

Selective and Rapid Water Translocation across Self-assembled Peptide-Diol Channel via the Formation of Dual Water Array

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1. General Methods

All reagents used for the synthesis were purchased from Sigma-Aldrich, Avra, TCI, Spectrochem, Alfa Aesar and used without further purification. Dry solvents, e.g., Toluene, Acetone, THF, CH₂Cl₂, and MeOH used for the synthesis, were purchased from Rankem, Merck and used without further drying. All the dry reactions were placed in oven-dried apparatus under atmospheric nitrogen conditions. The progress and completion of the reactions were monitored by performing thin layer chromatography experiments where the plates were visualized either by short-wave UV light or by different staining reagents (Ninhydrin, PMA, etc.). Column chromatography for purification of the compound was performed using distilled organic solvents on silica gel (100–200 or 230–400 mesh). HEPES buffer, HPTS dye, Triton X-100, Lucigenin dye, NaOH, and inorganic salts (NaCl, NaNO₃, KCl, LiCl, CsCl, NaBr, NaI, etc.) were used in molecular biology grade purchased from Sigma-Aldrich. Egg yolk phosphatidylcholine (EYPC) lipid (25 mg/mL in chloroform), mini-extruder, and polycarbonate membranes (100 and 200 nm) were obtained from Sigma-Aldrich (Avanti Polar Lipids).

2. Physical Measurements

All ¹H and ¹³C NMR spectra were recorded either on Bruker 400 MHz and Jeol 400 MHz spectrometers. The chemical shifts (δ) in ppm were referenced to the residual signal of deuterium solvents (¹H NMR CDCl₃: δ 7.26 ppm; ¹³C NMR CDCl₃: δ 77.2 ppm; ¹H NMR DMSO-*d*₆: δ 2.5 ppm; ¹³C NMR DMSO-*d*₆: δ 39.5 ppm). The multiplicities of the peaks are s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), m (multiplet). High-resolution mass spectra (HRMS) were acquired in the ESI (+ve) mode. The measurement of pH during preparation of buffer solution was done by pH meter purchased from Hanna instruments. Fluorescence spectra were recorded on a Fluoromax-4 from Horiba scientific, Jobin Yvon Edison equipped with an injector port and a magnetic stirrer. The experimental data obtained from fluorescence were processed in Origin 8.5 software. The field emission scanning electron microscopy (FESEM) images were obtained using FEI Quanta 3D dual beam ESEM at 3.0 kV. The high-resolution transmission electron microscopy (HRTEM) images were acquired on the Jeol USA JEM-2200 FS transmission electron microscope. The dynamic light scattering (DLS) measurement experiment was performed using the Nano ZS-90 setup from the Malvern instrument with a 636 nm laser as a light source. The light scattering intensity

was measured at a 90° angle by the detector and used further to calculate the diameter of the vesicles following the Stokes-Einstein equation.

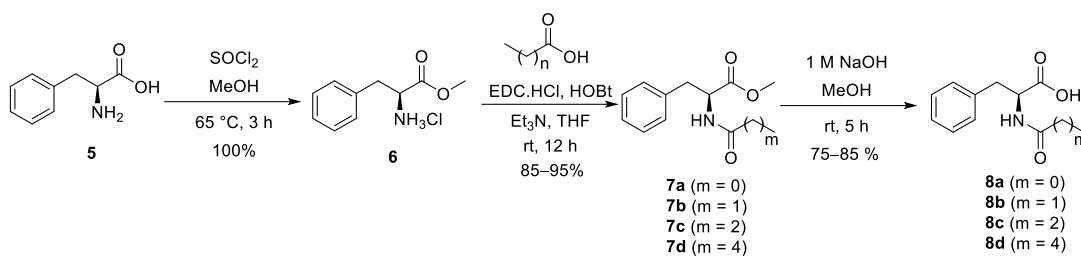
3. Synthesis of Compounds

3.1. Preparation of phenylalanine derivatives **8a–8d**.

To prepare the phenylalanine derivatives **8a–8d**, we have followed the reported literature procedure with slight modifications.¹ For the preparation of the methyl ester **6**, the *L*-phenylalanine **5** (1.24 g, 7.51 mmol) and methanol (90 mL) were taken in a 250 mL round bottom flask. The amino acid suspension was cooled to 0 °C by keeping it on an ice bath, and then SOCl₂ (2 mL) was added slowly to avoid the spontaneous heat generation. Then the reaction mixture was refluxed under stirring conditions for about 6 h under an inert atmosphere. After completion of the reaction, the reaction mixture was cooled to room temperature, solvent and unreacted SOCl₂ were evaporated to get the corresponding ester as a hydrochloride salt **6** in quantitative yield. Next, the methyl ester was used for the coupling reaction with different alkyl chains containing carboxylic acid to form the amides **7a–7d**.

In a 50 mL round bottom flask, methyl ester **6** (0.50 g, 2.32 mmol) and carboxylic acid (2.55 mmol) were taken and dissolved by 15 mL of dry THF. Then HOBt (0.38 g, 2.78 mmol), triethylamine (1 mL, 7.20 mmol), and EDC·HCl (0.54 g, 2.78 mmol) were added sequentially to the reaction mixture. The reaction mixture was then stirred at room temperature overnight under an inert atmosphere. After completion, the reaction mixture was washed sequentially with water and brine by extracting the compounds in CH₂Cl₂. Then the organic solvent was evaporated to yield the amide compounds **7a–7d** in moderate to good yield. The amide compounds were then used for the next steps without further purification.

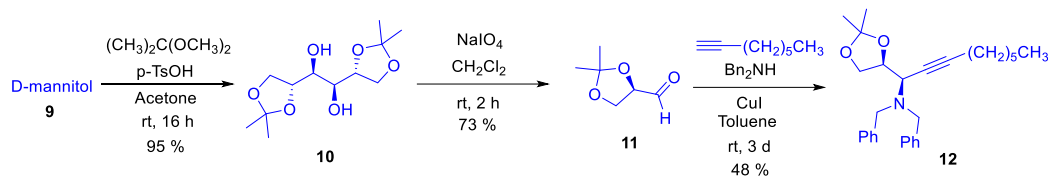
The hydrolysis reaction was done in the presence of sodium hydroxide as a base. For that, in a 25 mL round bottom flask, amides **7a–7d** (1.80 mmol) were taken and dissolved in 10 mL of THF : MeOH (1 : 1) solution. Then, NaOH (0.15 g, 3.62 mmol) was added into that reaction mixture by dissolving it in 2 mL of water. The reaction mixture was then heated at 65 °C for 5 h. After completion of the reaction, the reaction solution was cooled, acidified with 1 M HCl, and washed with water by extracting the compound in ethyl acetate (EtOAc). Then the volatiles were evaporated to get the crude compound which was then purified by column chromatography to get the pure acid compounds **8a–8d** in 75-85% yield. ¹H NMR spectra of the compounds were matched with the reported spectra.



