Supporting Information for

Immunomodulation and the quorum sensing molecule 3-oxo-C_{12}-homoserine lactone: The importance of chemical scaffolding for probe development

Amanda L. Garner, Jing Yu, Anjali K. Struss, Gunnar F. Kaufmann, Vladimir V. Kravchenko and Kim D. Janda

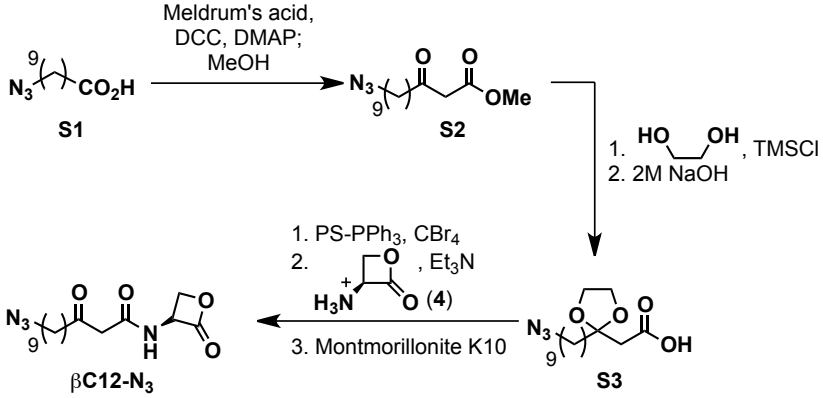
A. General Methods and Materials

General chemistry methods: Reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride. Yields refer to chromatographically and spectroscopically homogenous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25-mm EMD silica gel plates (60F-254) using permanganate or ninhydrin staining. Flash chromatography separations were performed on Silicycle silica gel (40-63 mesh). NMR spectra were recorded on Bruker 400 MHz spectrometer instruments and calibrated using a solvent peak as an internal reference. The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

General biology methods: All mammalian cell experiments were performed as previously described.[1] Reporter assay experiments using *P. aeruginosa* strain PAO-JP2 were performed as previously described with minor changes as described below.[2,3] All luminescence and absorbance readings were measured on a SpectraMax M2e Microplate Reader (Molecular Devices).

Materials: L-Serine-β-lactone tetrafluoroborate salt was purchased from Sigma Aldrich and used as received. Azidodecanoic acid was prepared according to literature precedent.[4,5]

B. Synthetic Procedures and Characterization Data



General synthetic protocol: A round-bottom flask was charged with carboxylic acid (decanoic acid **1** or azidodecanoic acid **S1**) (7.0 mmol, 1.0 equiv) and CH_2Cl_2 (50 mL) at 25 °C. Meldrum's acid (7.0 mmol, 1.0 equiv), DCC (7.7 mmol, 1.1 equiv) and DMAP (7.7 mmol, 1.1 equiv) were then added, and the solution was stirred at 25 °C overnight. The insoluble DCC by-product was then filtered off and the remaining solution was concentrated. The crude extract was then

dissolved in MeOH (50 mL) and refluxed overnight. Following concentration *in vacuo*, the crude material was purified via flash column chromatography (1:9 EtOAc in hexanes) to yield the coupled methyl ester (**2** or **S2**) (85–93% yield). The 3-oxo-substituent was then protected with ethylene glycol under standard conditions. The resulting crude protected methyl ester was hydrolyzed in 2N NaOH in MeOH. Extraction with EtOAc and concentration *in vacuo* yielded the protected acid as a white solid (**3** or **S3**) (60% yield). A fritted polypropylene tube was charged with protected acid (**3** or **S3**) (0.116 mmol, 1.0 equiv), polystyrene-supported PPh_3 (3 mmol/g, 0.255 mmol, 2.2 equiv), CBr_4 (0.127 mmol, 1.1 equiv) and CH_2Cl_2 (10 mL) at 25 °C. After shaking overnight at 25 °C, the resin was filtered off. The filtrate was concentrated *in vacuo*, and fresh CH_2Cl_2 (10 mL) was added followed by **4** (0.116 mmol, 1.0 equiv) and Et_3N (0.174 mmol, 1.5 equiv). The mixture was stirred overnight at 25 °C and the crude residue was purified by flash column chromatography (1:1 EtOAc in hexanes). Deprotection was afforded by stirring with Montmorillonite K10 (350 mg). **βC12** and **βC12-N_3** were obtained via preparative TLC (2:1 EtOAc in hexanes).

