Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Unprecedented inhibition of resistant Penicillin Binding Proteins by *bis*-2-oxoazetidinyl macrocycles

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Supplementary Information

Experimental Section

General

Experiments were performed under argon atmosphere in flame-dried glassware. All solvents, including anhydrous solvents, and reagents were purchased from Acros Organics, Alfa Aesar, Fluka, Sigma-Aldrich or VWR, and used without any further purification. TLC analyses were performed on aluminum plates coated with silica gel $60F_{254}$ (Merck) and visualized with a $KMnO_4$ solution and UV (254 nm) detection, and column chromatography was performed on silica gel (40-63 or 63-200 μm) purchased from Rocc. Melting points (mp) were determined on a Büchi B-540 apparatus calibrated with caffeine, vanillin, and phenacetin. $[\alpha]_D$ was measured on Perkin-Elmer 241 MC or 343 polarimeter, at 20 °C, in $CHCl_3$ or CH_3OH. Concentrations are given in g/100 mL. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded at 300 MHz for proton and 75 MHz for carbon (Bruker Avance 300) or 500 MHz for proton and 125 MHz for carbon (Bruker Avance 500) using deuterated chloroform ($CDCl_3$). Chemical shifts are reported in parts per million relative to residual $CHCl_3$ in $CDCl_3$ (7.26 and 77.16 ppm). NMR coupling constants (*J*) are reported in hertz. Infrared (IR) spectra were recorded using FTIR-8400S Shimadzu apparatus. Products were analyzed as thin films deposited on a Se-Zn crystal by evaporation from CH_2Cl_2 solutions. High Resolution Mass Spectrometry (HRMS) analyses were performed at the University of Mons Hainaut (Belgium) or at the University College London (UK).

General procedure for the preparation of bis-acylated compounds 3

Trifluoroacetic acid (13.57 mmol) was added to **2** (0.68 mmol) dissolved in CH_2Cl_2 (7 mL) at 0 °C. The mixture was warmed to rt and stirred for 2 h. Concentration of the reaction solution afforded the crude trifluoroacetate salt as a viscous oil. Then alkenoyl chloride (1.02 mmol) was added to a stirred solution of the crude trifluoroacetate salt and triethylamine (2.04 mmol) in CH_2Cl_2 (5 mL) cooled at 0 °C. The mixture was then warmed to r.t. and stirred overnight. The mixture was then diluted with CH_2Cl_2 (25 mL), and sequentially washed with HCl 2 M solution (30 mL), saturated aqueous $NaHCO_3$ (30 mL), and brine (40 mL). After drying over $MgSO_4$ and removing the solvent under reduced pressure, the residue was purified by flash column chromatography (hexane/EtOAc 3/2), to provide **3** as a white solid.

3a, **3e** and **3i** have been described previously.[1]
The isolated yields and the spectral data for **3b-d**, **3f-h** are as follows.

N-[(3S)-2-oxo-1-(pent-4-enoyl)azetidin-3-yl]hex-5-enamide (3b). Yield: 83%; mp 101.5-102.2 °C; $[\alpha]_D$ +16.4 (*c* 2.9 in $CHCl_3$); IR: ν 3298, 3078-2844, 1798, 1780, 1693, 1652, 1541 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.68-1.78 (m, 2H), 2.04-2.12 (m, 2H), 2.21-2.26 (m, 2H), 2.37-2.44 (m, 2H), 2.79-2.86 (m, 2H), 3.67 (dd, *J* = 3.9, 7.4 Hz, 1H), 3.85 (m, 1H), 4.70 (td, *J* = 3.8, 7.0 Hz, 1H), 4.96-5.10 (m, 4H), 5.68-5.89 (m, 2H), 6.38 (d, *J* = 7.3 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 24.2, 27.9, 33.6, 35.0, 35.9, 44.9, 55.9, 115.7, 115.9, 136.5, 137.6, 164.8, 170.6, 173.6; MS (ESI) *m/z* (%) 551 $[2M + Na]^+$ (36), 287 $[M + Na]^+$ (100), 265 $[M + H]^+$ (12); HRMS (ESI) calcd. $C_{14}H_{20}N_2O_3Na$ $[M + Na]^+$ 287.1372, found 287.1380.

N-[(3S)-2-oxo-1-(pent-4-enoyl)azetidin-3-yl]hept-6-enamide (3c). Yield: 85%; mp 98.5-99.7 °C; $[\alpha]_D$ +15.3 (*c* 3.4 in $CHCl_3$); IR: ν 3313-3300, 2980-2856, 1798, 1697, 1663, 1641, 1526 cm^{-1}; ^1H NMR (300

[1] A. Sliwa, G. Dive, J.-L. Habib Jiwan, J. Marchand-Brynaert, *Tetrahedron*, 2010, **66**, 9519.

MHz, $CDCl_3$, 20 °C): δ 1.37-1.47 (m, 2H), 1.60-1.70 (m, 2H), 2.03-2.10 (m, 2H), 2.22-2.27 (m, 2H), 2.39-2.46 (m, 2H), 2.75-2.93 (m, 2H), 3.68 (dd, *J* = 3.9, 7.4 Hz, 1H), 3.87 (m, 1H), 4.71 (td, *J* = 3.8, 7.0 Hz, 1H), 4.93-5.11 (m, 4H), 5.71-5.90 (m, 2H), 6.09 (d, *J* = 7.0 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 24.8, 28.0, 28.5, 33.5, 35.8, 36.0, 45.1, 55.9, 115.0, 115.9, 136.5, 138.3, 164.7, 170.6, 173.6; MS (ESI) *m/z* (%) 579 $[2M + Na]^+$ (27), 301 $[M + Na]^+$ (100), 279 $[M + H]^+$ (31); HRMS (ESI) calcd. $C_{15}H_{22}N_2O_3Na$ $[M + Na]^+$ 301.1528, found 301.1534.

N-[(3S)-1-(hex-5-enoyl)-2-oxoazetidin-3-yl]pent-4-enamide (3d). Yield: 85%; mp 95.1-96.3 °C; $[\alpha]_D$ +17.8 (*c* 3.1 in $CHCl_3$); IR: ν 3300, 3080-2872, 1798, 1778, 1693, 1654, 1537 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.70-1.80 (m, 2H), 2.07-2.14 (m, 2H), 2.29-2.40 (m, 4H), 2.69-2.74 (td, *J* = 2.8, 7.4 Hz, 2H), 3.65 (dd, *J* = 3.9, 7.4 Hz, 1H), 3.84 (m, 1H), 4.70 (td, *J* = 3.9, 7.0 Hz, 1H), 4.95-5.09 (m, 4H), 5.70-5.86 (m, 2H), 6.41 (br s, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.2, 29.2, 33.1, 35.0, 36.0, 44.9, 55.8, 115.5, 116.2, 136.5, 137.7, 164.7, 171.3, 173.0; MS (ESI) *m/z* (%) 551 $[2M + Na]^+$ (85), 287 $[M + Na]^+$ (100), 265 $[M + H]^+$ (21); HRMS (ESI) calcd. $C_{14}H_{20}N_2O_3Na$ $[M + Na]^+$ 287.1372, found 287.1367.

N-[(3S)-1-(hex-5-enoyl)-2-oxoazetidin-3-yl]hept-6-enamide (3f). Yield: 82%; mp 85.8-86.5 °C; $[\alpha]_D$ +15.3 (*c* 3.3 in $CHCl_3$); IR: ν 3296, 3080-2854, 1797, 1780, 1693, 1651, 1537 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.36-1.46 (m, 2H), 1.59-1.69 (m, 2H), 1.71-1.80 (m, 2H), 2.02-2.15 (m, 4H), 2.21-2.26 (m, 2H), 2.69-2.75 (m, 2H), 3.66 (dd, *J* = 3.9, 7.4 Hz, 1H), 3.85 (m, 1H), 4.70 (td, *J* = 3.9, 7.0 Hz, 1H), 4.92-5.06 (m, 4H), 5.70-5.84 (m, 2H), 6.24 (d, *J* = 7.1 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.2, 24.8, 28.4, 33.1, 33.5, 35.8, 36.0, 45.0, 55.8, 115.0, 115.5, 137.7, 138.3, 164.7, 171.2, 173.6; MS (ESI) *m/z* (%) 607 $[2M + Na]^+$ (21), 315 $[M + Na]^+$ (100), 293 $[M + H]^+$ (24); HRMS (ESI) calcd. $C_{16}H_{24}N_2O_3Na$ $[M + Na]^+$ 315.1685, found 315.1684.

N-[(3S)-1-(hept-6-enoyl)-2-oxoazetidin-3-yl]pent-4-enamide (3g). Yield: 70%; mp 105.2-106.6 °C; $[\alpha]_D$ +17.3 (*c* 3.3 in $CHCl_3$); IR: ν 3296, 2978-2852, 1799, 1780, 1691, 1653, 1541 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.39-1.49 (m, 2H), 1.62-1.72 (m, 2H), 2.03-2.10 (m, 2H), 2.30-2.41 (m, 4H), 2.69-2.75 (m, 2H), 3.65 (dd, *J* = 3.9, 7.4 Hz, 1H), 3.85 (m, 1H), 4.71 (td, *J* = 3.9, 7.0 Hz, 1H), 4.91-5.10 (m, 4H), 5.71-5.87 (m, 2H), 6.30 (d, *J* = 7.0 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.5, 28.4, 29.2, 33.5, 35.1, 36.5, 45.0, 55.8, 114.8, 116.2, 136.5, 138.5, 164.7, 171.3, 173.0; MS (ESI) *m/z* (%) 579 $[2M + Na]^+$ (53), 301 $[M + Na]^+$ (100), 279 $[M + H]^+$ (22); HRMS (ESI) calcd. $C_{15}H_{22}N_2O_3Na$ $[M + Na]^+$ 301.1528, found 301.1530.

N-[(3S)-1-(hept-6-enoyl)-2-oxoazetidin-3-yl]hex-5-enamide (3h). Yield: 79%; mp 87.1-87.9 °C; $[\alpha]_D$ +14.1 (*c* 3.0 in $CHCl_3$); IR: ν 3296, 3065-2852, 1798, 1780, 1693, 1655, 1535 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.39-1.49 (m, 2H), 1.62-1.78 (m, 4H), 2.03-2.12 (m, 4H), 2.21-2.26 (m, 2H), 2.69-2.75 (m, 2H), 3.66 (dd, *J* = 3.9, 7.4 Hz, 1H), 3.85 (m, 1H), 4.70 (td, *J* = 3.9, 7.0 Hz, 1H), 4.91-5.04 (m, 4H), 5.68-5.85 (m, 2H), 6.26 (d, *J* = 7.0 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.5, 24.2, 28.4, 33.0, 33.5, 35.0, 36.5, 45.0, 55.8, 114.8, 115.7, 137.6, 138.4, 164.8, 171.3, 173.5; MS (ESI) *m/z* (%) 607 $[2M + Na]^+$ (100), 315 $[M + Na]^+$ (78), 293 $[M + H]^+$ (11); HRMS (ESI) calcd. $C_{16}H_{24}N_2O_3Na$ $[M + Na]^+$ 315.1685, found 315.1693.

General procedure of RCM for the preparation of bis-acylated compounds 4

Grubbs catalyst (second generation) (0.05 equiv) was added to a stirred solution of **3** (1 equiv) in dry CH_2Cl_2 (5 mM) and the solution was stirred at reflux under argon for 4 h. Then a second addition of Grubbs catalyst (0.05 equiv) was made and the mixture was additionally stirred at reflux for 20 h. Then the solvent was removed under reduced pressure and the crude product was purified thrice by column chromatography (EtOAc/MeOH 98/2), to provide **4** as pale-brown oil.

4e and **4i** have been described previously.[1]

The isolated yields and the spectral data for **4b-d**, **4f-h** are as follows.

Non-symmetrical cyclic dimer (4b). Yield: 35%; $[\alpha]_D$ -44.7 (*c* 1.0 in $CHCl_3$); IR: ν 3350-3255, 2983-2925, 1796, 1744, 1724, 1720, 1709, 1668, 1537, 1444 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 20 °C): δ 1.61-1.81 (m, 4H), 1.98-2.50 (m, 12H), 2.64-3.16 (m, 4H), 3.48-3.69 (m, 2H), 3.77-3.97 (m, 2H), 4.71-5.11 (m, 2H), 5.29-5.52 (m, 4H), 6.80-7.11 and 7.35-7.48 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.9, 24.3, 27.2, 27.4, 31.0, 31.7, 34.1, 35.0, 35.8, 36.0, 44.9, 45.5, 55.3, 129.2, 129.7, 130.2, 130.5, 130.7, 130.8, 165.4, 165.6, 171.2, 171.5, 173.7, 174.0; MS (ESI) *m/z* (%) 495 $[M + Na]^+$ (100), 481 $[M + Na - CH_2]^+$ (16), 473 $[M + H]^+$ (50), 459 $[M + H - CH_2]^+$ (8), 445 $[M + H - CO]^+$ (11); HRMS (ESI) calcd. $C_{24}H_{32}N_4O_6Na$ $[M + Na]^+$ 495.2220, found 495.2221.

Non-symmetrical cyclic dimer (4c). Yield: 33%; $[\alpha]_D$ -10.5 (*c* 2.0 in $CHCl_3$); IR: ν 3284, 2981, 1780, 1693, 1512 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 20 °C): δ 1.24-1.39 (m, 4H), 1.54-1.62 (m, 4H), 1.99-2.08 (m, 4H), 2.21-2.36 (m, 8H), 2.67-2.93 (m, 4H), 3.53-3.64 (m, 2H), 3.82-3.95 (m, 2H), 4.83-5.01 (m, 2H), 5.34-5.41 (m, 4H), 6.92-7.13 (m, 2H); ^{13}C (125 MHz, $CDCl_3$, 20 °C): δ 22.4, 24.3, 24.6, 25.1, 26.2, 26.8, 27.4, 27.9, 28.4, 28.9, 29.5, 29.8, 31.7, 31.8, 35.4, 35.9, 36.1, 36.4, 45.2, 45.6, 55.5, 55.6, 127.9, 128.7, 129.8, 128.9, 130.7, 131.3, 131.8, 165.3, 165.5, 171.1, 171.4, 173.9, 174.0; MS (ESI) *m/z* (%) 523 $[M + Na]^+$ (100); HRMS (ESI) calcd. $C_{26}H_{36}N_4O_6Na$ $[M + Na]^+$ 523.2533, found 523.2513.

Non-symmetrical cyclic dimer (4d). Yield: 21%; $[\alpha]_D$ -43.0 (*c* 1.1 in $CHCl_3$); IR: ν 3390-3258, 2962-2858, 1796, 1745, 1734, 1712-1647, 1539, 1437 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 20 °C): δ 1.56-2.44 (m, 16H), 2.55-3.05 (m, 4H), 3.39-3.67 (m, 2H), 3.77-3.92 (m, 2H), 4.83-5.15 (m, 2H), 5.29-5.60 (m, 4H), 6.82-7.21 and 7.30-7.40 (m, 2H); ^{13}C (125 MHz, $CDCl_3$, 20 °C): δ 22.5, 23.0, 23.1, 24.0, 26.0, 26.1, 28.1, 28.3, 31.0, 31.2, 34.7, 35.0, 35.1, 35.9, 36.4, 36.8, 44.8, 45.3, 45.7, 55.2, 55.4, 55.5, 55.7, 129.0, 130.0, 130.2, 130.5, 130.8, 130.9, 131.0, 165.2, 165.3, 171.3, 171.5, 173.5, 173.6; MS (ESI) *m/z* (%) 495 $[M + Na]^+$ (100), 481 $[M + Na - CH_2]^+$ (17), 473 $[M + H]^+$ (9), 445 $[M + H - CO]^+$ (9); HRMS (ESI) calcd. $C_{24}H_{32}N_4O_6Na$ $[M + Na]^+$ 495.2220, found 495.2221.

Non-symmetrical cyclic dimer (4f). Yield: 57%; $[\alpha]_D$ -11.6 (*c* 1.0 in $CHCl_3$); IR: ν 3408-3231, 2960-2833, 1789, 1720, 1693, 1674, 1531, 1456 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.24-1.81 (m, 12H), 1.95-2.34 (m, 12H), 2.56-2.77 (m, 4H), 3.48-3.69 (m, 2H), 3.79-3.93 (m, 2H), 4.65-4.99 (m, 2H), 5.23-5.47 (m, 4H), 6.82-7.24 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 22.4, 22.9, 24.3, 24.7, 26.4, 28.3, 28.8, 31.3, 31.4, 31.6, 31.9, 35.1, 35.2, 35.7, 35.9, 36.1, 36.4, 44.8, 45.3, 55.2, 55.3, 129.3, 129.9, 130.8, 131.9, 165.2, 165.5, 171.4, 171.7, 174.2, 174.4; MS (ESI) *m/z* (%) 551 $[M + Na]^+$ (100), 537 $[M + Na - CH_2]^+$ (25), 529 $[M + H]^+$ (9); HRMS (ESI) calcd. $C_{28}H_{40}N_4O_6Na$ $[M + Na]^+$ 551.2846, found 551.2851.

Non-symmetrical cyclic dimer (4g). Yield: 41%; $[\alpha]_D$ -3.8 (*c* 3.6 in $CHCl_3$); IR: ν 3310, 2924, 1794, 1689, 1529 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.24-1.43 (m, 4H), 1.54-1.66 (m, 4H), 1.96-2.14 (m, 4H), 2.20-3.07 (m, 12H), 3.41-3.89 (m, 4H), 4.67-5.14 (m, 2H), 5.35-5.61 (m, 4H), 6.64-7.20 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.0, 23.4, 27.6, 27.7, 28.1, 28.2, 28.4, 28.5, 29.8, 31.4, 31.8, 35.3, 36.1, 36.8, 45.0, 45.3, 55.2, 55.6, 129.3, 129.9, 130.7, 131.2, 132.0, 165.2, 171.7, 171.8, 171.9, 173.3, 173.4; MS (ESI) *m/z* (%) 523 $[M + Na]^+$ (100), 509 $[M + Na - CH_2]^+$ (42), 501 $[M + H]^+$ (39), 487 $[M + H - CH_2]^+$ (21), 473 $[M + H - CO]^+$ (21), 459 $[M + H - CH_2 - CO]^+$ (8); HRMS (ESI) calcd. $C_{26}H_{36}N_4O_6Na$ $[M + Na]^+$ 523.2533, found 523.2533.

Non-symmetrical cyclic dimer (4h). Yield: 31%; $[\alpha]_D$ -5.2 (*c* 1.0 in $CHCl_3$); IR: ν 3389-3221, 2986-2885, 1796, 1747, 1720, 1697, 1683, 1539, 1521 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 20 °C): δ 1.35-1.87 (m, 12H), 1.96-2.31 (m, 12H), 2.59-2.79 (m, 4H), 3.43-3.68 (m, 2H), 3.75-3.89 (m, 2H), 4.63-4.98 (m, 1H), 5.18-5.49 (m, 5H), 6.57-7.18 and 7.30-7.53 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 22.6, 23.6, 26.1, 26.7, 27.9, 28.1, 31.1, 31.4, 32.0, 34.1, 34.5, 36.2, 36.4, 36.6, 45.4, 55.0, 130.1, 130.5, 130.7, 131.9, 165.7, 172.2, 173.3; MS (ESI) *m/z* (%) 551 $[M + Na]^+$ (100), 537 $[M + Na - CH_2]^+$ (45), 529 $[M + H]^+$ (32), 515 $[M + H - CH_2]^+$ (13), 501 $[M + H - CO]^+$ (23); HRMS (ESI) calcd. $C_{28}H_{40}N_4O_6Na$ $[M + Na]^+$ 551.2846, found 551.2826.

General Procedure for Hydrogenation

To a stirred solution of compound obtained by RCM (1 eq.) in methanol (0.03 M) was added 10% Pd/C (0.05 eq.). After being stirred under hydrogen atmosphere (P = 1 atm) for 3 h at room temperature, the mixture was filtered through a short pad of Celite and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/MeOH 98/2), to provide products as colourless oils.

5e and **5i** have been described previously.[1]

The isolated yields and the spectral data for **5b-d**, **5f-h** are as follows.

