 24.92, 25.60, 29.20, 29.22, 29.28, 29.36, 29.39, 29.57, 29.63, 29.66, 29.68, 29.70, 31.93, 40.14, 68.84, 176.65, 192.40, 198.12. ES-MS *m/z* 337.2367 [M-H], $C_{20}H_{33}O_4^-$ requires 337.2379.

3-(8-Methylnonoyl)tetronic acid [(8Me-C9)-TOA)] S17



8-Methyl-7-oxononanoic acid[4]
A solution of isobutyryl chloride (5.25 g, 0.0492 mol) in dry chloroform (10 ml) was added to a mixture of 1-morpholinocyclohexene (7.9 g, 0.0472 mol), triethylamine (5.16 g, 0.606 mol) in chloroform (35 ml) over 30 min at room temperature. The slurry formed was stirred overnight at room temperature. 20% Aqueous HCl (25 ml) was added and the mixture was heated at reflux for 5 h. The solution was cooled and the aqueous layer was removed and the pH was checked to ensure it remained acidic. The organic layer was washed with saturated $NaHCO_3$ (2 x 20 ml) and brine (20 ml), dried over magnesium sulphate and concentrated to dryness to give the crude diketone, 2-isobutyrylcyclohexanone as an orange oil (6.791 g).

The crude diketone was refluxed with a solution of NaOH (6.0 g, 0.15 mol) in water (10 ml) for 1 h. The mixture was cooled and poured into ice water (50 ml) and acidified with conc. HCl (14 ml) to pH 1-3. The aqueous solution was extracted with DCM (2 x 25 ml) and the combined extracts were washed with brine (25 ml), dried over magnesium sulphate and concentrated to dryness to give crude product as a brown oil (4.876 g). This was purified by high vacuum (1 to 2 mbar) distillation to give pure 8-methyl-7-oxononanoic acid as a yellow oily solid (3.1 g).

^{1}H NMR ($CDCl_3$) δ 1.11 (6H, d, Me$_2$), 1.38 (2H, m, (CH$_2$)$_2$C*H*$_2$(CH$_2$)$_2$), 1.65 (4H, m, CH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$), 2.38 (2H, t, C*H*$_2$COCHMe$_2$), 2.47 (2H, t, C*H*$_2$CO$_2$H), 2.61 (1H, m, CHMe$_2$)

8-Methylnonanoic acid

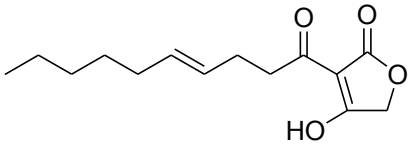
A solution of 8-methyl-7-oxononanoic acid (4.5 g, 0.0242 mol), hydrazine monohydrate (7.71 ml) and potassium hydroxide pellets (1.59 g, 0.0242 mol) in diethylene glycol (23 ml) were heated at reflux for 7 h. Another lot of potassium hydroxide pellets (7.98 g, 0.121 mol) and diethylene glycol (23 ml) were added to the mixture and further heated at reflux overnight. The mixture was cooled to room temperature and poured into ice water (400 ml) and acidified to pH 1-2 with conc. HCl (25 ml). The resulting white solid was collected by filtration and washed with water (4 x 10 ml) to furnish 8-methylnonanoic acid (3.14 g, 75%).

^{1}H NMR ($CDCl_3$) δ 0.89 (6H, d, Me_2), 1.18 (2H, m, CH_2CHMe_2), 1.28-1.37 (6H, m, CO$(CH_2)_2$(CH_2)$_3$, 1.54 (1H, m, C$H$$Me_2$), 1.66 (2H, quintet, $COCH_2CH_2$), 2.37 (2H, t, $COCH_2$).

The use of 8-methylnonanoic acid in the **GP2** gave **S17** as a grey/white solid in 66% yield.