Scheme S1. Preparation of the acid derivatives **8a–8d**.

3.2. Preparation of diol-protected compound **12**.

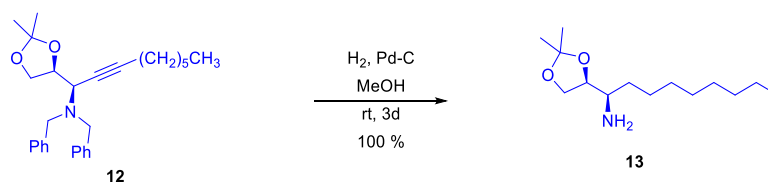
For the preparation of molecule **12**, we have followed the literature procedure reported from our group.^{2, 3}



Scheme S2. Preparation of the diol-protected compound **12**.

3.3. Synthesis procedure for diol-protected amine **13**.

In a 100 mL round bottom flask, 2.0 g of diol-protected compound **12** was taken and dissolved in 30 mL of MeOH. The compound solution was then degassed for about 1 h using N₂ gas balloon. Then into that degassed solution one small pinch of 10% Pd-C was added. The reaction mixture was then stirred under a hydrogen gas balloon for 3 days at room temperature. After completion of the reaction, the whole solution was passed through celite bed while washing with MeOH. Then the solvent was evaporated to get the amine **13** as a colourless sticky liquid in quantitative yield.



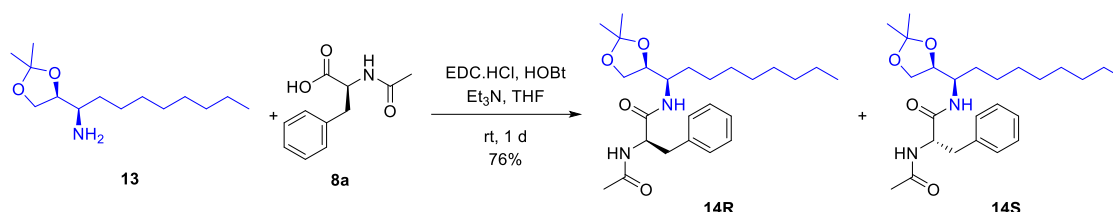
Scheme S3. Synthesis of compound **13**.

Yield: 100%; **¹H NMR (400 MHz, CDCl₃):** δ 4.03 – 3.96 (m, 1H), 3.87 (q, J = 6.8 Hz, 1H), 3.66 (dd, J = 8.1, 7.0 Hz, 1H), 2.69 (t, J = 7.5 Hz, 1H), 1.76 (s, 2H), 1.41 (s, 3H), 1.34 (s,

3H), 1.32 – 1.17 (m, 14H), 0.86 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3 : $\text{MeOH-}d_4$ = 4 : 1): δ 109.1, 80.4, 67.1, 54.2, 34.1, 31.9, 29.8, 29.6, 29.4, 26.8, 26.1, 25.5, 22.8, 14.2; HRMS (ESI): Calcd. $\text{C}_{14}\text{H}_{30}\text{NO}_2$ $[\text{M}+\text{H}]^+$: 244.2276, Found: 244.2281.

3.4. Synthesis of the compound 1R, 1S, 2R, 2S, 3R, 3S, 4R and 4S

14R and 14S:



Scheme S4. Synthesis of compound **14R** and **14S**.

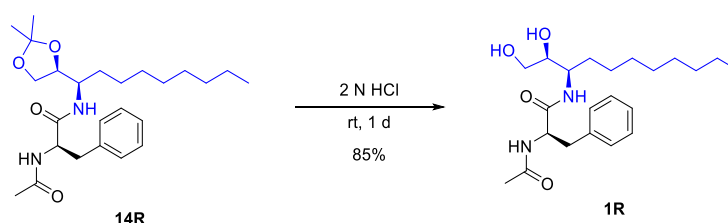
In a 25 mL round bottom flask the amine **13** (0.14 g, 0.58 mmol) was taken and dissolved in 6 mL of dry THF. Then into the reaction mixture acetyl-*L*-phenylalanine **8a** (0.12 g, 0.58 mmol), HOBT (0.08 g, 0.58 mmol) and triethylamine (0.5 mL) were added. At last EDC·HCl (0.13 g, 0.69 mmol) was added and the reaction mixture was then stirred at room temperature overnight under an inert atmosphere. After completion of the reaction, the reaction mixture was washed with water (3×10 mL) and followed by brine solution (1×10 mL) by extracting the compound in CHCl_3 (50 mL). The organic layer was then dried over Na_2SO_4 and the solvent was evaporated in a rotary evaporator to get the crude product. The crude product was purified by silica gel column chromatography using ethyl acetate (EtOAc) in petroleum ether (PE) as a solvent system. Two diastereomeric amide compounds **14R** and **14S**, were collected separately in 37% yield (**14R** in 45% EtOAc/PE) and 39% yield (**14S** 75% EtOAc/PE).

(R)-2-acetamido-*N*-((R)-1-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)-3-phenylpropanamide ($\text{C}_{25}\text{H}_{40}\text{N}_2\text{O}_4$) 14R: ^1H NMR (400 MHz, CDCl_3): δ 7.33 – 7.18 (m, 5H), 6.11 – 5.96 (m, 2H), 4.64 (q, $J = 7.7$ Hz, 1H), 4.07 (td, $J = 7.0, 2.0$ Hz, 1H), 3.90 (q, $J = 8.4$ Hz, 1H), 3.82 (dd, $J = 8.2, 6.7$ Hz, 1H), 3.20 (t, $J = 7.8$ Hz, 1H), 3.11 (dd, $J = 13.7, 7.6$ Hz, 1H), 3.01 (dd, $J = 13.8, 6.5$ Hz, 1H), 1.96 (s, 3H), 1.67 – 1.60 (m, 2H), 1.55 – 1.43 (m, 2H), 1.29 (s, 3H), 1.29 (s, 3H), 1.27 – 1.24 (m, 10H), 0.87 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3): δ 171.1, 170.1, 136.5, 129.4, 128.8, 127.2, 109.1, 76.6, 66.2, 55.0, 49.5, 38.2, 33.1, 32.0, 29.6, 29.5,

29.4, 26.4, 26.1, 25.2, 23.2, 22.8, 14.2; **HRMS (ESI):** Calcd. C₂₅H₄₁N₂O₄ [M+H]⁺: 433.3066, Found: 433.3068.

