Supporting Information

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

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Content:

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:Et_2O = 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[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 H_2O (3 mL). The orange reaction mixture was stirred at ambient temperature. After 16 h, the starting material was completely consumed (TLC cyclohexane:Et_2O = 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 H_2O (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%). [α_D] -33.3 (C = 3.9, $CHCl_3$). 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 cm^{-1}. ^1H NMR & ^{13}C NMR see Table 1. EIMS (70 eV) *m/z* 196 [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 $C_{12}H_{23}O_3$ [M+H$^+$] 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 β (1.508 g) was added to a stirred solution of olefin **10** (228 mg, 1 mmol) in ^{t}BuOH (3 mL) and H_2O (3 mL). The orange reaction mixture was further stirred at ambient temperature. After 22 h, the starting material was completely consumed (TLC cyclohexane:Et2O = 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_2O (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_D] +37.3 (C = 2.1, $CHCl_3$). 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^{-1}. ^{1}H NMR & ^{13}C NMR see Table 1. EIMS (70 eV) see above. HRMS (ESI) calculated for $C_{12}H_{23}O_3$ [M+H$^+$] m/z = 215.1642; found: 215.1641.

*(R)-1-Benzyloxydecan-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*-butylmethylether (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%). [α_D] -4.4 (C = 1.2, $CHCl_3$). 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, $CDCl_3$) 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). ^{13}C NMR: (176 MHz, $CDCl_3$) 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 $C_{17}H_{29}O_2$ [$M+H^+$] m/z = 265.2161, found: 265.2161.

*(R)-(1-Benzyloxymethyl-nonyloxy)-tert-butyldimethylsilane (**12**).* A solution of *tert*-butyldimethylsilyl chloride (50% w/w in CH_2Cl_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_2Cl_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_2Cl_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_2Cl_2 (2x20 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue (cyclohexane: *tert*-butylmethylether = 98:2 → 97:3) yielded the product as colorless oil (813 mg 89%). [α_D] +11.3 (C = 1.3, $CHCl_3$). 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}. ^1H NMR: (700 MHz, $CDCl_3$) 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). ^{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 $C_{23}H_{43}O_2Si$ [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 $C_{16}H_{37}O_2Si$ [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_2Cl_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_2Cl_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-butyldimethylsilanyloxy)-3-(S)-hydroxydodecanoyl]oxazolidin-2-one (**15**).* Solution of dibutylboron triflate (1 M in CH_2Cl_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_2Cl_2 (10 mL, freshly distilled from CaH_2) at 0 °C. After 1 h, a solution of **14** (~ 1.06 mmol) in dry CH_2Cl_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_2Cl_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). $[\alpha_D]$ +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}. ^1H 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 $C_{28}H_{48}NO_5Si$ [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, $CHCl_3$). 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.

*(S)-1-Benzyloxydecan-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*-butylmethylether (4x20 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 (1.33 g, 87%). [α_D]

+6.8 (C = 1.2, $CHCl_3$). 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, $CDCl_3$) 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). ^{13}C NMR: (176 MHz, $CDCl_3$) 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 $C_{17}H_{29}O_2$ [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 CH_2Cl_2, 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 CH_2Cl_2 (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 CH_2Cl_2, 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 CH_2Cl_2 (2x30 mL). the combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue (cyclohexane: *tert*-butylmethylether = 98:2 → 97:3) yielded the product as colorless oil (1.63 g, 88%). [α_D] -10.3 (C = 2.7, $CHCl_3$). 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}. ^1H NMR: (700 MHz, $CDCl_3$) 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). ^{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 $C_{23}H_{43}O_2Si$ [M+H$^+$] m/z = 379.3027, found: 379.3024.

*(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, $CHCl_3$). 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) calculcated for $C_{16}H_{37}O_2Si$ [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), CH_2Cl_2 (1 mL) and pH 7 Phosphate buffer (0.5 mL). The resulting mixture was stirred at ambient temperature and became turbid within < 1min. After 20 min, the starting material was

completely consumed (TLC cyclohexane:*tert*-butylmethylether = 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_2Cl_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_2Cl_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_2Cl_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_2Cl_2 (1 + 1 mL) was added slowly at 0 °C. After 1.25 h, the aldehyde was (almost) completely consumed (TLC cyclohexane:*tert*-butylmethylether = 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_2Cl_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*-butylmethylether = 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}. ^1H 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 $C_{28}H_{48}NO_5Si$ [M+H$^+$] m/z = 506.3296, found: 506.3295.

*4-(R)-Hydroxy-5-(S)-octyldihydrofuran-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 $CDCl_3$)

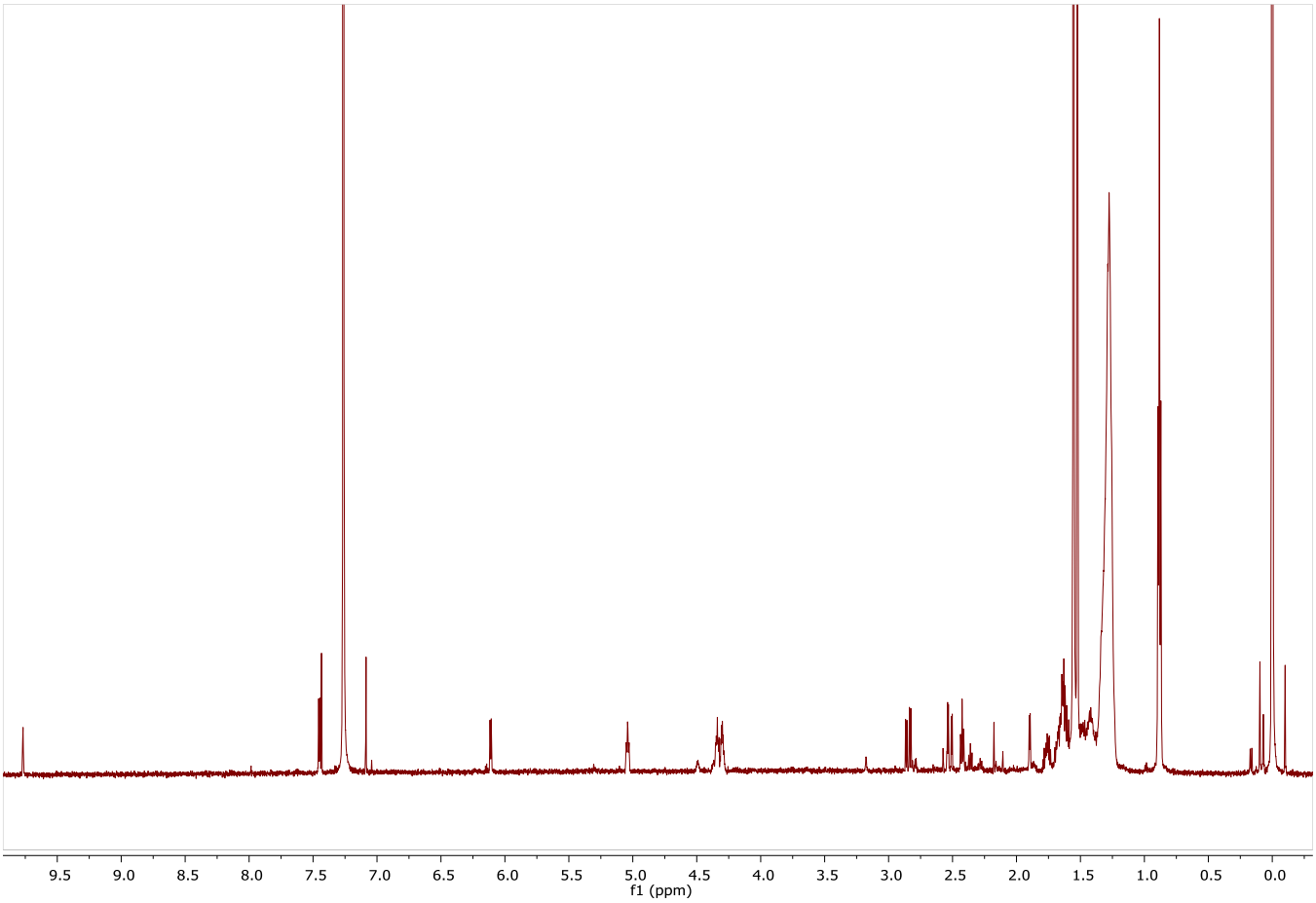


Figure S2.

Two-dimensional NMR spectra of the authentic purified secretion, containing natural compound (*4S,5R*)-**1**). A) COSY spectrum with the resonances labeled in ω1 and ω2 (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 **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 $CDCl_3$)

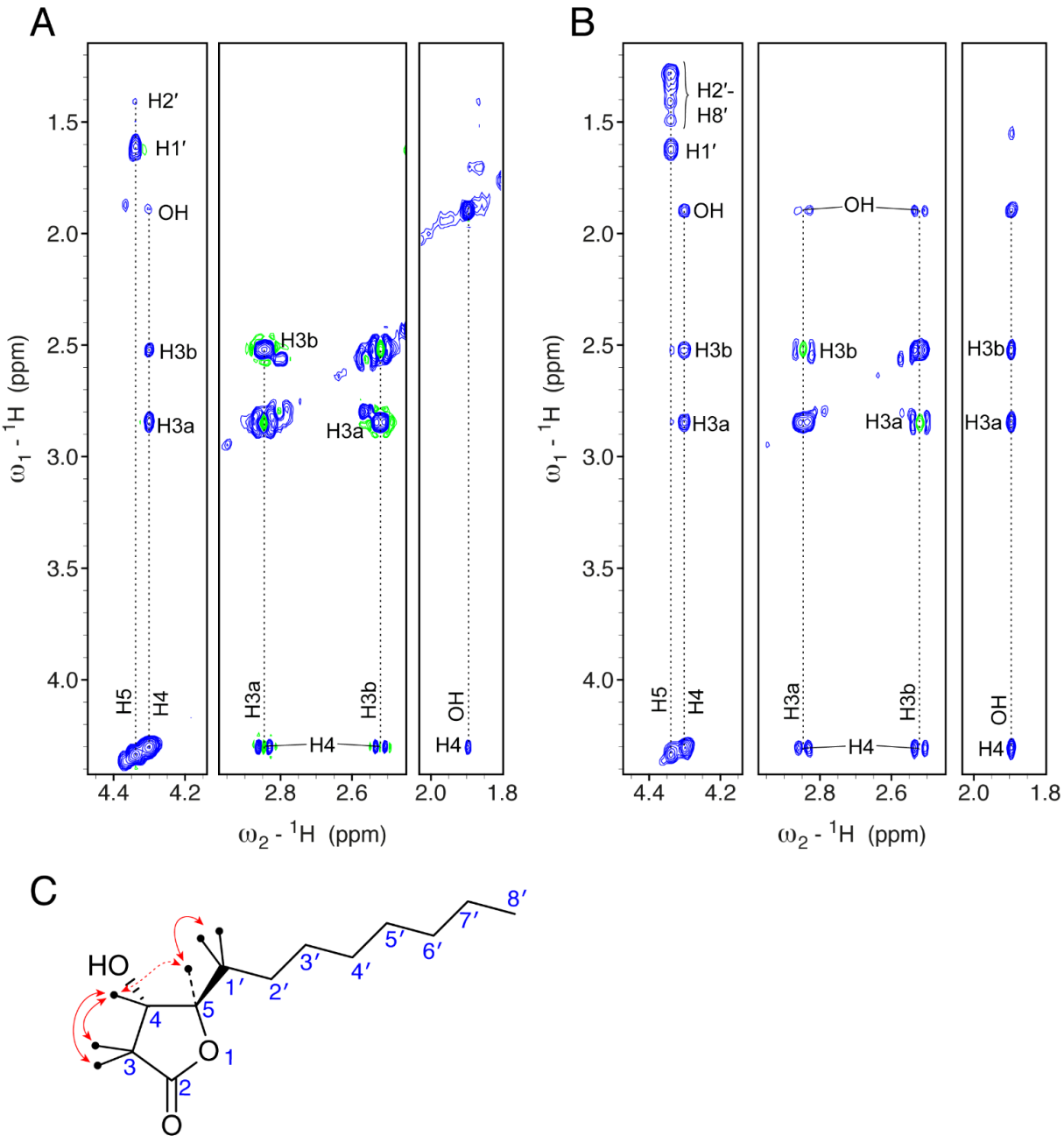


Figure S3. Synthetic compound (*4R,5S*)-**1**

^{1}H NMR (300 MHz $CDCl_3$)