Non-symmetrical cyclic dimer (5b). Yield: 48%; $[\alpha]_D$ -26.1 (*c* 1.0 in $CHCl_3$); IR: ν 3294, 2924, 1791, 1742, 1720, 1705, 1647, 1533, 1435 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 20 °C): δ 1.23-1.40 (m, 12H), 1.58-1.80 (m, 8H), 2.18-2.29 (m, 4H), 2.52-2.66 (m, 2H), 2.76-2.92 (m, 2H), 3.56-3.67 (m, 2H), 3.64-3.95 (m, 2H), 4.89-5.03 (m, 2H), 6.92-7.14 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.4, 24.3, 24.5, 24.9, 26.8, 27.1, 27.2, 27.3, 27.4, 27.5, 27.7, 35.4, 35.6, 35.7, 36.2, 45.2, 45.6, 55.5, 165.2, 165.3, 171.8, 172.1, 174.1, 174.2; MS (ESI) *m/z* (%) 499 $[M + Na]^+$ (100), 485 $[M + Na - CH_2]^+$ (15), 477 $[M + H]^+$ (7); HRMS (ESI) calcd. $C_{24}H_{36}N_4O_6Na$ $[M + Na]^+$ 499.2533, found 499.2516.

Non-symmetrical cyclic dimer (5c). Yield: 52%; $[\alpha]_D$ +7.9 (*c* 1.6 in $CHCl_3$); IR: ν 3287, 2923, 1796, 1726, 1693, 1672, 1535 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.27-1.32 (m, 16H), 1.62-1.74 (m, 8H), 2.22-2.27 (m, 4H), 2.54-2.85 (m, 4H), 3.57-3.69 (m, 2H), 3.85-3.98 (m, 2H), 4.76-4.81 (m, 1H), 4.92-4.98 (m, 1H), 6.64-6.85 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.9, 24.2, 25.0, 27.4, 27.7, 28.1, 28.2, 28.6, 35.5, 35.9, 36.0, 36.3, 45.1, 45.7, 55.6, 55.8, 165.1, 165.2, 171.8, 172.1, 174.0, 174.2; MS (ESI) *m/z* (%) 527 $[M + Na]^+$ (100), 505 $[M + H]^+$ (11); HRMS (ESI) calcd. $C_{26}H_{40}N_4O_6Na$ $[M + Na]^+$ 527.2846, found 527.2864.

Non-symmetrical cyclic dimer (5d). Yield: 22%; $[\alpha]_D$ -17.7 (*c* 0.6 in $CHCl_3$); IR: ν 3263, 2924, 1796, 1747, 1731, 1718, 1705, 1647, 1541, 1437 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.26-1.47 (m, 10H), 1.56-1.87 (m, 10H), 2.21-2.32 (m, 4H), 2.49-2.67 (m, 2H), 2.80-3.01 (m, 2H), 3.57-3.68 (m, 2H), 3.84-3.94 (m, 2H), 4.82-5.20 (m, 2H), 6.50-7.09 (m, 2H); ^{13}C (125 MHz, $CDCl_3$, 20 °C): δ 23.3, 23.5, 23.6, 23.8, 23.9, 24.1, 24.3, 24.4, 24.5, 24.6, 26.5, 26.7, 26.8, 26.9, 27.0, 27.0, 27.1, 27.2, 27.3, 27.4, 27.5, 27.7, 35.2, 35.4, 35.6, 35.7, 35.8, 36.1, 45.3, 45.5, 55.4, 55.5, 55.6, 55.7, 165.1, 165.2, 165.3, 171.8, 173.8, 173.9, 174.0, 174.1; MS (ESI) *m/z* (%) 499 $[M + Na]^+$ (100), 485 $[M + Na - CH_2]^+$ (17), 477 $[M + H]^+$ (10), 449 $[M + H - CO]^+$ (7); HRMS (ESI) calcd. $C_{24}H_{36}N_4O_6Na$ $[M + Na]^+$ 499.2533, found 499.2518.

Non-symmetrical cyclic dimer (5f). Yield: 63%; $[\alpha]_D$ -17.3 (*c* 1.0 in $CHCl_3$); IR: ν 3369-3258, 2935-2850, 1790, 1745, 1731, 1724, 1710, 1649, 1533, 1456 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.20-1.39 (m, 20H), 1.52-1.76 (m, 8H), 2.18-2.27 (m, 4H), 2.52-2.85 (m, 4H), 3.58-3.68 (m, 2H), 3.80-3.92 (m, 2H), 4.66-4.88 (m, 2H), 6.81-6.99 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.8, 24.9, 25.0, 27.9, 28.0, 28.1, 28.2, 28.3, 28.4, 35.7, 35.8, 36.2, 36.4, 45.2, 45.3, 55.7, 165.0, 165.1, 171.8, 174.2; MS (ESI) *m/z* (%) 555 $[M + Na]^+$ (100), 541 $[M + Na - CH_2]^+$ (27), 533 $[M + H]^+$ (20); HRMS (ESI) calcd. $C_{28}H_{44}N_4O_6Na$ $[M + Na]^+$ 555.3159, found 555.3134.

Non-symmetrical cyclic dimer (5g). Yield: 60%; $[\alpha]_D$ -23.9 (*c* 1 in $CHCl_3$); IR: ν 3355, 2938, 1794, 1776, 1716, 1682, 1535 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.21-1.41 (m, 16H), 1.52-1.77 (m, 8H), 2.21-2.26 (m, 4H), 2.51-2.84 (m, 4H), 3.61-3.73 (m, 2H), 3.83-3.89 (m, 4H), 4.71-4.92 (m, 2H), 6.86-7.08 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.9, 24.5, 24.7, 24.9, 25.0, 27.7, 27.8, 28.0, 28.1, 28.2, 35.7, 35.9, 36.3, 45.1, 45.3, 55.6, 55.8, 165.2, 171.8, 174.1, 174.2; MS (ESI) *m/z* (%) 527 $[M + Na]^+$ (100), 513 $[M + Na - CH_2]^+$ (72), 505 $[M + H]^+$ (14); HRMS (ESI) calcd. $C_{26}H_{40}N_4O_6Na$ $[M + Na]^+$ 527.2846, found 527.2874.

Non-symmetrical cyclic dimer (5h). Yield: 36%; $[\alpha]_D$ -18.5 (*c* 0.5 in $CHCl_3$); IR: ν 3362-3223, 2924-2860, 1794, 1695, 1683, 1535, 1461 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 1.20-1.43 (m, 20H), 1.57-1.77 (m, 8H), 2.21-2.30 (m, 4H), 2.53-2.68 (m, 2H), 2.75-2.83 (m, 2H), 3.62-3.69 (m, 2H), 3.84-3.92 (m, 2H), 4.73-4.90 (m, 2H), 6.40-6.61 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 23.8, 23.9, 24.8, 24.9, 27.7, 27.9, 28.0, 28.1, 28.2, 28.3, 35.8, 36.4, 45.3, 55.7, 55.8, 165.0, 171.8, 171.9, 174.0, 174.1; MS (ESI) *m/z* (%) 555 $[M + Na]^+$ (100), 541 $[M + Na - CH_2]^+$ (47), 533 $[M + H]^+$ (7); HRMS (ESI) calcd. $C_{28}H_{44}N_4O_6Na$ $[M + Na]^+$ 555.3159, found 555.3162.

N-[(3S)-2-oxo-1-(pent-4-enoyl)azetidin-3-yl]heptanamide (6). Following the general procedure for the preparation of bis-acylated compounds, **6** was obtained as a white solid. Yield: 80%; mp 115.7-116.5 °C; $[\alpha]_D$ +18.2 (*c* 1.5 in $CHCl_3$); IR: ν 3294-3288, 2951-2867, 1797, 1780, 1693, 1651, 1539 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 0.84-0.88 (m, 3H), 1.23-1.43 (m, 6H), 1.55-1.65 (m, 2H), 2.19-2.25 (m, 2H), 2.37-2.44 (m, 2H), 2.73-2.88 (m, 2H), 3.67 (dd, *J* = 3.8, 7.4 Hz, 1H), 3.85 (m, 1H), 4.69 (td, *J* = 3.8, 6.9 Hz, 1H), 4.97-5.10 (m, 2H), 5.75-5.89 (m, 1H), 6.37 (br d, *J* = 7.3 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 14.1, 22.6, 25.3, 28.0, 28.9, 31.6, 36.0, 45.0, 55.9, 115.9, 136.5, 164.8, 170.6, 173.8; MS (ESI) *m/z* (%) 303 $[M + Na]^+$ (31), 281 $[M + H]^+$ (100), 253 $[M + H - CO]^+$ (17); HRMS (ESI) calcd. $C_{15}H_{24}N_2O_3Na$ $[M + Na]^+$ 303.1685, found 303.1681.

General procedure of CM for the preparation of non-cyclic dimers

Grubbs catalyst (second generation) (0.05 equiv) was added to a stirred solution of **6** or **9** (1 equiv) in dry CH_2Cl_2 (10 mM) and the solution was stirred at reflux under argon for 4 h. Then a second addition of Grubbs catalyst (0.05 equiv) was made and the mixture was additionally stirred at reflux for 8 h. Then the solvent was removed under reduced pressure and the crude product was purified thrice by column chromatography (hexane/EtOAc 2/8), to provide **7** or **10** as a pale-brown solid.

N-[(3S)-1-{8-[(3S)-3-heptanamido-2-oxoazetidin-1-yl]-8-oxooct-4-enoyl}-2-oxoazetidin-3-yl]heptanamide (7). Yield : 77%; mp 135.4-136.2 °C; $[\alpha]_D$ +3.3 (*c* 1.0 in $CHCl_3$); IR: ν 3310, 2920, 1798, 1724, 1688, 1666, 1529 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 0.85-0.89 (m, 6H), 1.28-1.35 (m, 12H), 1.57-1.67 (m, 4H), 2.20-2.25 (m, 4H), 2.33-2.39 (m, 4H), 2.72-2.86 (m, 4H), 3.60 (m, 2H), 3.90 (m, 2H), 4.88-4.94 (m, 2H), 5.46 (m, 2H), 6.53 and 6.63 (2 br d, *J* = 7.5 Hz, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 14.1, 22.6, 25.4, 27.1, 29.0, 31.6, 36.0, 36.1, 45.5, 55.6, 129.2, 165.2, 171.0, 173.8; MS (ESI) *m/z* (%) 555 $[M + Na]^+$ (100), 533 $[M + H]^+$ (7); HRMS (ESI) calcd. $C_{28}H_{44}N_4O_6Na$ $[M + Na]^+$ 555.3159, found 555.3157.

N-[(3S)-1-{8-[(3S)-3-heptanamido-2-oxoazetidin-1-yl]-8-oxooctanoyl}-2-oxoazetidin-3-yl]heptanamide (8). Following the general procedure for hydrogenation, compound **8** was obtained as a pale-brown solid. Yield: 60%; mp 145.8-146.4 °C; $[\alpha]_D$ +6.4 (*c* 2.0 in $CHCl_3$); IR: ν 3283, 2930, 1789, 1697, 1650, 1547 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 0.84-0.88 (m, 6H), 1.27-1.36 (m, 16H), 1.56-1.65 (m, 8H), 2.19-2.26 (m, 4H), 2.63-2.76 (m, 4H), 3.60-3.65 (m, 2H), 3.84-3.90 (m, 2H), 4.78-4.85 (m, 2H), 6.56-6.70 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 14.1, 22.6, 23.9, 25.4, 28.5, 29.0, 31.6, 36.0, 36.3, 45.2, 55.7, 165.0, 171.5, 174.0; MS (ESI) *m/z* (%) 557 $[M + Na]^+$ (100); HRMS (ESI) calcd. $C_{28}H_{46}N_4O_6Na$ 557.3315, found 557.3323.

tert-butyl N-[(3S)-2-oxo-1-pentanoylazetidin-3-yl]carbamate. To a stirred solution of **1** (553 mg, 2.97 mmol) in dry CH_2Cl_2 (25 mL) were added pyridine (0.48 mL, 5.94 mmol) and valeroyl chloride (0.71 mL, 5.94 mmol). The mixture was stirred for 12 h at rt and then diluted with CH_2Cl_2 (30 mL) and the organic layer was washed with HCl 2 M solution (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL). After drying over $MgSO_4$ and removing the solvent under reduced pressure, the residue was purified by flash column chromatography (hexane/EtOAc 4/1), to provide the product as a white solid (321 mg, 40%). mp 114.8-115.3 °C; $[\alpha]_D$ +10.1 (*c* 1.0 in $CHCl_3$); IR: ν 3362, 2976-2933, 1797, 1780, 1688, 1537 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 0.91 (t, *J* = 7.4Hz, 3H), 1.33-1.43 (m, 11H), 1.64 (m, 2H), 2.72 (m, 2H), 3.64 (dd, *J* = 3.8, 7.4 Hz, 1H), 3.86 (m, 1H), 4.45 and 4.68 (2 br s, 1H, rotamers), 5.17 (br d, *J* = 7.5Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 13.9, 22.4, 26.1, 28.3, 36.5, 45.6, 56.6, 81.2, 154.7, 165.1, 171.5; MS (ESI) *m/z* (%) 293 $[M + Na]^+$ (100), 279 $[M + H]^+$ (8); HRMS (ESI) calcd. $C_{13}H_{21}N_2O_4$ $[M - H]^+$ 269.1501, found 269.1494.

N-[(3S)-2-oxo-1-pentanoylazetidin-3-yl]hept-6-enamide (9). Following the general procedure for the preparation of bis-acylated compounds, starting from tert-butyl N-[(3S)-2-oxo-1-pentanoylazetidin-3-yl]carbamate, compound **9** was obtained as a white solid. Yield: 89%; mp 101.0-101.9 °C; $[\alpha]_D$ +15.8 (*c* 1.0 in $CHCl_3$); IR: ν 3282, 2959-2923, 1780, 1728, 1718, 1693, 1655, 1537 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 20 °C): δ 0.91 (t, *J* = 7.3 Hz, 3H), 1.31-1.46 (m, 4H), 1.59-1.69 (m, 4H), 2.02-2.09 (m, 2H), 2.24 (t, *J* = 7.6 Hz, 2H), 2.68-2.74 (m, 2H), 3.66 (dd, *J* = 3.9, 7.4 Hz, 1H), 3.85 (m, 1H), 4.71 (td, *J* = 3.8, 6.8

Hz, 1H), 4.93-5.02 (m, 2H), 5.70-5.84 (m, 1H), 6.21 (br d, *J* = 7.0 Hz, 1H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 13.9, 22.4, 24.8, 26.2, 28.5, 33.5, 35.8, 36.4, 45.0, 55.8, 115.0, 138.3, 164.7, 171.5, 173.6; MS (ESI) *m/z* (%) 303 $[M + Na]^+$ (100), 281 $[M + H]^+$ (65), 253 $[M + H - CO]^+$ (22); HRMS (ESI) calcd. $C_{15}H_{24}N_2O_3Na$ $[M + Na]^+$ 303.1685, found 303.1670.

N,N'-bis[(3S)-2-oxo-1-pentanoylazetidin-3-yl]dodec-6-enediamide (10). Following the general procedure for CM, compound **10** was obtained as a pale-brown solid. Yield: 74%; mp 146.1-146.8 °C; $[\alpha]_D$ +5.7 (*c* 1.0 in $CHCl_3$); IR: ν 3292, 2962-2868, 1796, 1693, 1676, 1656, 1537 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ 0.91 (t, *J* = 7.4 Hz, 6H), 1.24-1.43 (m, 8H), 1.58-1.72 (m, 8H), 2.00 (m, 4H), 2.20-2.25 (m, 4H), 2.68-2.73 (m, 4H), 3.63-3.67 (m, 2H), 3.82-3.87 (m, 2H), 4.70 (m, 2H), 5.34 (m, 2H), 6.54-7.03 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 13.9, 22.4, 24.7, 26.2, 28.7, 32.0, 34.3, 35.9, 36.4, 44.9, 55.8, 130.1, 130.5, 131.3, 164.8, 165.0, 171.6, 174.0, 174.1; MS (ESI) *m/z* (%) 555 $[M + Na]^+$ (100), 541 $[M + Na - CH_2]^+$ (57); HRMS (ESI) calcd. $C_{28}H_{44}N_4O_6Na$ $[M + Na]^+$ 555.3159, found 555.3160.

N,N'-bis[(3S)-2-oxo-1-pentanoylazetidin-3-yl]dodecanediamide (11). Following the general procedure for hydrogenation, compound **11** was obtained as a pale-brown solid. Yield: 82%; mp 165.4-166.3 °C; $[\alpha]_D$ +12.9 (*c* 1.0 in $CHCl_3$); IR: ν 3300, 2930-2849, 1780, 1730, 1693, 1654, 1539 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ 0.91 (t, *J* = 7.3 Hz, 6H), 1.26-1.42 (m, 16H), 1.58-1.68 (m, 8H), 2.19-2.24 (m, 4H), 2.67-2.72 (m, 4H), 3.63-3.67 (m, 2H), 3.82-3.86 (m, 2H), 4.67-4.73 (m, 2H), 6.53-6.70 (m, 2H); ^{13}C (75 MHz, $CDCl_3$, 20 °C): δ 13.9, 22.3, 25.2, 25.3, 26.2, 27.8, 28.8, 29.0, 29.1, 35.8, 35.9, 36.4, 44.9, 55.8, 165.0, 171.6, 174.1; MS (ESI) *m/z* (%) 557 $[M + Na]^+$ (100), 541 $[M + Na - CH_2]^+$ (70); HRMS (ESI) calcd. $C_{28}H_{46}N_4O_6Na$ $[M + Na]^+$ 557.3315, found 557.3314.

Computational chemistry

All the calculations have been performed with the Gaussian03 suite of programs.[2] The B3LYP/6-31G(d) optimization of the dimers has been made with redundant internal coordinates in order to ensure a rapid convergence of each component of the gradient lower than 2.10-6 a.u. For the localization of the TS at the RHF/MINI-1' level, 3N-6 variables of the Z-matrix have been used. The eigenvector components associated to the negative eigenvalue of the Hessian matrix well combine the variables describing the pseudo 8-membered ring of the H transfer from Ser49 to the nitrogen of the β-lactam.

Acyl-enzymes modeling

The crystal structure of R39 complexed with a monobactam (unpublished results) was used as a template to model the acyl-enzyme between R39 and compound **5e**. The interactions made by the compound amide group with Asn300 side chain on one side, and with the backbone carbonyl of Thr413 on the other side were preserved. The remaining dihedral angles were adapted to avoid steric clashes between the compound and the protein. A similar procedure was used to model the acyl-enzyme between PBP2a and **5e**.

[2] Gaussian 03, Revision D.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

Biochemical evaluation

Assay with resistant PBPs

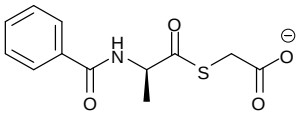
Purified PBP5 from *Enterococcus faecium* D63r and PBP2a from methicillin-resistant *Staphylococcus aureus* ATCC 43300 were used as target proteins to test inhibitory activity of synthesized β-lactams. Each of the purified PBPs (2.5 µM) were first incubated with 1 mM potential inhibitor in 100 mM phosphate buffer, 0.01% Triton X-100,[3] pH 7, for 4 h at 30 °C. Then, 25 µM fluorescein-labeled ampicillin was added to detect the residual penicillin binding activity (RA). The samples were further incubated for 30 min at 37 °C in a total volume of 20 µL. Denaturation buffer was added (0.1 M Tris/HCl, pH 6.8, containing 25% glycerol, 2% SDS, 20% β-mercaptoethanol and 0.02% bromophenol blue) and the samples were heated to 100 °C for 1 min. The samples were then loaded onto a 10% SDS-acrylamide gel (10 x 7 cm) and electrophoresis was performed for 45 min at 180 V (12 mA). Detection and quantification of the RAs were done with Molecular Image FX equipment and Quantity One software (BioRad, Hercules, CA, USA). Three independent experiments were carried out for each inhibitor.

[3] B. Y. Feng, B. K. Shoichet, *Nat. Protoc.* 2006, **1**, 550.