^{1}H NMR ($CDCl_3$) δ 0.89 (6H, d, Me_2), 1.33 (2H, m, CH_2CH Me_2), 1.38-1.76 (9H, m, C$H$$CH_2$(C$H_2$)$_4$, 2.94 (2H, t, $COCH_2$), 4.54 & 4.68 (2H, 2 x br s, ring CH_2). ^{13}C NMR ($CDCl_3$) δ 22.62, 27.12, 27.18, 27.93, 27.94, 29.11, 29.22, 29.47, 38.90, 69.14, 177.99, 192.36, 197.78. ES-MS *m/z* 253.1462 [M-H], $C_{14}H_{21}O_4^-$ requires 253.1440. RP-HPLC R_T 6.28 min.

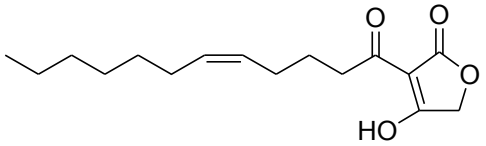
3-[(*E*)-4-Decenoyl]tetronic acid (Δ^{4E}-C10-TOA) S18



The use of *trans*-4-decenoic acid (described as for **S14**) in **GP2** gave **S18** as a pale yellow solid in 65% yield.

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.22-1.38 (6H, m, C$H_2$$CH_2$CO & (C$H_2$)$_2$Me), 1.99 (2H, m, C$H_2$$(CH_2)_2$CO), 2.43 (2H, m, C$H_2$$(CH_2)_2$Me), 3.02 (2H, t, CH_2CO), 4.58 & 4.70 (2H, 2 x s, ring CH_2), 5.44 (1H, m, C$H$$(CH_2)_2$CO), 5.52 (1H, m, C$HCH(CH_2)_2$CO). ^{13}C NMR ($CDCl_3$) δ 14.37, 22.41, 27.31, 29.05, 31.23, 32.33, 33.80, 67.92, 99.66, 129.30, 131.03, 171.47, 189.14, 193.10. ES-MS *m/z* 251.1286 [M-H], $C_{14}H_{19}O_4^-$ requires 251.1283. RP-HPLC R_T 4.58 min.

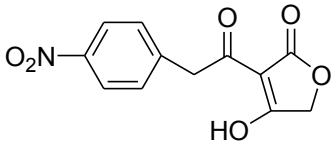
3-[(*Z*)-5-Dodecenoyl]tetronic acid (Δ^{5Z}-C12-TOA) S19



The use of *cis*-5-dodecenoic acid in **GP2** gave **S19** as a cream solid in 76% yield.

^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, Me), 1.30-1.39 (8H, m, (C*H*$_2$)$_4$Me), 1.79 (2H, m, C*H*$_2$CH$_2$CO), 2.04 (2H, m, C*H*$_2$(CH$_2$)$_2$CO), 2.17 (2H, m, C*H*$_2$(CH$_2$)$_4$Me), 2.97 (2H, m, CH$_2$CO), 4.58 & 4.70 (2H, 2 x s, ring CH$_2$), 5.35 (1H, m, C*H*(CH$_2$)$_3$CO), 5.44 (1H, m, C*H*CH(CH$_2$)$_3$CO).
^{13}C NMR ($CDCl_3$) δ 14.10, 22.64, 24.90, 27.24, 27.29, 29.61, 29.66, 31.75, 49.30, 68.16, 100.23, 127.79, 130.89, 177.26, 192.7, 197.70. ES-MS *m/z* 279.1618[M-H], $C_{16}H_{23}O_4^-$ requires 279.1596. RP-HPLC R_T 8.42 min.

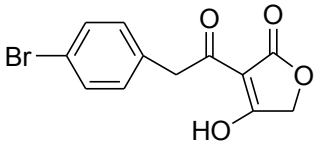
3-(*p*-Nitrophenyl)acetyltetronic acid (pNPC2-TOA) S20



Use of 4-nitrophenylacetic acid in **GP2** gave **S20** as a white solid, after trituration with acetone, in 44% yield.

^{1}H NMR ($CDCl_3$) δ 4.35 (2H, s, CH$_2$CO), 4.65 & 4.78 (2H, 2 x s, ring CH$_2$), 7.58 (2H, ABq, Ar 2H & 6H), 8.23 (2H, ABq, Ar 3H & 5H). ^{13}C NMR (DMSO-d_6) δ 46.40, 68.00, 98.56, 123.56, 131.40, 144.81, 146.59, 172.34, 189.42, 189.97. ES-MS *m/z* 262.0333 [M-H], $C_{12}H_8NO_6^-$ requires 262.0352.