Data for βC12: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.09 (br s, 1H), 5.21 (ddd, *J* = 8.0 Hz, 4.0 Hz, 1.2 Hz, 1H), 4.47–4.49 (m, 2H), 3.51 (s, 2H), 2.54 (t, *J* = 4.0 Hz, 2H), 1.58–1.63 (m, 4H), 1.26–1.31 (m, 11H), 0.89 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 207.4, 168.3, 166.5, 65.9, 58.5, 47.5, 44.3, 32.0, 29.6, 29.5, 29.4, 29.2, 23.6, 22.9, 24.3; HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{26}NO_4$ $[M+H]^+$ 284.3633, found 284.2819.

Data for βC12-N_3: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.05 (br s, 1H), 5.20 (ddd, *J* = 7.2 Hz, 6.0 Hz, 1.2 Hz, 1H), 4.45–4.47 (m, 2H), 3.49 (s, 2H), 2.52 (t, *J* = 7.2 Hz, 2H), 1.55–1.61 (m, 7H), 1.25–1.38 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 207.0, 168.2, 166.3, 65.7, 58.3, 51.5, 47.3, 44.0, 29.2, 28.9, 28.8, 26.7, 23.3, 21.1, 14.2; HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{25}N_4O_4$ $[M+H]^+$ 325.3758, found 325.4001.

C. Reporter Assay for Autoinducer Activity in *Pseudomonas aeruginosa*

PAO-JP2 (*lasI/rhlI*-deleted) strain, harboring plasmid pKD201 containing a *LasI* reporter coupled to the *luxCDABE* luminescence system, was incubated overnight in LB medium containing 300 μg/mL trimethprim. 500 μL of overnight culture was diluted in 50 mL fresh LB/trimethoprim. The diluted culture was then allowed to grow at 37 °C (250 rpm) until the culture reached an optical density (OD_{600}) of 0.4–0.6. Aliquots (99 μL) of this culture were then plated into black, clear-bottom 96-well assay plates, and treated with compounds (1.0 μL of 100 μM DMSO stock, 1.0 μM final concentration). DMSO (1.0 μL) was used as a negative control. All samples were examined in triplicate. The plates were then incubated for 4 h at 37 °C (150 rpm) after which both luminescence and absorbance were measured. Relative luminescence units were normalized with respect to cell viability ([luminescence read-out]/[absorbance read-out]).

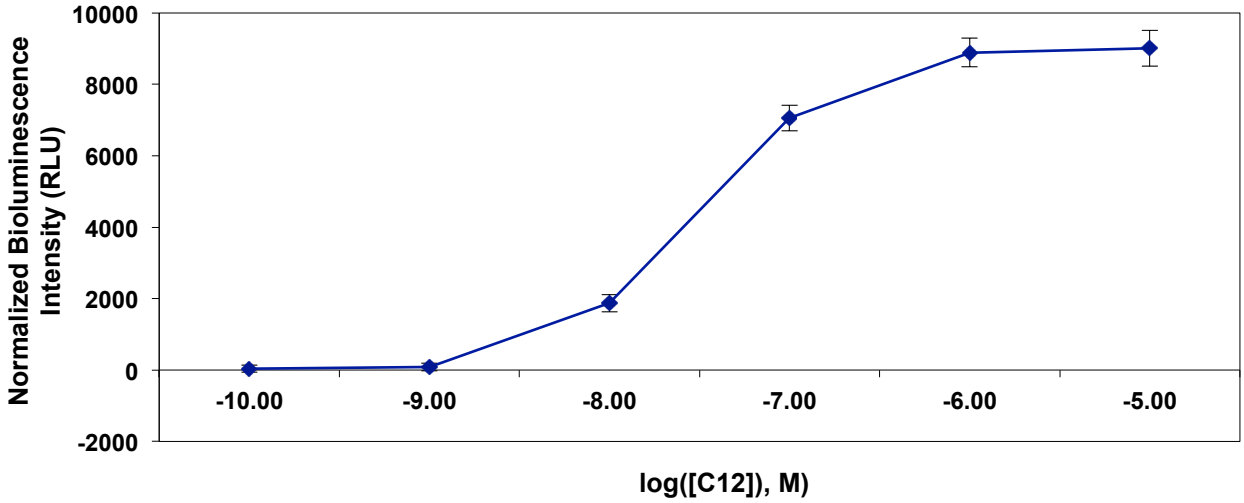


Figure S1. Dose-dependent autoinducer activity of **C12**.