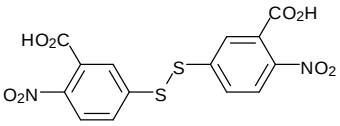
Assay with R39

All assays with R39 have been done in microtiter plates 96-wells (Brand, Wertheim, Germany). 20 mM of the tested compounds have been solved in DMF. Finally 7.5 µL of the solution have been used in the assay. The final concentration of the compounds in the assays was 100 µM. The final concentration of DMF in the assays was 0.25%. R39 (3.5 nM) was incubated in the presence of the potential inhibitors in 10 mM sodium phosphate buffer (pH 7.2) with 100 mM NaCl, 100 mM D-alanine, 0.01 mg/ml BSA and 0.01% Triton for 60 min at 25 °C. This preincubation was realized, in order to detect also slow binding inhibitors. After the preincubation the residual activity RA of R39 was determined by observing the hydrolysis of the thiolester S2d substrate (*i.e.* N-benzoyl-D-Alanyl-thioglycolate), in the presence of DTNB, catalyzed by the non-inhibited enzyme. The initial rate of hydrolysis of 1 mM S2d in the presence of 1 mM DTNB was determined by monitoring the increase of absorbance at 412 nm (DTNB: $\varepsilon[\Delta\varepsilon] = 13600\ M^{-1}\ s^{-1}$) using a microplate absorbance reader (Power Wave X, Biotek Instruments, Winooski, U.S.A.). The rate of spontaneous hydrolysis of S2d in the presence of the inhibitors was also determined in absence of R39. All assays have been done three times. The determination of RA of R39 in absence of inhibitors has been done six times on each plate. In order to detect false positives which could be slow binding non-competitive promiscuous inhibitors, the assays have been done in the presence of 0.01% Triton-X-100. [3,4]

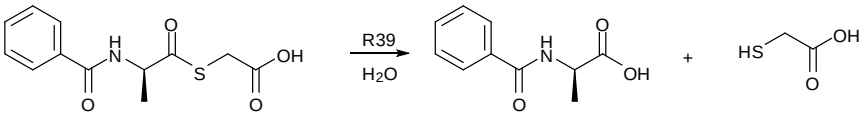
N-benzoyl-D-alanyl-thioglycolate (S2d):



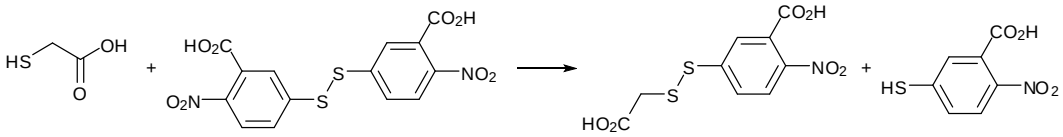
5-(3-Carboxy-4-nitrophenyl)disulfanyl-2-nitrobenzoic acid (DTNB):



Hydrolysis of the thiolester S2d substrate by R39:



Formed thiol reacts with DTNB, affording 2-nitro-5-thiobenzoate, which ionizes in water at neutral and alkaline pH. This ion has a yellow color and is quantified by measuring the absorbance:



[4] B. K. Shoichet, *Drug Discovery Today* 2006, **11**, 607.

Absolute energies of the precursors in the selected conformations

Compound	Absolute Energy of Open Precursors (a.u.)			
	i	*ii*	*iii*	*iv*
3a	-841.345666490	-841.344443521	-841.353210506	-841.352690676
3b	-880.656567829	-880.653009341	-880.663916481	-880.661489845
3c	-919.971821898	-919.970354436	-919.979335270	-919.978698126
3d	-880.656474399	-880.655707513	-880.664624120	-880.664457265
3e	-919.971291898	-919.968972012	-919.978967831	-919.977524714
3f	-959.284718263	-959.283092592	-959.292076753	-959.291873437
3g	-919.971522432	-919.970387861	-919.979169246	-919.979055387
3h	-959.285225983	-959.283693496	-959.292508879	-959.292301476
3i	-998.596454880	-998.595138399	-998.604239590	-998.603651658

Absolute energies of HH and HT unsaturated and saturated dimers

Unsaturated Dimer	Saturated Dimer	Absolute Energy of HH Unsaturated Dimer (a.u.)	Absolute Energy of HT Unsaturated Dimer (a.u.)	Absolute Energy of HH Saturated Dimer (a.u.)	Absolute Energy of HT Saturated Dimer (a.u.)
4b	**5b**	-1604.15438264	-1604.14594029	-1606.61653396	-1606.60549081
4c	**5c**	-1682.76433472	-1682.78664441	-1685.22705340	-1685.24077746
4d	**5d**	-1604.12946450	-1604.15983383	-1606.59401209	-1606.61375676
4e	**5e**	-1682.78001976	-1682.79530381	-1685.23800902	-1685.25186658
4f	**5f**	-1761.40880740	-1761.39708436	-1763.86072030	-1763.84755629
4g	**5g**	-1682.75924146	-1682.78188747	-1685.21531345	-1685.23860424
4h	**5h**	-1761.39571506	-1761.39276448	-1763.85536192	-1763.85467780
4i	**5i**	-1840.03971085	-1840.03584304	-1842.49592968	-1842.49350722

Absolute and relative energies of the precursors of linear dimers in the selected conformations

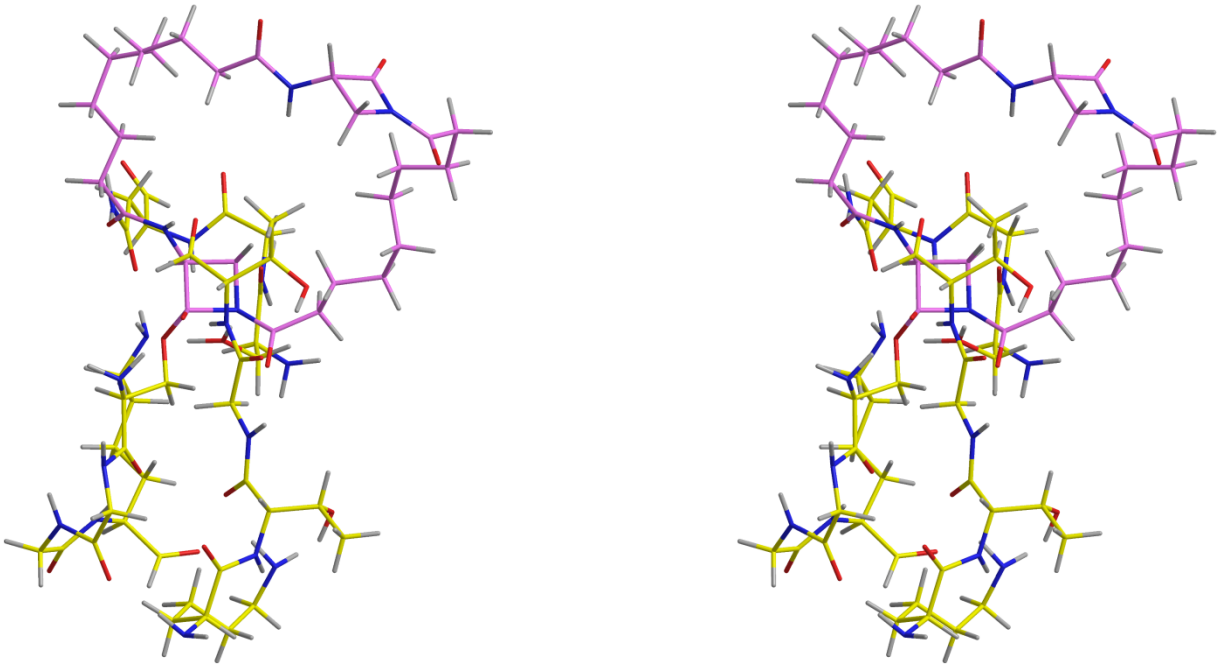
Geometry	Absolute Energy of **6** (a.u.)	Relative Energy of **6** (kcal/mol)	Absolute Energy of **9** (a.u.)	Relative Energy of **9** (kcal/mol)
i	-921.208638229	4.93	-921.209144878	4.56
ii	-921.207482127	5.66	-921.207458348	5.62
iii	-921.216498978	0.00	-921.216415416	0.00
iv	-921.215812176	0.43	-921.216085773	0.21

Absolute, relative energies and heat of formation of linear dimers 7 and 10

Compound	Absolute Energy (a.u.)	Relative Energy (kcal/mol)	Heat of Formation (kcal/mol)
7	-1763.84693322	0.00	0.15
10	-1763.84628580	0.41	0.45

The heats of formation of the dimers have been calculated with respect to the most stable conformation of their respective open precursor which is the *iii* conformation.

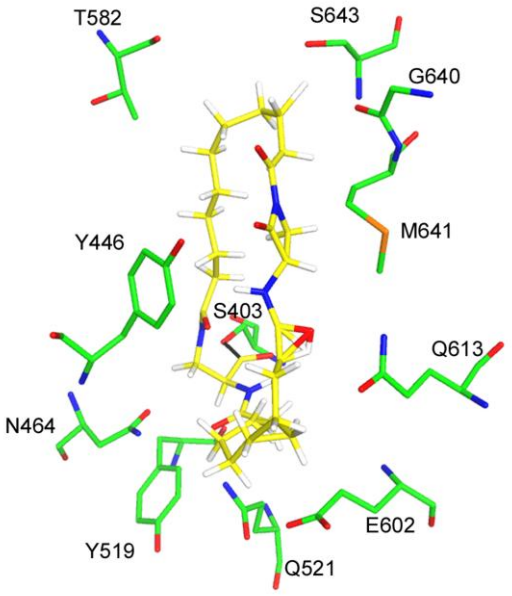
Stereo view of compound 5e in the model of R39 active site



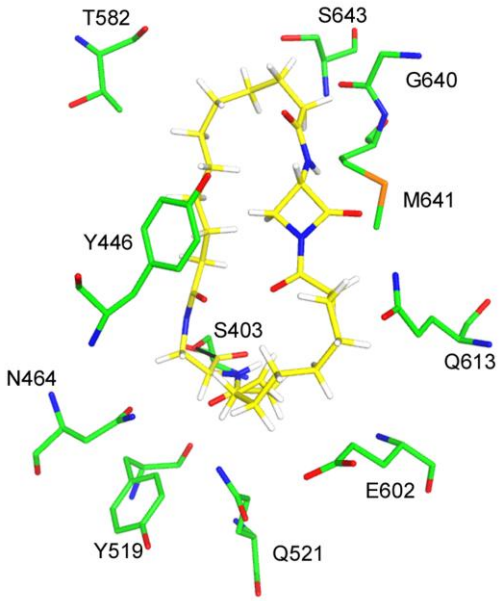
Legend: C of **5e** are purple, others in yellow, H are in grey, O in red, N in blue

Molecule 5f docked as an acyl-enzyme complex in PBP2a cavity

a)



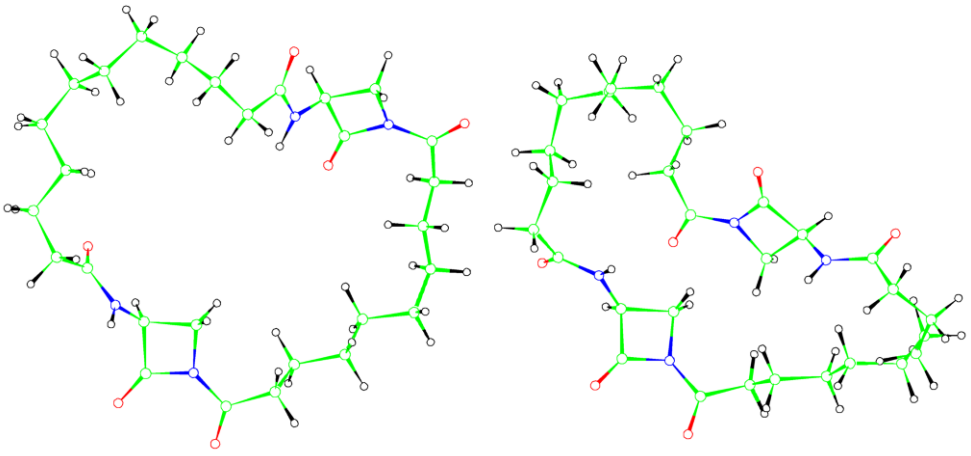
b)



Acyl-enzyme complexes between **5f** and PBP2a. a) **5f**, HH conformer b) **5f**, HT conformer.
Legend: Residues surrounding the alkyl chain of the macrocycle at the bottom of the active site are highlighted with carbon atoms coloured green, nitrogen blue and oxygen red. The carbon atoms of **5f** are coloured yellow and hydrogen white. The active serine 403 is behind the ligand and the covalent bond between the serine and the ligand is coloured black.

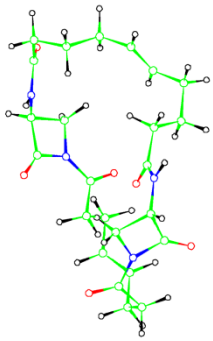
The flexibility of the macrocycle **5f** of both HH and HT isomers allows its docking in the active site as acyl-enzymes. The docking highlights the possibility for the whole macrocycle to completely fill the active cavity with the closed lactam cycle sandwiched between Tyr446 and Met641. The figures show the macrocycle lying between a series of residues, especially Glu602 and Gln613 at the entrance of the cavity, without making hydrogen bonds with these residues. The important catalytic residues of the conserved motifs (Ser403, Lys406, Ser462 and Lys597) lie behind the macrocycle and are not shown on the figures.

A modification of the geometry has been applied to dock this molecule into PBP2a. Starting from these two different geometries, a reoptimization at the B3LYP/6-31G(d) level leads to two other local minima with relative energies of 4.56 kcal and 5.16 kcal (Fig. below).

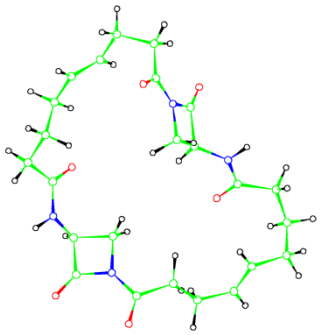


HH $\Delta E = 4.56$ HT $\Delta E = 5.16$

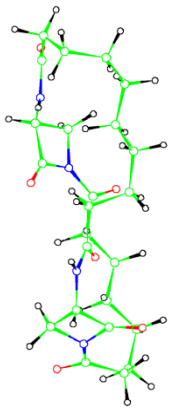
Dimers 4 and 5. Relative energies are given in kcal/mol



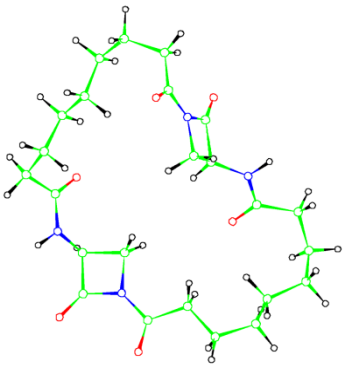
4b HH ΔE = 3.42



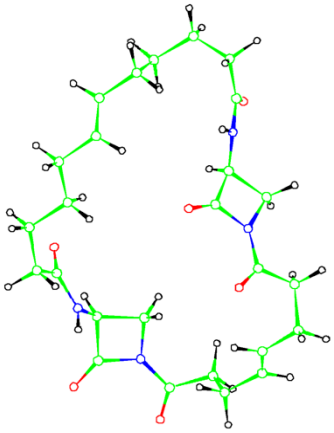
4b HT ΔE = 8.72



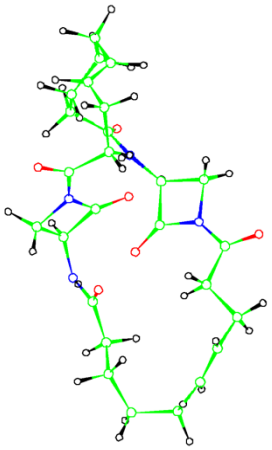
5b HH ΔE = 0.00



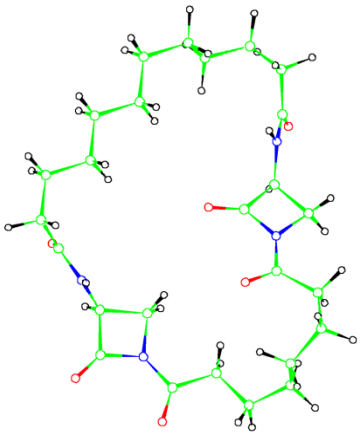
5b HT ΔE = 6.93



4c HH ΔE = 19.43



4c HT ΔE = 5.43



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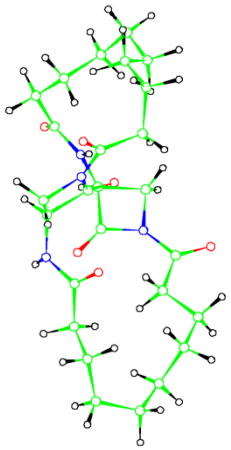
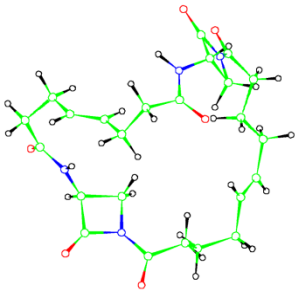
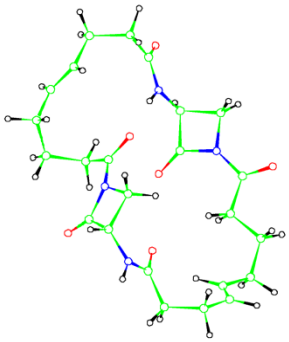


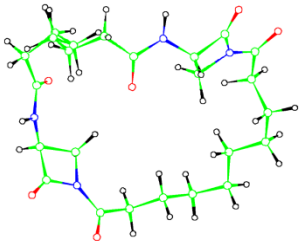
5c HT ΔE = 6.96



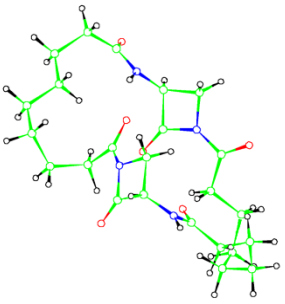
4d HH ΔE = 19.06



4d HT ΔE = 0.00



5d HH ΔE = 16.02



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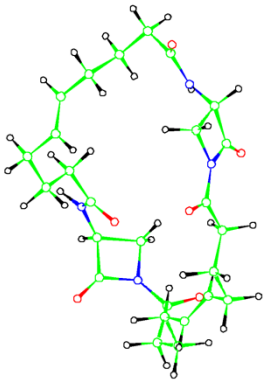
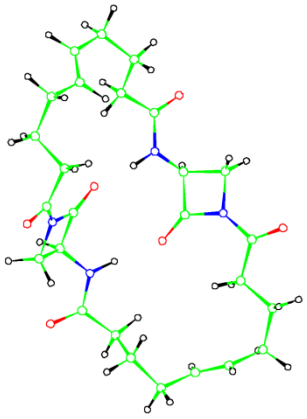
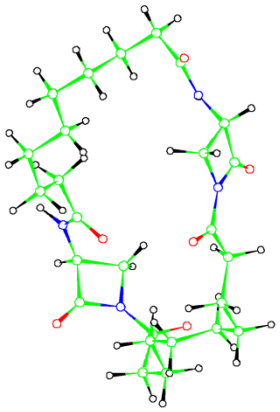


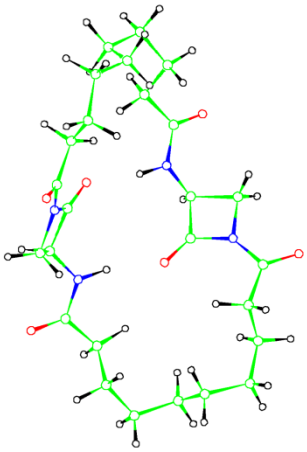
4e HH ΔE = 9.59



4e HT ΔE = 0.00



5e HH ΔE = 8.70



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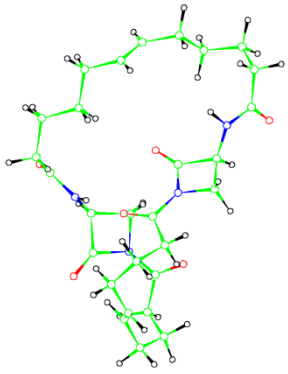
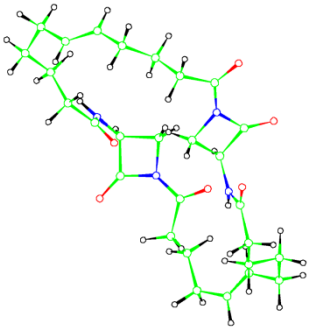
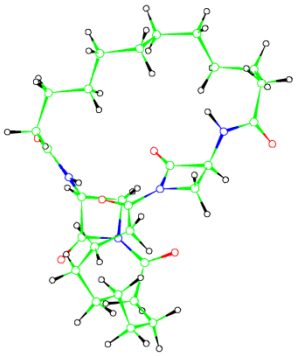


