Supporting Information

A novel class of defensive compounds in harvestmen: hydroxy-γ-lactones from the phalangiid *Egaenus convexus*

Günther Raspotnig, Felix Anderl, Olaf Kunert, Miriam Schaider, Adrian Brückner, Mario Schubert, Stefan Dötterl, Roman Fuchs, and Hans-Jörg Leis

1Institute of Biology, University of Graz, 8010 Graz, Austria

2Research Unit of Osteology and Analytical Mass Spectrometry, Medical University, University Children’s Hospital, 8036 Graz, Austria

3Institute of Pharmaceutical Sciences, University of Graz, Austria

4Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, United States of America

5Department of Biosciences, University of Salzburg, 5020 Salzburg, Austria

Content:

1) Synthesis of reference materials: page 3

2) Supporting Figures (NMR-spectra of reference materials): page 13

Figure S1. $^1$H NMR (600 MHz CDCl$_3$) authentic purified secretion containing (4S,5R)-1

Figure S2. COSY and TOCSY (600 MHz CDCl$_3$) authentic purified secretion containing (4S,5R)-1

Figure S3. $^1$H NMR (300 MHz CDCl$_3$) compound (4R,5S)-1

Figure S4. $^{13}$C NMR (75 MHz CDCl$_3$) compound (4R,5S)-1

Figure S5. COSY (300 MHz CDCl$_3$) compound (4R,5S)-1

Figure S6. HSQC (300/75 MHz CDCl$_3$) compound (4R,5S)-1
Figure S7. HMBC (300/75 MHz CDCl$_3$) compound (4R,5S)-1
Figure S8. $^1$H NMR (300 MHz CDCl$_3$) compound (4R,5R)-1
Figure S9. $^{13}$C NMR (75 MHz CDCl$_3$) compound (4R,5R)-1
Figure S10. $^1$H NMR (700 MHz CDCl$_3$) compound 15: 4-benzyl-3-[4-(R)-(tert-butyldimethylsilyl)oxy]-3-(S)-hydroxydodecanoyl]oxazolidin-2-one
Figure S11. $^{13}$C NMR (175 MHz CDCl$_3$) compound 15: 4-benzyl-3-[4-(R)-(tert-butyldimethylsilyl)oxy]-3-(S)-hydroxydodecanoyl]oxazolidin-2-one
Figure S12. $^1$H NMR (700 MHz CDCl$_3$) compound 13: (S)-2-(tert-butyldimethylsilyl)oxazolidin-2-one
Figure S13. $^{13}$C NMR (175 MHz CDCl$_3$) compound 13: (S)-2-(tert-butyldimethylsilyl)oxazolidin-2-one
Figure S14. $^1$H NMR (700 MHz CDCl$_3$) compound 12: (S)-(1-benzyloxymethyl)nonyl]-tert-butyldimethylsilane
Figure S15. $^{13}$C NMR (175 MHz CDCl$_3$) compound 12: (S)-(1-benzyloxymethyl)nonyl]-tert-butyldimethylsilane
Figure S16. $^1$H NMR (700 MHz CDCl$_3$) compound 11: (S)-1-benzyloxydecan-2-one
Figure S17. $^{13}$C NMR (175 MHz CDCl$_3$) compound 11: (S)-1-benzyloxydecan-2-one

3) Supporting figures (mass spectra of microderivatives): page 29

Figure S18. El-mass spectrum of compound 2 (5-octyl-4-(trimethylsilyl)oxy)dihydro-3H-furan-2-one
Figure S19. El-mass spectrum of compound 3 (4-(tert-butyldimethylsilyl)oxy)-5-octyldihydro-3H-furan-2-one
Figure S20. El-mass spectrum of compound 5 (trimethylsilyl 3,4-bis((trimethylsilyl)oxy)dodecanoate)
Figure S21. El-mass spectrum of compound 6 (methyl 3,4-bis((trimethylsilyl)oxy)dodecanoate)
Figure S22. El-mass spectrum of compound 8a (1,3,4-tris((trimethylsilyl)oxy)dodecane)
Figure S23. El-mass spectrum of compound 8b (1,3,4-tris((trimethylsilyl)oxy)-1,1-dideuteriododecane)

4) Supporting figures (Comparison of sampling techniques): page 35

Figure S24. Total ion chromatogram of whole body-extracts of _Egaenus convexus_ vs. direct sampling of secretion
Figure S25. $^1$H NMR (700 MHz CDCl$_3$) whole body-extract of _Egaenus convexus_
1) Synthesis of Reference Materials (see scheme 2):

*Dodec-3-enoic acid ethyl ester (10).* Triethylamine (4.2 mL, 30 mmol) was added to a mixture of mono ethyl malonate (3.54 mL, 30 mL) and decanal (3.86 mL, 20 mmol). The resulting mixture was heated (without any additional solvent) under nitrogen atmosphere to 90-100 °C (oil bath). Immediately after reaching this temperature, vigorous gas evolution started. After 5 h, the reaction mixture was cooled to ambient temperature. The reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:Et₂O = 19:1 → 14:1) yielded the product as pale yellow liquid (1.92 g, 42%). The physical properties of the product were in good accordance with the reported values\(^\text{13}\).