(S)-2-acetamido-N-((R)-1-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)-3-phenylpropanamide (C₂₅H₄₀N₂O₄) 14S: ¹H NMR (400 MHz, CDCl₃): δ 7.33 – 7.22 (m, 5H), 6.20 (d, *J* = 7.9 Hz, 1H), 5.69 (d, *J* = 9.5 Hz, 1H), 4.68 – 4.58 (m, 1H), 4.10 (t, *J* = 7.6 Hz, 1H), 3.96 – 3.82 (m, 2H), 3.49 – 3.41 (m, 1H), 3.14 (dd, *J* = 13.6, 5.6 Hz, 1H), 2.98 (dd, *J* = 13.7, 8.6 Hz, 1H), 1.98 (s, 3H), 1.46 – 1.32 (m, 2H), 1.30 (s, 3H), 1.27 (s, 3H), 1.25 – 1.11 (m, 12H), 0.89 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃): δ 170.9, 170.0, 136.9, 129.4, 128.9, 127.2, 109.2, 76.6, 66.3, 54.9, 49.6, 38.5, 33.0, 32.0, 29.8, 29.6, 29.4, 26.2, 26.1, 24.9, 23.3, 22.8, 14.3; **HRMS (ESI):** Calcd. C₂₅H₄₁N₂O₄ [M+H]⁺: 433.3066, Found: 433.3055.

(R)-2-acetamido-N-((2S,3R)-1,2-dihydroxyundecan-3-yl)-3-phenylpropanamide (C₂₂H₃₆N₂O₄) 1R:



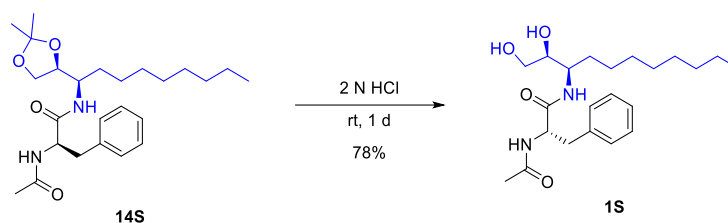
Scheme S5. Synthesis of compound **1R**.

In a 25 mL round bottom flask compound **14R** (0.06 g, 0.14 mmol) was taken and dissolved in 3 mL of THF : MeOH (1 : 1) solution. Then into that solution, 2 mL of 2 N HCl was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO₃ solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na₂SO₄ and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (3% MeOH/CHCl₃) solvent system to obtain pure product **1R** as a white solid in 85% yield. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.11 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 7.30 – 7.12 (m, 5H), 4.72 (d, *J* = 5.2 Hz, 1H), 4.53 – 4.45 (m, 1H), 4.38 (t, *J* = 5.7 Hz, 1H), 3.82 – 3.69 (m, 1H), 3.46 – 3.39 (m, 1H), 3.23 – 3.09 (m, 2H), 2.98 (dd, *J* = 13.8, 4.6 Hz, 1H), 2.72 (dd, *J* = 13.7, 10.1 Hz, 1H), 1.73 (s, 3H), 1.49 – 1.37 (m, 2H), 1.27 – 1.21 (d, *J* = 4.6 Hz, 12H), 0.91 – 0.81 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.5, 169.0, 138.1, 129.1, 128.0, 126.2, 72.3, 62.9, 54.2, 49.9, 37.7, 31.3,

30.9, 29.1, 29.1, 28.7, 25.8, 22.4, 22.1, 14.0; **HRMS (ESI):** Calcd. C₂₂H₃₇N₂O₄ [M+H]⁺: 393.2753, Found: 393.2740.

(S)-2-acetamido-N-((2S,3R)-1,2-dihydroxyundecan-3-yl)-3-phenylpropanamide

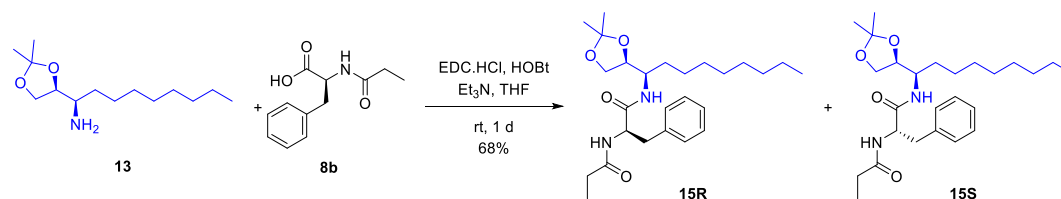
(C₂₂H₃₆N₂O₄) 1S:



Scheme S6. Synthesis of compound **1S**.

In a 25 mL round bottom flask compound **14S** (0.07 g, 0.16 mmol) was taken and dissolved in 4 mL of THF : MeOH (1 : 1) solution. Then into that solution, 3 mL of 2 N HCl was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO₃ solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na₂SO₄ and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (4% MeOH/CHCl₃) solvent system to obtain pure product **1S** as a white solid in 78% yield. **¹H NMR (400 MHz, DMSO-*d*₆):** δ 8.10 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 9.0 Hz, 1H), 7.29 – 7.20 (m, 4H), 7.19 – 7.12 (m, 1H), 4.72 (d, *J* = 4.8 Hz, 1H), 4.53 (td, *J* = 9.3, 5.3 Hz, 1H), 4.40 (t, *J* = 5.9 Hz, 1H), 3.73 (q, *J* = 8.7 Hz, 1H), 3.47 – 3.40 (m, 1H), 3.21 (td, *J* = 6.2, 2.0 Hz, 2H), 2.92 (dd, *J* = 13.6, 5.3 Hz, 1H), 2.71 (dd, *J* = 13.5, 9.6 Hz, 1H), 1.74 (s, 3H), 1.37 (q, *J* = 7.6 Hz, 2H), 1.30 – 1.08 (m, 12H), 0.88 – 0.80 (m, 3H); **¹³C NMR (100 MHz, DMSO-*d*₆):** δ 171.7, 168.9, 138.0, 129.2, 128.0, 126.2, 72.5, 62.9, 54.2, 49.7, 38.0, 31.3, 30.7, 29.0, 28.9, 28.7, 25.6, 22.5, 22.1, 14.0; **HRMS (ESI):** Calcd. C₂₂H₃₇N₂O₄ [M+H]⁺: 339.2753, Found: 339.2756.