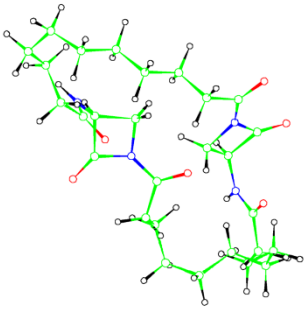
4f HH ΔE = 0.00



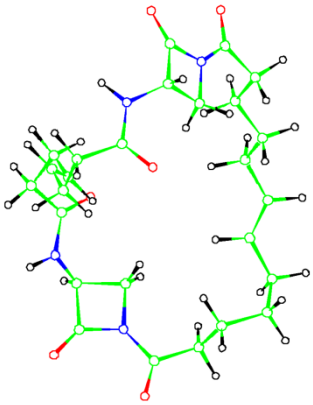
4f HT ΔE = 7.36



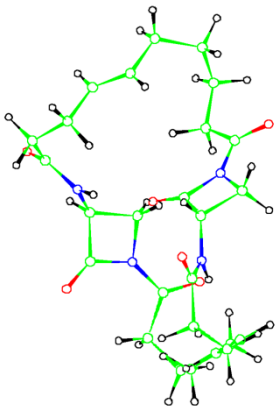
5f HH ΔE = 0.00



5f HT ΔE = 8.26



4g HH ΔE = 22.63



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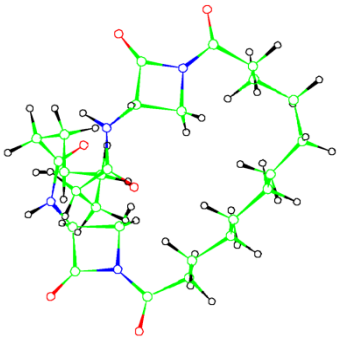

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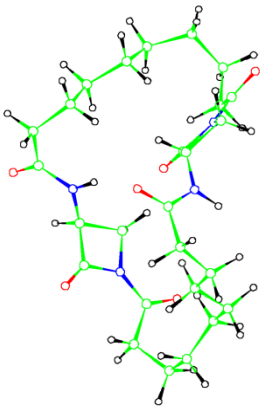

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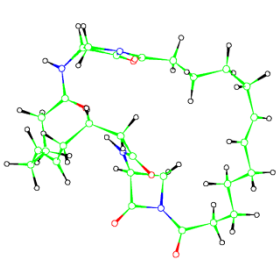

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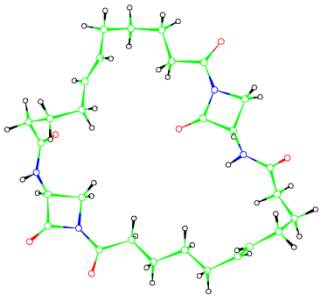
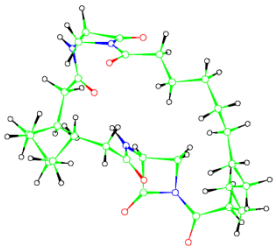
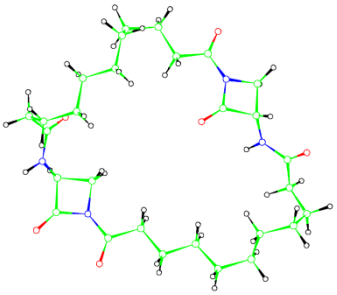


4h HT ΔE = 10.07



5h HH ΔE = 3.36



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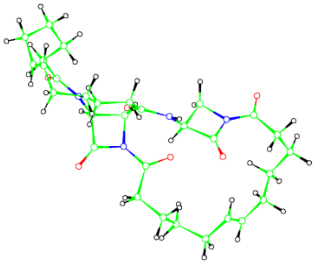
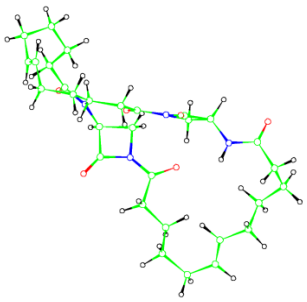
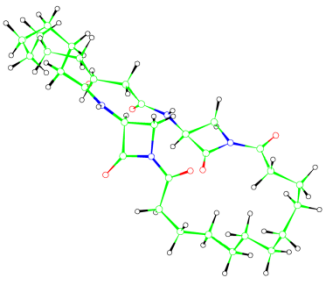


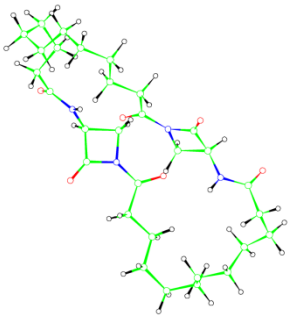
4i HH ΔE = 0.00



4i HT ΔE = 2.43



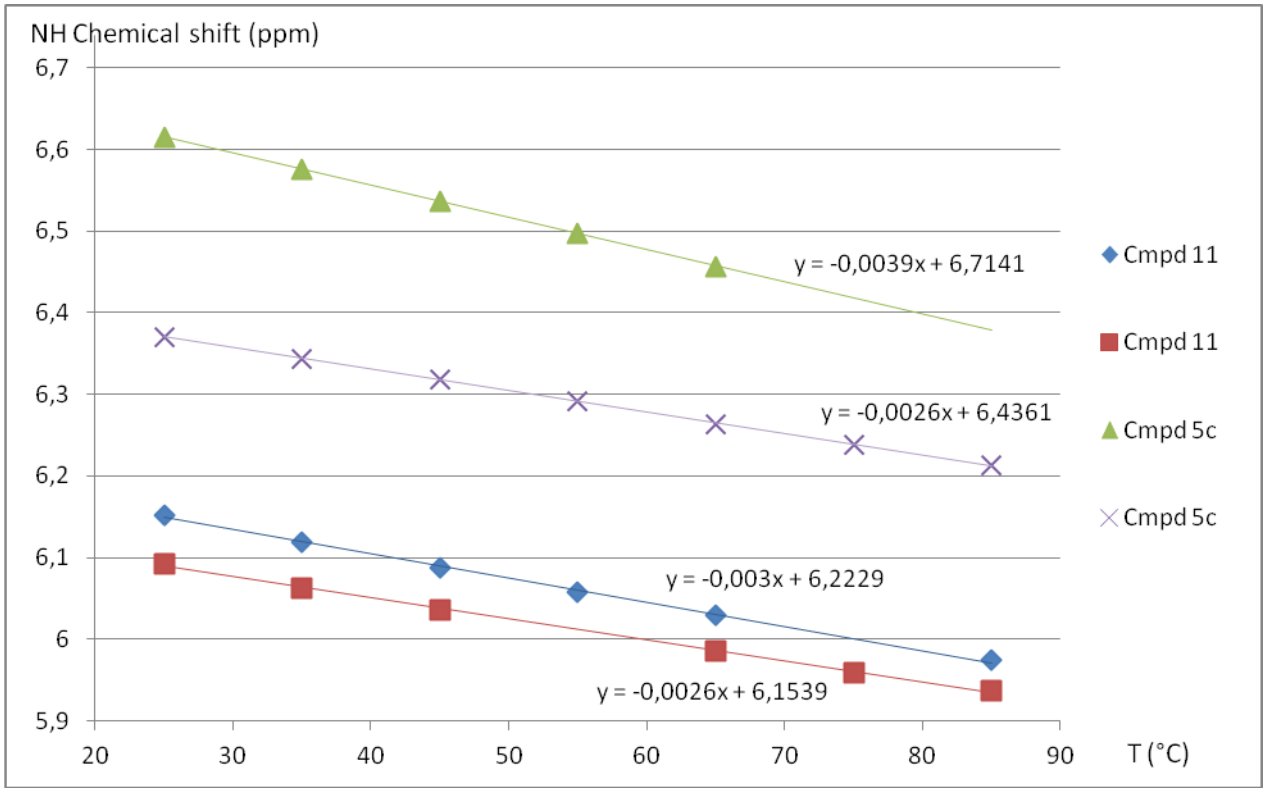
5i HH ΔE = 0.00



5i HT ΔE = 1.52

Experiments of temperature coefficients for amide protons

Temperature coefficients of the NH chemical shifts were recorded at 500 MHz in 1,1,2,2-tetrachloroethane-d_2 by heating at 10°C intervals from 298 to 358 K.



The temperature coefficients of the two amide protons of compound **11** are -3.0 and -2.6 ppb/°C.
The temperature coefficients of the two amide protons of compound **5c** are -2.6 and -3.9 ppb/°C.

In non-competitive solvents, intramolecular hydrogen-bonded amide protons typically exhibit a relatively large temperature dependence of the chemical shift relative to free amide protons which exhibit a small temperature dependence of the chemical shift.[5]

We can observe two different evolutions for the NH of the compound **5c**, it could be explained by the presence of one intramolecular hydrogen-bonded amide proton since the second amide proton is not involved in an intramolecular H bond.

[5] T. K. Chakraborty, A. Ghosh, R. Nagaraj, A. R. Sankar, A. C. Kunwar, Tetrahedron 2001, **57**, 9169 and references therein.


ppm
173,602
170,625
164,801
137,609
136,463
115,927
115,722
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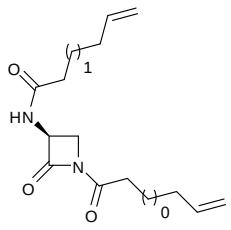
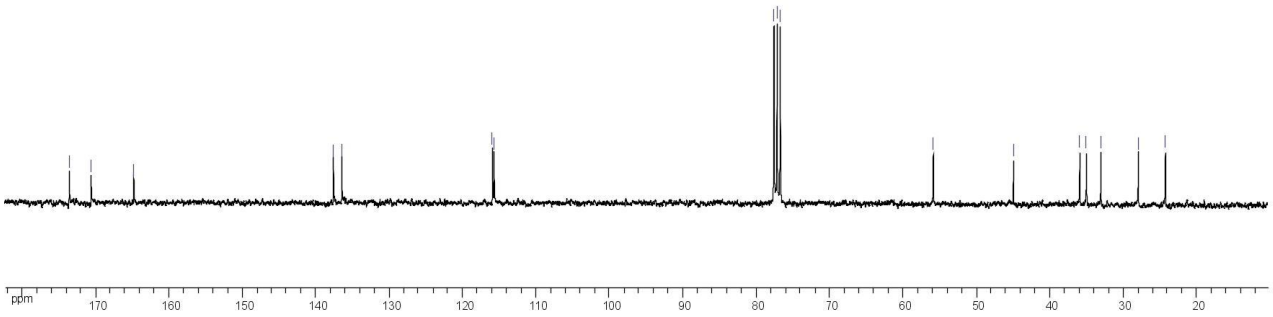
77,160
76,737
55,879
44,954
35,927
35,000
33,051
27,950
24,250


O
1
HN
N
O
O
0

3b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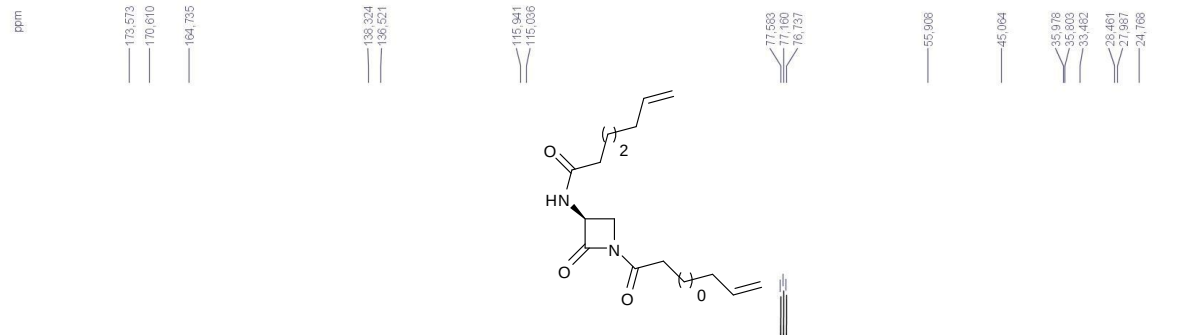
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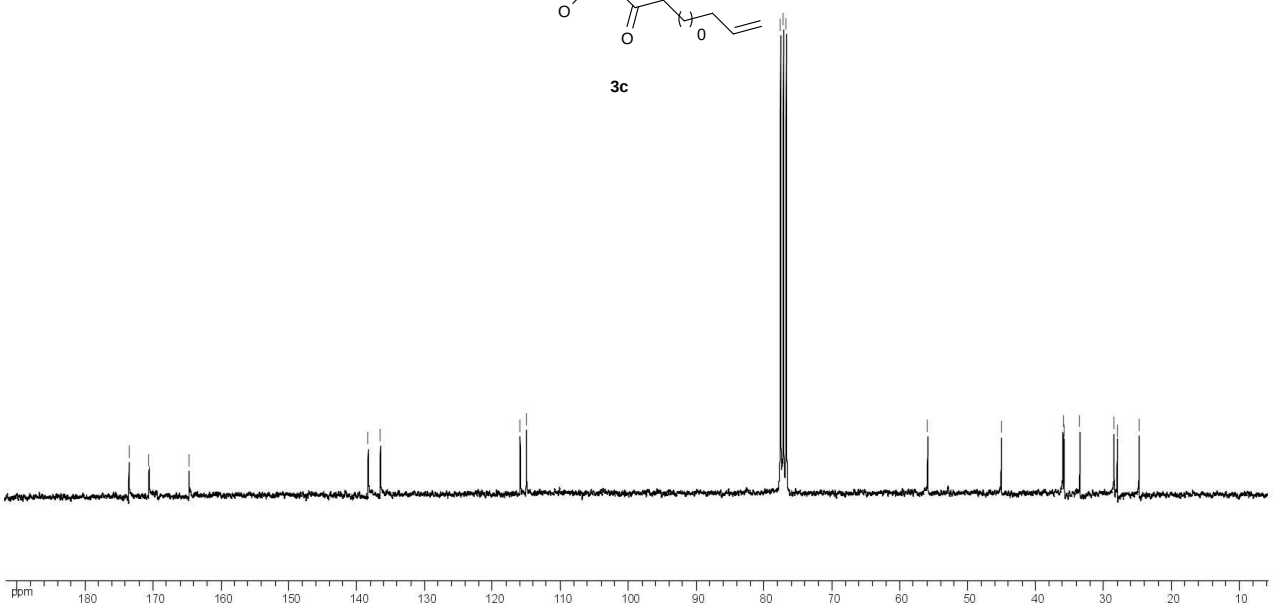
O
2
HN
N
O
O
0

3c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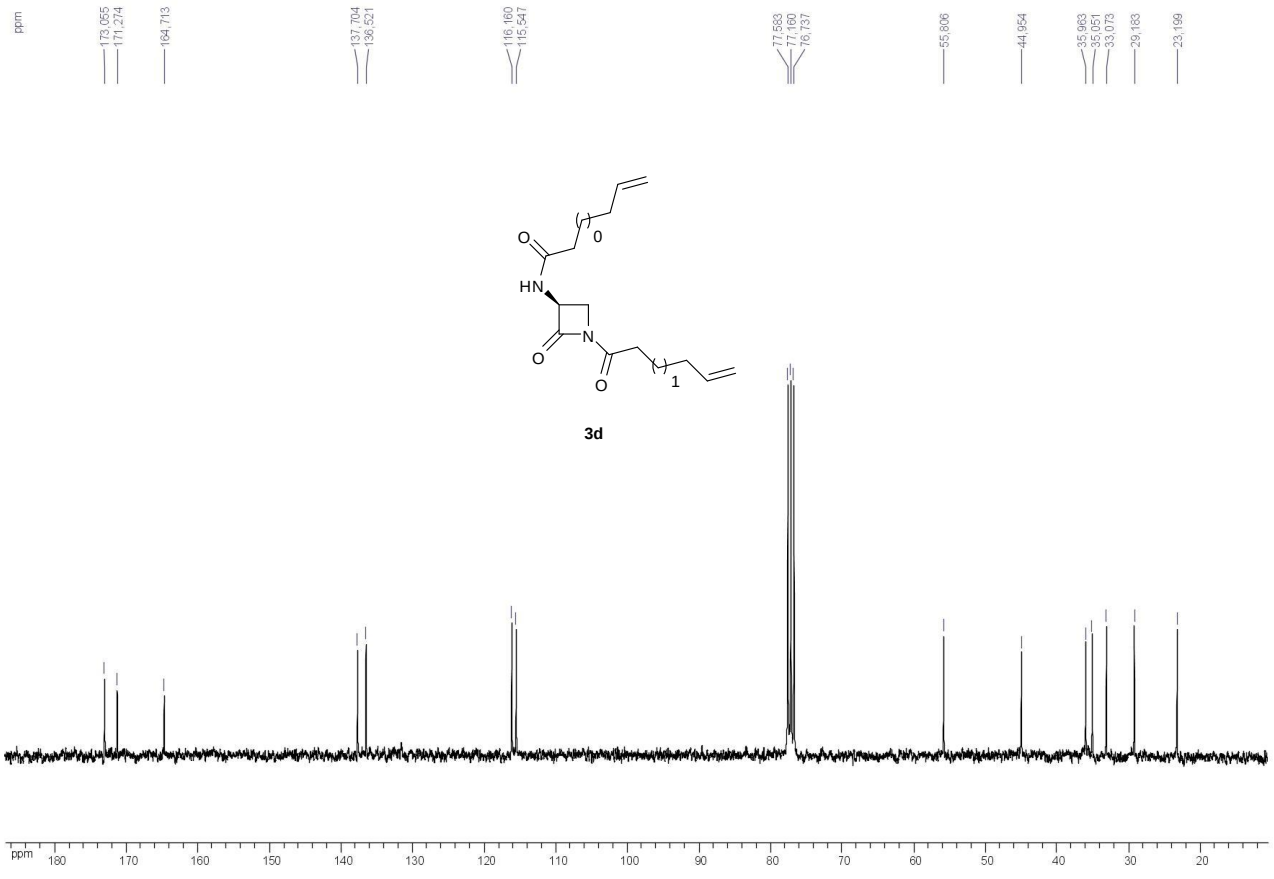
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10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171,274
164,713
137,704
136,521
116,160
115,547
77,583
77,160
76,737
55,806
44,954
35,963
35,051
33,073
29,183
23,199
O
HN
N
O
O
0
1
ppm
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170
160
150
140
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120
110
100
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80
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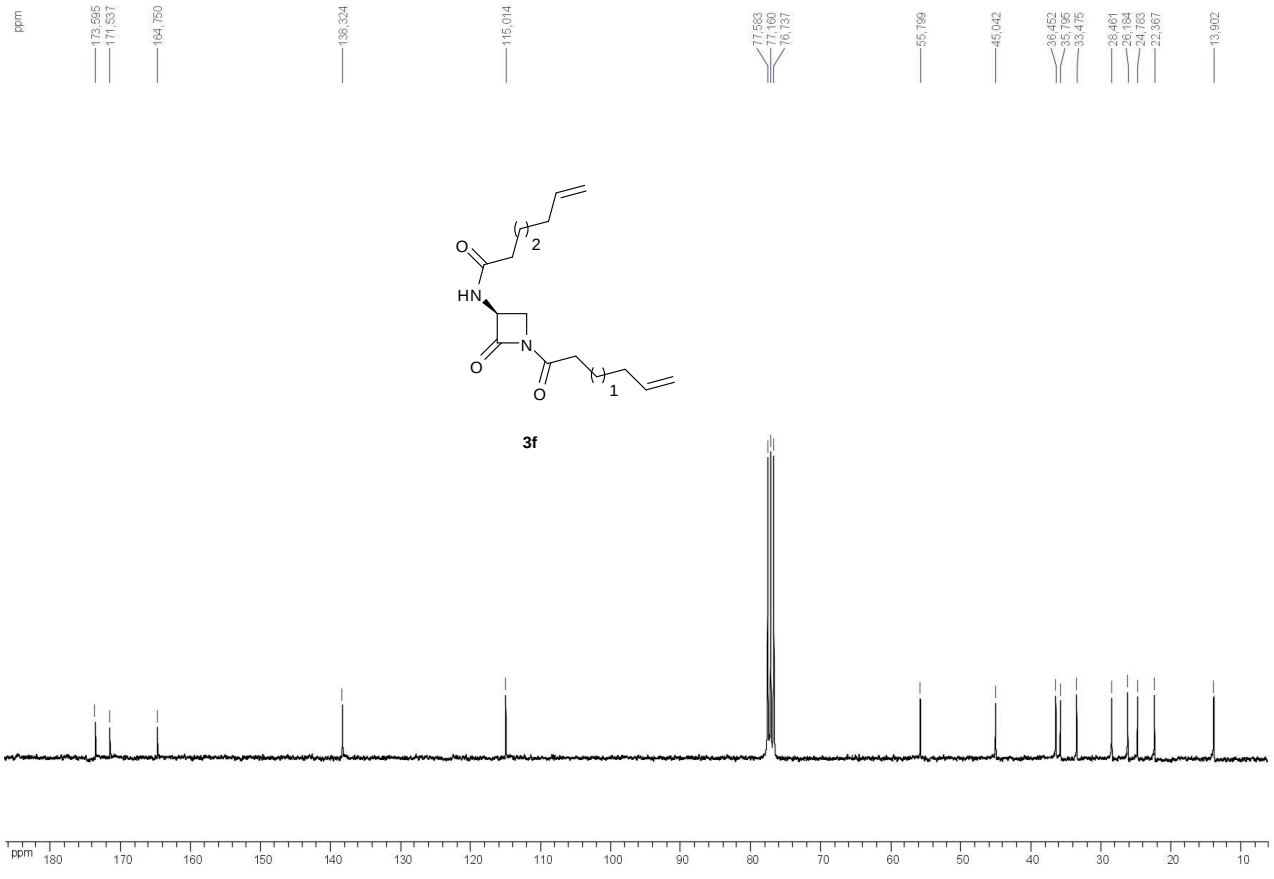
20