*(4S,5S)-4-Hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one ((4S,5S)-1).* AD-mix α for Sharpless asymmetric dihydroxylation (1.508 g) was added to a stirred solution of olefin 10 (229 mg, 1 mmol) in tBuOH (3 mL) and H₂O (3 mL). The orange reaction mixture was stirred at ambient temperature. After 16 h, the starting material was completely consumed (TLC cyclohexane:Et₂O = 5:1, PMA stain), the reaction mixture had turned yellow. Sodium bisulfite (499 mg, 2.6 mmol) was added to the reaction mixture, which was further stirred at ambient temperature. After 30 min, the gray suspension was diluted with H₂O (5 mL) and was extracted with EtOAc (4x10 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:EtOAc = 4:5) yielded the product as colorless wax (150 mg, 70%). \([\alpha]_D -33.3 (C = 3.9, \text{CHCl}_3)\). IR(film): 3470, 2953, 2922, 2849, 1741, 1458, 1403, 1379, 1348, 1329, 1297, 1236, 1201, 1171, 1142, 1097, 1044, 1036, 1015, 970, 898, 797, 759, 724, 688, 556, 487, 443, 411 \text{cm}^{-1}. \(^1\text{H} \text{NMR & } ^{13}\text{C} \text{NMR see Table 1. EIMS (70 eV) } m/z 196 [\text{M}^+ - 18] (1), 144 (7), 143 (30), 142 (42), 136 (8), 124 (30), 115 (8), 111(14), 98 (18),}\)
97 (21), 95 (14), 89 (11), 83 (75), 82 (19), 71 (19), 69 (100), 67 (14), 57 (35), 55 (62), 44 (25), 43 (29), 41 (25). HRMS (ESI) calculated for C\textsubscript{12}H\textsubscript{23}O\textsubscript{3} [M+H\textsuperscript{+}] m/z = 215.1647; found: 215.1642.

(4R,5R)-4-Hydroxy-5-octyl-4,5-dihydro-3H-furan-2-one ((4R,5R)-1). AD-mix B (1.508 g) was added to a stirred solution of olefin 10 (228 mg, 1 mmol) in \textsuperscript{1}BuOH (3 mL) and H\textsubscript{2}O (3 mL). The orange reaction mixture was further stirred at ambient temperature. After 22 h, the starting material was completely consumed (TLC cyclohexane:EtOAc = 5:1, PMA stain), the reaction mixture has turned yellow. Sodium bisulfite (560 mg, 3 mmol) was added to the reaction mixture, which was further stirred at ambient temperature. After 30 min, the gray suspension was diluted with H\textsubscript{2}O (5 mL) and was extracted with EtOAc (4x10 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:EtOAc = 1:1) yielded the product as colorless wax (134 mg, 63%). [α\textsubscript{D}] +37.3 (C = 2.1, CHCl\textsubscript{3}). IR(film): 3474, 2953, 2922, 2849, 1741, 1458, 1403, 1379, 1348, 1329, 1297, 1236, 1201, 1171, 1142, 1097, 1044, 1036, 1015, 970, 898, 797, 759, 724, 688, 556, 488, 444, 410 cm\textsuperscript{-1}. \textsuperscript{1}H NMR & \textsuperscript{13}C NMR see Table 1. EIMS (70 eV) see above. HRMS (ESI) calculated for C\textsubscript{12}H\textsubscript{23}O\textsubscript{3} [M+H\textsuperscript{+}] m/z = 215.1642; found: 215.1641.