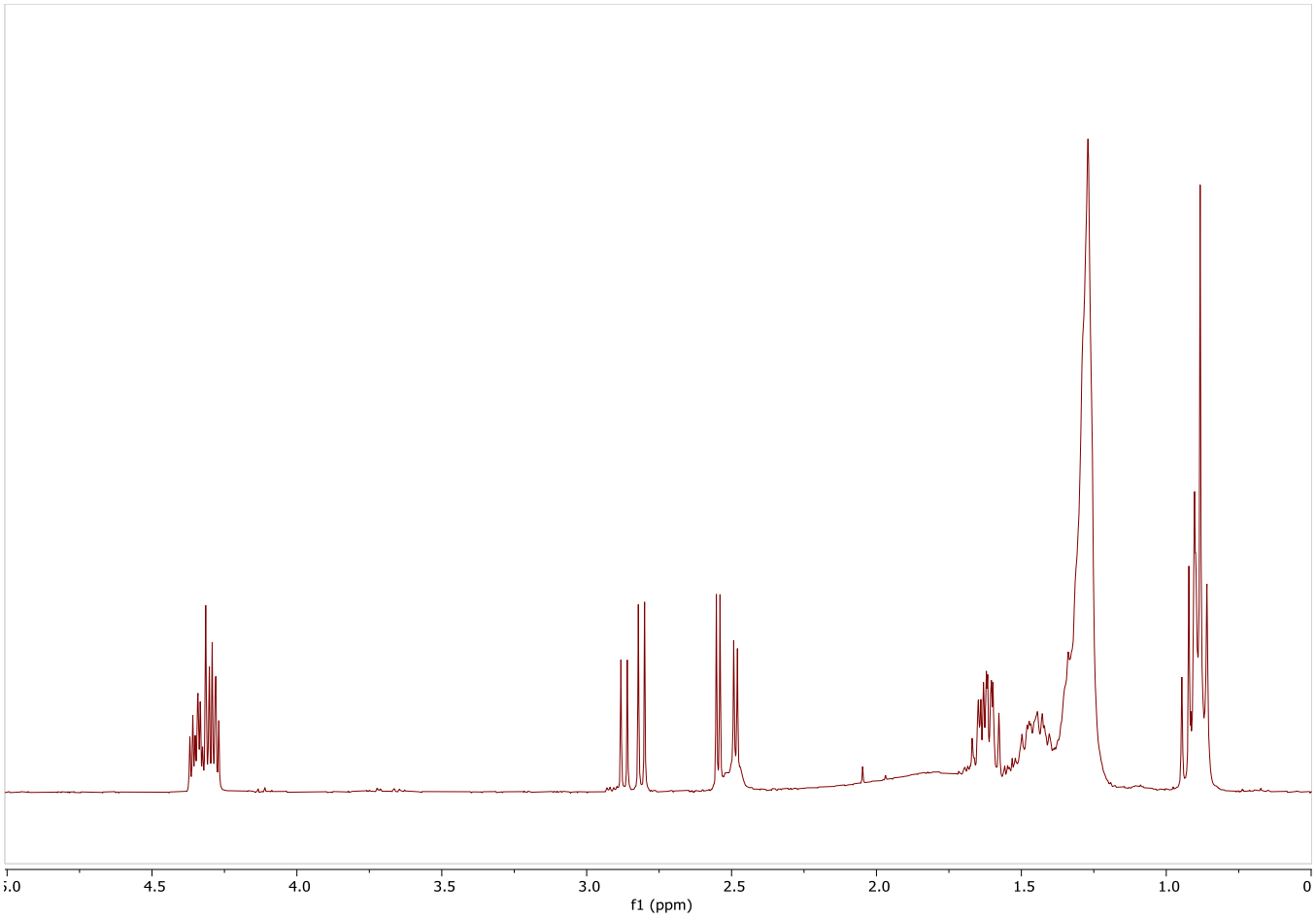


Figure S4. Synthetic compound (*4R,5S*)-**1**

^{13}C NMR (75 MHz $CDCl_3$)

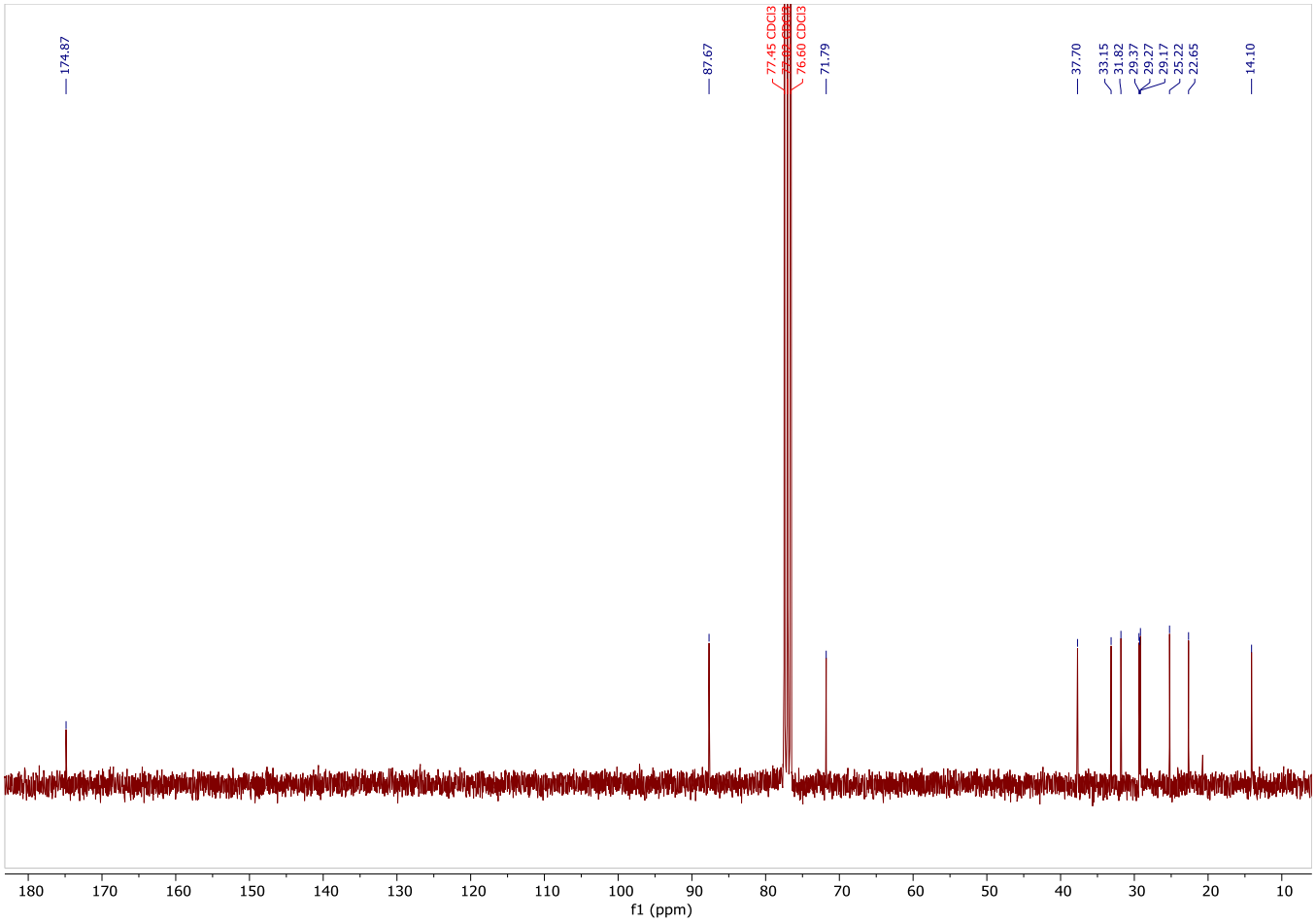


Figure S5. Synthetic compound (*4R,5S*)-**1**

COSY (300 MHz $CDCl_3$)

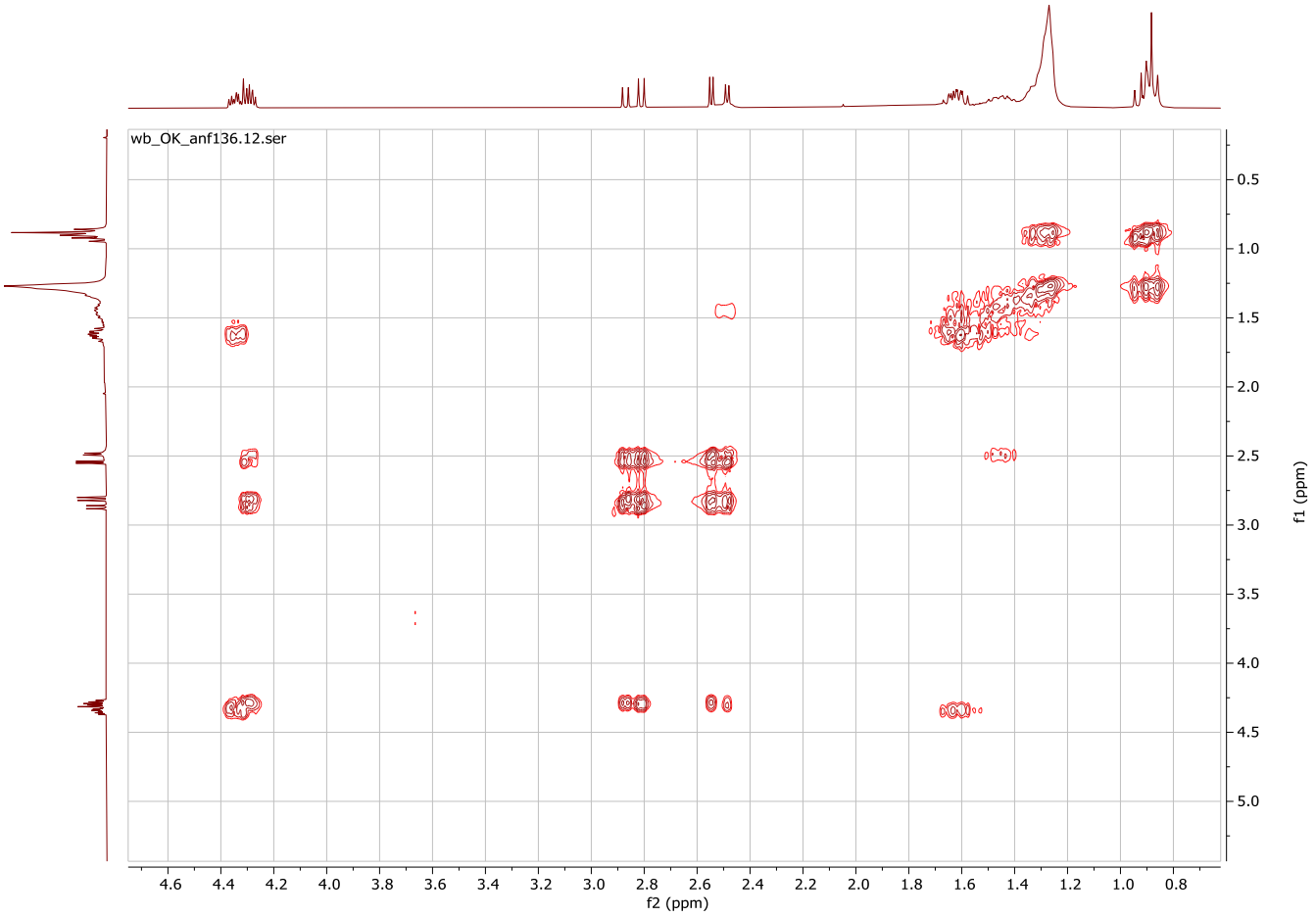


Figure S6. Synthetic compound (*4R,5S*)-**1**

HSQC (300/75 MHz $CDCl_3$)