15R and 15S:



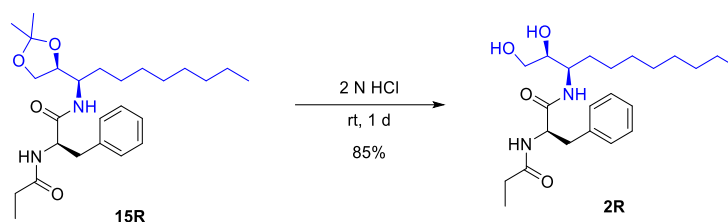
Scheme S7. Synthesis of compound **15R** and **15S**.

In a 25 mL round bottom flask the amine **13** (0.18 g, 0.74 mmol) was taken and dissolved in 7 mL of dry THF. Then into the reaction mixture propionyl-L-phenylalanine **8b** (0.17 g, 0.77 mmol), HOBt (0.10 g, 0.74 mmol) and triethylamine (0.5 mL) were added. At last, EDC·HCl (0.17 g, 0.89 mmol) was added, and the reaction mixture was then stirred at room temperature overnight under an inert atmosphere. After completion of the reaction, the reaction mixture was washed with water (2 × 10 mL) and followed by brine solution (1 × 10 mL) by extracting the compound in CHCl₃ (60 mL). The organic layer was then dried over Na₂SO₄, and the solvent was evaporated in a rotary evaporator to get the crude product. The crude product was purified by silica gel column chromatography using ethyl acetate (EtOAc) in petroleum ether (PE) as a solvent system. Two diastereomeric amide compounds **15R** and **15S**, were collected separately in 35% yield (**15R** in 25% EtOAc/PE) and 33% yield (**15S** 40% EtOAc/PE).

(R)-N-((R)-1-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)-3-phenyl-2-propionamido-propanamide (C₂₆H₄₂N₂O₄) 15R: ¹H NMR (400 MHz, CDCl₃): δ 7.34 – 7.15 (m, 5H), 6.00 (dd, *J* = 32.6, 8.6 Hz, 2H), 4.65 (q, *J* = 7.8 Hz, 1H), 4.06 (t, *J* = 7.0 Hz, 1H), 3.90 (q, *J* = 8.4 Hz, 1H), 3.83 (t, *J* = 7.4 Hz, 1H), 3.21 (t, *J* = 7.8 Hz, 1H), 3.16 – 3.08 (m, 1H), 3.01 (dd, *J* = 13.7, 6.6 Hz, 1H), 2.18 (q, *J* = 7.6 Hz, 2H), 1.53 – 1.44 (m, 2H), 1.29 (s, 3H), 1.28 (s, 3H), 1.27 – 1.12 (m, 12H), 1.09 (t, *J* = 7.6 Hz, 3H), 0.87 (t, *J* = 6.3 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃): δ 173.8, 171.2, 136.6, 129.4, 128.8, 127.2, 109.1, 76.7, 66.3, 54.8, 49.5, 38.1, 33.1, 32.0, 29.8, 29.7, 29.5, 29.4, 26.4, 26.1, 25.2, 22.8, 14.2, 9.8; **HRMS (ESI):** Calcd. C₂₆H₄₃N₂O₄ [M+H]⁺: 447.3222, Found: 447.3219.

(S)-N-((R)-1-((S)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)-3-phenyl-2-propionamido-propanamide (C₂₆H₄₂N₂O₄) 15S: ¹H NMR (400 MHz, CDCl₃): δ 7.32 – 7.20 (m, 5H), 6.19 (d, *J* = 7.6 Hz, 1H), 5.74 (d, *J* = 9.4 Hz, 1H), 4.66 (td, *J* = 8.2, 5.8 Hz, 1H), 4.10 (t, *J* = 6.8 Hz, 1H), 3.94 – 3.83 (m, 2H), 3.44 (dd, *J* = 8.2, 7.0 Hz, 1H), 3.14 (dd, *J* = 13.7, 5.8 Hz, 1H), 2.99 (dd, *J* = 13.7, 8.5 Hz, 1H), 2.21 (q, *J* = 7.6 Hz, 2H), 1.29 (s, 3H), 1.27 (s, 3H), 1.26 – 1.15 (m, 14H), 1.11 (t, *J* = 7.6 Hz, 3H), 0.89 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃): δ 173.7, 171.0, 136.9, 129.4, 128.9, 127.2, 109.2, 76.7, 66.4, 54.7, 49.6, 38.6, 33.1, 32.0, 29.8, 29.7, 29.6, 29.4, 26.3, 26.1, 24.9, 22.8, 14.3, 9.8; **HRMS (ESI):** Calcd. C₂₆H₄₃N₂O₄ [M+H]⁺: 447.3222, Found: 447.3219.

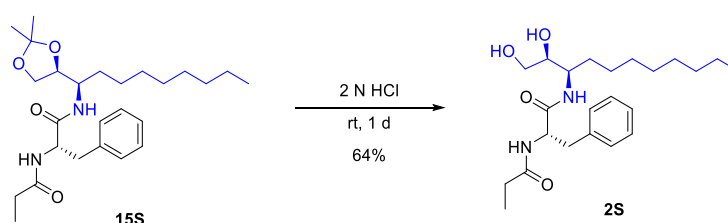
(R)-N-((2S,3R)-1,2-dihydroxyundecan-3-yl)-3-phenyl-2-propionamidopropanamide (C₂₃H₃₈N₂O₄) 2R:



Scheme S8. Synthesis of compound **2R**.