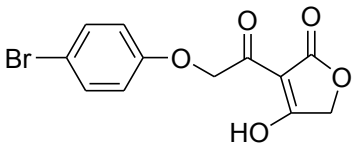
3-(*p*-Bromophenyl)acetyltetronic acid (pBrPC2-TOA) S21



Use of 4-bromophenylacetic acid in **GP2** gave **S21** as an off white solid in 81% yield.

^{1}H NMR ($CDCl_3$) δ 4.18 (2H, s, COCH$_2$), 4.72 (2H, s, ring CH$_2$), 7.26 (2H, ABq, Ar 2H & 6H), 7.50 (2H, ABq, Ar 3H & 5H). ^{13}C NMR (DMSO-d_6) δ 45.57, 67.95, 98.81, 119.93, 131.40, 131.52, 132.18, 132.31, 135.69, 172.14, 189.76, 190.32. ES-MS *m/z* 294.9580 & 296.9546 [M-H], $C_{12}H_8BrO_4^-$ requires 294.9606 & 296.9585. RP-HPLC R_T 0.98 min.

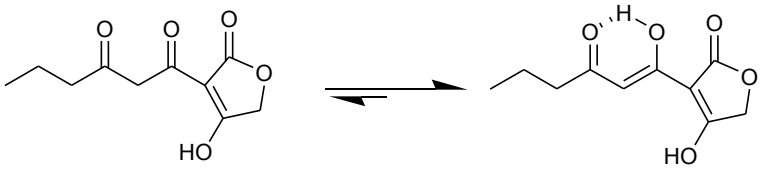
3-(*p*-Bromophenoxyacetyl)tetronic acid (pBrPOC2-TOA) S22



Use of 4-bromophenoxyacetic acid in **GP2** gave **S22** as an off white solid in 68% yield.

^{1}H NMR ($CDCl_3$) δ 4.86 (2H, s, ring CH_2), 5.24 (2H, s, OCH_2), 6.86 (2H, ABq, Ar 2H & 6H), 7.43 (2H, ABq, Ar 3H & 5H). ^{13}C NMR ($CDCl_3$) δ 68.63, 71.53, 96.74, 112.14, 117.19, 132.37, 158.20, 190.57. ES-MS *m/z* 310.9520 & 312.9538 [M-H], $C_{12}H_8BrO_5^-$ requires 310.9555 & 312.9535.

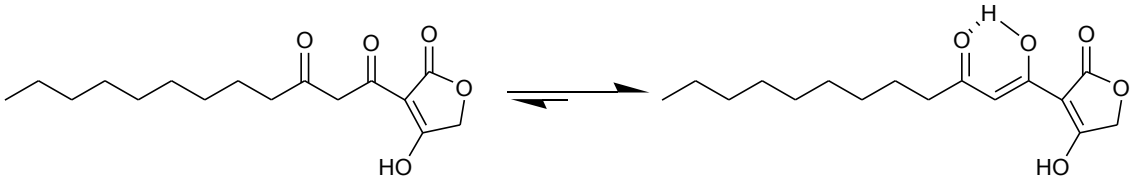
3-(3-Oxohexanoyl)tetronic acid (3O-C6-TOA) S23



Use of 3-oxohexanoic acid[5] in **GP2** gave **S23** as a cream solid, after trituration with ether, in 10% yield.

^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t, Me), 1.71 (2H, m, C*H*$_2$Me), 2.36 & 2.61 (2H, m, C*H*$_2$CH$_2$Me), 4.04 & 14.36 (1H, 2 x s, COC*H*COH), 4.68 & 4.77 (2H, 2 x s, ring CH_2), 6.36 (1H, s, ring OH) ES-MS *m/z* 211.0592 [M-H], $C_{10}H_{11}O_5^-$ requires 211.0606.