3d


ppm
173,595
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164,750
138,324
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77,160
76,737
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45,042
36,452
35,795
33,475
28,461
26,184
24,783
22,367
13,902
O
HN
N
O
O
2
1
ppm
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150
140
130
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110
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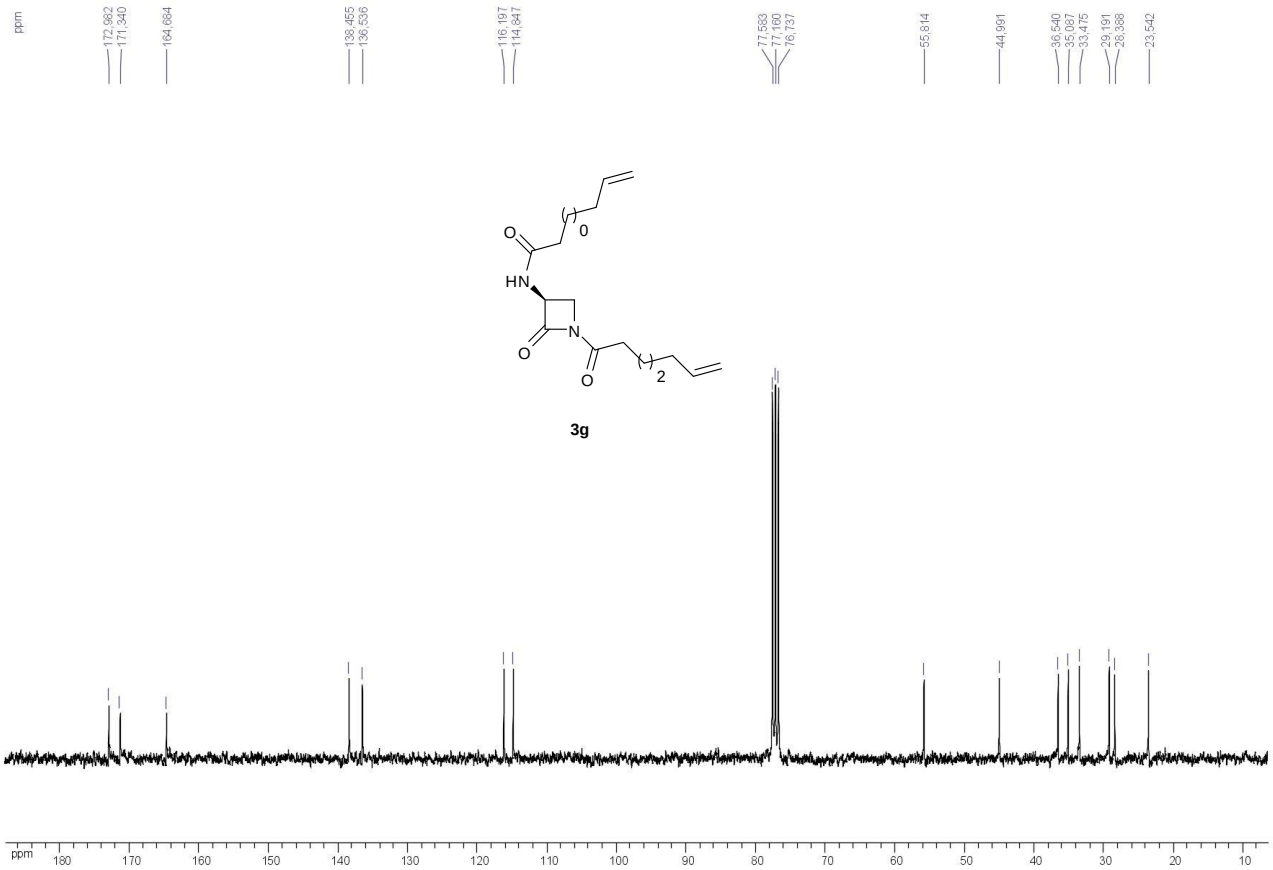
40
30
20
10

3f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138,455
136,536
116,197
114,847
77,583
77,160
76,737
55,814
44,991
36,540
35,067
33,475
29,191
28,388
23,542
O
0
HN
N
O
O
2
ppm
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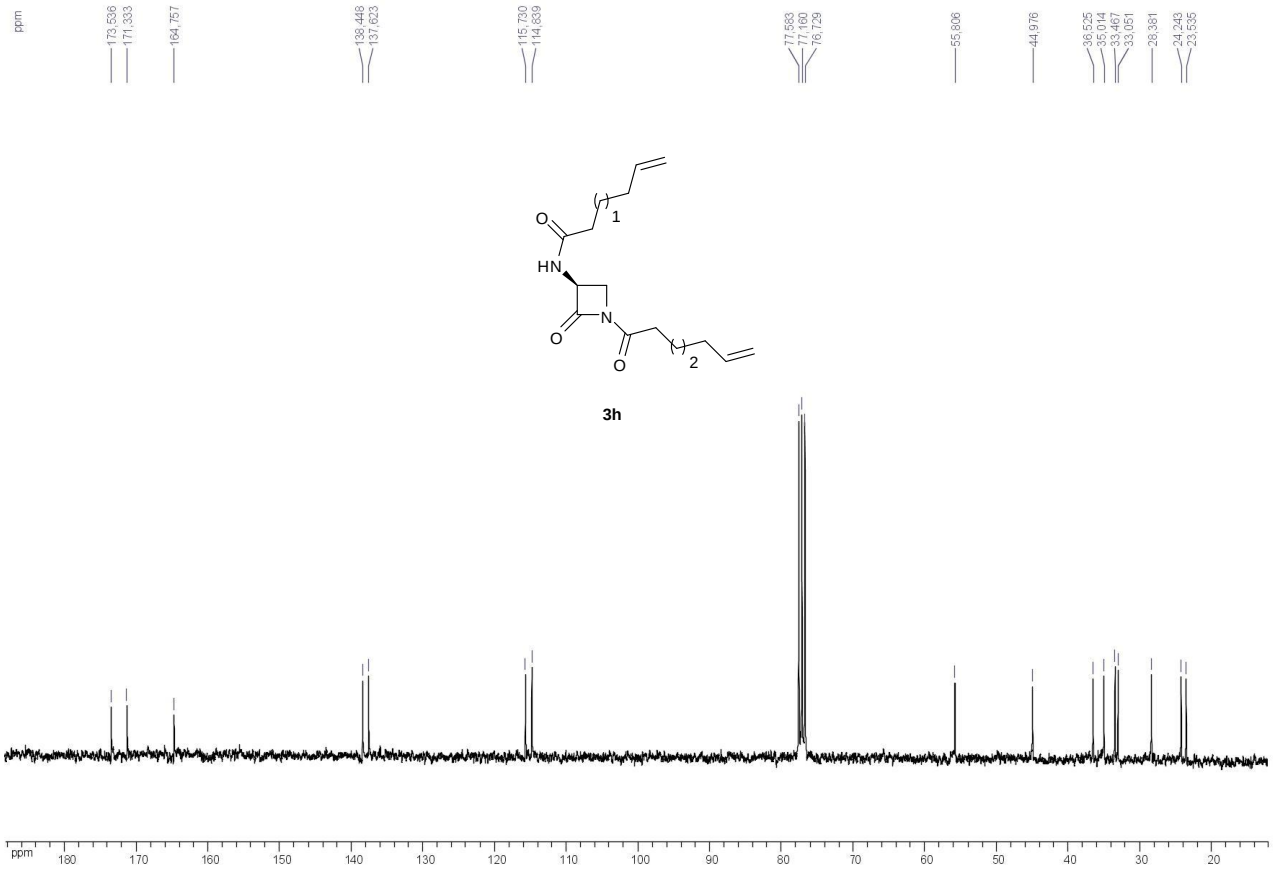
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

3g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138,448
137,623
115,730
114,839
77,583
77,160
76,729
55,806
44,976
36,525
35,014
33,467
33,051
28,381
24,243
23,536
O
1
HN
N
O
O
2
ppm
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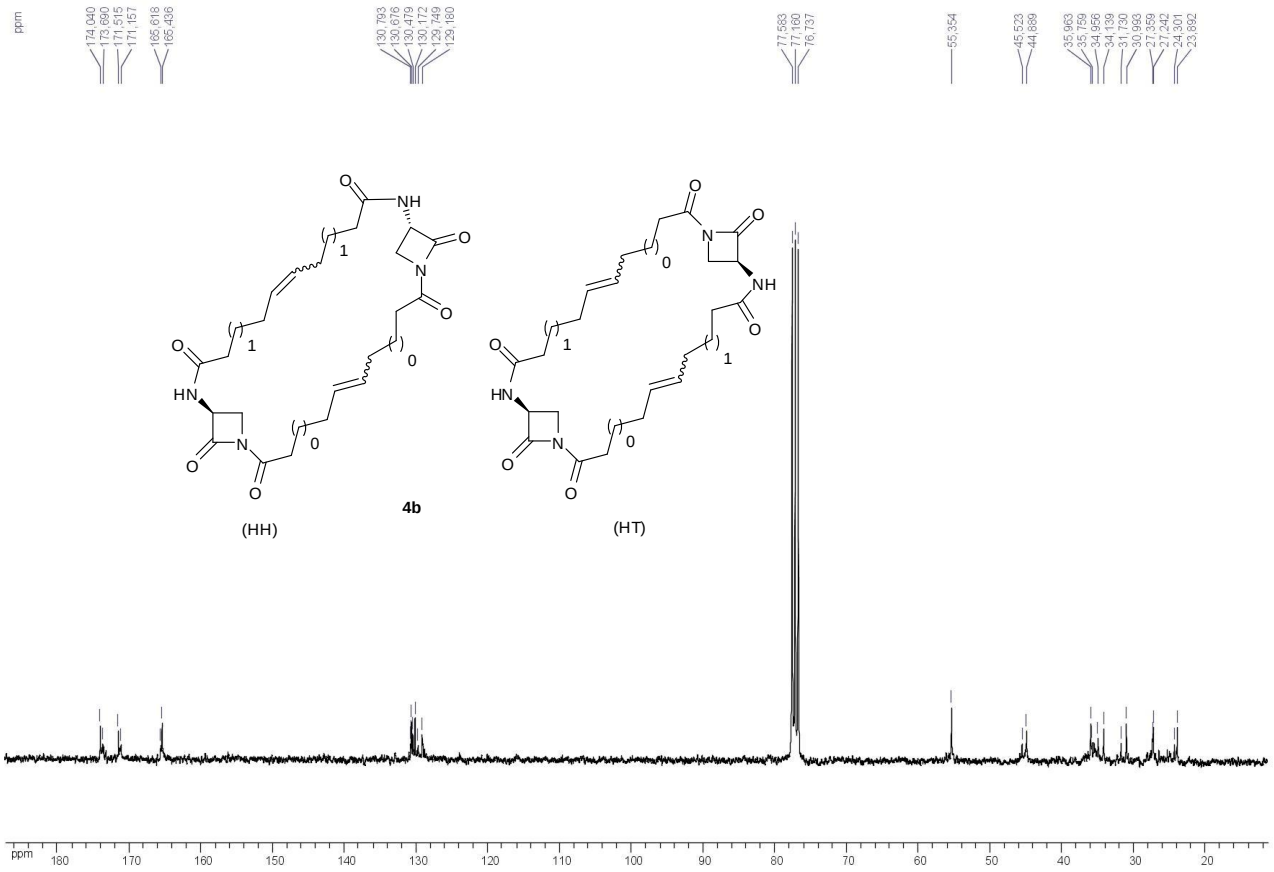
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

3h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171.515
171.157
165.618
165.436
130.793
130.676
130.479
130.172
129.749
129.180
77.583
77.160
76.737
55.354
45.523
44.889
35.963
35.759
34.956
34.139
31.730
30.993
27.359
27.242
24.301
23.892
NH
HN
N
O
1
0
4b
(HH)
(HT)
ppm
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
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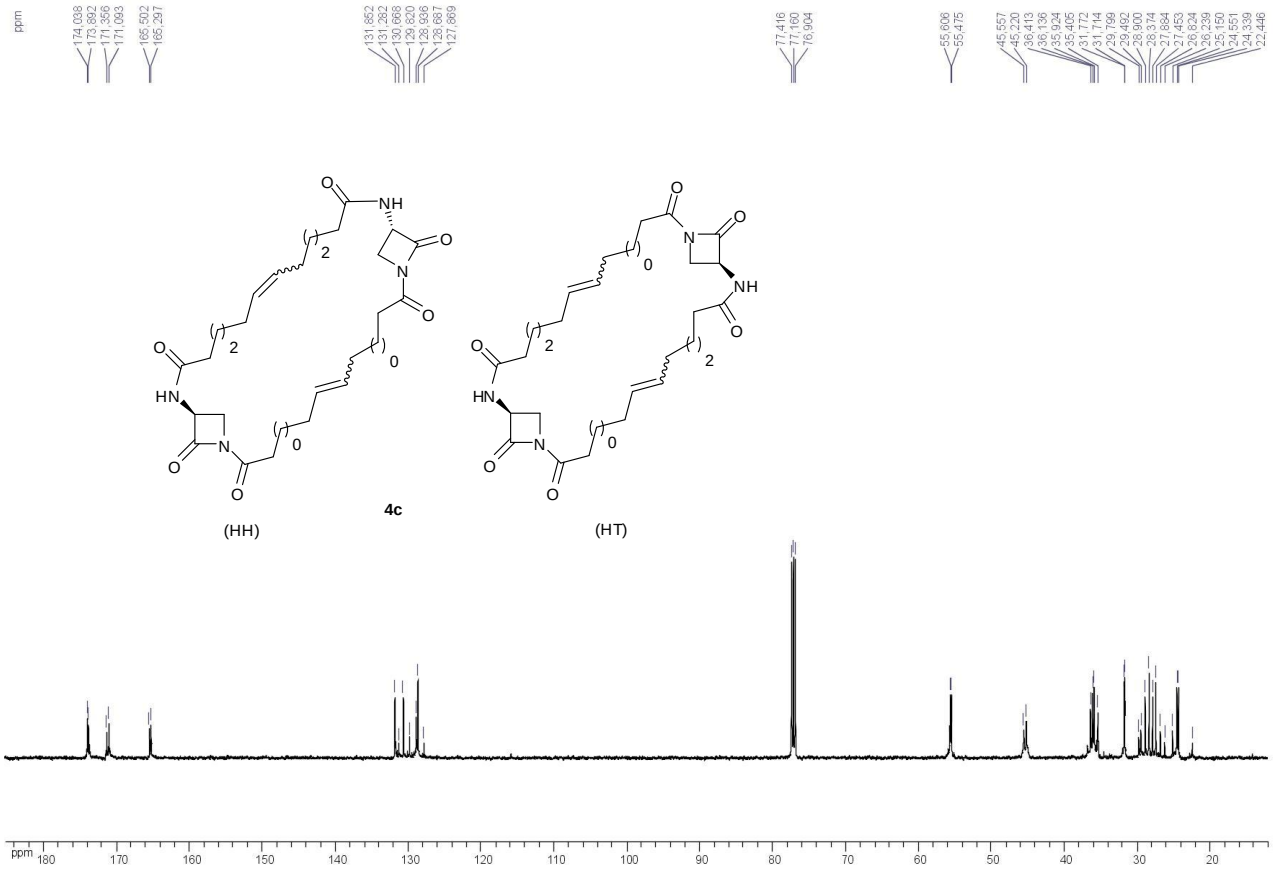
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171.356
171.093
165.502
165.297
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131.282
130.668
129.820
128.936
128.687
127.869
77.416
77.160
76.904
55.606
55.475
45.557
45.220
36.413
36.136
35.924
35.405
31.772
31.714
29.799
29.492
28.900
28.374
27.884
27.453
26.824
26.239
25.150
24.551
24.339
22.446
NH
HN
N
O
2
0
4c
(HH)
(HT)
ppm
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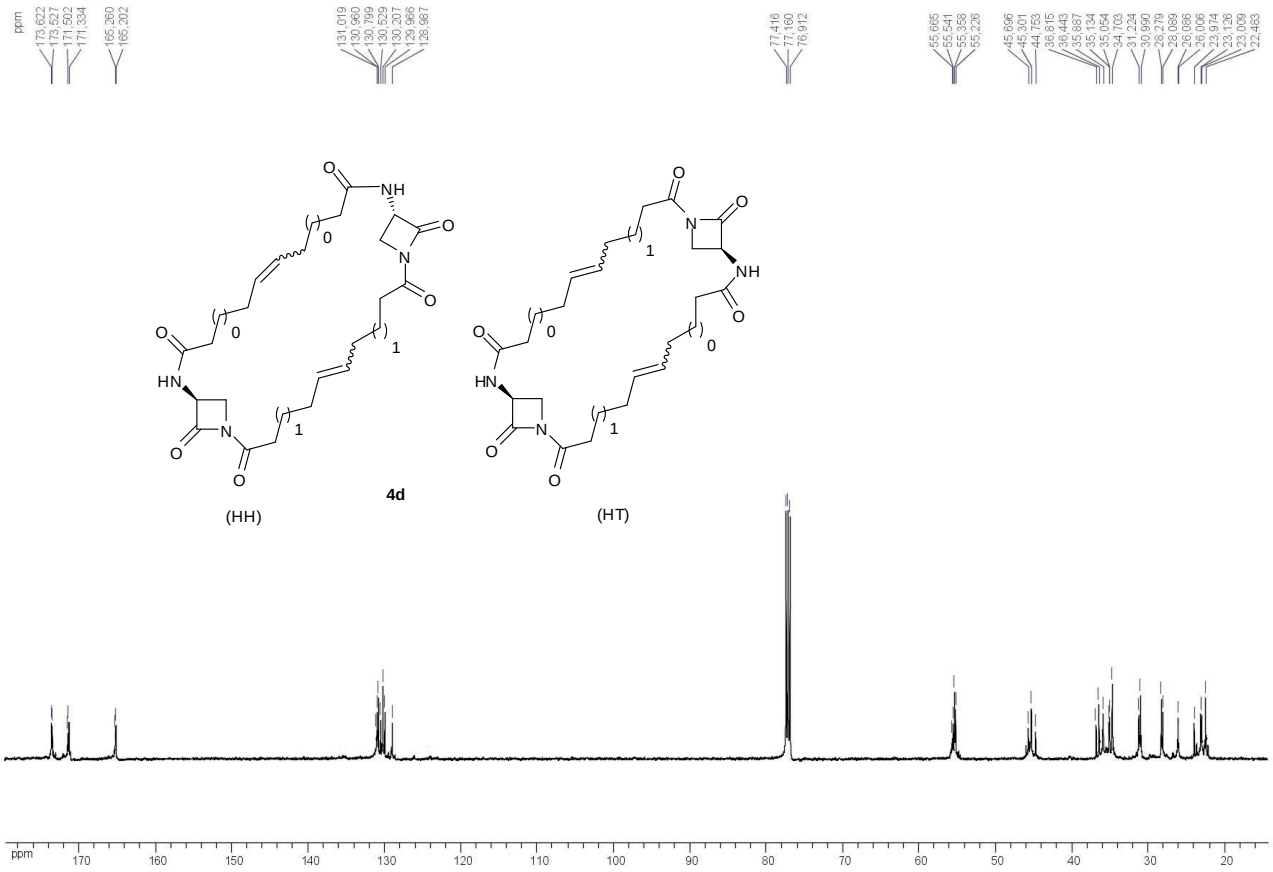
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20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173.527
171.502
171.334
165.260
165.202
131.019
130.960
130.799
130.529
130.207
129.966
128.987
77.416
77.160
76.912
55.685
55.541
55.358
55.226
45.696
45.301
44.753
36.815
36.443
35.697
35.054
34.703
31.224
30.990
28.279
28.089
26.086
26.006
23.974
23.126
23.009
22.483
4d
(HH)
(HT)
ppm
170
160
150
140
130
120
110
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90
80
70
60
50
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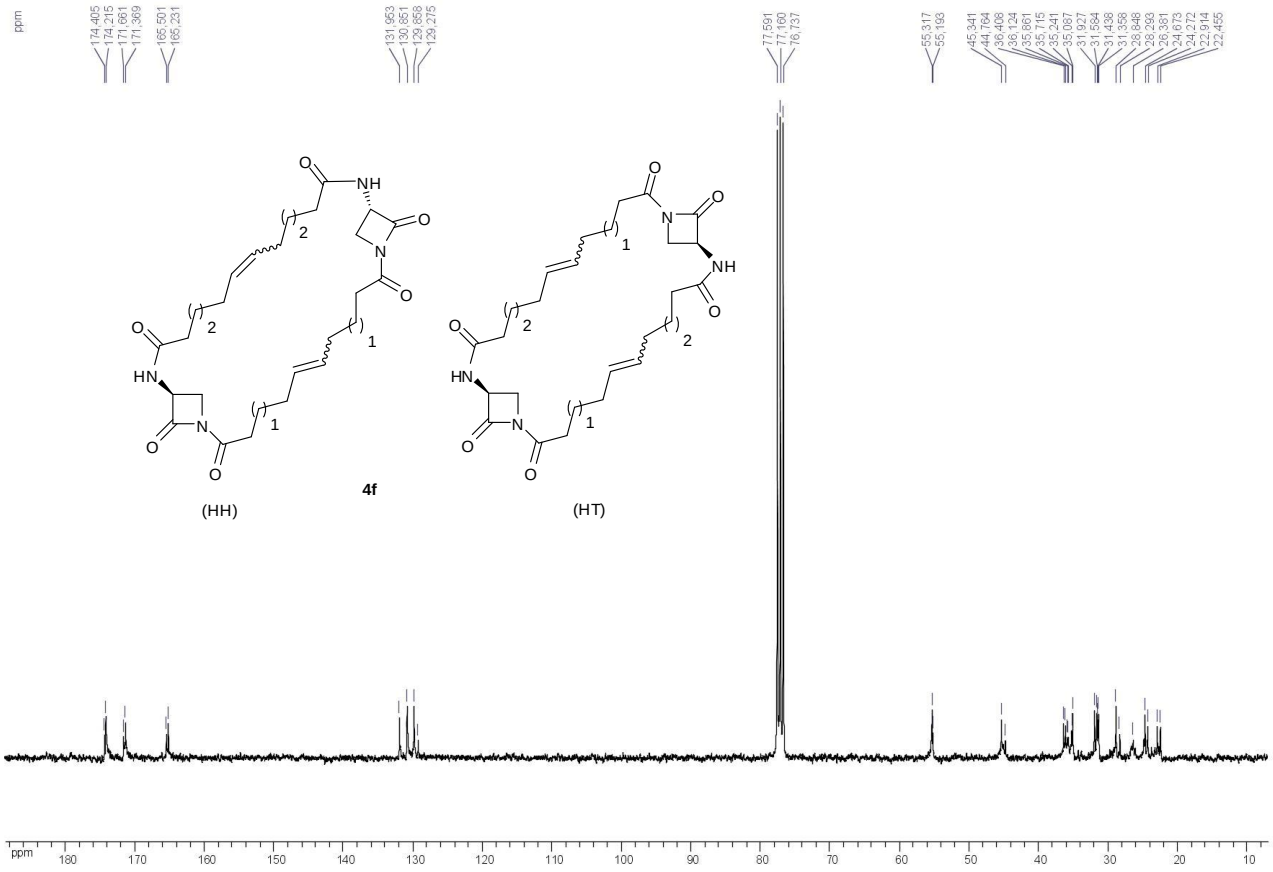
30
20