(R)-1-Benzylxydecan-2-ol (11). Heptyl magnesium bromide (1 M in THF, 8 mL, 8 mmol) was added to a stirred solution suspension of copper(I) cyanide (15 mg, 0.17 mmol) and (R)-(−)-glycidyl benzyl ether (0.5 mL, 3.3 mmol) in THF (10 mL) at ~ -10 °C (ice/NaCl cooling bath). After 1 h, the starting material was completely consumed (TLC cyclohexane:EtOAc = 3:1, PMA stain). The reaction mixture was treated with saturated ammonium chloride solution (15 mL) and the resulting mixture was extracted with tert-butylmethyleneether (3x20 mL). The combined organic layers were dried over sodium sulfate. Purification of the residue by flash chromatography (cyclohexane:tert-
butylmethylether = 4:1) yielded the product as colorless oil (657 mg, 75%). \([\alpha_D] -4.4 \quad (C = 1.2, \text{CHCl}_3)\). IR(film): 3428, 3064, 3030, 2922, 2853, 1496, 1453, 1363, 1306, 1255, 1204, 1091, 1027, 907, 733, 697, 611, 476 cm\(^{-1}\). \(^1\)H NMR(700 MHz, CDCl\(_3\)) \(\delta 7.48 – 7.27 \quad (m, 5H), 4.55 \quad (s, 2H), 3.81 \quad (dt, J = 10.9, 7.3, 3.2 Hz, 1H), 3.50 \quad (dd, J = 9.4, 3.0 Hz, 1H), 3.34 \quad (q, J = 3.3 Hz, 1H), 1.50 – 1.21 \quad (m, 14H), 0.88 \quad (t, J = 7.0 Hz, 3H). \(^{13}\)C NMR: (176 MHz, CDCl\(_3\)) \(\delta 138.0, 128.5, 127.8, 127.7, 127.7, 77.2, 77.0, 76.9, 74.7, 73.3, 70.5, 33.2, 31.9, 29.7, 29.5, 29.3, 25.5, 22.7, 14.1\). HRMS (ESI) calculated for C\(_{17}\)H\(_{29}\)O\(_2\) [M+H\(^+\)] \(m/z = 265.2161\), found: 265.2161.

(R)-(1-Benzylxomethyl-nonyloxy)-tert-butyldimethylsilane (12). A solution of tert-butyldimethylsilyl chloride (50% w/w in CH\(_2\)Cl\(_2\), 1.2 mL, \~ 0.6 g, 4 mmol) was added to a solution of alcohol 11 (637 mg, 2.4 mmol) and imidazole (0.41 g, 6 mmol) in CH\(_2\)Cl\(_2\) (25 mL). The reaction mixture turned immediately turbid and was stirred at ambient temperature. After 1.5 h still a trace of starting material remained (TLC cyclohexane:EtOAc = 9:1, PMA). A second batch of a solution of tert-butyldimethylsilyl chloride (50% w/w in CH\(_2\)Cl\(_2\), 0.25 mL, 0.83 mmol) was added. After 3 h, the starting material was completely consumed. The reaction mixture was poured onto a saturated ammonium chloride solution (15 mL). The layers were separated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (2x20 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue (cyclohexane: tert-butyldimethylether = 98:2 \rightarrow 97:3) yielded the product as colorless oil (813 mg 89%). \([\alpha_D] +11.3 \quad (C = 1.3, \text{CHCl}_3)\). IR(film): 3030, 2953, 2925, 2854, 1496, 1462, 1407, 1361, 1251, 1204, 1114, 1028, 1005, 967, 939, 833, 811, 774, 733, 696, 665, 612, 467 cm\(^{-1}\). \(^1\)H NMR: (700 MHz, CDCl\(_3\)) \(\delta 7.33 \quad (s, 5H), 4.52 \quad (s, 2H), 3.81 \quad (qd, J = 6.6, 3.2 Hz, 1H), 3.37 \quad (qdd, J = 9.8, 5.5, 1.2 Hz, 2H), 1.59 – 1.47 \quad (m, 1H), 1.46 – 1.33 \quad (m, 2H), 1.27 \quad (d, J = 15.6 Hz, 12H), 0.88 \quad (d, J = 1.6 Hz, 15H), 0.05 \quad (dd, J = 6.4, 1.3
Hz, 6H). $^{13}$C NMR: (176 MHz, CDCl$_3$) δ 138.6, 128.3, 128.3, 127.6, 127.5, 77.2, 77.0, 76.9, 74.9, 73.3, 71.6, 34.8, 31.9, 29.8, 29.6, 29.3, 25.9, 25.7, 25.3, 22.7, 18.2, 14.1, -4.3, -4.7. HRMS (ESI) calculated for C$_{23}$H$_{43}$O$_2$Si [M+H$^+$] m/z = 379.3027, found: 379.3024.

(R)-2-(tert-Butyldimethylsilanyloxy)decan-1-ol (13). A solution/suspension of benzyl ether 12 (790 mg, 2.1 mmol) and palladium on carbon (10%, 215 mg) in EtOAc (50 mL) was stirred under hydrogen atmosphere at ambient temperature. After 18 h, the starting material was completely consumed (TLC cyclohexane:tert-butylmethylether = 97:3, PMA stain). The reaction mixture was filtered through a plug of florisil® (~ 2 cm) and the residue was rinsed with EtOAc (4x3 mL). The clear filtrate was concentrated under reduced pressure to yield the product as colorless oil (607 mg, quant.) [α]D -9.4 (C = 3.7, CHCl$_3$). IR(film): 3379, 2954, 2926, 2855, 1463, 1378, 1361, 1252, 1102, 1042, 1004, 960, 939, 833, 810, 774, 722, 666, 573, 463 cm$^{-1}$. $^1$H NMR: (700 MHz, CDCl$_3$) δ 3.73 (qd, $J$ = 6.2, 3.7 Hz, 1H), 3.56 (ddd, $J$ = 10.9, 6.0, 3.6 Hz, 1H), 3.44 (dt, $J$ = 11.1, 5.6 Hz, 1H), 1.93 (q, $J$ = 5.6, 4.3 Hz, 1H), 1.48 (dt, $J$ = 8.1, 5.6 Hz, 2H), 1.27 (s, 14H), 0.89 (d, $J$ = 16.9 Hz, 12H), 0.09 (s, 6H). $^{13}$C NMR: (176 MHz, CDCl$_3$) δ 77.22, 77.04, 76.86, 72.98, 66.31, 34.00, 31.88, 29.80, 29.54, 29.26, 25.87, 25.36, 22.68, 18.11, 14.11, -0.00, -4.43, -4.55. HRMS (ESI) calculated for C$_{16}$H$_{37}$O$_2$Si [M+H$^+$] m/z = 289.2557, found: 289.2555.