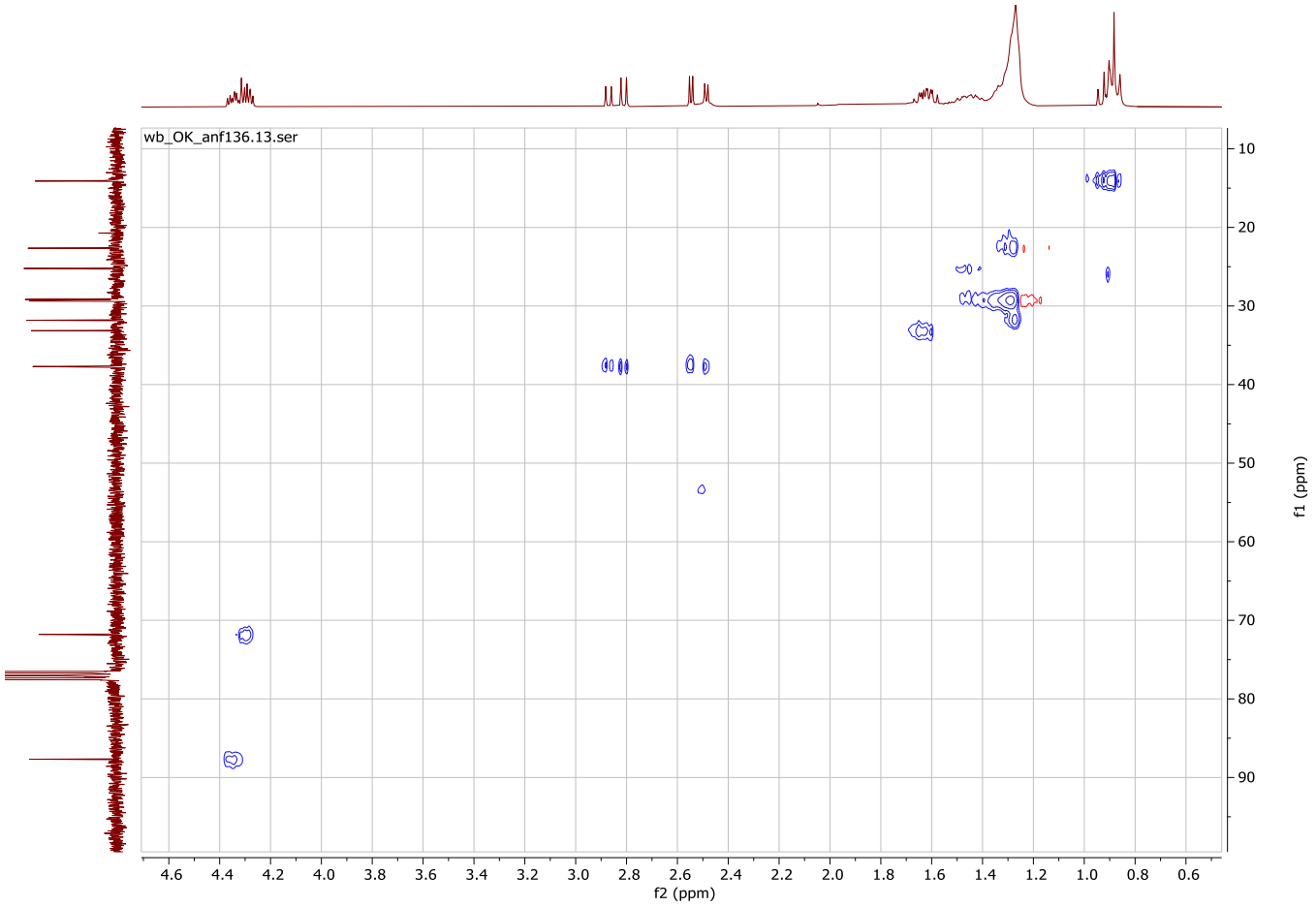


Figure S7. Synthetic compound (*4R,5S*)-**1**

HMBC (300/75 MHz $CDCl_3$)

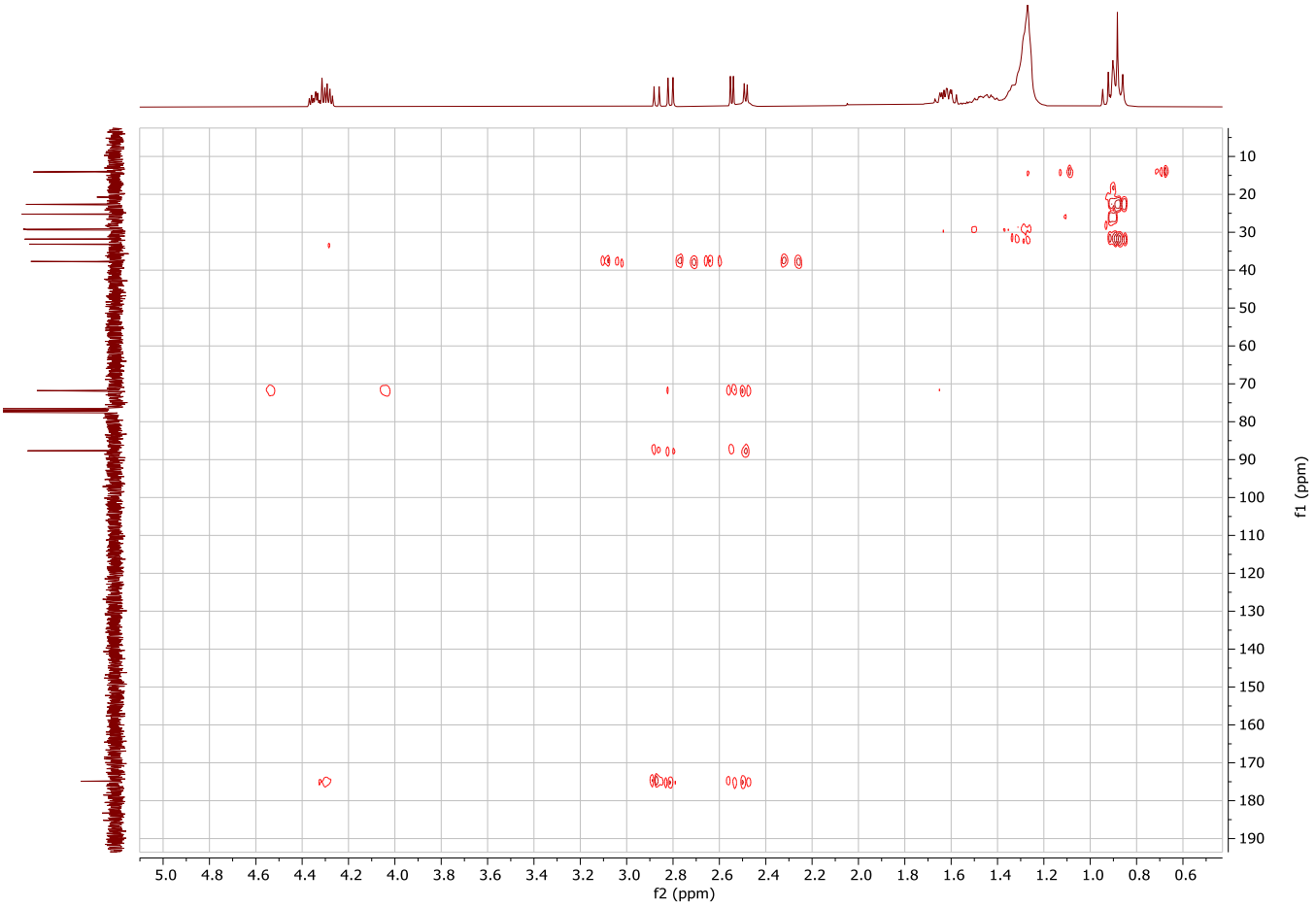


Figure S8. Synthetic compound (*4R,5R*)-**1**

^{1}H NMR (300 MHz $CDCl_3$)

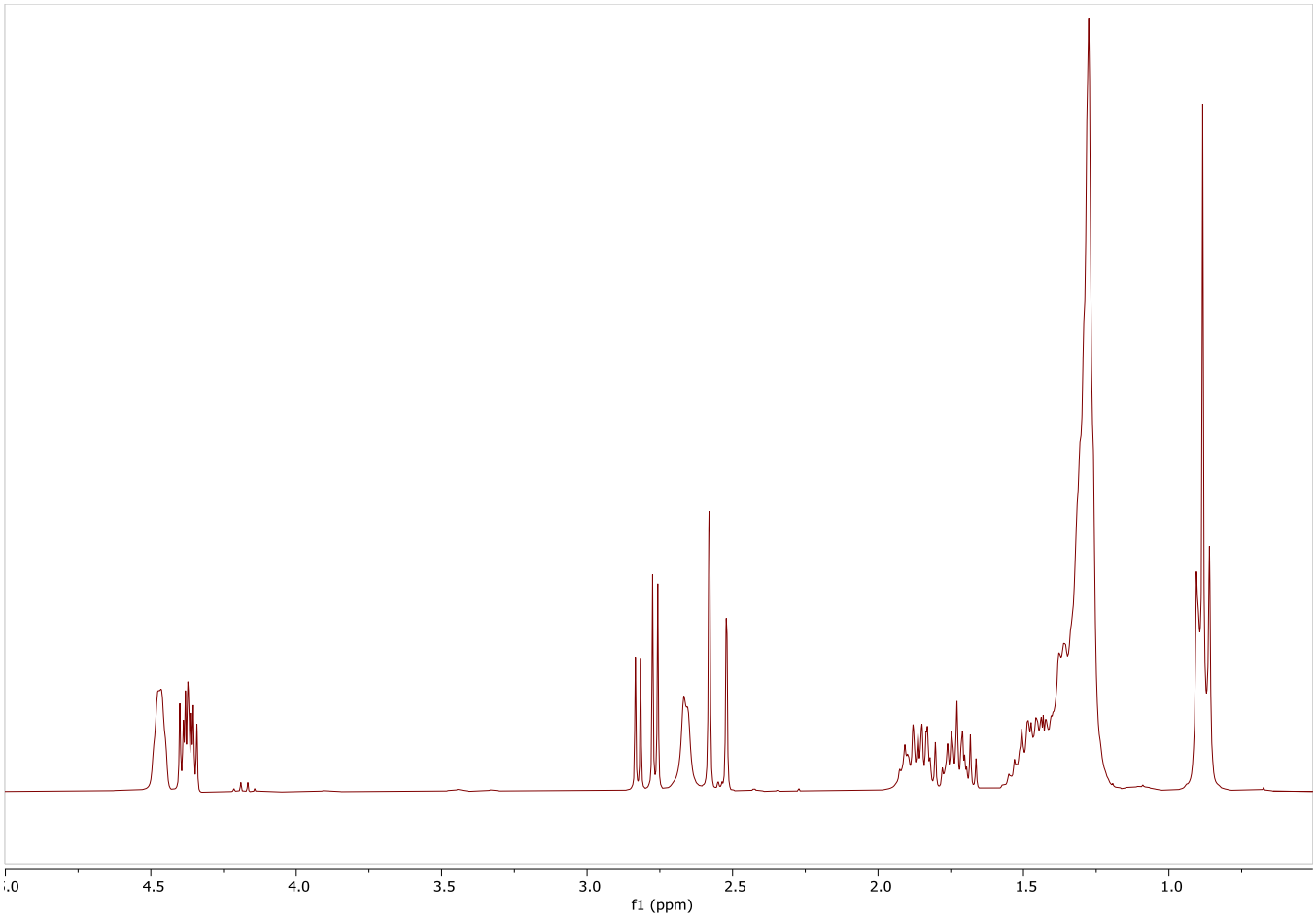


Figure S9. Synthetic compound (*4R,5R*)-**1**

^{13}C NMR (75 MHz $CDCl_3$)