ppm
174.405
174.215
171.661
171.369
165.501
165.231
131.963
130.851
129.858
129.275
77.591
77.160
76.737
55.317
55.193
45.341
44.764
36.408
36.124
35.861
35.715
35.241
35.087
31.927
31.584
31.438
31.358
28.848
28.293
26.381
24.673
24.272
22.914
22.455
4f
(HH)
(HT)
ppm
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160
150
140
130
120
110
100
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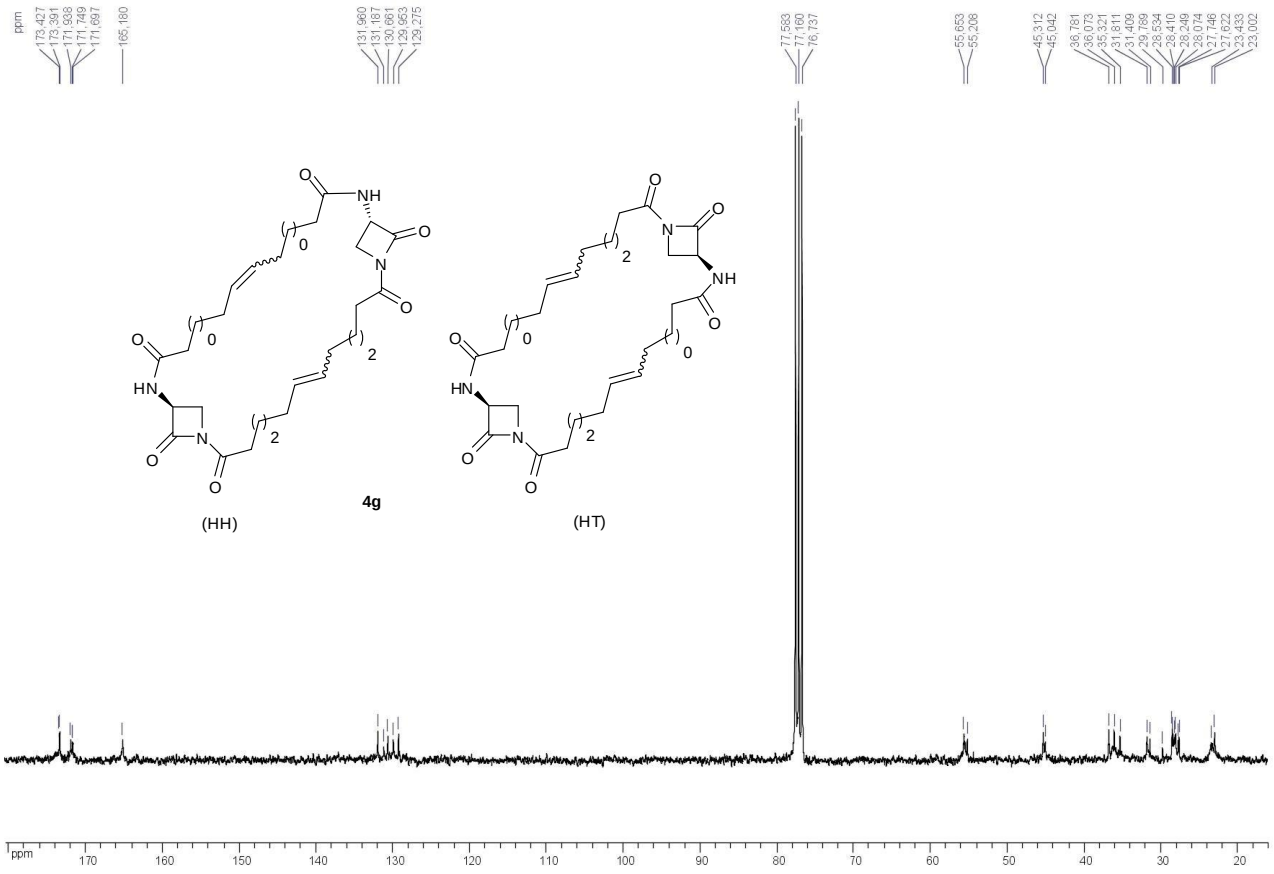
80
70
60
50
40
30
20
10


ppm
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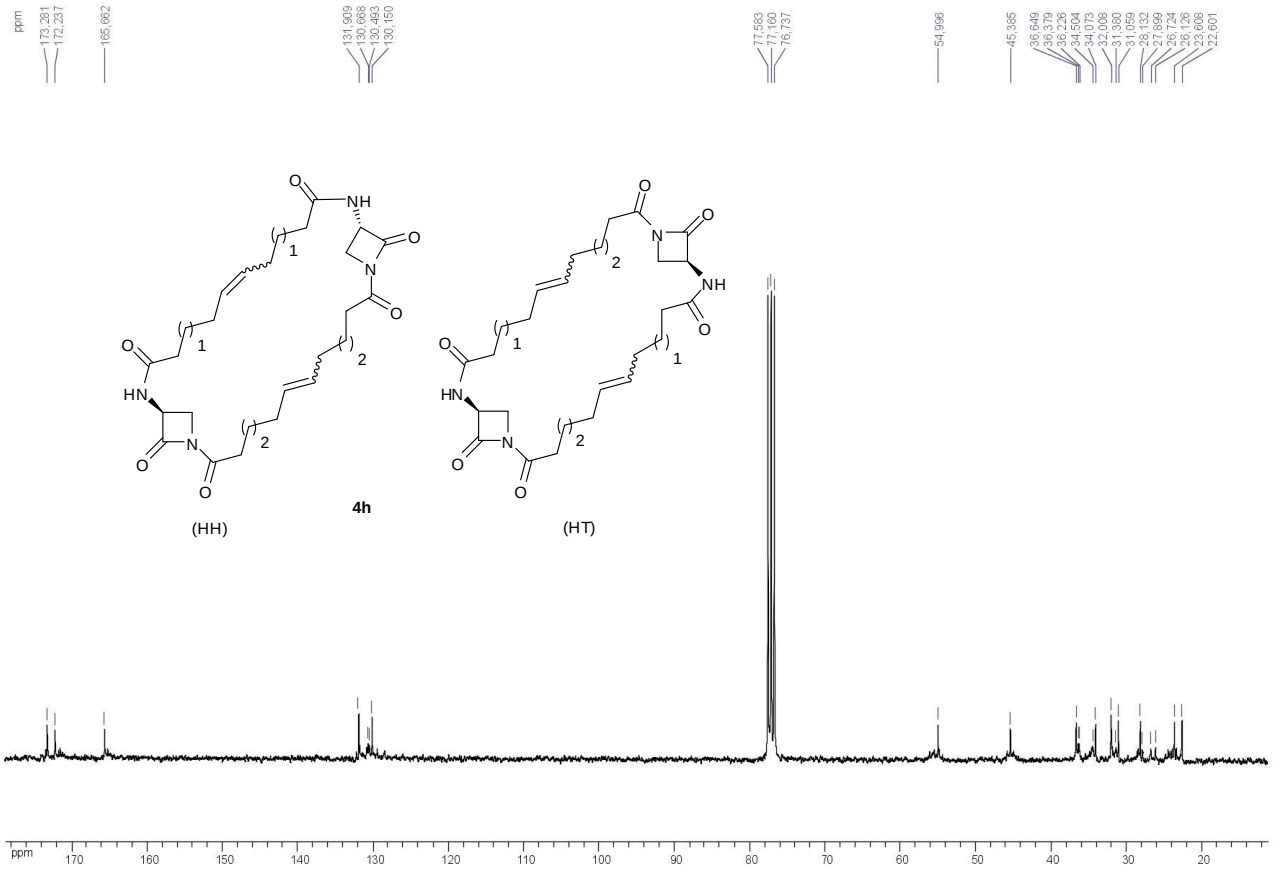
171,749
171,697
165,180
131,960
131,187
130,661
129,953
129,275
77,583
77,160
76,737
55,653
55,208
45,312
45,042
36,781
36,073
35,321
31,811
31,409
29,789
28,534
28,410
28,249
28,074
27,746
27,622
23,433
23,002
NH
HN
N
O
0
2
4g
(HH)
(HT)
ppm
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


ppm
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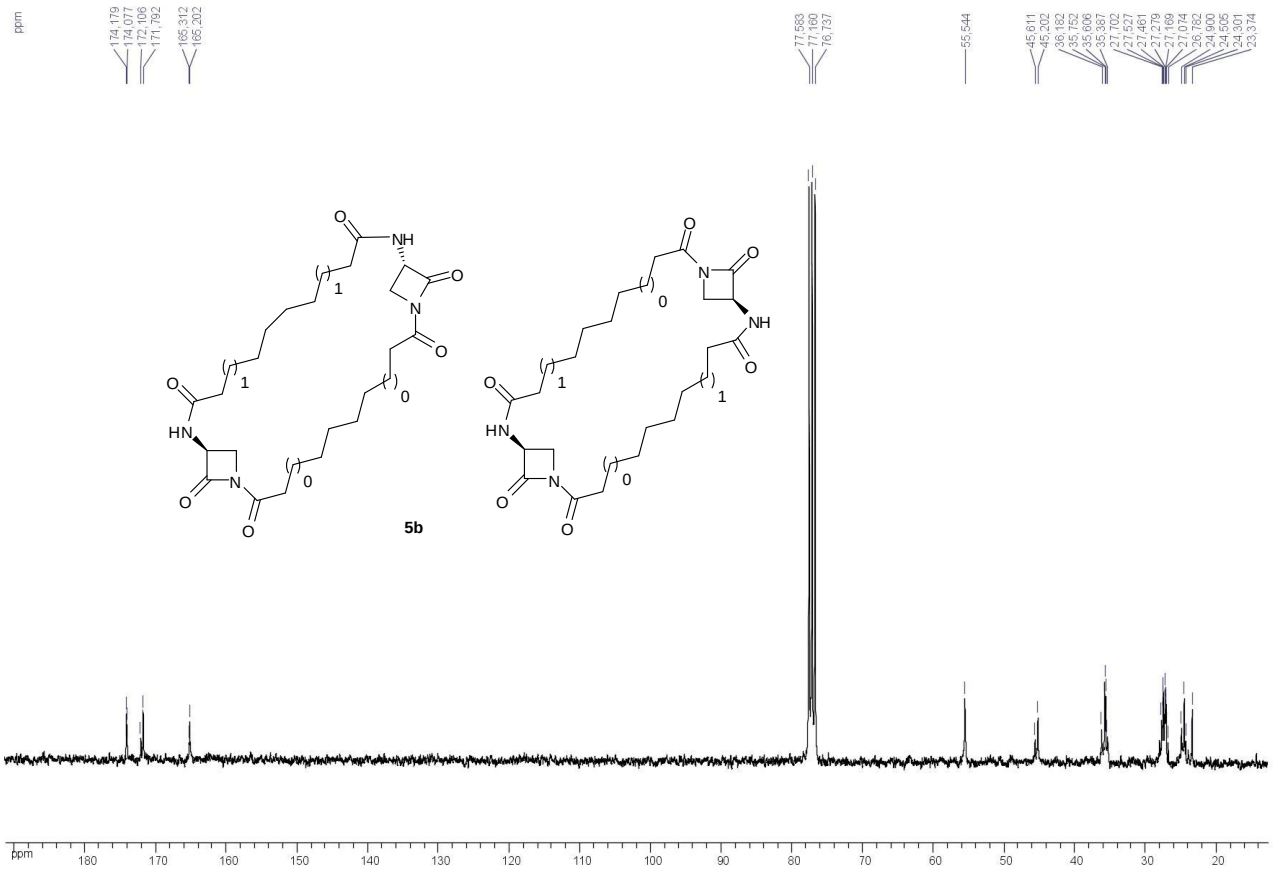
165,662
131,909
130,668
130,493
130,150
77,583
77,160
76,737
54,996
45,385
36,649
36,378
36,228
34,504
34,073
32,008
31,380
31,059
28,132
27,899
26,734
26,126
23,608
22,601
NH
HN
N
O
1
2
4h
(HH)
(HT)
ppm
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


ppm
174,179
174,077
172,106
171,792
165,312
165,202
77,583
77,160
76,737
55,544
45,611
45,202
36,182
35,752
35,606
35,387
27,702
27,527
27,461
27,279
27,169
27,074
26,782
24,900
24,505
24,301
23,374
5b
ppm
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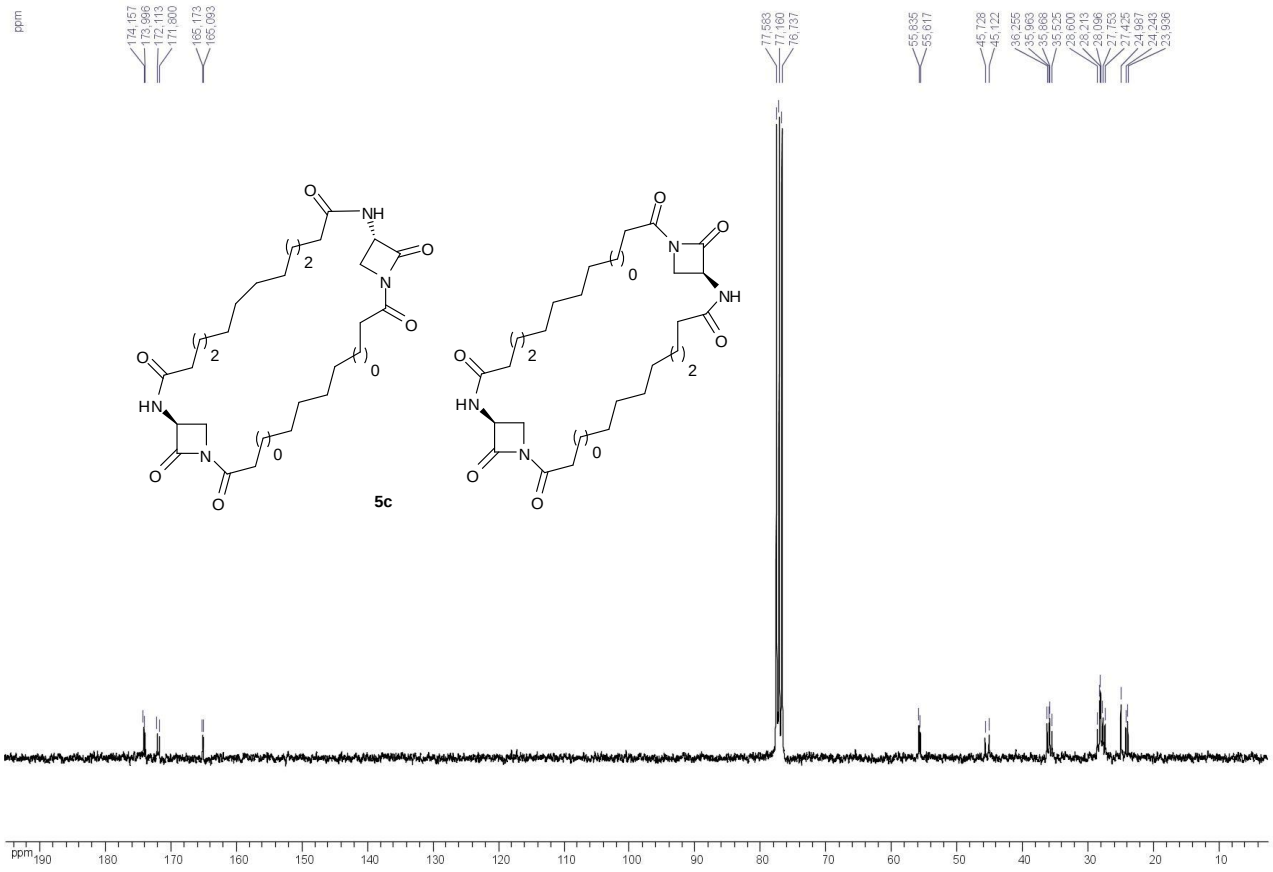
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