In a 25 mL round bottom flask compound **15R** (0.08 g, 0.18 mmol) was taken and dissolved in 4 mL of THF : MeOH (1 : 1) solution. Then into that solution, 3 mL of 2 N HCl was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO₃ solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na₂SO₄ and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (3% MeOH/CHCl₃) solvent system to obtain pure product **2R** as a white solid in 85% yield. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.99 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 9.1 Hz, 1H), 7.29 – 7.13 (m, 5H), 4.73 (d, *J* = 4.3 Hz, 1H), 4.53 (td, *J* = 9.4, 5.2 Hz, 1H), 4.39 (bs, 1H), 3.74 (q, *J* = 6.7 Hz, 1H), 3.43 (bs, 1H), 3.21 (bs, 2H), 2.94 (dd, *J* = 13.6, 5.2 Hz, 1H), 2.72 (dd, *J* = 13.5, 9.7 Hz, 1H), 2.02 (q, *J* = 7.6 Hz, 2H), 1.38 (q, *J* = 7.4 Hz, 2H), 1.30 – 1.10 (m, 12H), 0.87 (t, *J* = 7.6 Hz, 3H), 0.84 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆): δ 172.7, 171.6, 138.0, 129.2, 127.9, 126.1, 72.5, 62.9, 54.0, 49.7, 37.9, 31.3, 30.8, 28.9, 28.9, 28.7, 28.3, 25.6, 22.1, 14.0, 9.9; HRMS (ESI): Calcd. C₂₃H₃₉N₂O₄ [M+H]⁺: 407.2909, Found: 407.2901.

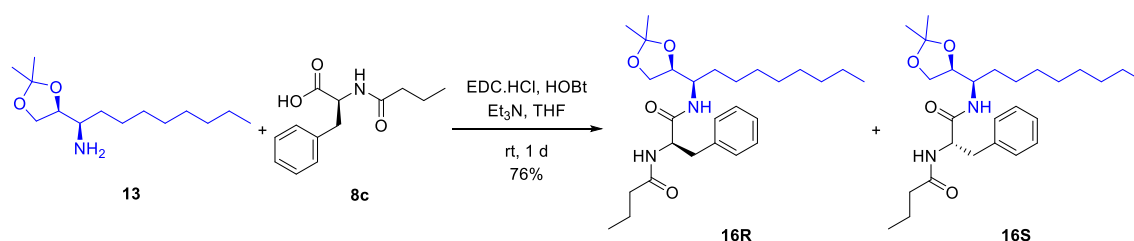
(S)-N-((2S,3R)-1,2-dihydroxyundecan-3-yl)-3-phenyl-2-propionamidopropanamide (C₂₃H₃₈N₂O₄) 2S:



Scheme S9. Synthesis of compound **2S**.

In a 25 mL round bottom flask compound **15S** (0.07 g, 0.16 mmol) was taken and dissolved in 4 mL of THF : MeOH (1 : 1) solution. Then into that solution, 3 mL of 2 N HCl was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO₃ solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na₂SO₄ and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (4% MeOH/CHCl₃) solvent system to obtain pure product **2S** as a white solid in 64% yield. **¹H NMR (400 MHz, DMSO-*d*₆):** δ 7.99 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.27 – 7.15 (m, 5H), 4.73 (d, *J* = 4.8 Hz, 1H), 4.53 (td, *J* = 9.4, 5.2 Hz, 1H), 4.39 (t, *J* = 5.9 Hz, 1H), 3.74 (q, *J* = 6.5 Hz, 1H), 3.47 – 3.40 (m, 1H), 3.21 (td, *J* = 6.0, 3.0 Hz, 2H), 2.95 (dd, *J* = 13.6, 5.2 Hz, 1H), 2.72 (dd, *J* = 13.5, 9.7 Hz, 1H), 2.02 (q, *J* = 7.8, 7.2 Hz, 2H), 1.38 (q, *J* = 7.6 Hz, 2H), 1.27 – 1.18 (m, 12H), 0.87 (t, *J* = 7.6 Hz, 3H), 0.85 (t, *J* = 6.8 Hz, 3H); **¹³C NMR (100 MHz, DMSO-*d*₆):** δ 172.7, 171.6, 138.0, 129.2, 127.9, 126.1, 72.5, 62.9, 54.0, 49.7, 37.9, 31.3, 30.8, 28.9, 28.9, 28.7, 28.3, 25.6, 22.1, 14.0, 9.9; **HRMS (ESI):** Calcd. C₂₃H₃₉N₂O₄ [M+H]⁺: 407.2909, Found: 407.2901.

16R and 16S:



Scheme S10. Synthesis of compounds **16R** and **16S**.

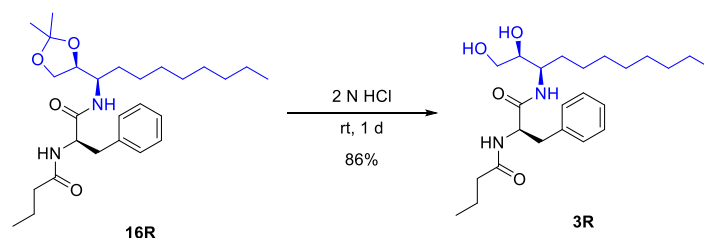
In a 50 mL round bottom flask, the amine **13** (0.27 g, 1.13 mmol) was taken and dissolved in 10 mL of dry THF. Then into the reaction mixture butyryl-L-phenylalanine **8c** (0.26 g, 1.13 mmol), HOBt (0.15 g, 1.13 mmol) and triethylamine (1 mL) were added. At last, EDC·HCl (0.26 g, 1.35 mmol) was added, and the reaction mixture was then stirred at room temperature overnight under an inert atmosphere. After completion of the reaction, the reaction mixture was washed with water (3 × 10 mL) and followed by brine solution (1 × 10 mL) by extracting the compound in CHCl₃ (40 mL). The organic layer was then dried over Na₂SO₄, and the solvent was evaporated in a rotary evaporator to get the crude product. The crude

product was purified by silica gel column chromatography using ethyl acetate (EtOAc) in petroleum ether (PE) as a solvent system. Two diastereomeric amide compounds **16R** and **16S**, were collected separately in 38% yield (**16R** in 25% EtOAc/PE) and 38% yield (**16S** 40% EtOAc/PE).