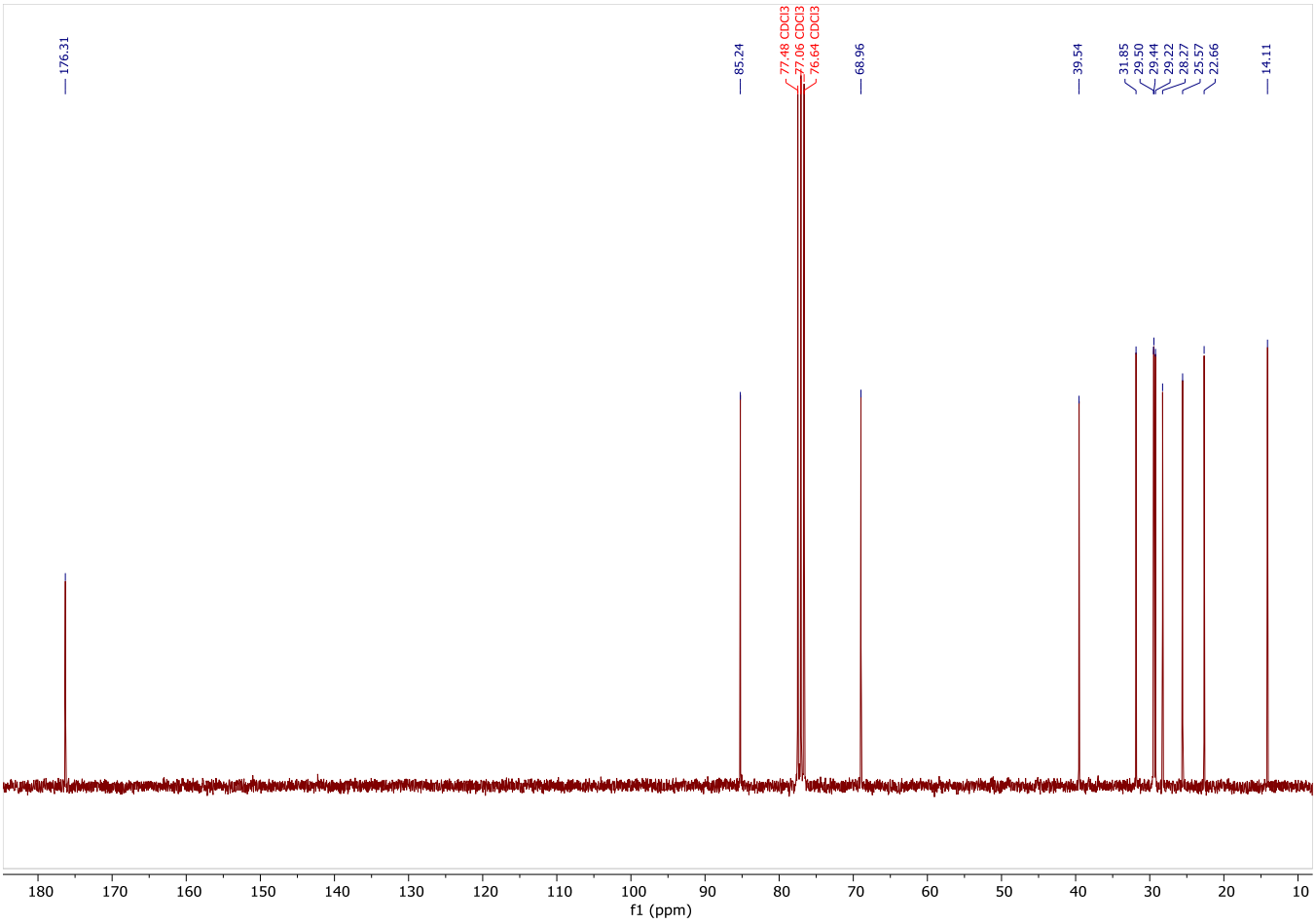


Figure S10. Compound **15:** *4-benzyl-3-[4-(R)-(tert-butyldimethylsilanyloxy)-3-(S)-hydroxydodecanoyl]oxazolidin-2-one*)

^{1}H NMR (700 MHz $CDCl_3$)

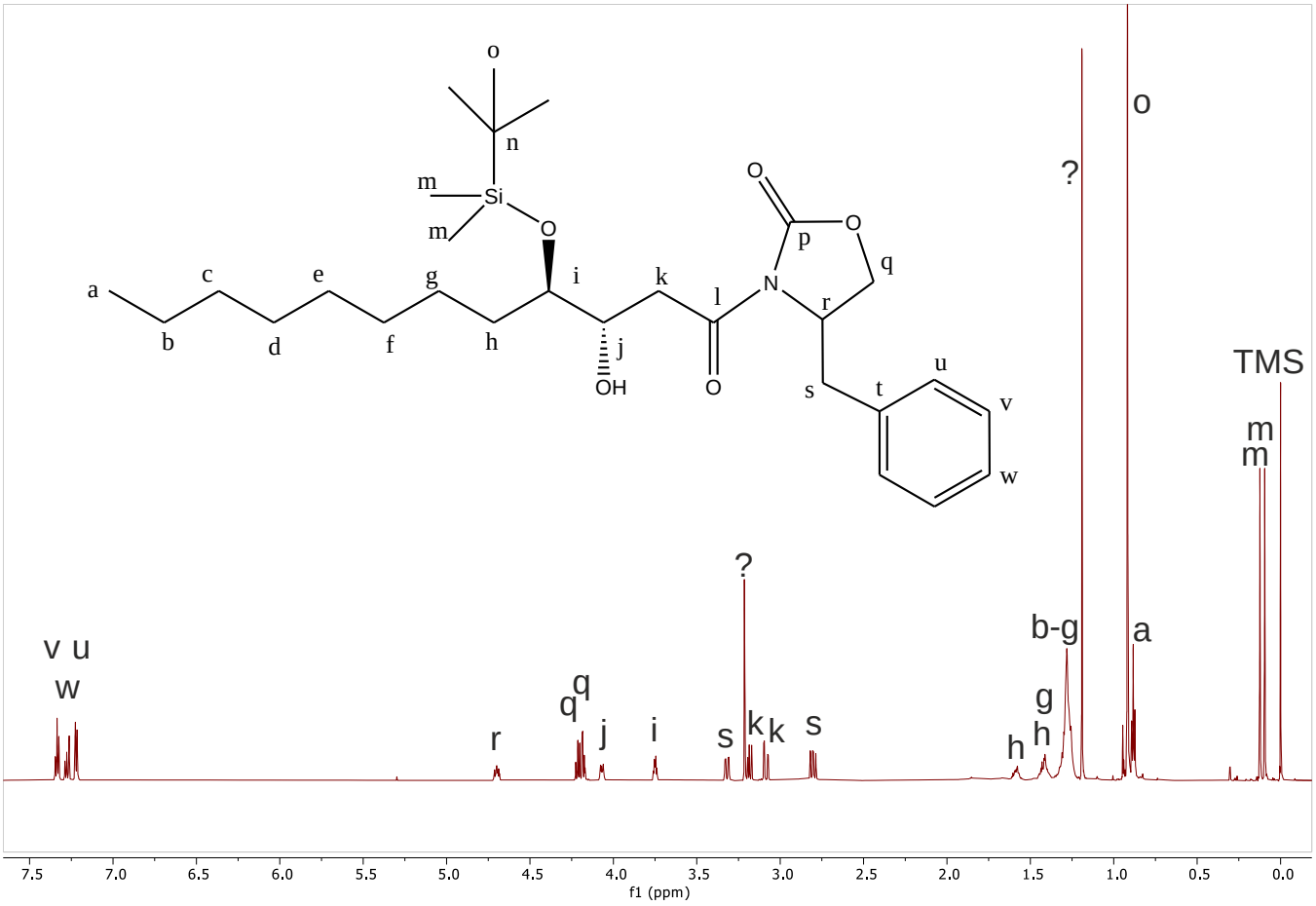


Figure S11. Compound **15:** *4-benzyl-3-[4-(R)-(tert-butyldimethylsilanyloxy)-3-(S)-hydroxydodecanoyl]oxazolidin-2-one*)

^{13}C NMR (175 MHz $CDCl_3$)

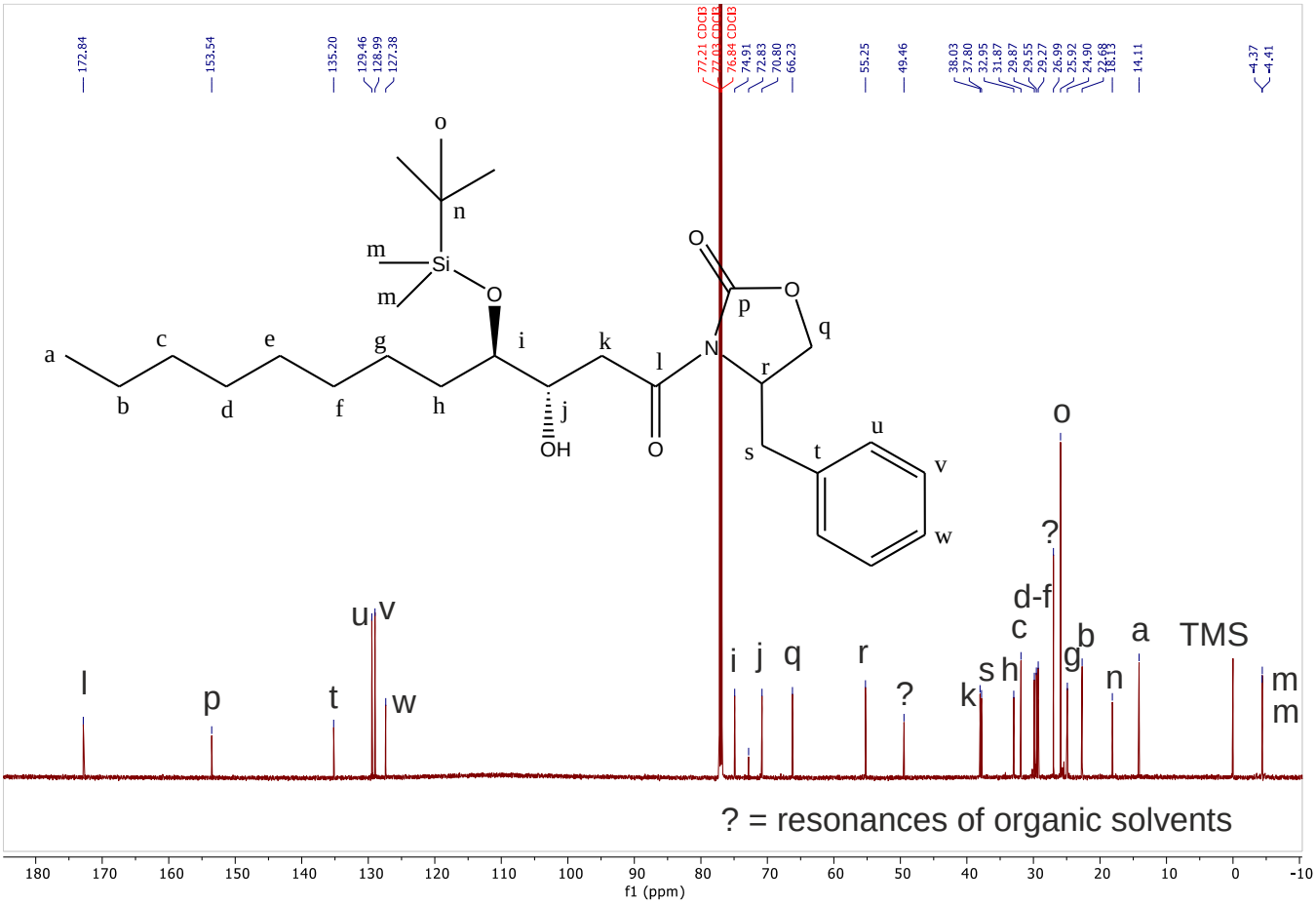


Figure S12. Compound **13:** *(S)-2-(tert-butyldimethylsilanyloxy)decan-1-ol*

^{1}H NMR (700 MHz $CDCl_3$)