(R)-2-(tert-Butyldimethylsilanyloxy)decanal (14). BAIB ([bis(acetoxy)iodo]benzene: 402 mg, 1.25 mmol) was added to a stirred solution of alcohol 13 (304 mg, 1.06 mmol) and TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxyl: 30 mg, 0.2 mmol) in MeCN (4.5 mL), CH$_2$Cl$_2$ (3 mL) and pH 7 phosphate buffer (1.67 M, 1.5 mL). The resulting mixture was stirred at ambient temperature and became turbid within < 1min. After 45 min, the starting material was completely consumed (TLC cyclohexane:tert-butylmethylether = 14:1, PMA stain). After 1 h, the reaction mixture was poured
onto saturated sodium thiosulfate solution (10 mL) and the resulting mixture was extracted with CH$_2$Cl$_2$ (4x10 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was used directly in the next step.

4-Benzyl-3-[4-(R)-(tert-butyldimethylsilyloxy)-3-(S)-hydroxydodecanoyl]oxazolidin-2-one (15).

Solution of dibutylboron triflate (1 M in CH$_2$Cl$_2$, 2.5 mL, 2.5 mmol) followed by triethyl amine (360 µL, 2.6 mmol) were added to a stirred solution of oxazolidinone 16 (548 mg, 2.5 mmol) in dry CH$_2$Cl$_2$ (10 mL, freshly distilled from CaH$_2$) at 0 °C. After 1 h, a solution of 14 (~ 1.06 mmol) in dry CH$_2$Cl$_2$ (2 + 1 mL) was added slowly at 0 °C. After 45 min, the aldehyde was completely consumed (TLC cyclohexane:tert butylmethylether = 14:1, PMA stain). The reaction mixture was diluted with a cloudy mixture of EtOH (30 mL), pH 7 phosphate buffer (1.67 M, 15 mL) and aqueous hydrogen peroxide (30%, 5 mL). The resulting biphasic mixture was stirred for further 10 min at 0 °C before the layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (1x50 + 3x20 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:tert butylmethylether = 3:1) yielded the product as colorless oil (270 mg, 50% over 2 steps). [α$_D$] +16.8 (C = 0.8, CHCl$_3$).

IR(film): 3522, 3029, 2952, 2925, 2854, 1783, 1695, 1604, 1497, 1462, 1386, 1357, 1289, 1250, 1210, 1196, 1084, 1051, 1030, 1004, 938, 834, 776, 748, 701, 671, 631, 594, 504 cm$^{-1}$. $^1$H NMR: (700 MHz, CDCl$_3$) δ 7.40 – 7.10 (m, 5H), 4.70 (ddt, $J = 9.7$, 7.6, 3.1 Hz, 1H), 4.25 – 4.12 (m, 2H), 4.07 (ddd, $J = 10.0$, 4.3, 2.8 Hz, 1H), 3.75 (dt, $J = 6.5$, 4.4 Hz, 1H), 3.32 (dd, $J = 13.5$, 3.4 Hz, 1H), 3.19 (dd, $J = 16.5$, 10.0 Hz, 1H), 3.08 (dd, $J = 16.4$, 2.7 Hz, 0H), 2.80 (dd, $J = 13.5$, 9.5 Hz, 1H), 1.63 – 1.55 (m, 1H), 1.42 (qt, $J = 10.6$, 6.0 Hz, 1H), 1.36 – 1.24 (m, 12H), 0.92 (s, 9H), 0.88 (t, $J = 7.0$ Hz, 3H), 0.12 (s, 3H), 0.10 (s, 3H). $^{13}$C NMR: (176 MHz, CDCl$_3$) δ 172.84, 153.54, 135.20, 129.46, 128.99,
127.38, 77.21, 77.03, 76.84, 74.91, 72.83, 70.80, 66.23, 55.25, 49.46, 38.03, 37.80, 32.95, 31.87, 29.87, 29.55, 29.27, 26.99, 25.92, 24.90, 22.68, 18.13, 14.11, -4.37, -4.41. HRMS (ESI) calculated for C_{28}H_{48}NO_{5}Si [M+H^+] m/z = 506.3296, found: 506.3296.