***N*-((*R*)-1-(((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)amino)-1-oxo-3-phenylpropan-2-yl)butyramide (C₂₇H₄₄N₂O₄) **16R**:** ¹H NMR (400 MHz, CDCl₃): δ 7.32 – 7.18 (m, 5H), 6.09 (bs, 1H), 6.01 (bs, 1H), 4.67 (q, *J* = 7.5 Hz, 1H), 4.06 (td, *J* = 7.1, 2.1 Hz, 1H), 3.90 (dd, *J* = 17.5, 8.4 Hz, 1H), 3.82 (dd, *J* = 8.2, 6.6 Hz, 1H), 3.20 (t, *J* = 7.8 Hz, 1H), 3.12 (dd, *J* = 13.7, 7.6 Hz, 1H), 2.99 (dd, *J* = 13.8, 6.8 Hz, 1H), 2.13 (dd, *J* = 16.0, 8.8 Hz, 2H), 1.66 – 1.53 (m, 2H), 1.29 (s, 3H), 1.28 (s, 3H), 1.27 – 1.17 (m, 14H), 0.87 (td, *J* = 7.2, 2.0 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃): δ 173.1, 171.2, 136.6, 129.4, 128.8, 127.2, 109.1, 76.8, 66.3, 54.7, 49.4, 38.6, 38.2, 33.2, 32.0, 29.6, 29.6, 29.4, 26.4, 26.1, 25.2, 22.8, 19.1, 14.2, 13.7; **HRMS (ESI)**: Calcd. C₂₇H₄₅N₂O₄ [M+H]⁺: 461.3379, Found: 461.3377.

***N*-((*S*)-1-(((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)amino)-1-oxo-3-phenylpropan-2-yl)butyramide (C₂₇H₄₄N₂O₄) **16S**:** ¹H NMR (400 MHz, CDCl₃): δ 7.34 – 7.18 (m, 5H), 6.17 (d, *J* = 6.7 Hz, 1H), 5.73 (d, *J* = 9.1 Hz, 1H), 4.66 (td, *J* = 8.1, 6.0 Hz, 1H), 4.09 (td, *J* = 6.8, 1.9 Hz, 1H), 3.93 – 3.86 (m, 2H), 3.44 (dd, *J* = 8.2, 7.0 Hz, 1H), 3.14 (dd, *J* = 13.7, 5.9 Hz, 1H), 2.99 (dd, *J* = 13.7, 8.5 Hz, 1H), 2.15 (td, *J* = 7.4, 2.5 Hz, 2H), 1.67 – 1.55 (m, 2H), 1.45 – 1.32 (m, 4H), 1.28 (s, 3H), 1.27 (s, 3H), 1.26 – 1.13 (m, 10H), 0.88 (td, *J* = 7.2, 2.3 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃): δ 172.9, 171.0, 136.9, 129.4, 128.9, 127.2, 109.2, 77.4, 76.7, 66.4, 54.7, 49.5, 38.6, 33.1, 32.0, 29.8, 29.6, 29.4, 26.3, 26.1, 24.9, 22.8, 19.1, 14.3, 13.8; **HRMS (ESI)**: Calcd. C₂₇H₄₅N₂O₄ [M+H]⁺: 461.3379, Found: 461.3376.

***N*-((*R*)-1-(((2*S*,3*R*)-1,2-dihydroxyundecan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)butyramide (C₂₄H₄₀N₂O₄) **3R**:**

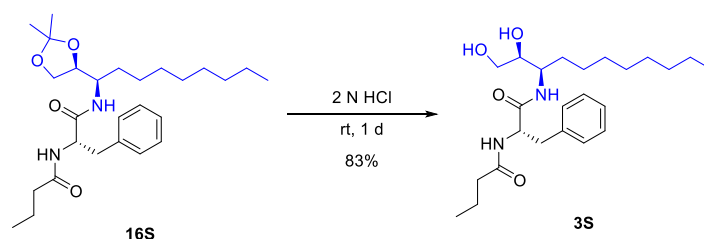


Scheme S11. Synthesis of compound **3R**.

In a 25 mL round bottom flask compound **16R** (0.10 g, 0.22 mmol) was taken and dissolved in 5 mL of THF : MeOH (1 : 1) solution. Then into that solution, 2 mL of 2 N HCl

was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO₃ solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na₂SO₄ and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (2% MeOH/CHCl₃) solvent system to obtain pure product **3R** as a white solid in 86% yield. **¹H NMR (400 MHz, DMSO-*d*₆):** δ 8.04 (d, *J* = 8.6 Hz, 1H), 7.37 (d, *J* = 9.1 Hz, 1H), 7.29 – 7.12 (m, 5H), 4.71 (d, *J* = 5.1 Hz, 1H), 4.51 (td, *J* = 9.9, 4.6 Hz, 1H), 4.37 (t, *J* = 5.7 Hz, 1H), 3.77 (dd, *J* = 16.4, 8.5 Hz, 1H), 3.42 (q, *J* = 6.2 Hz, 1H), 3.23 – 3.09 (m, 2H), 2.99 (dd, *J* = 13.7, 4.6 Hz, 1H), 2.73 (dd, *J* = 13.7, 10.3 Hz, 1H), 1.98 (t, *J* = 7.4 Hz, 2H), 1.52 – 1.31 (m, 2H), 1.30 – 1.13 (m, 14H), 0.85 (t, *J* = 6.8 Hz, 3H), 0.70 (t, *J* = 7.4 Hz, 3H); **¹³C NMR (100 MHz, DMSO-*d*₆):** δ 171.8, 171.5, 138.1, 129.1, 128.0, 126.1, 79.2, 72.2, 62.9, 53.9, 49.8, 37.5, 37.09, 31.3, 31.1, 29.1, 28.7, 25.7, 22.1, 18.6, 14.0, 13.4; **HRMS (ESI):** Calcd. C₂₄H₄₁N₂O₄ [M+H]⁺: 421.3066, Found: 421.3063.

***N*-((*S*)-1-(((2*S*,3*R*)-1,2-dihydroxyundecan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)butyramide (C₂₄H₄₀N₂O₄) **3S**:**

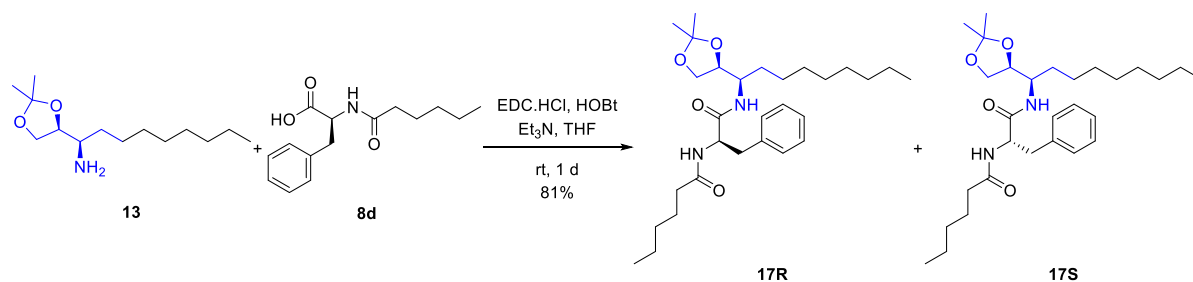


Scheme S12. Synthesis of compound **3S**.