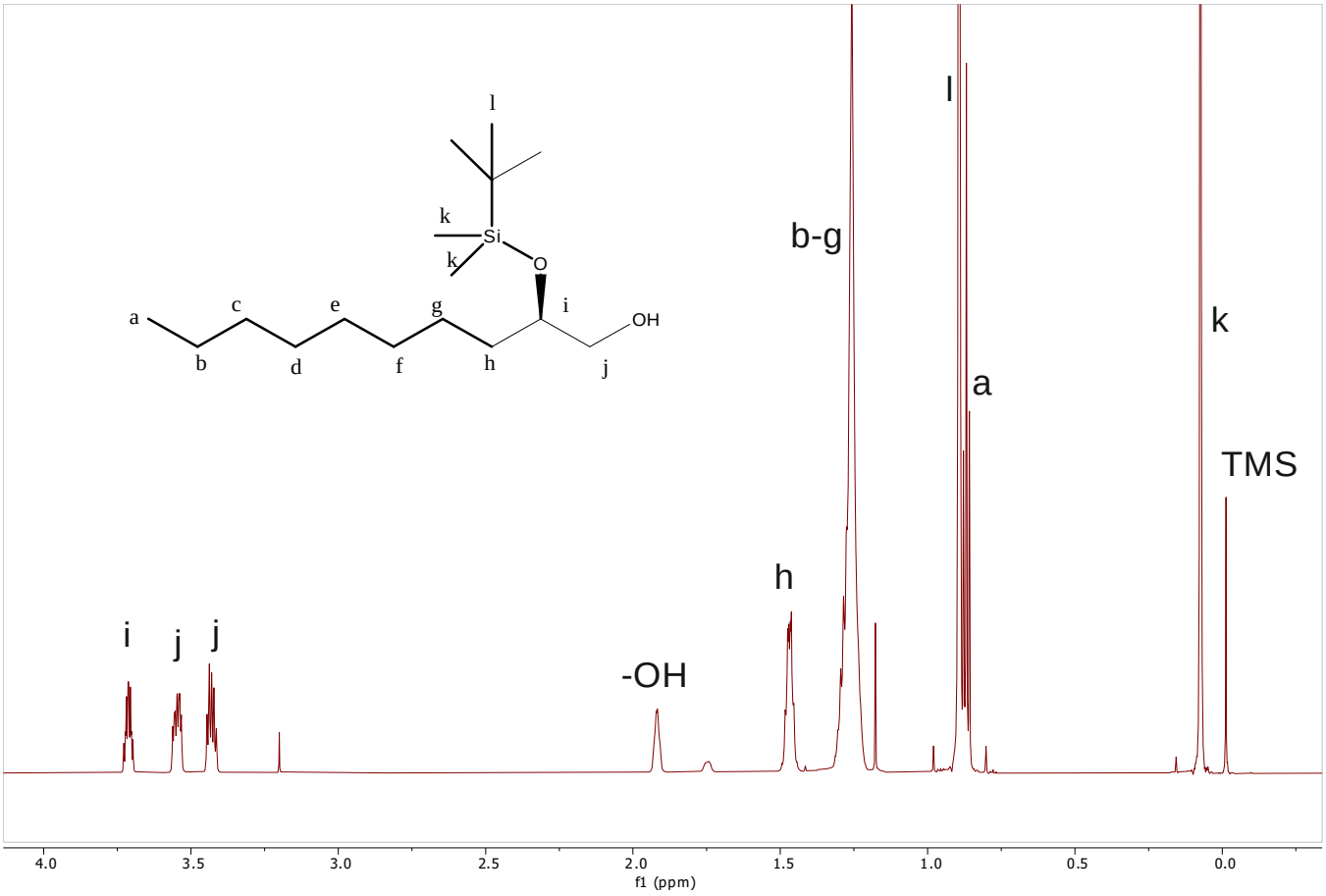


Figure S13. Compound **13:** *(S)-2-(tert-butyldimethylsilanyloxy)decan-1-ol*

^{13}C NMR (175 MHz $CDCl_3$)

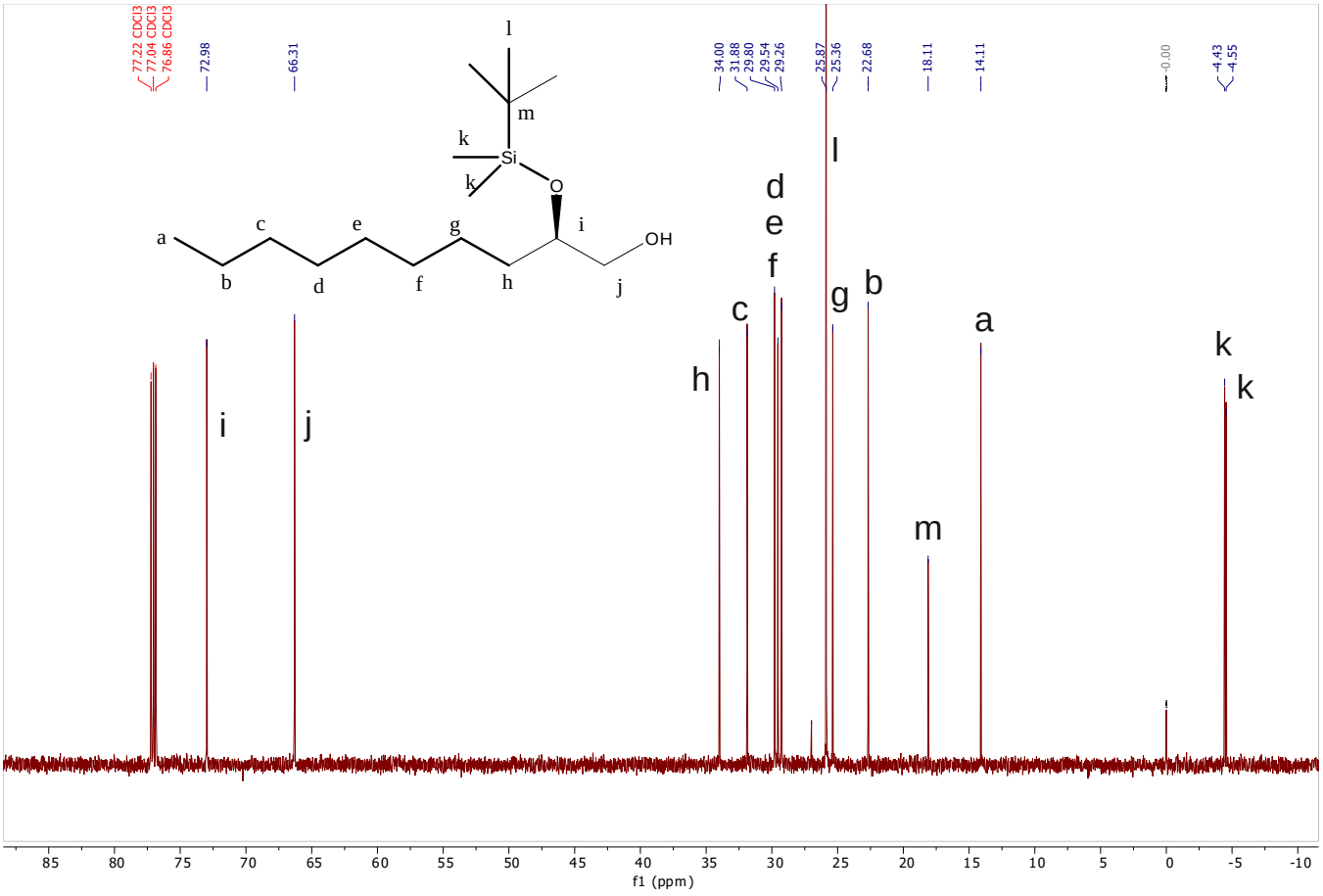


Figure S14. Compound **12:** *(S)-(1-benzyloxymethylnonyloxy)-tert-butyldimethylsilane*

^{1}H NMR (700 MHz $CDCl_3$)

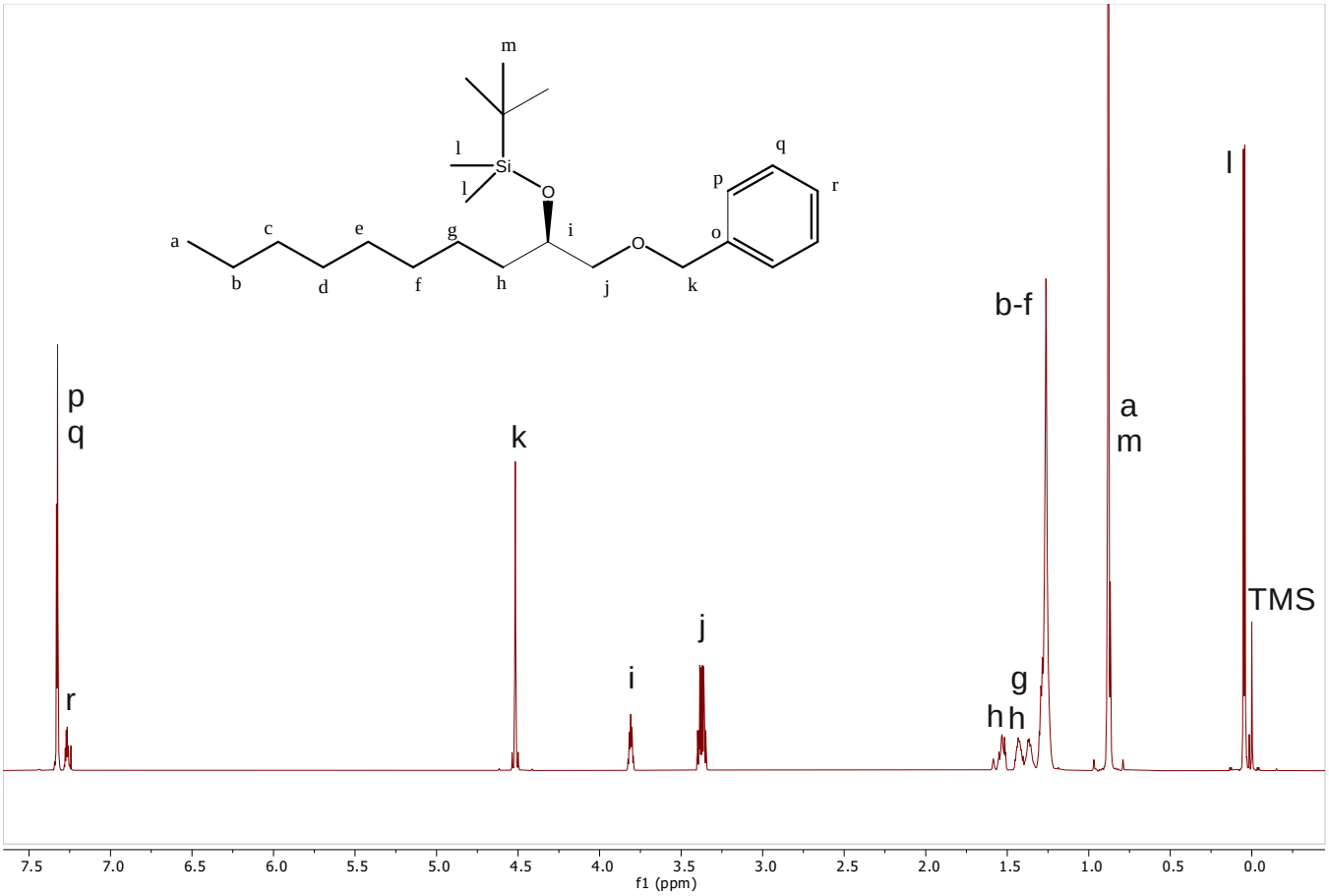


Figure S15. Compound **12:** *(S)-(1-benzyloxymethylnonyloxy)-tert-butyldimethylsilane*

^{13}C NMR (175 MHz $CDCl_3$)