4-(S)-Hydroxy-5-(R)-octyldihydrofuran-2-one (S,R-1). A solution of tetrabutylammonium fluoride (1 M in THF, 1.5 mL, 1.5 mmol) was added to a stirred solution of TBS ether 15 (254 mg, 0.5 mmol) in THF (3 mL). The yellow reaction mixture was further stirred at ambient temperature. After 1 h, the starting material was completely consumed (TLC cyclohexane:EtOAc = 6:4, PMA stain) and a much more polar product was formed. The reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:EtOAc = 6:4) yielded the product as colorless wax (76 mg, 71%).

[α]D +25.2 (C = 0.33, CHCl3). IR(film): 3423, 2954, 2917, 2851, 1758, 1470, 1359, 1329, 1283, 1245, 1181, 1122, 1087, 1049, 1033, 994, 979, 953, 908, 877, 756, 718, 692, 570, 513, 436, 409 cm\(^{-1}\). \(^1\)H NMR & \(^{13}\)C NMR: see Table 1. HRMS (ESI) calculated for C_{12}H_{23}O_{3} [M+H^+] m/z = 215.1642, found: 215.1642.

(S)-1-Benzylxodecan-2-ol (ent-11). Heptyl magnesium bromide (1 M in THF, 15 mL, 15 mmol) was added to a stirred solution suspension of copper(II) acetate monohydrate (78 mg, 0.4 mmol) and (S)-(+)glycidyl benzyl ether (0.88 mL, 5.8 mmol) in THF (20 mL) at ~ -10 °C (ice/NaCl cooling bath). After 1 h, the starting material was completely consumed (TLC cyclohexane:EtOAc = 3:1, PMA stain). The reaction mixture was treated with saturated ammonium chloride solution (30 mL) and the resulting mixture was extracted with tert-butylmethyl ether (4x20 mL). The combined organic layers were dried over sodium sulfate. Purification of the residue by flash chromatography (cyclohexane:tert-butylmethyl ether = 4:1) yielded the product as colorless oil (1.33 g, 87%). [α]D
+6.8 (C = 1.2, CHCl₃). IR(film): 3428, 3064, 3030, 2922, 2853, 1496, 1453, 1363, 1306, 1255, 1204, 1091, 1027, 907, 733, 697, 611, 476 cm⁻¹. ¹H NMR(700 MHz, CDCl₃) δ 7.48 – 7.27 (m, 5H), 4.55 (s, 2H), 3.81 (dtt, J = 10.9, 7.3, 3.2 Hz, 1H), 3.50 (dd, J = 9.4, 3.0 Hz, 1H), 3.34 (q, J = 3.3 Hz, 1H), 1.50 – 1.21 (m, 14H), 0.88 (t, J = 7.0 Hz, 3H). ¹³C NMR: (176 MHz, CDCl₃) δ 138.0, 128.5, 127.8, 127.7, 127.7, 77.2, 77.0, 76.9, 74.7, 73.3, 70.5, 33.2, 31.9, 29.7, 29.5, 29.3, 25.5, 22.7, 14.1. HRMS (ESI) calculated for C₁₇H₂₉O₂ [M+H⁺] m/z = 265.2161, found: 265.2161.