In a 25 mL round bottom flask compound **16S** (0.12 g, 0.26 mmol) was taken and dissolved in 6 mL of THF : MeOH (1 : 1) solution. Then into that solution, 3 mL of 2 N HCl was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO₃ solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na₂SO₄ and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (2.5% MeOH/CHCl₃) solvent system to obtain pure product **3S** as a white solid in 83% yield. **¹H NMR (400 MHz, DMSO-*d*₆):** δ 8.01 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.29 – 7.11 (m, 5H), 4.74 (d, *J* = 4.7 Hz,

1H), 4.55 (td, $J = 9.6, 5.1$ Hz, 1H), 4.39 (t, $J = 5.9$ Hz, 1H), 3.75 (q, $J = 8.6$ Hz, 1H), 3.48 – 3.40 (m, 1H), 3.26 – 3.16 (m, 2H), 2.95 (dd, $J = 13.6, 5.1$ Hz, 1H), 2.72 (dd, $J = 13.6, 9.9$ Hz, 1H), 1.99 (t, $J = 7.4$ Hz, 2H), 1.46 – 1.31 (m, 4H), 1.28 – 1.11 (m, 12H), 0.88 – 0.81 (m, 3H), 0.70 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 171.8, 171.7, 138.0, 129.2, 127.9, 126.1, 79.2, 72.4, 62.9, 54.0, 49.7, 37.9, 37.1, 31.6, 30.8, 28.9, 28.7, 25.6, 22.1, 18.6, 14.0, 13.4; HRMS (ESI): Calcd. $\text{C}_{24}\text{H}_{41}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 421.3066, Found: 421.3057.

17R and 17S:



Scheme S13. Synthesis of compound **17R** and **17S**.

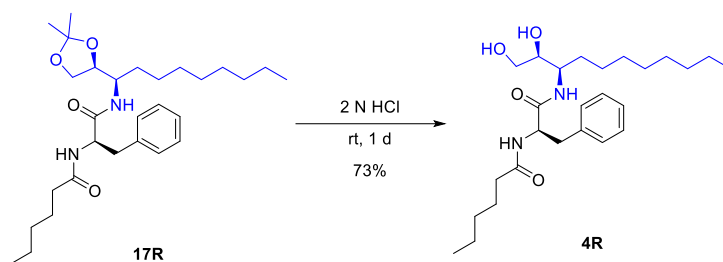
In a 50 mL round bottom flask, the amine **13** (0.30 g, 1.23 mmol) was taken and dissolved in 12 mL of dry THF. Then into the reaction mixture, hexanoyl-*L*-phenylalanine **8d** (0.32 g, 1.23 mmol), HOBt (0.17 g, 1.23 mmol), and triethylamine (1 mL) were added. At last, EDC·HCl (0.29 g, 1.48 mmol) was added, and the reaction mixture was then stirred at room temperature overnight under an inert atmosphere. After completion of the reaction, the reaction mixture was washed with water (3×10 mL) and followed by brine solution (1×10 mL) by extracting the compound in CHCl_3 (50 mL). The organic layer was then dried over Na_2SO_4 , and the solvent was evaporated in a rotary evaporator to get the crude product. The crude product was purified by silica gel column chromatography using ethyl acetate (EtOAc) in petroleum ether (PE) as a solvent system. Two diastereomeric amide compounds **17R** and **17S**, were collected separately in 40% yield (**17R** in 20% EtOAc/PE) and 41% yield (**17S** 25% EtOAc/PE).

***N*-((*R*)-1-(((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)amino)-1-oxo-3-phenylpropan-2-yl)hexanamide ($\text{C}_{29}\text{H}_{48}\text{N}_2\text{O}_4$) **17R**:** ^1H NMR (400 MHz, CDCl_3): δ 7.32 – 7.19 (m, 5H), 6.11 – 6.02 (m, 1H), 5.97 (t, $J = 7.9$ Hz, 1H), 4.66 (dd, $J = 15.0, 7.6$ Hz, 1H), 4.06 (td, $J = 7.1, 2.1$ Hz, 1H), 3.90 (dd, $J = 17.2, 8.7$ Hz, 1H), 3.83 (dd, $J = 8.2, 6.7$ Hz, 1H), 3.24 – 3.17 (m, 1H), 3.12 (dd, $J = 13.8, 7.6$ Hz, 1H), 3.00 (dd, $J = 13.8, 6.8$ Hz, 1H), 2.14 (dd, $J = 14.5, 6.9$ Hz, 2H), 1.61 – 1.54 (m, 2H), 1.53 – 1.46 (m, 2H), 1.29 (s, 3H), 1.29 (s, 3H), 1.27 – 1.19

(m, 16H), 0.86 (t, $J = 7.1$ Hz, 6H); ^{13}C NMR (101 MHz, CDCl_3): δ 173.2, 171.2, 136.6, 129.4, 128.8, 127.2, 109.1, 76.7, 66.3, 54.7, 49.4, 38.1, 36.7, 33.2, 32.0, 31.4, 29.6, 29.6, 29.4, 26.4, 26.1, 25.4, 25.2, 22.8, 22.5, 14.3, 14.0; HRMS (ESI): Calcd. $\text{C}_{29}\text{H}_{49}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 489.3692, Found: 489.3691.