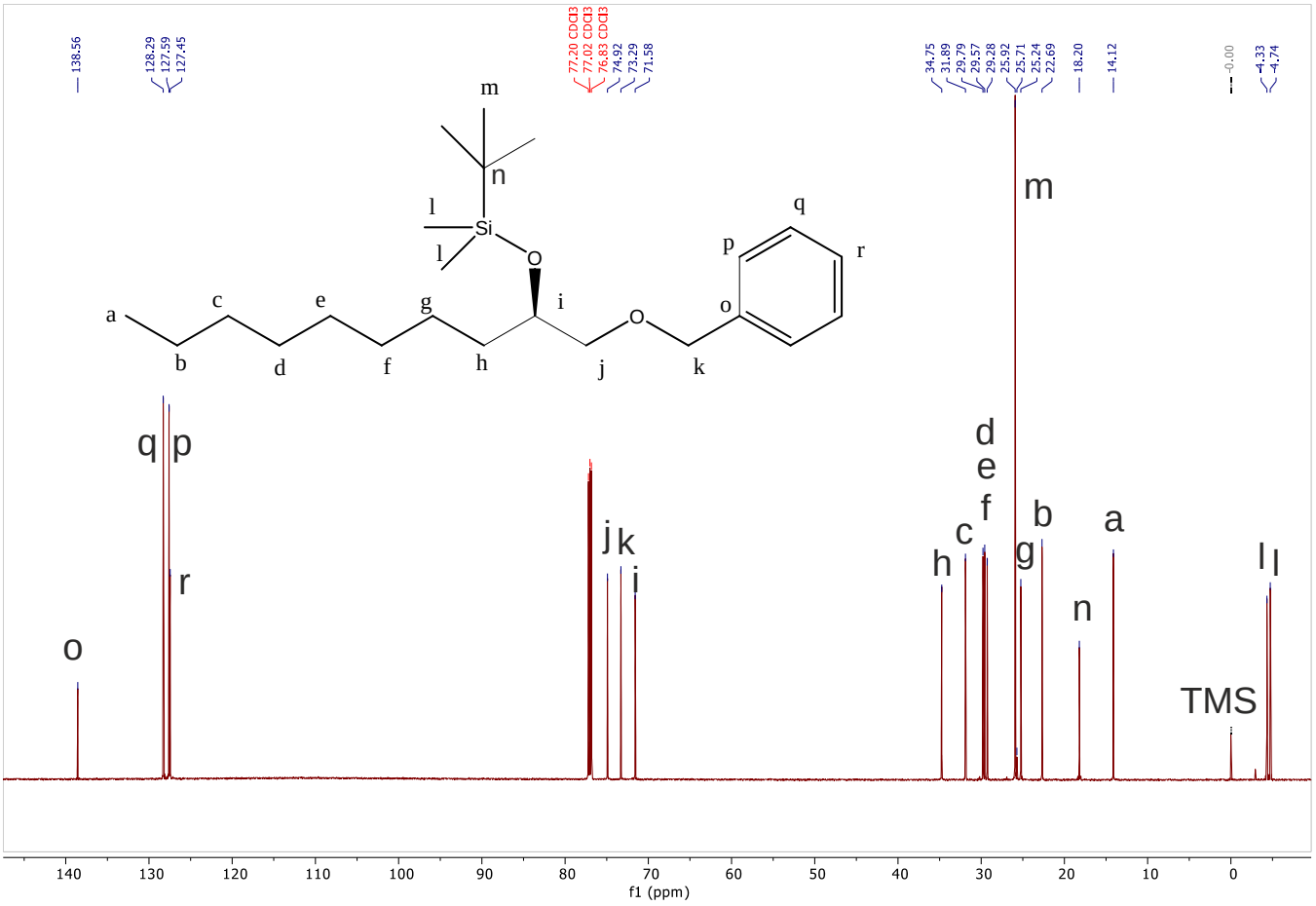


Figure S16. Compound **11:** *(S)-1-benzyloxydecan-2-ol*

^{1}H NMR (700 MHz $CDCl_3$)

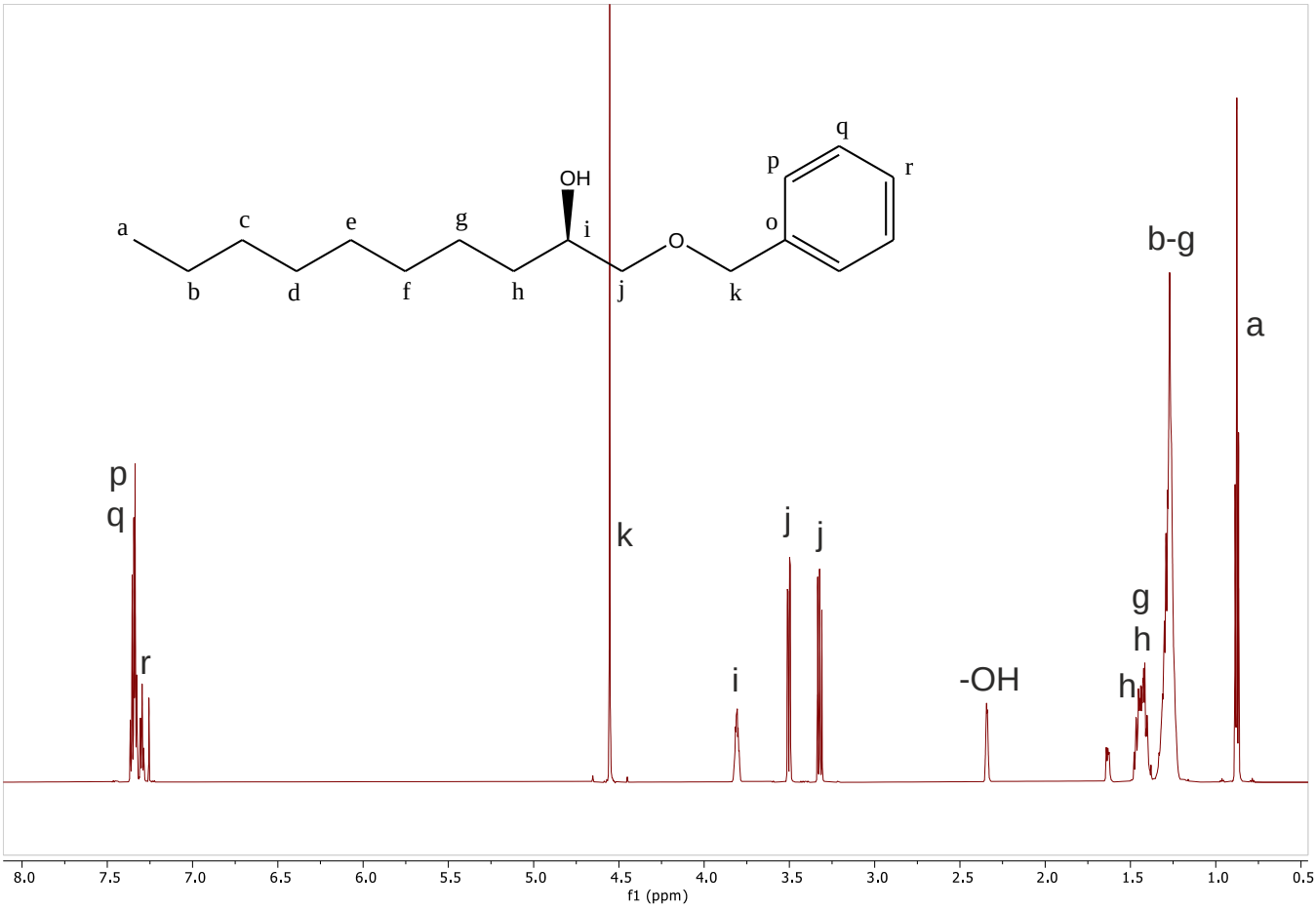
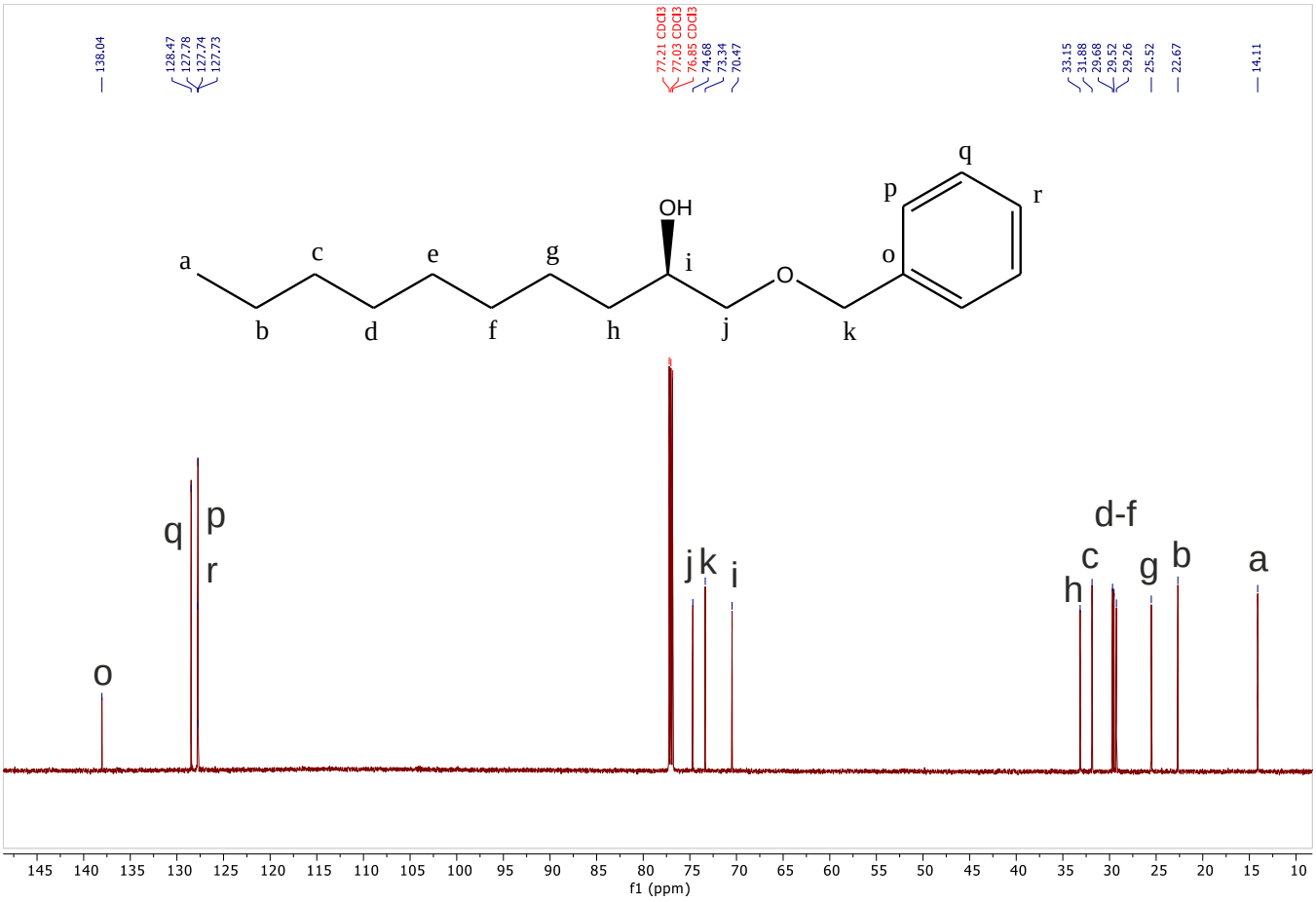