ppm
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172,113
171,800
165,173
165,093
77,583
77,160
76,737
55,835
55,617
45,728
45,122
36,255
35,963
35,868
35,525
28,600
28,213
28,096
27,753
27,425
24,987
24,243
23,936
5c
ppm
190
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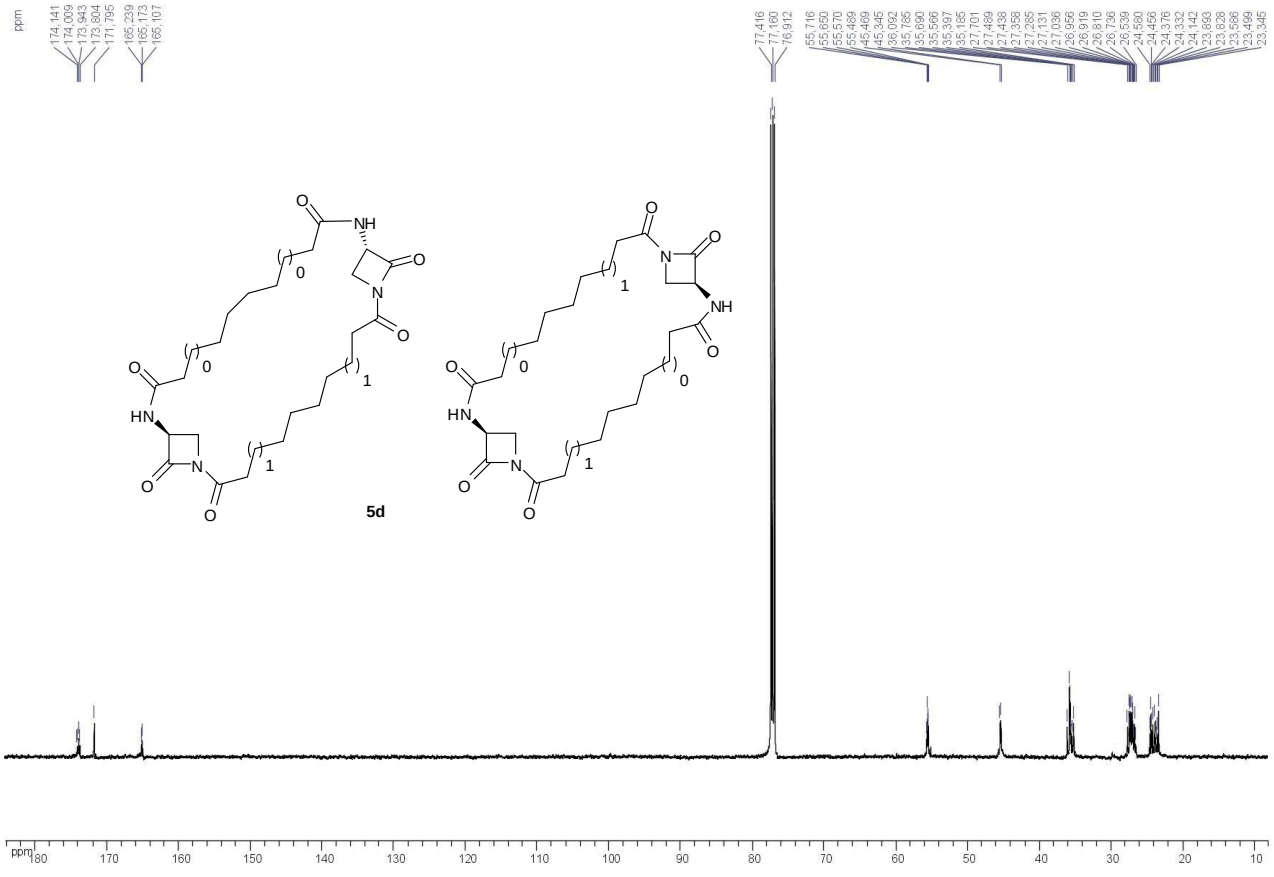
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


ppm
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173,943
173,804
171,795
166,239
166,173
166,107
77,416
77,160
76,912
55,716
55,650
55,570
55,489
45,469
45,345
36,092
35,785
35,690
35,566
35,397
35,185
27,701
27,489
27,438
27,358
27,285
27,131
27,036
26,956
26,919
26,810
26,736
26,539
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24,456
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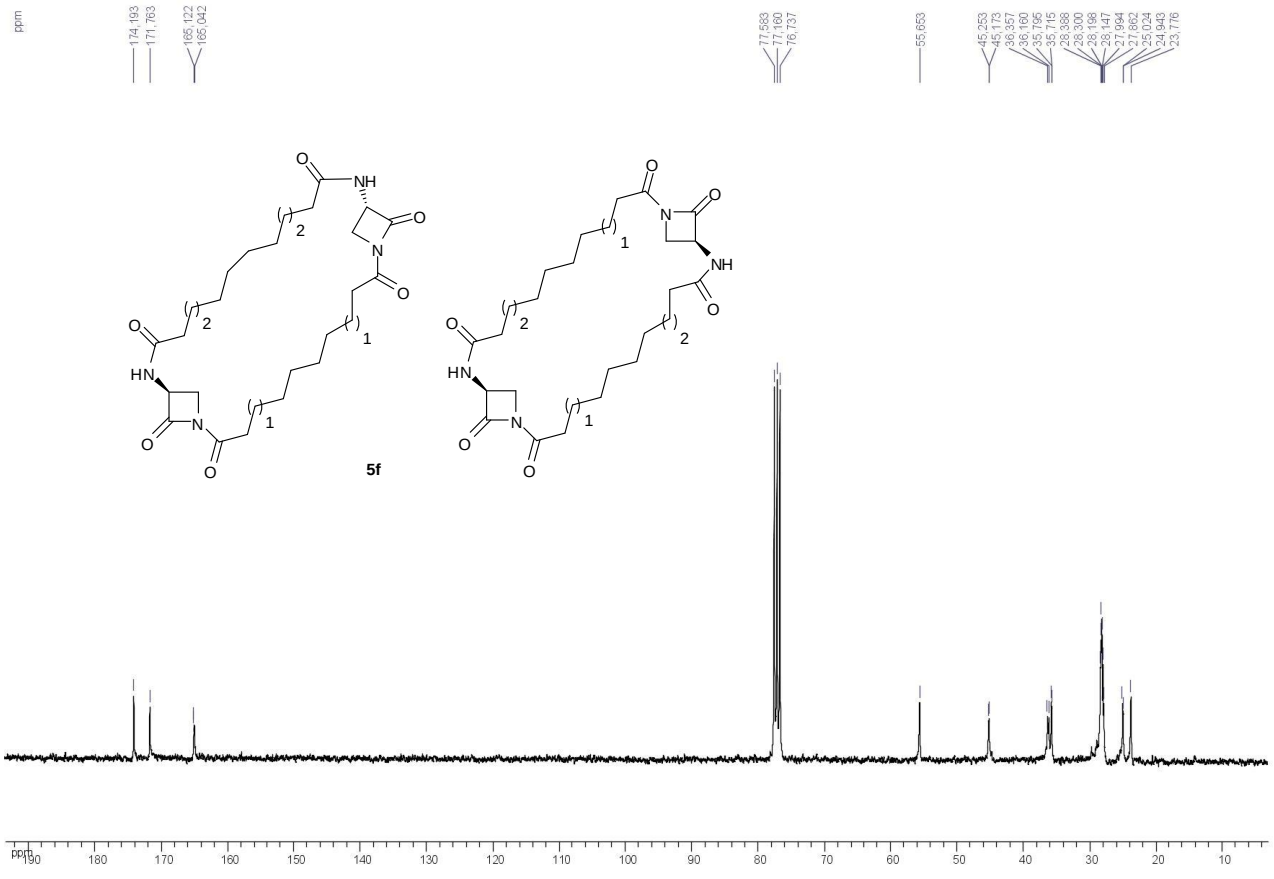
5d
ppm


ppm
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166,042
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77,160
76,737
55,653
45,253
45,173
36,357
36,160
35,795
35,715
28,388
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27,994
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25,004
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23,776
NH
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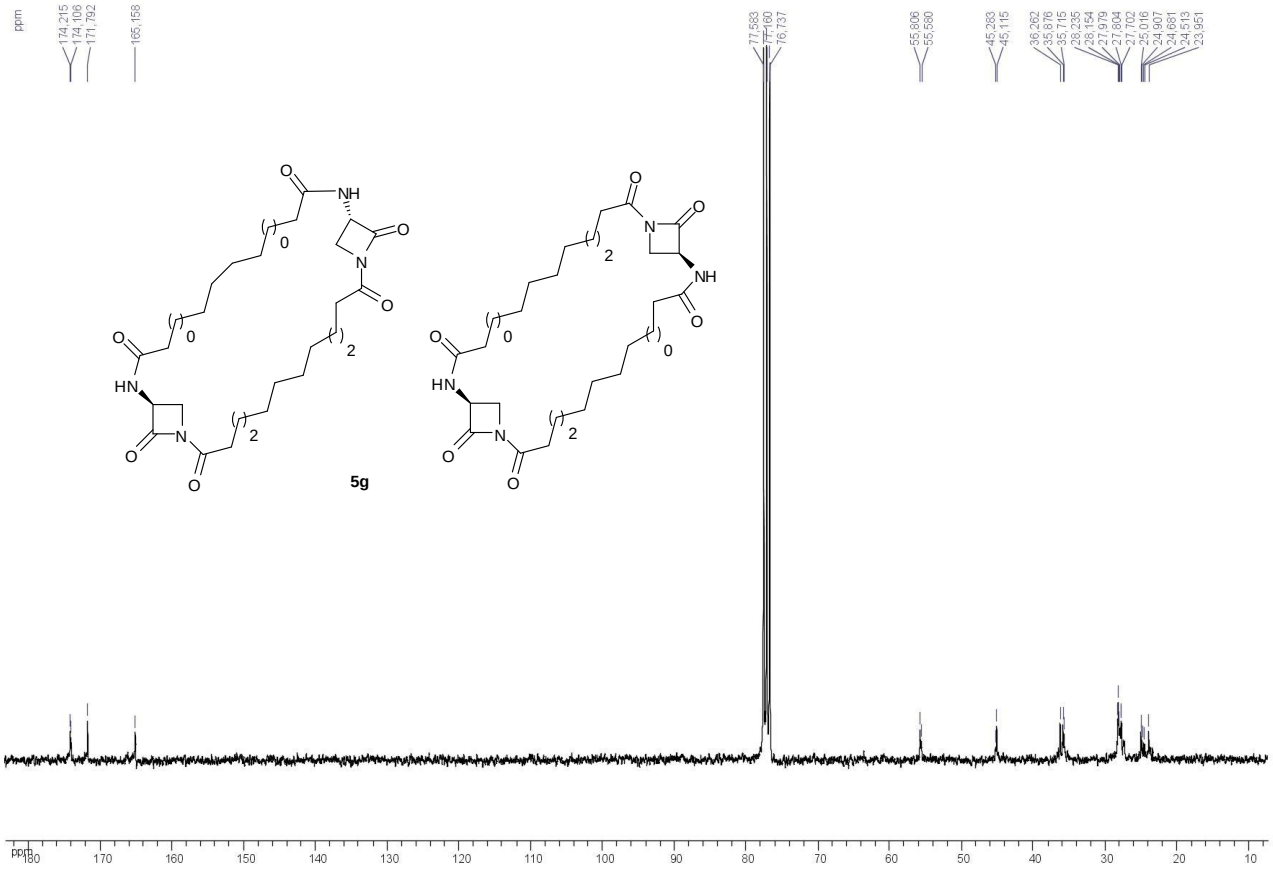
HN
5f
ppm


ppm
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77,160
76,737
55,806
55,580
45,283
45,115
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35,876
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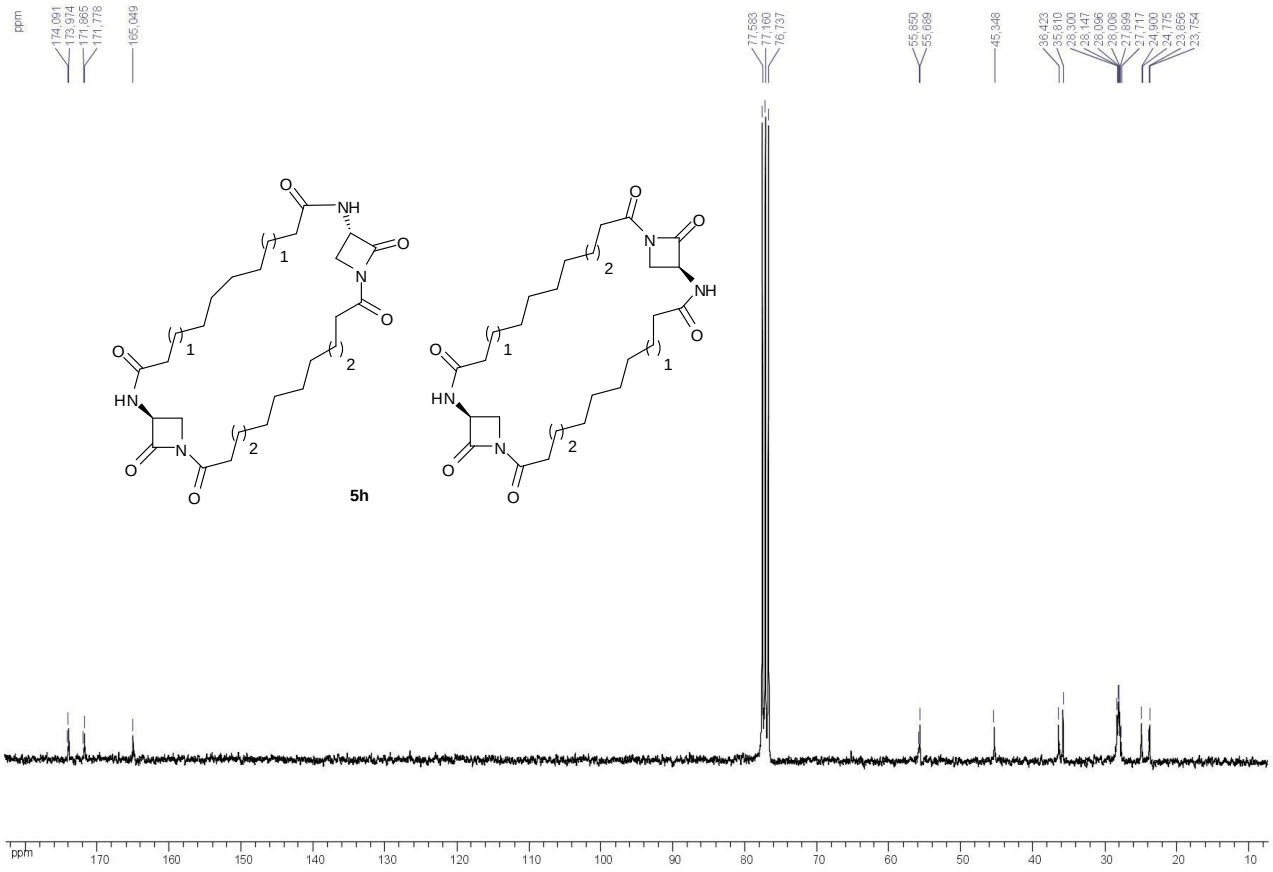
5g