(S)-(1-Benzyloxymethylnonyloxy)-tert-butyldimethylsilane (ent-12). A solution of tert-butyldimethylsilyl chloride (50% w/w in CH₂Cl₂, 2 mL, ~ 1 g, 6.5 mmol) was added to a solution of alcohol ent-11 (1.30 g, 4.9 mmol) and imidazole (0.68 g, 10 mmol) in CH₂Cl₂ (25 mL). The reaction mixture turned immediately turbid and was stirred at ambient temperature. After 1 h, some of the starting material remained (TLC cyclohexane:EtOAc = 9:1, PMA stain), thus a second batch of a solution of tert-butyldimethylsilyl chloride (50% w/w in CH₂Cl₂, 0.8 mL, ~ 0.4 g, 2.7 mmol) was added. After 4 h, the starting material was completely consumed. The reaction mixture was poured onto a saturated ammonium chloride solution (20 mL). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (2x30 mL). the combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue (cyclohexane: tert-butyldimethylether = 98:2 → 97:3) yielded the product as colorless oil (1.63 g, 88%). [α]D -10.3 (C = 2.7, CHCl₃). IR(film): 3030, 2953, 2925, 2854, 1496, 1462, 1407, 1361, 1251, 1204, 1114, 1028, 1005, 967, 939, 833, 811, 774, 733, 696, 665, 612, 467 cm⁻¹. ¹H NMR: (700 MHz, CDCl₃) δ 7.33 (s, 5H), 4.52 (s, 2H), 3.81 (qd, J = 6.6, 3.2 Hz, 1H), 3.37 (qdd, J = 9.8, 5.5, 1.2 Hz, 2H), 1.59 – 1.47 (m, 1H), 1.46 – 1.33 (m, 2H), 1.27 (d, J = 15.6 Hz, 12H), 0.88 (d, J = 1.6 Hz, 15H), 0.05 (dd, J = 6.4, 1.3 Hz, 6H). ¹³C NMR: (176 MHz, CDCl₃) δ 138.6, 128.3, 128.3, 127.6, 127.5, 77.2, 77.0, 76.9,
(S)-2-((tert-Butyldimethylsilanyloxy)decan-1-ol (ent-13). A solution/suspension of benzyl ether ent-12 (810 mg, 2.1 mmol) and palladium on carbon (10%, 107 mg) in EtOAc (10 mL) was stirred under hydrogen atmosphere at ambient temperature. After 20 h, the starting material was ~½ converted to a more polar product (TLC cyclohexane:tert-butylmethylether = 97:3, PMA stain). The hydrogen atmosphere was replaced by nitrogen and a second batch of palladium on carbon (10%, 105 mg) was added. The nitrogen was again replaced by hydrogen atmosphere and the reaction mixture was further stirred at ambient temperature. After 2 d, the starting material was almost completely consumed. The reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:tert-butylmethylether = 11:1) yielded the product as colorless oil (589 mg, 97%). [α]D +10.0 (C = 3.7, CHCl3). IR(film): 3379, 2954, 2926, 2855, 1463, 1378, 1361, 1252, 1102, 1042, 1004, 960, 939, 833, 810, 774, 722, 666, 573, 463 cm⁻¹. ¹H NMR: (700 MHz, CDCl3) δ 3.73 (qd, J = 6.2, 3.7 Hz, 1H), 3.56 (ddd, J = 10.9, 6.0, 3.6 Hz, 1H), 3.44 (dt, J = 11.1, 5.6 Hz, 1H), 1.93 (q, J = 5.6, 4.3 Hz, 1H), 1.48 (dt, J = 8.1, 5.6 Hz, 2H), 1.27 (s, 14H), 0.89 (d, J = 16.9 Hz, 12H), 0.09 (s, 6H). ¹³C NMR: (176 MHz, CDCl3) δ 77.22, 77.04, 76.86, 72.98, 66.31, 34.00, 31.88, 29.80, 29.54, 29.26, 25.87, 25.36, 22.68, 18.11, 14.11, -0.00, -4.43, -4.55. HRMS (ESI) calculated for C_{16}H_{37}O_{2}Si [M+H⁺] m/z = 289.2557, found: 289.2555.

(S)-2-((tert-Butyldimethylsilanyloxy)decanal (ent-14). BAIB (99 mg, 0.3 mmol) was added to a stirred solution of alcohol ent-13 (56 mg, 0.2 mmol) and TEMPO (6 mg, 0.04 mmol) in MeCN (1.5 mL), CH₂Cl₂ (1 mL) and pH 7 Phosphate buffer (0.5 mL). The resulting mixture was stirred at ambient temperature and became turbid within < 1 min. After 20 min, the starting material was
completely consumed (TLC cyclohexane:tert-butylmethyl ether = 14:1, PMA stain). After 30 min, the reaction mixture was poured onto saturated sodium thiosulfate solution (5 mL) and the resulting mixture was extracted with CH$_2$Cl$_2$ (3x10 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was used directly in the next step.