Figure S17. Compound **11:** *(S)-1-benzyloxydecan-2-ol*

^{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)*

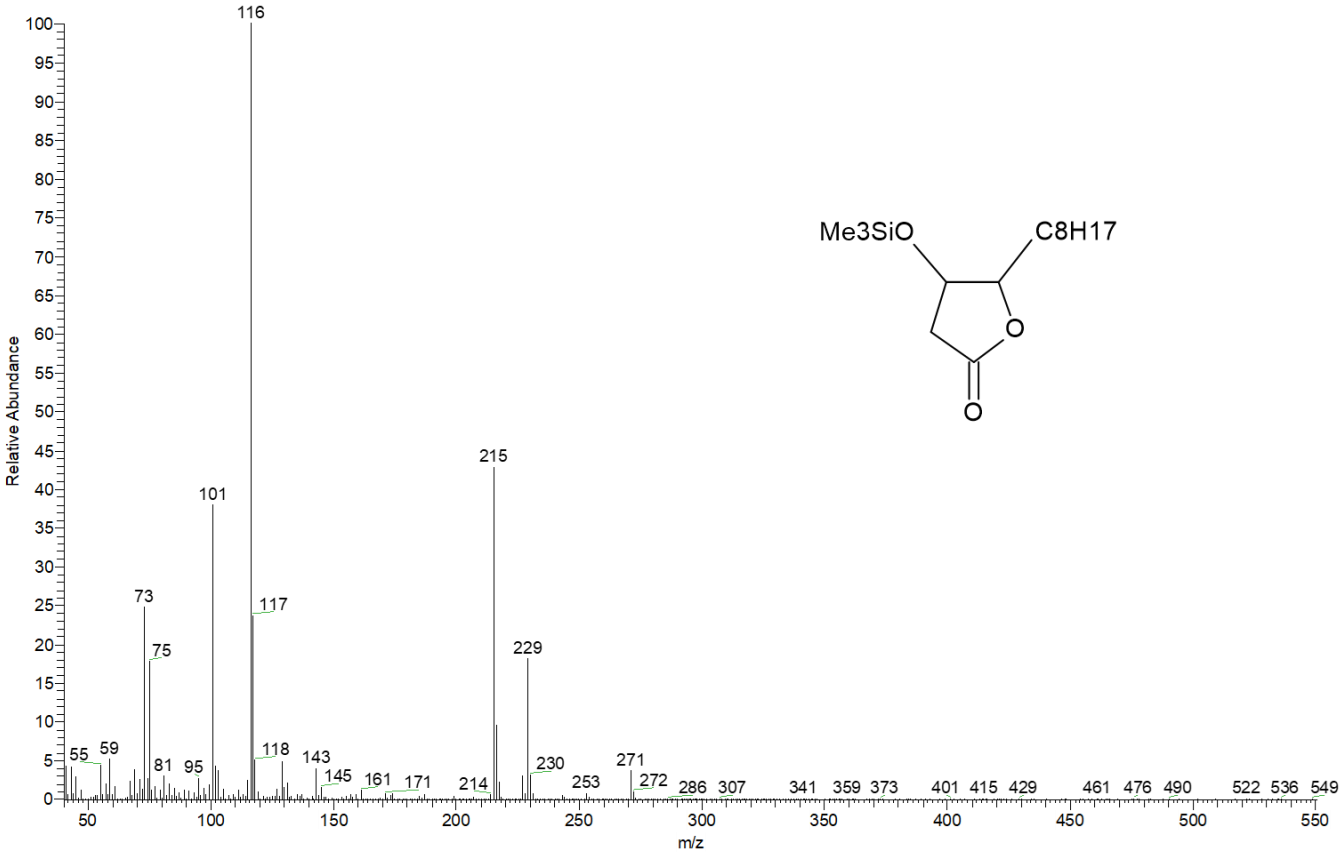


Figure S19. EI-mass spectrum of compound **3** (*4-(tert-butyldimethylsilyloxy)-5-octyldihydro-3H-furan-2-one)*

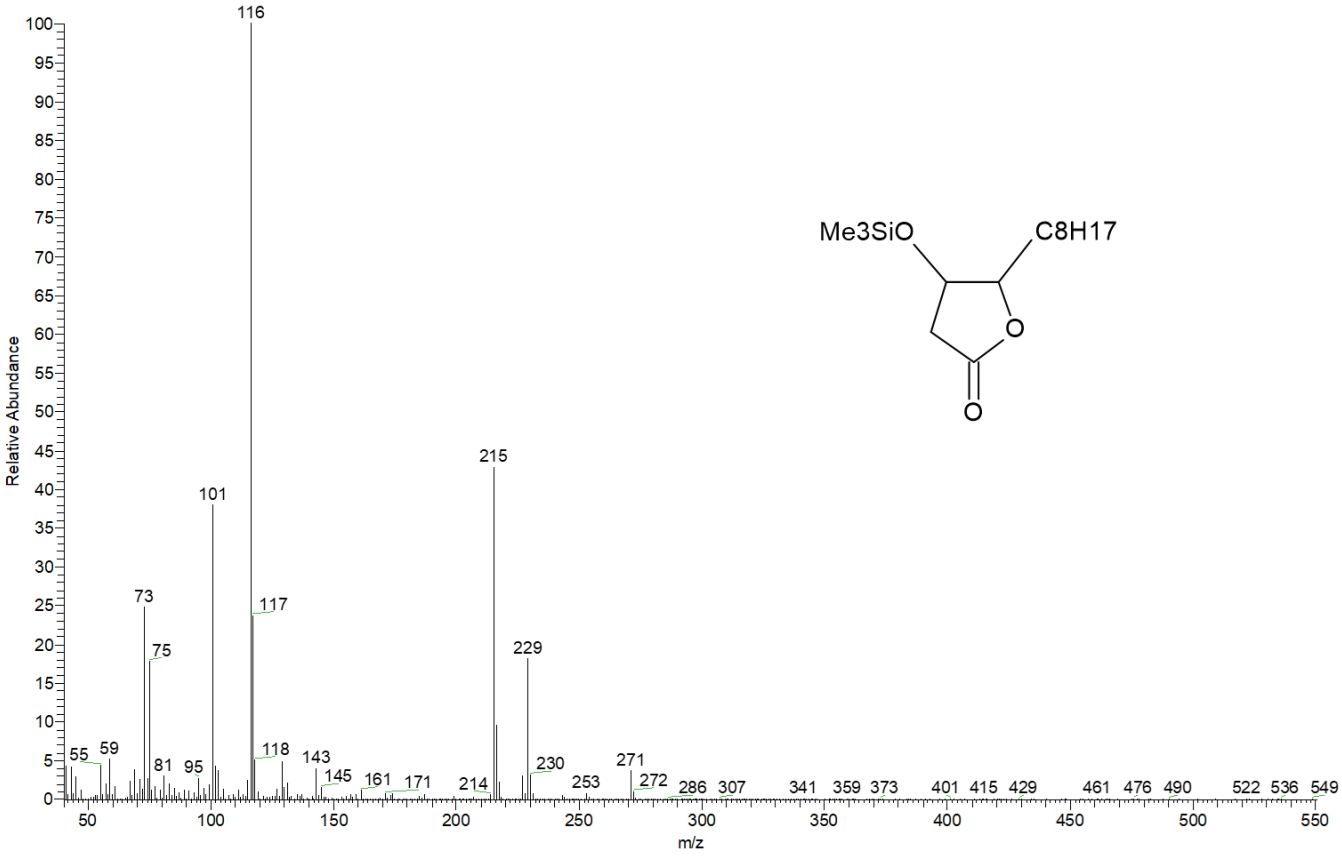


Figure S20. EI-mass spectrum of compound **5** (*trimethylsilyl 3,4-bis((trimethylsilyl)oxy)dodecanoate)*

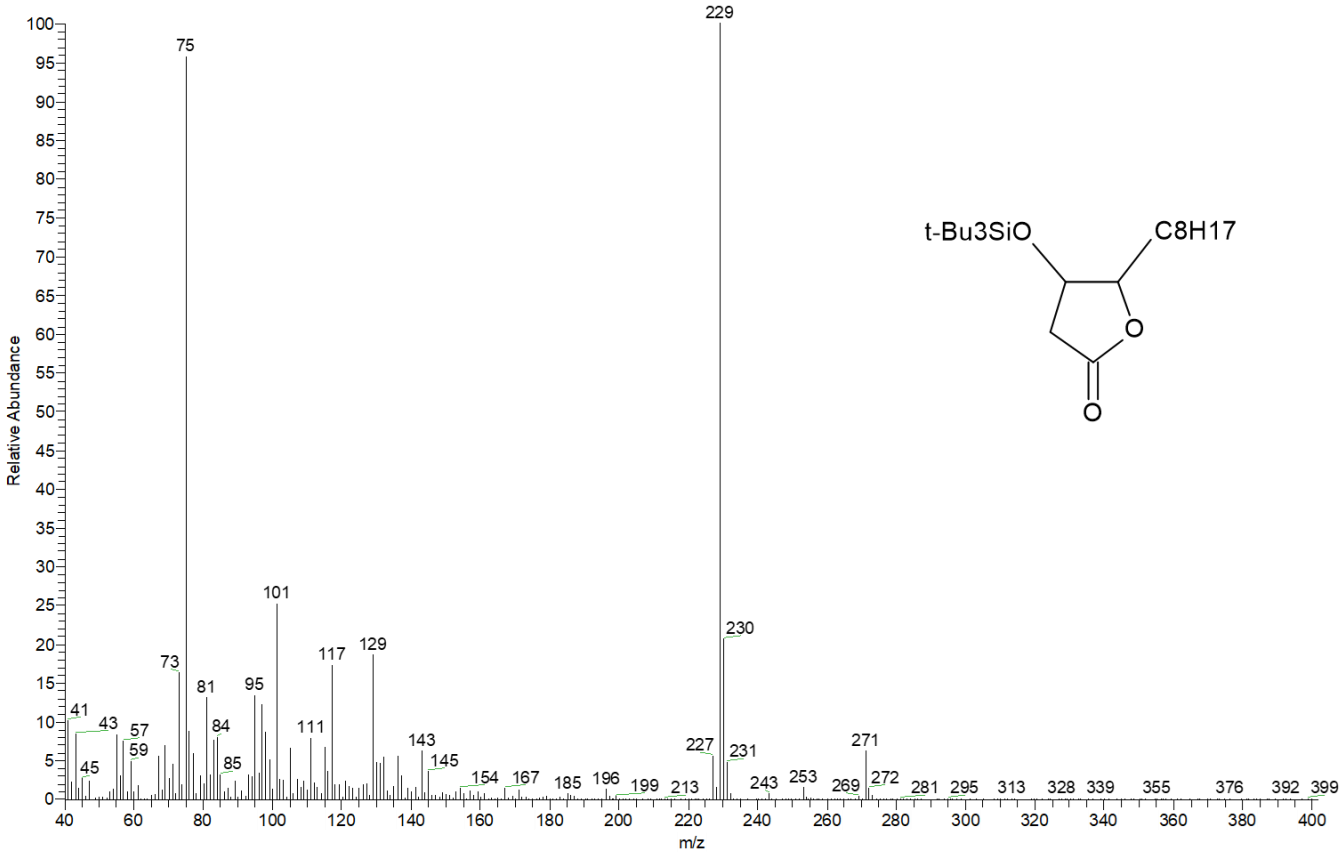


Figure S21. EI-mass spectrum of compound **6** (*methyl 3,4-bis((trimethylsilyl)oxy)dodecanoate)*

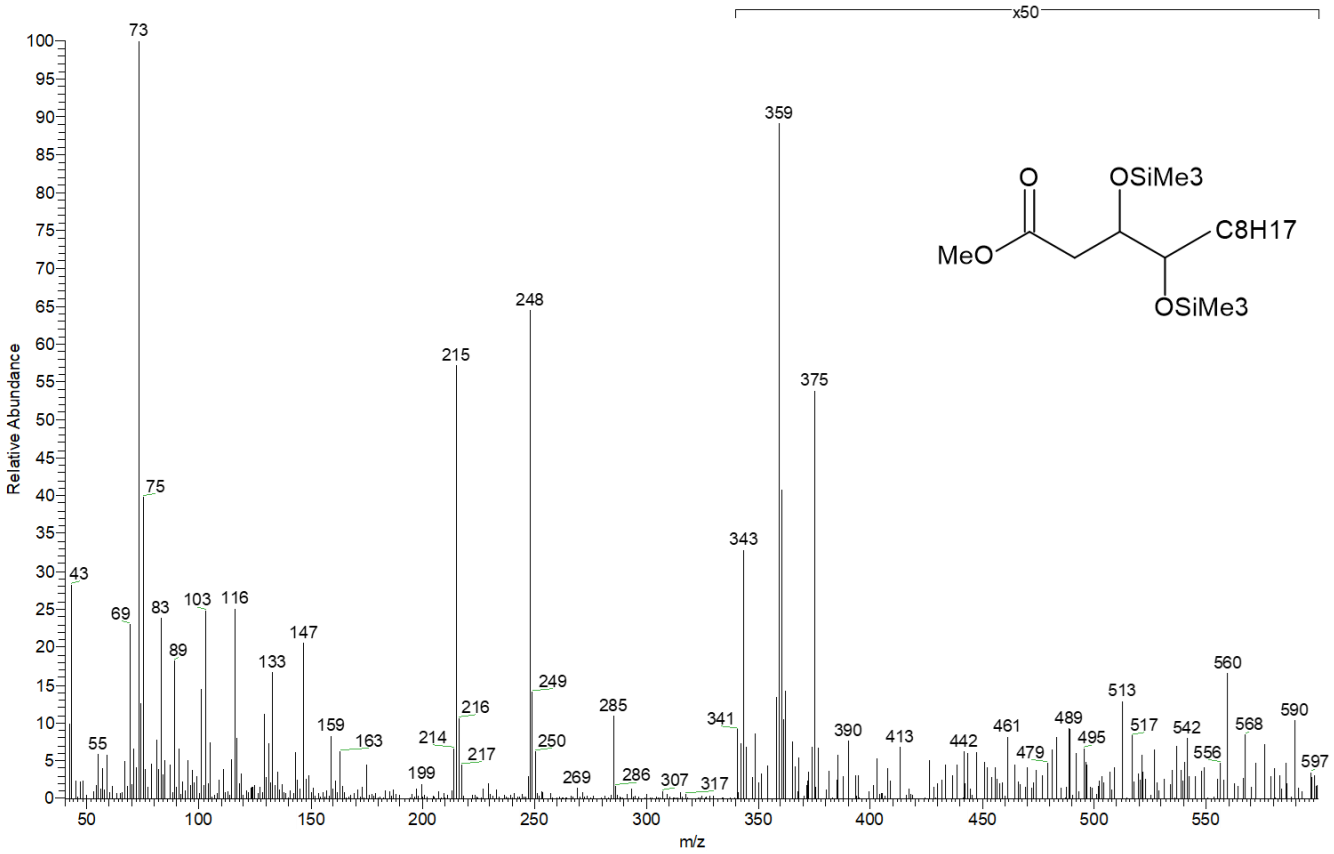


Figure S22. EI-mass spectrum of compound **8a** (*1,3,4-tris((trimethylsilyl)oxy)dodecane)*