ppm
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165,049
77,583
77,160
76,737
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55,689
45,346
36,423
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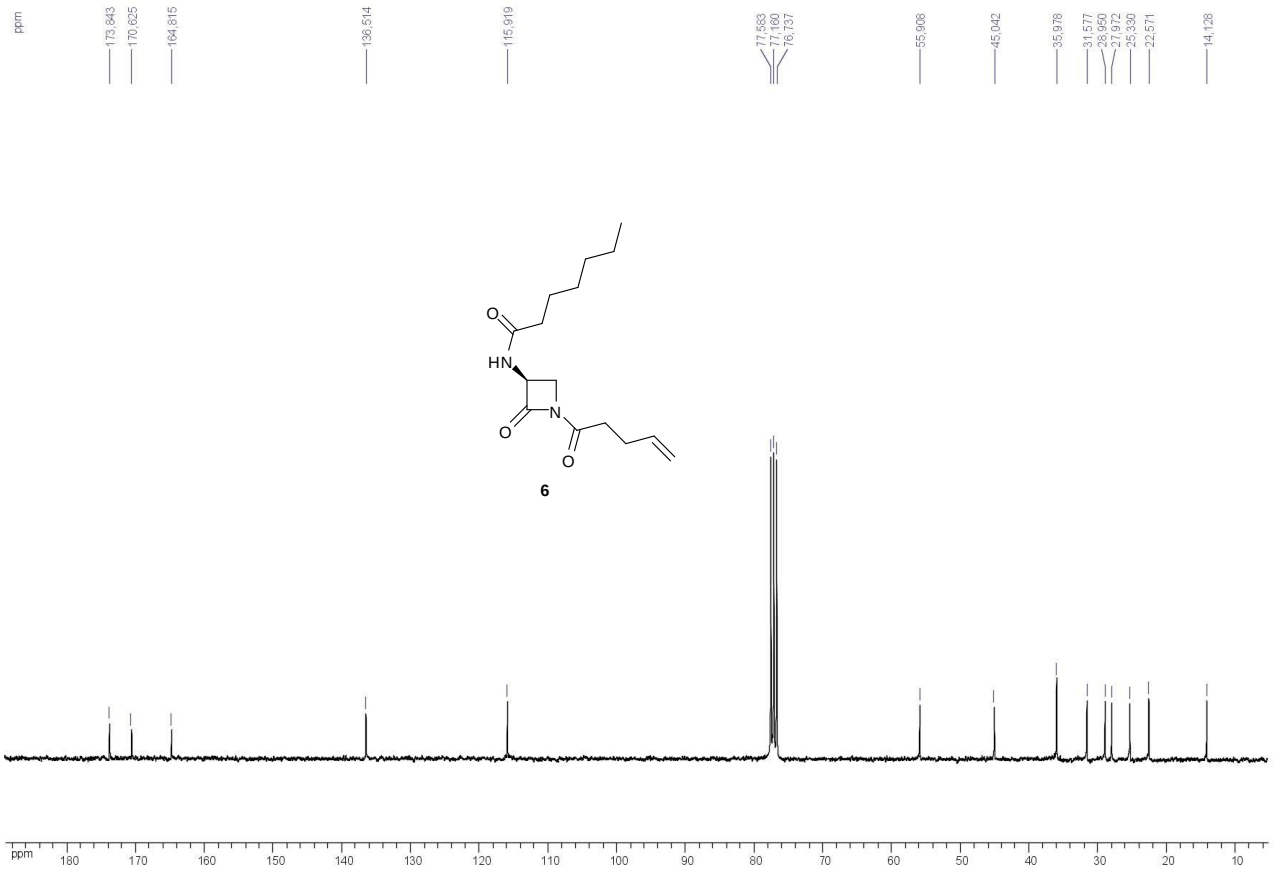
ppm
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136,514
115,919
77,583
77,160
76,737
55,908
45,042
35,978
31,577
28,950
27,972
25,330
22,571
14,128
HN
O
N
O
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ppm
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160
150
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120
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80
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20
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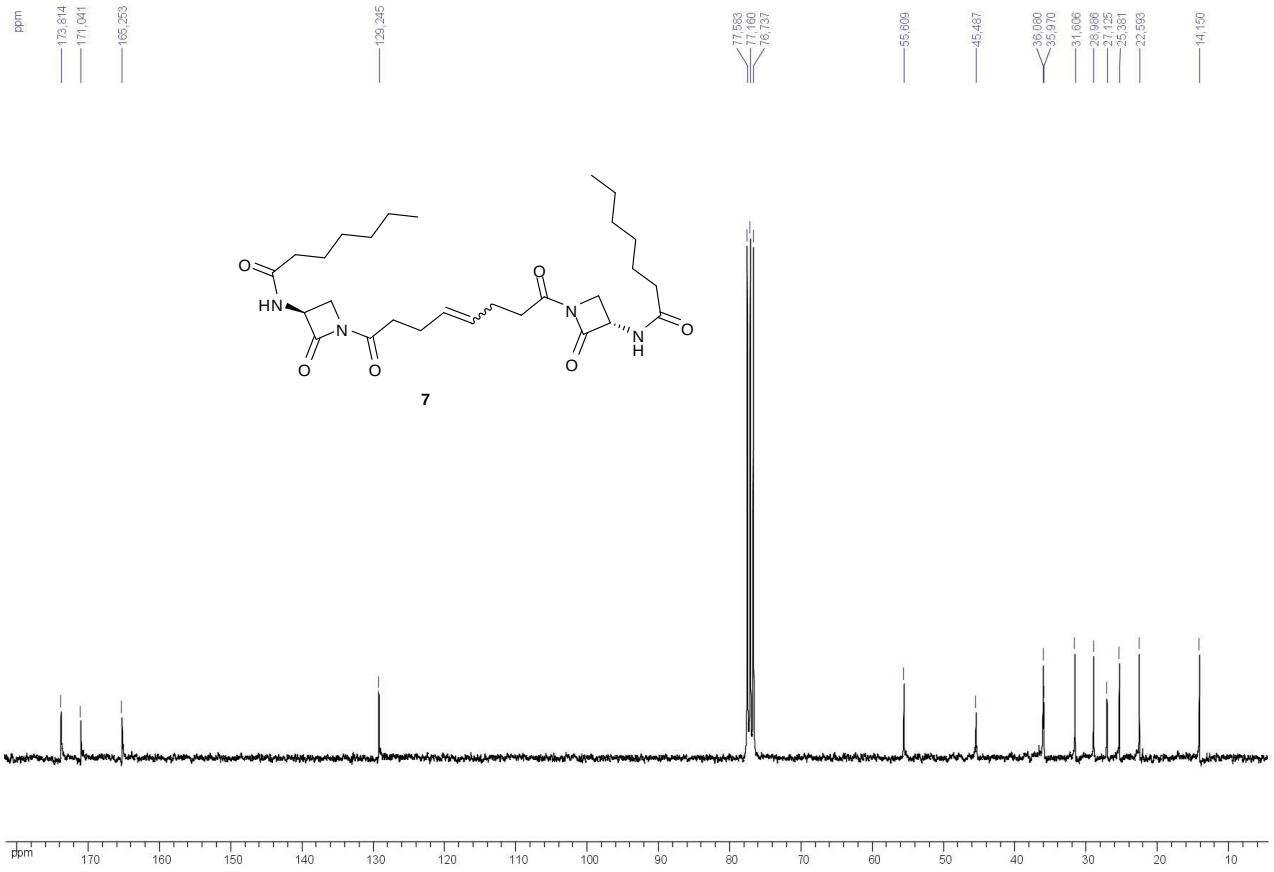
ppm
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129,245
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77,160
76,737
55,609
45,487
36,080
35,970
31,606
28,986
27,125
25,381
22,593
14,150
HN
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O
O
O
O
N
O
N
H
O
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ppm
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160
150
140
130
120
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80
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40
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20
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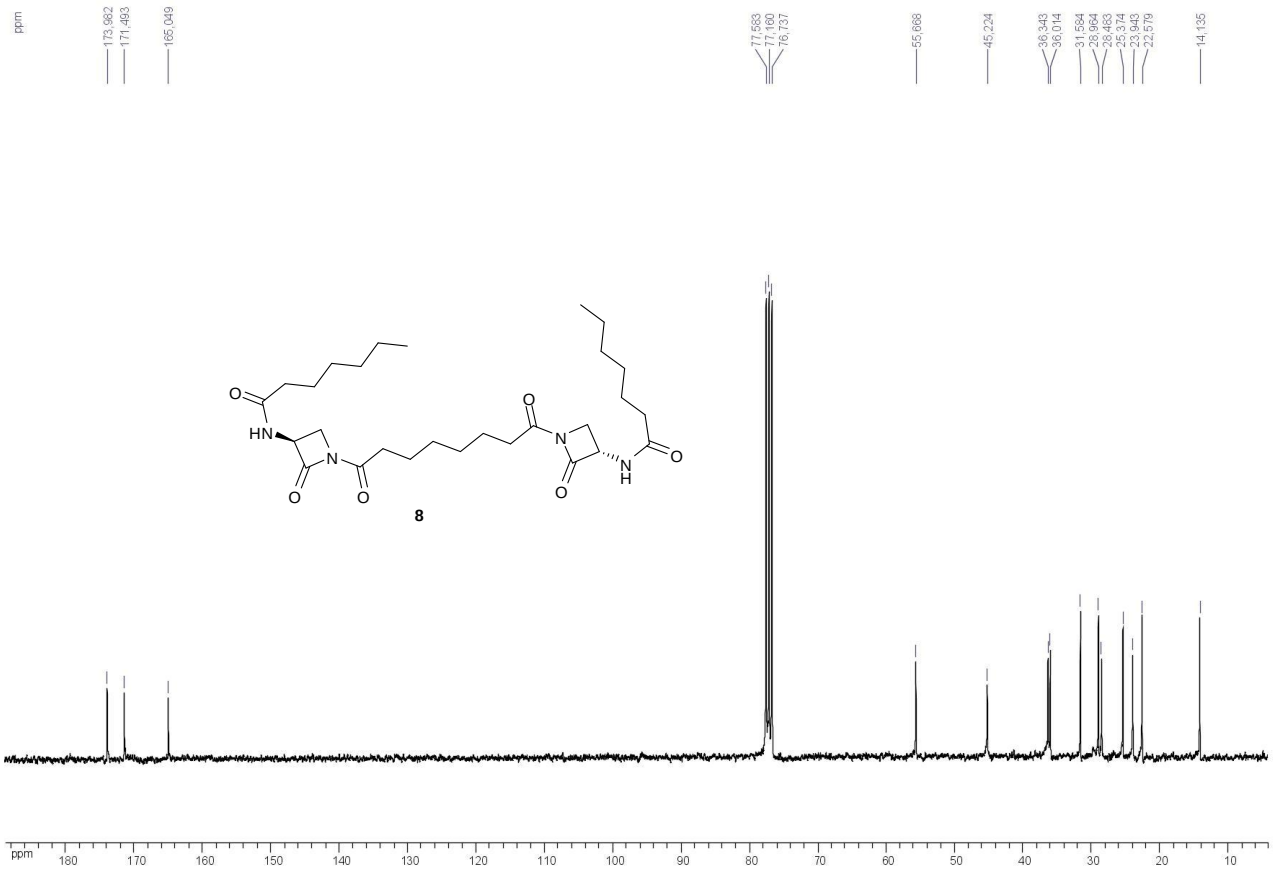
ppm
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171,493
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77,583
77,160
76,737
55,668
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36,343
36,014
31,584
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N
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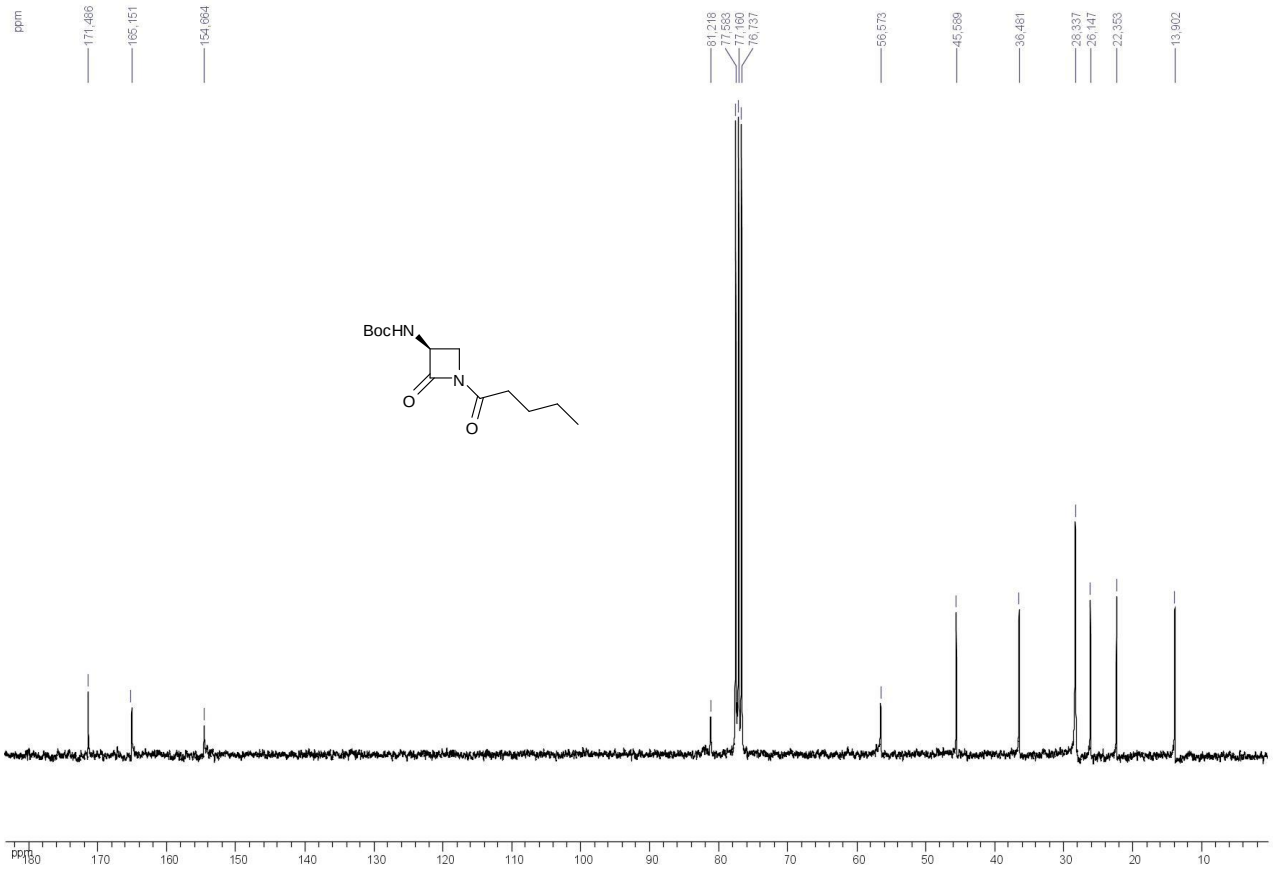
ppm
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77,583
77,160
76,737
56,573
45,589
36,481
28,337
26,147
22,353
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BocHN
N
O
O
ppm
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160
150
140
130
120
110
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80
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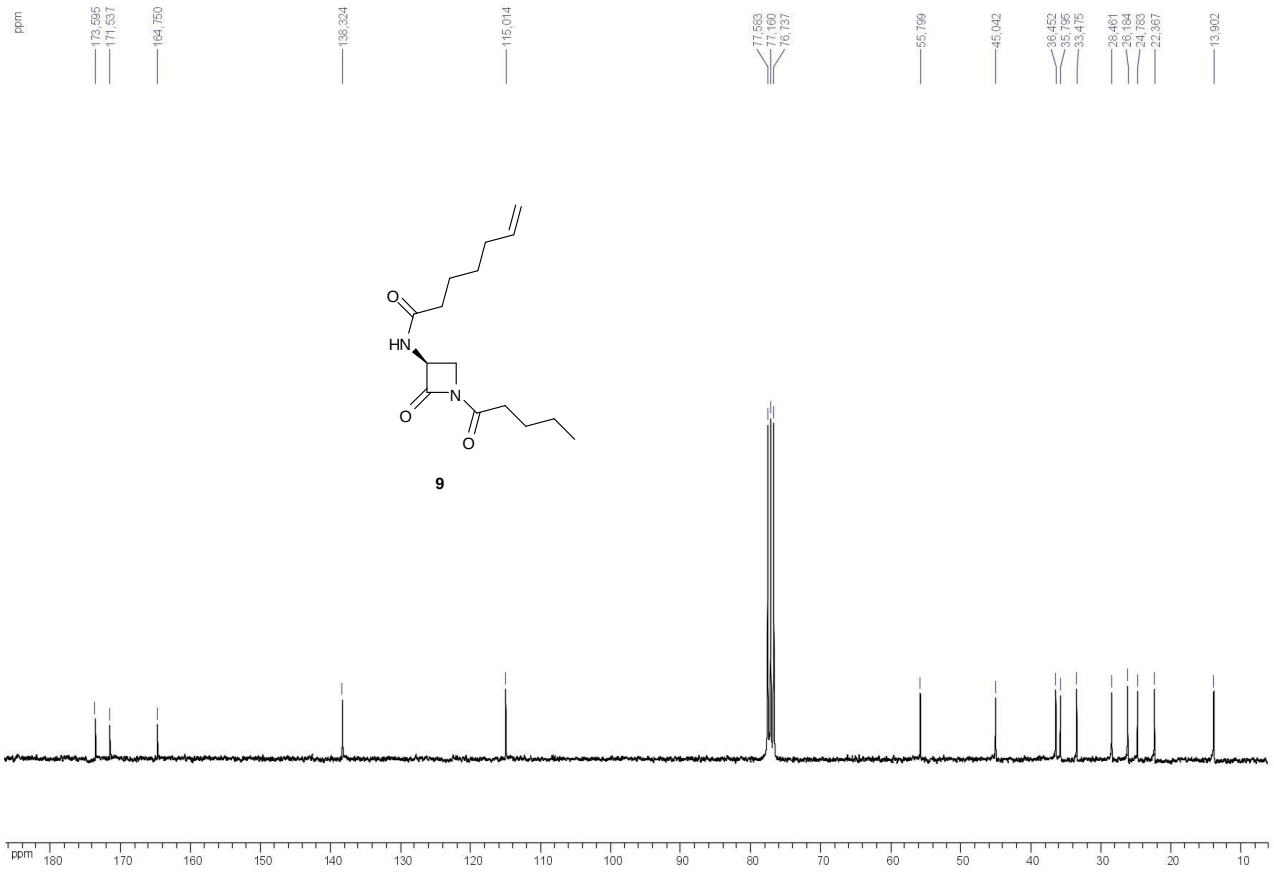
10


ppm
173,595
171,537
164,750
138,324
115,014
77,583
77,160
76,737
55,799
45,042
36,452
35,795
33,475
28,461
26,184
24,783
22,367
13,902
O
HN
N
O
O
ppm
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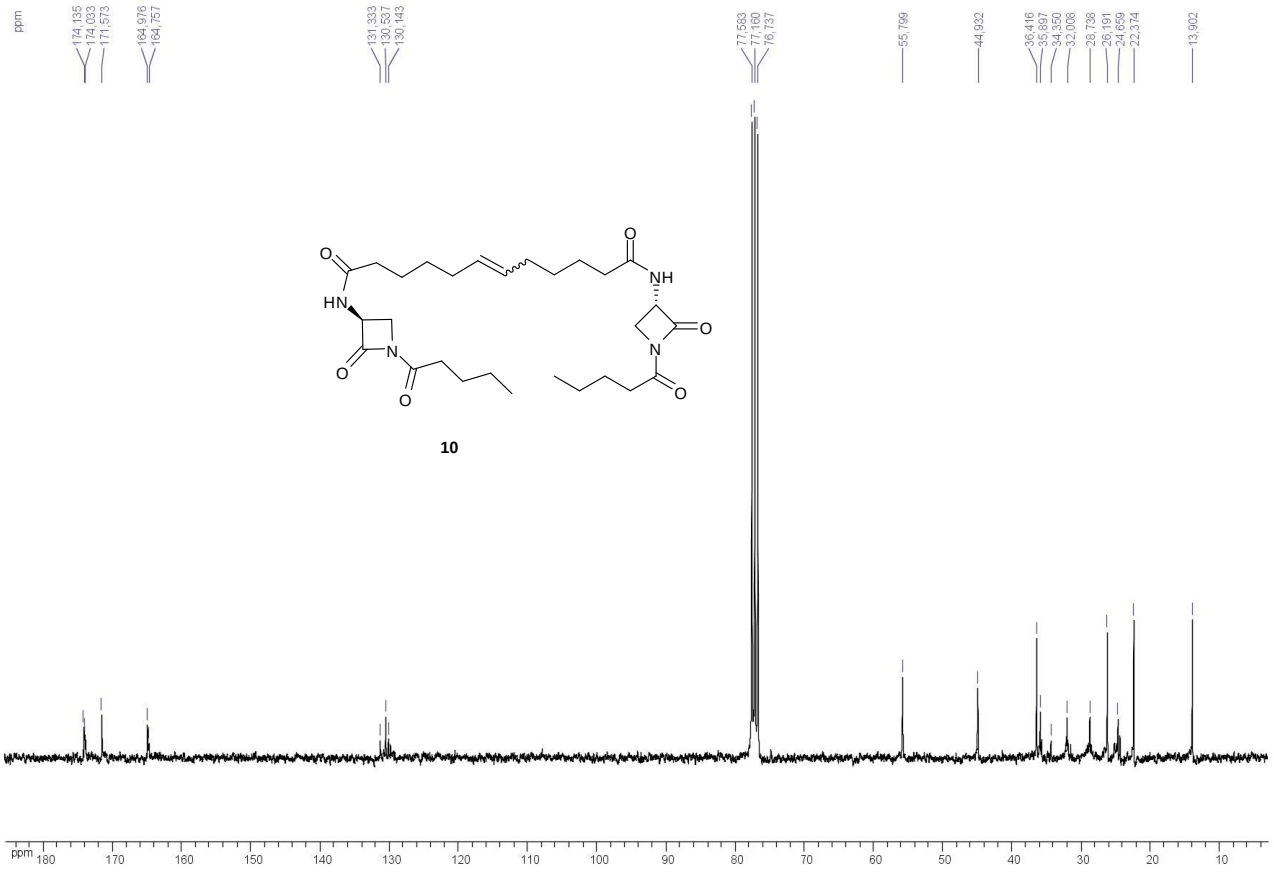
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

9


ppm
174,135
174,033
171,573
164,976
164,757
131,333
130,537
130,143
77,583
77,160
76,737
55,799
44,932
36,416
35,897
34,350
32,008
28,738
26,191
24,659
22,374
13,902
O
NH
HN
N
O
O
ppm
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170
160
150
140
130
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110
100
90
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70
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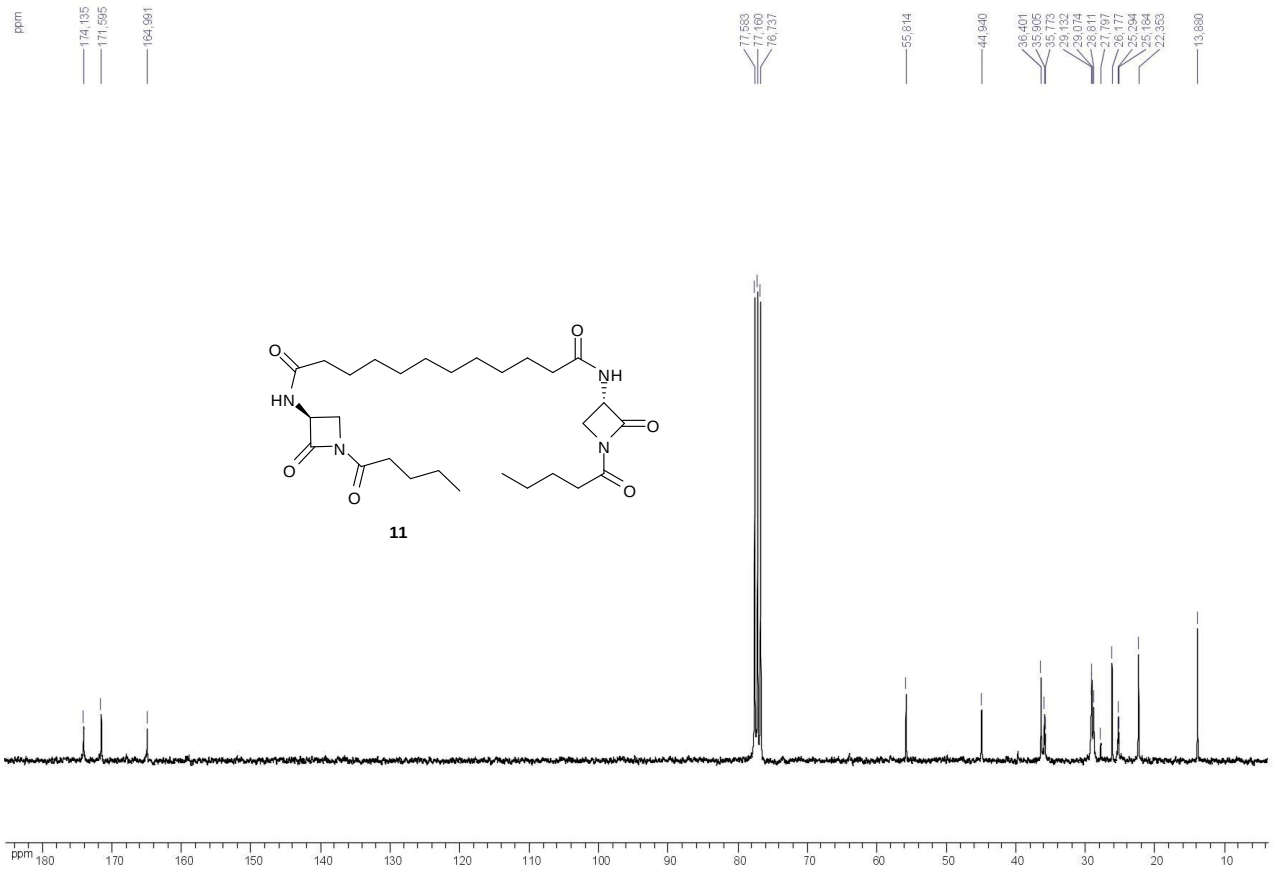
50
40
30
20
10

10


ppm
174,135
171,595
164,991
77,583
77,160
76,737
55,814
44,940
36,401
35,905
33,773
29,132
29,074
28,811
27,197
26,177
25,494
25,184
22,953
13,880
HN
NH
O
N
ppm
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

11