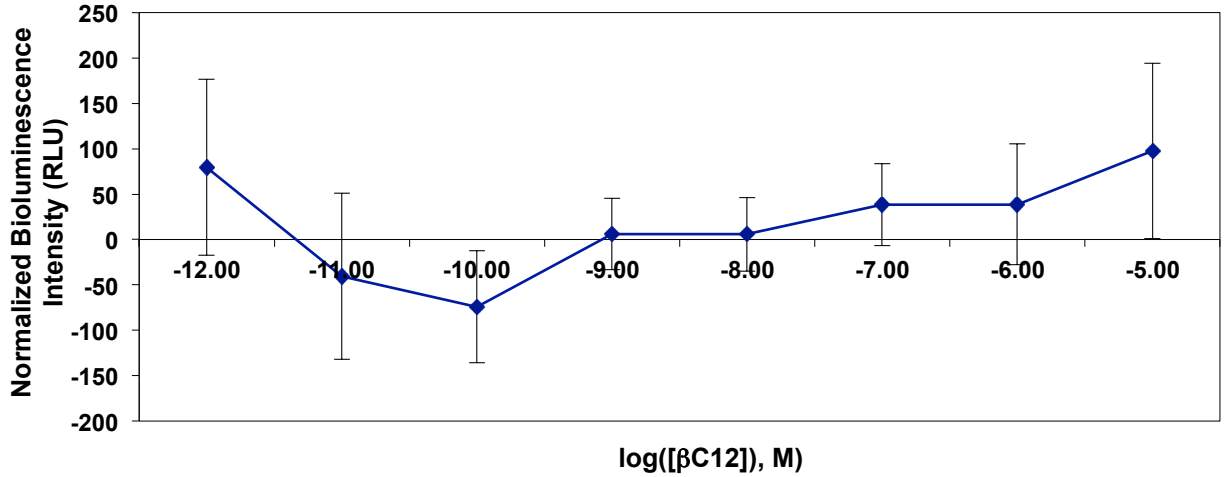


Figure S2. Dose-dependent autoinducer activity of **βC12**.

D. References

1. Kravchenko, V. V.; Kaufmann, G. F.; Mathison, J. C.; Scott, D. A.; Katz, A. Z.; Wood, M. R.; Brogan, A. P.; Lehmann, M.; Mee, J. M.; Iwata, K.; Pan, Q.; Fearns, C.; Knaus, U. G.; Meijler, M. M.; Janda, K. D.; Ulevitch, R. J. *J. Biol. Chem.* **2006**, *281*, 28822.
2. Duan, K.; Surette, M. G. *J. Bacteriol.* **2007**, *189*, 4827.
3. Amara, N.; Mashiach, R.; Amar, D.; Krief, P.; Spieser, S. A. H.; Bottomely, M. J.; Aharoni, A.; Meijler, M. M. *J. Am. Chem. Soc.* **2009**, *131*, 10610.
4. Nagarajan, S. R.; Devadas, B.; Zupec, M. E.; Freeman, S. K.; Brown, D. L.; Lu, H.-F.; Mehta, P. P.; Kishoe, N. S.; McWherter, C. A.; Getman, D. P.; Gordon, J. I.; Sikorski, J. A. *J. Med. Chem.* **1997**, *40*, 1422.
5. Garner, A. L.; Yu, J.; Struss, A. K.; Lowery, C. A.; Zhu, J.; Kim, S. K.; Park, J.; Mayorov, A. V.; Kaufmann, G. F.; Kravchenko, V. V.; Janda, K. D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2702.