4-Benzyl-3-[4-(S)-(tert-butyldimethylsilanyloxy)-3-(R)-hydroxydodecanoyl]oxazolidin-2-one (ent-15). A solution of dibutylboron triflate (1 M in CH$_2$Cl$_2$, 0.4 mL, 0.4 mmol) follow by triethyl amine (60 µL, 0.4 mmol) were added to a stirred solution of oxazolidinone ent-16 (88 mg, 0.4 mmol) in dry CH$_2$Cl$_2$ (4 mL, freshly distilled from CaH$_2$) at 0 °C. After 3 h, a solution of crude aldehyde ent-14 (~ 0.2 mmol) in dry CH$_2$Cl$_2$ (1 + 1 mL) was added slowly at 0 °C. After 1.25 h, the aldehyde was (almost) completely consumed (TLC cyclohexane:tert-butylmethyl ether = 9:1, PMA stain). The reaction mixture was diluted with a cloudy mixture of EtOH (6 mL), pH 7 phosphate buffer (1.67 M, 3 mL) and aqueous hydrogen peroxide (30%, 1 mL). The resulting biphasic mixture was stirred for further 10 min at 0 °C before the layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (3x10 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:tert-butylmethyl ether = 3:1) yielded the product as colorless oil (41 mg, 41% over 2 steps). $[\alpha]_D$ -13.6 (C = 0.8, CHCl$_3$). IR(film): 3522, 3029, 2952, 2925, 2854, 1783, 1695, 1604, 1497, 1462, 1386, 1357, 1289, 1250, 1210, 1196, 1084, 1051, 1030, 1004, 938, 834, 776, 748, 701, 671, 631, 594, 504 cm$^{-1}$. $^1$H NMR: (700 MHz, CDCl$_3$) $\delta$ 7.40 – 7.10 (m, 5H), 4.70 (ddt, $J = 9.7, 7.6, 3.1$ Hz, 1H), 4.25 – 4.12 (m, 2H), 4.07 (ddd, $J = 10.0, 4.3, 2.8$ Hz, 1H), 3.75 (dt, $J = 6.5, 4.4$ Hz, 1H), 3.32 (dd, $J = 13.5, 3.4$ Hz, 1H), 3.19 (dd, $J = 16.5, 10.0$ Hz, 1H), 3.08 (dd, $J = 16.4, 2.7$ Hz, 0H), 2.80 (dd, $J = 16.5, 10.0$ Hz, 1H), 2.56 (ddd, $J = 16.0, 10.0, 7.5$ Hz, 1H), 2.42 (dd, $J = 16.5, 3.4$ Hz, 1H), 2.08 (dd, $J = 17.0, 11.0$ Hz, 1H), 1.98 (s, 3H), 1.96 (s, 3H), 1.85 (s, 3H), 1.18 (s, 9H), 1.08 (s, 9H), 1.04 (s, 9H), 0.88 (s, 9H).
= 13.5, 9.5 Hz, 1H), 1.63 – 1.55 (m, 1H), 1.42 (qt, J = 10.6, 6.0 Hz, 1H), 1.36 – 1.24 (m, 12H), 0.92 (s, 9H), 0.88 (t, J = 7.0 Hz, 3H), 0.12 (s, 3H), 0.10 (s, 3H). $^{13}$C NMR: (176 MHz, CDCl$_3$) δ 172.84, 153.54, 135.20, 129.46, 128.99, 127.38, 77.21, 77.03, 76.84, 74.91, 72.83, 70.80, 66.23, 55.25, 49.46, 38.03, 37.80, 32.95, 31.87, 29.87, 29.55, 29.27, 26.99, 25.92, 24.90, 22.68, 18.13, 14.11, -4.37, -4.41. HRMS (ESI) calculated for C$_{28}$H$_{48}$NO$_5$Si [M+H$^+$] m/z = 506.3296, found: 506.3295.

4-(R)-Hydroxy-5-(S)-octylidihydrofuran-2-one (R,S-1): A solution of tetrabutylammonium fluoride (1 M in THF, 0.1 mL, 0.1 mmol) was added to a stirred solution of TBS ether ent-15 (10 mg, 0.02 mmol) in THF (0.5 mL). The yellow reaction mixture was further stirred at ambient temperature. After 1.5 h, the starting material was completely consumed (TLC cyclohexane:EtOAc = 7:3, PMA stain) a much more product, which was by TLC identical with the racemate ANF-096, was formed. The reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography (cyclohexane:EtOAc = 6:4) yielded the product as colorless film/wax (3 mg, 70%). [α]$_D$ -32.3 (C = 0.13, CHCl$_3$). IR(film): 3423, 2954, 2917, 2851, 1758, 1470, 1359, 1329, 1283, 1245, 1181, 1122, 1087, 1049, 1033, 994, 979, 953, 908, 877, 756, 718, 692, 570, 513, 436, 409 cm$^{-1}$. $^1$H NMR & $^{13}$C NMR: see Table 1. HRMS (ESI) calculated for C$_{12}$H$_{23}$O$_3$ [M+H$^+$] m/z = 215.1642, found: 215.1642.
2) Supporting figures (NMR-spectra)

Figure S1.

Authentic material (containing natural compound (4S,5R)-1)) after purification (see also Table 1), for comparison with whole body extract see Figure S25.

$^1$H NMR (600 MHz CDCl$_3$)
Figure S2.

Two-dimensional NMR spectra of the authentic purified secretion, containing natural compound \((4S,5R)-\textbf{1}\). A) COSY spectrum with the resonances labeled in \(\omega_1\) and \(\omega_2\) (label rotated by 90°). B) TOCSY spectrum recorded with a mixing time of 120 ms. C) Key correlations observed in the COSY spectrum are indicated as red arrows on the structure of \(\textbf{1}\). The cross-peak corresponding to the arrow with the dotted line was too close to the diagonal and could not be observed (chemical shifts of H-4 and H-5 are too similar). Both spectra were recorded at 293 K, using 4 scans and 1024x256 complex points.