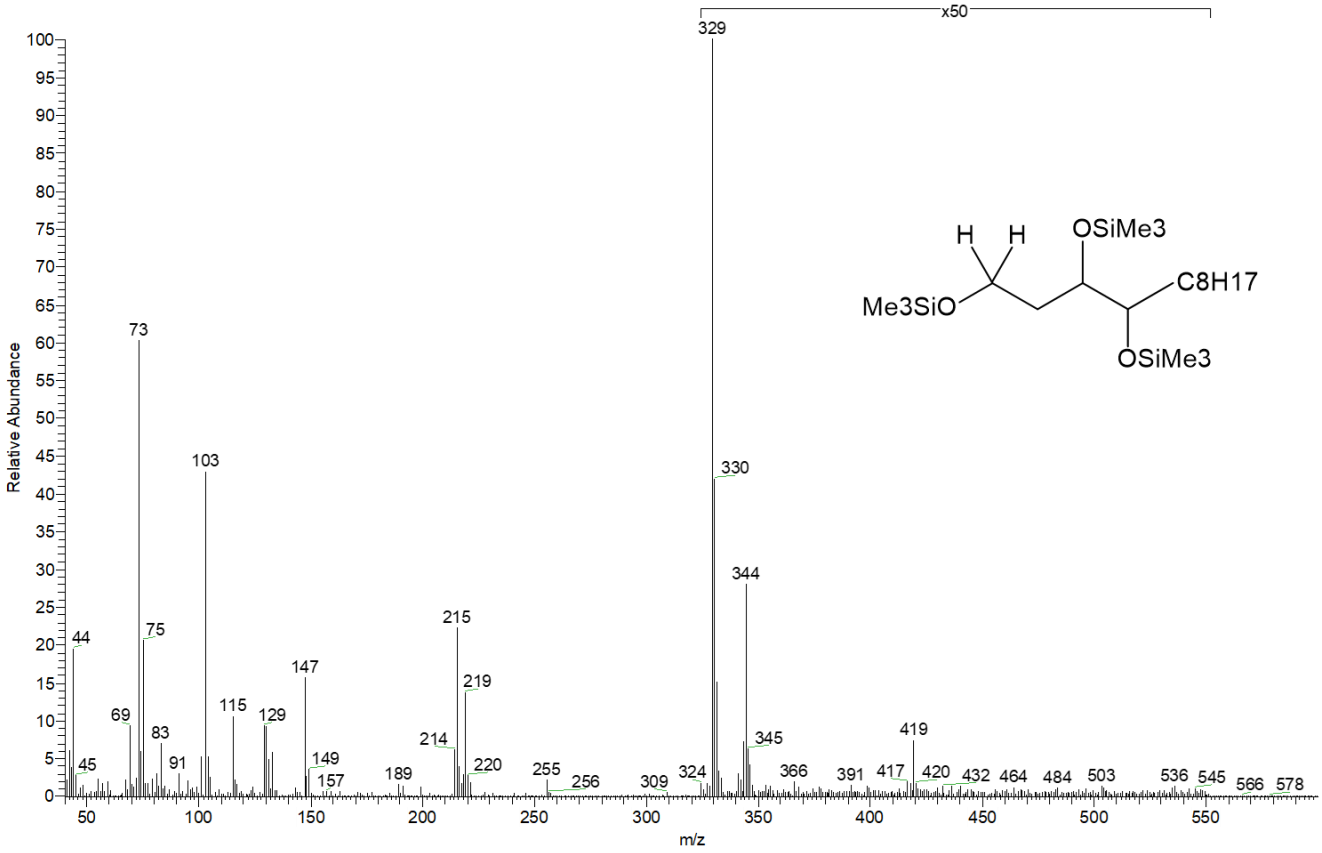
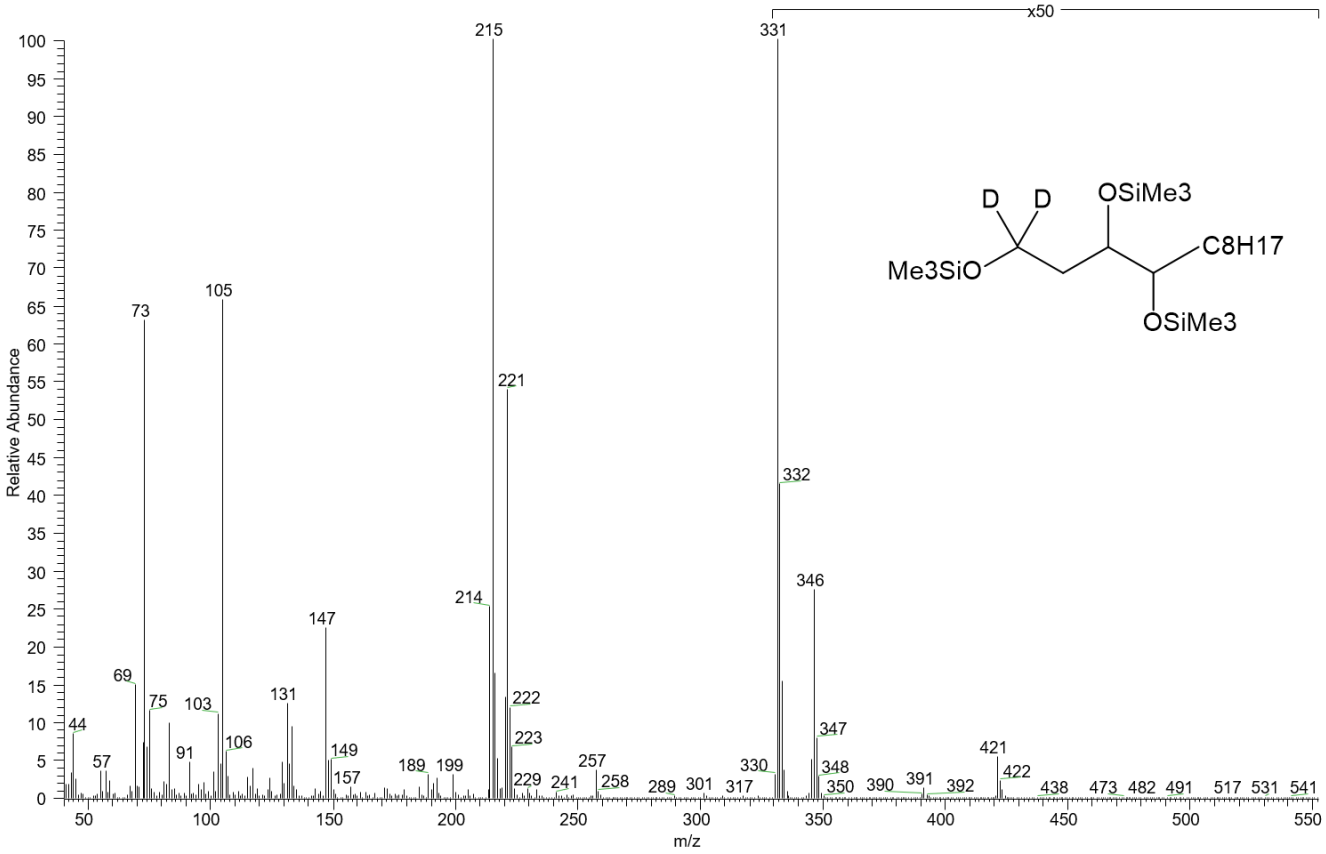


Figure S23. EI-mass spectrum of compound **8b** (*1,3,4-tris((trimethylsilyl)oxy)-1,1-dideuteriododecane)*



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=17.56-17.61min (=(*4S,5R*)-**1**)) and t_R=17.80-17.85min (=(*4S,5S*)-**1**). Cuticular hydrocarbons (CHCs) can be observed at longer retention times (from t_R=20.60 on). Major hydrocarbons are tricosane (t_R=21.49min), pentacosane (t_R=22.31min) and heptacosane (t_R=23.10min). The remaining CHCs are methyl-branched or/and unsaturated. The compound at t_R=23.53min (chromatogram below) is an artifact.

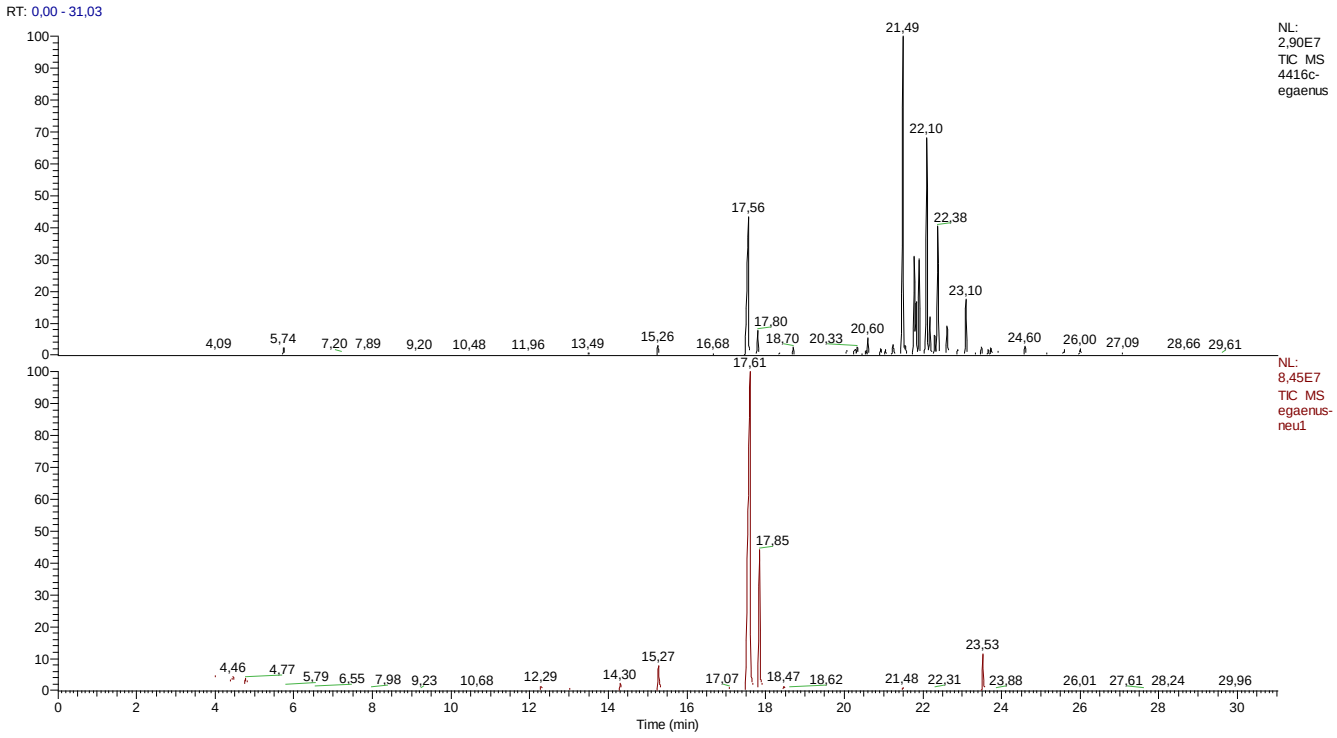


Figure S25.

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).

1H NMR (700MHz $CDCl_3$)