3-(3-Oxododecanoyl)tetronic acid (3O-C12-TOA) S24



Use of 3-oxododecanoic acid (described as for **S13**) in **GP2** gave **S24** as a yellow solid in 27% yield.

^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, Me), 1.28-1.31 (12H, m, (C*H*$_2$)$_6$Me), 1.72 (2H, m, C*H*$_2$(CH$_2$)$_6$Me), 2.37 & 2.62 (2H, 2 x m, C*H*$_2$(CH$_2$)$_7$Me), 4.03 & 14.37 (1H, 2 x s, COC*H*COH), 4.72 & 4.77 (2H, 2 x s, ring CH_2), 6.35 (1H, s, ring OH). ES-MS *m/z* 295.1568 [M-H], $C_{16}H_{23}O_5^-$ requires 295.1545. RP-HPLC R_T 4.83 min.

Additional experimental methods

Production of α-hemolysin. *S. aureus* strain USA300 was grown in brain heart infusion broth at 37 ºC for 6 h in the absence or presence of a range of concentrations of 5-HE-C8-TMA **4** (or C14-TOA **17)**, the bacteria removed and the cell free culture supernatant precipitated with trichloroacetic acid. The protein pellet obtained was subjected to SDS-PAGE and immunoblotting as described previously.[6] α-Hemolysin was detected by incubating with a monoclonal antibody followed by a protein A–horse radish peroxidase conjugate (Sigm-Aldrich). Western blots were developed with an ECL chemiluminescence kit (GE Healthcare).

Chrome Azurol S (CAS) iron chelation assay. A 0.5 mL aliquot of PBS (pH 7.4) without (as a reference) or with the relevant compound (50 μM) was mixed with 0.5 ml of CAS assay solution prepared according to Schwyn and Neilands.[7] The sample (s) and reference (r) absorbances were determined at 630 nm after 15 min incubation at room temperature. The percentage of iron-chelating activity was calculated by subtracting the sample A_{630} from that of the reference A_{630} value.

Siderophore units are defined as $[A_{(r)} - A_{(s)} / A_{(r)}] \times 100$.

Membrane dipole potential. *S. aureus* membranes were prepared and labelled with the dipole potential fluorescent sensor di-8-ANEPPS (Invitrogen) and the K_d estimated for each compound determined using the dual wavelength ratiometric method as described previously.[6,8]

Bacterial adherence to desqamated nasal epithelial cells. Cells were harvested from the anterior nares of healthy donors by vigorous swabbing of the moist squamous epithelium on the inside of the nasal septum.[9] Swabs from both nostrils were taken and agitated in 3 ml of PBS to suspend the cells which were washed in sterile PBS and the numbers adjusted to 5 x 10^4 cells/mL in PBS and incubated with *S. aureus* at 37 ºC for 1 h with or without test compound, fixed, stained and the numbers of attached bacteria counted as described by O'Brien *et al.*[9]

Septic arthritis model. The *S. aureus* murine arthritis infection model described in detail by Shaw *et al*[10] and by Gjertsson *et al*[11] was carried out in the experimental animal facility at the University of Gothenburg, Sweden according to local ethical and husbandry standards.
Female NMRI mice (n = 30) were inoculated into the tail vein with 200 μL of bacterial suspension (~ 0.6–1 x 10^7 bacteria per mouse; *S. aureus* strain Newman) in phosphate buffered saline (PBS; pH 7.4) containing 5% w/v BSA and 10% v/v DMSO. Mice were dosed daily over 14 days with either C14-TOA **17** (10 mg/kg body weight) or PBS as a control. During the course of the experiments, mice were weighed and examined for general appearance, weight change, mortality, and the development of arthritis, before being sacrificed. Joints were examined histopathologically and the numbers of bacteria present in the kidneys were determined by viable counts as total bacteria/cell. Clinical arthritis, defined by visible erythema and/or swelling of at least one joint, was scored from 0 to 3 for each limb (1, mild swelling and/or erythema; 2, moderate swelling and erythema; 3, marked swelling and erythema). An arthritic index was generated by adding the scores for each limb of a given animal.

References

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