COSY and TOCSY (600 MHZ CDCl$_3$)
Figure S3. Synthetic compound (4R,5S)-1

$^1$H NMR (300 MHz CDCl$_3$)
Figure S4. Synthetic compound ($4R,5S$)-1

$^{13}$C NMR (75 MHz CDCl$_3$)
Figure S5. Synthetic compound (4R,5S)-1

COSY (300 MHz CDCl₃)
Figure S6. Synthetic compound (4R,5S)-1

HSQC (300/75 MHz CDCl₃)
Figure S7. Synthetic compound (4R,5S)-1

HMBC (300/75 MHz CDCl₃)
Figure S8. Synthetic compound (4R,5R)-1

$^1$H NMR (300 MHz CDCl$_3$)
Figure S9. Synthetic compound (4R,5R)-1

$^{13}$C NMR (75 MHz CDCl$_3$)
Figure S10. Compound 15: 4-benzyl-3-[4-\((R)\)-(tert-butyldimethylsilyloxy)-3-(S)-hydroxydodecanoyl]oxazolidin-2-one

$^1$H NMR (700 MHz CDCl$_3$)
Figure S11. Compound 15: 4-benzyl-3-[4-(R)-(tert-butyldimethylsilanyloxy)-3-(S)-hydroxydodecanoyl]oxazolidin-2-one

$^{13}$C NMR (175 MHz CDCl$_3$)
Figure S12. Compound 13: (S)-2-(tert-butyldimethylsilyloxy)decan-1-ol

$^1$H NMR (700 MHz CDCl$_3$)
Figure S13. Compound **13**: (S)-2-(tert-butyl(dimethyl)silyloxy)decan-1-ol

$^{13}$C NMR (175 MHz CDCl$_3$)
Figure S14. Compound 12: (S)-(1-benzyloxymethylnonyloxy)-tert-butyl(dimethyl)silane

$^1$H NMR (700 MHz CDCl$_3$)
Figure S15. Compound **12**: \((S)-(1\text{-benzyloxymethylnonyloxy})\text{-}\text{tert-butyl(dimethyl)silane}\)

$^{13}$C NMR (175 MHz CDCl$_3$)
Figure S16. Compound 11: (S)-1-benzyloxydecan-2-ol

$^1$H NMR (700 MHz CDCl$_3$)
Figure S17. Compound **11**: (S)-1-benzylxydecan-2-ol

$^{13}$C NMR (175 MHz CDCl$_3$)
3) Supporting figures (mass spectra of microderivatives)

Figure S18. EI-mass spectrum of compound 2 (5-octyl-4-(trimethylsilyloxy)dihydro-3H-furan-2-one)
Figure S19. EI-mass spectrum of compound 3 (4-(tert-butyldimethylsilyloxy)-5-octyldihydro-3H-furan-2-one)
Figure S20. E1-mass spectrum of compound 5 (trimethylsilyl 3,4-bis((trimethylsilyl)oxy)dodecanoate)
Figure S21. El-mass spectrum of compound 6 (methyl 3,4-bis((trimethylsilyl)oxy)dodecanoate)
Figure S22. EI-mass spectrum of compound 8a (1,3,4-tris((trimethylsilyl)oxy)dodecane)
Figure S23. El-mass spectrum of compound 8b (1,3,4-tris((trimethylsilyl)oxy)-1,1-dideuteriododecane)
4) Supporting figures (Comparison of sampling techniques)

Figure S24.

Total ion chromatogram of whole body-extracts of *Egaenus convexus*, showing gland-derived lactones as well as cuticle-derived hydrocarbons (above). Total ion chromatogram of directly sampled secretion (i.e. secretion dabbed from ozopores on filter paper) showing lactones only, and lacking hydrocarbons (below).

Under the given chromatographic conditions (see experimental section), lactones were observed at $t_R=17.56-17.61\text{min} = (4S,5R)\cdot 1$ and $t_R=17.80-17.85\text{min} = (4S,5S)\cdot 1$. Cuticular hydrocarbons (CHCs) can be observed at longer retention times (from $t_R=20.60$ on). Major hydrocarbons are tricosane ($t_R=21.49\text{min}$), pentacosane ($t_R=22.31\text{min}$) and heptacosane ($t_R=23.10\text{min}$). The remaining CHCs are methyl-branched or/and unsaturated. The compound at $t_R=23.53\text{min}$ (chromatogram below) is an artifact.
Figure S25.

Authentic material obtained by whole-body-extraction of 14 individuals of *Egaenus convexus* (comp. Figure S1). Origin of additional compounds (a, b) is uncertain; long-chain alkanes (c) are probably cuticle-derived (comp. Figure S24).

$1^H$ NMR (700MHz CDCl$_3$)

- a = resonances of benzyl methyl ketone
- b = resonances of ethyl acetate
- c = resonances of long-chain alkanes