***N*-((*S*)-1-(((*R*)-1-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)nonyl)amino)-1-oxo-3-phenylpropan-2-yl)hexanamide ($\text{C}_{29}\text{H}_{48}\text{N}_2\text{O}_4$) **17S**:** ^1H NMR (400 MHz, CDCl_3): δ 7.33 – 7.21 (m, 5H), 6.14 (d, $J = 7.3$ Hz, 1H), 5.72 (d, $J = 9.3$ Hz, 1H), 4.65 (td, $J = 8.1, 6.0$ Hz, 1H), 4.09 (td, $J = 6.7, 1.6$ Hz, 1H), 3.94 – 3.80 (m, 2H), 3.49 – 3.39 (m, 1H), 3.14 (dd, $J = 13.7, 5.9$ Hz, 1H), 2.99 (dd, $J = 13.7, 8.4$ Hz, 1H), 2.16 (td, $J = 7.4, 2.4$ Hz, 2H), 1.64 – 1.51 (m, 2H), 1.45 – 1.30 (m, 4H), 1.28 (s, 3H), 1.27 (s, 3H), 1.26 – 1.12 (m, 14H), 0.92 – 0.84 (m, 6H); ^{13}C NMR (101 MHz, CDCl_3): δ 173.2, 171.2, 136.6, 129.4, 128.8, 127.2, 109.1, 76.7, 66.3, 54.7, 49.4, 38.1, 36.7, 33.2, 32.0, 31.4, 29.6, 29.4, 26.4, 26.1, 25.4, 25.2, 24.9, 22.8, 22.5, 14.2, 14.0; HRMS (ESI): Calcd. $\text{C}_{29}\text{H}_{49}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 489.3692, Found: 489.3691.

***N*-((*R*)-1-(((2*S*,3*R*)-1,2-dihydroxyundecan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)hexanamide ($\text{C}_{26}\text{H}_{44}\text{N}_2\text{O}_4$) **4R**:**

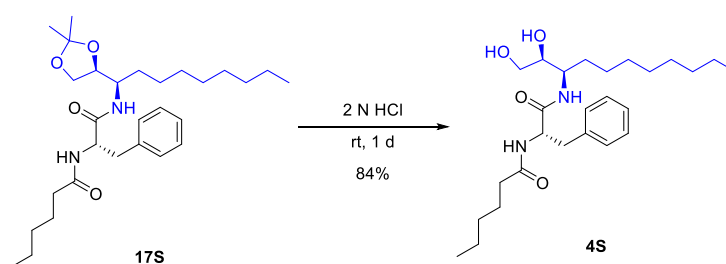


Scheme S14. Synthesis of compound **4R**.

In a 25 mL round bottom flask compound **17R** (0.16 g, 0.32 mmol) was taken and dissolved in 6 mL of THF : MeOH (1 : 1) solution. Then into that solution, 3 mL of 2 N HCl was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO_3 solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na_2SO_4 and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (2% MeOH/ CHCl_3) solvent system to obtain pure product **4R** as a white solid in 73% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.04 (d, $J = 8.5$ Hz, 1H), 7.35 (d, $J = 9.1$ Hz, 1H), 7.29 – 7.08 (m, 5H), 4.71 (bs, 1H), 4.50 (td, $J = 10.0, 4.7$ Hz, 1H), 4.37 (bs, 1H), 3.77 (q, $J = 8.3, 7.5$ Hz, 1H), 3.42 (d, $J = 5.6$ Hz, 1H), 3.25 –

3.08 (m, 2H), 2.99 (dd, $J = 13.8, 4.6$ Hz, 1H), 2.73 (dd, $J = 13.7, 10.3$ Hz, 1H), 1.99 (t, $J = 7.2$ Hz, 2H), 1.48 – 1.30 (m, 4H), 1.29 – 1.12 (m, 14H), 1.11 – 0.98 (m, 2H), 0.85 (t, $J = 6.7$ Hz, 3H), 0.80 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 172.0, 171.5, 138.2, 129.1, 128.0, 126.1, 79.2, 72.2, 62.9, 53.9, 49.8, 37.5, 35.2, 31.4, 31.1, 30.7, 29.1, 28.7, 25.8, 24.9, 22.1, 21.9, 14.0, 13.8; HRMS (ESI): Calcd. $\text{C}_{26}\text{H}_{45}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 449.3379, Found: 449.3381.

***N*-((*S*)-1-(((2*S*,3*R*)-1,2-dihydroxyundecan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)hexanamide ($\text{C}_{26}\text{H}_{44}\text{N}_2\text{O}_4$) **4S**:**



Scheme S15. Synthesis of compound **4S**.

In a 25 mL round bottom flask compound **17S** (0.24 g, 0.50 mmol) was taken and dissolved in 8 mL of THF : MeOH (1 : 1) solution. Then into that solution, 4 mL of 2N HCl was added and the reaction mixture was then stirred at room temperature overnight. After completion of the reaction, the reaction solution was neutralized by the dilute NaHCO_3 solution. The neutralized solution was then washed with water and brine by extracting the compound in ethyl acetate (EtOAc). The organic layer was dried over Na_2SO_4 and the solvent was then evaporated to get the crude product. The crude product was purified by silica gel column chromatography using methanol in chloroform (2.5% MeOH/ CHCl_3) solvent system to obtain pure product **4S** as a white solid in 84% yield. ^1H NMR (400 MHz, DMSO- d_6): δ 8.01 (d, $J = 8.5$ Hz, 1H), 7.54 (d, $J = 9.1$ Hz, 1H), 7.31 – 7.11 (m, 5H), 4.74 (bs, 1H), 4.61 – 4.49 (m, 1H), 4.38 (bs, 1H), 3.75 (q, $J = 7.0$ Hz, 1H), 3.44 (bs, 1H), 3.27 – 3.14 (m, 2H), 2.95 (dd, $J = 13.6, 4.9$ Hz, 1H), 2.72 (dd, $J = 13.3, 9.8$ Hz, 1H), 2.00 (t, $J = 7.3$ Hz, 2H), 1.43 – 1.31 (m, 4H), 1.28 – 1.12 (m, 14H), 1.09 – 1.01 (m, 2H), 0.82 (dt, $J = 17.4, 6.9$ Hz, 6H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 171.9, 171.8, 138.0, 129.2, 127.9, 126.1, 79.2, 72.4, 62.9, 54.0, 49.7, 37.9, 35.2, 31.3, 30.8, 30.6, 29.0, 28.7, 25.6, 24.9, 22.1, 21.9, 14.0, 13.8; HRMS (ESI): Calcd. $\text{C}_{26}\text{H}_{45}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$: 449.3379, Found: 449.3385.

4. Field Emission Scanning Electron Microscopy (FESEM)

4.1. FESEM Studies for Compound **4S** in CHCl₃ and MeOH

The surface morphology followed by the aggregation pattern of the compound **4S** in solid-state was examined by field emission scanning electron microscopy (FESEM) studies. For that, the compound **4S** was dissolved in CHCl₃ or moist MeOH (2% water in methanol solvent) to prepare a 100 μ M solution. The compound solution was drop-casted on a silicon wafer, dried, and then used for FESEM studies.

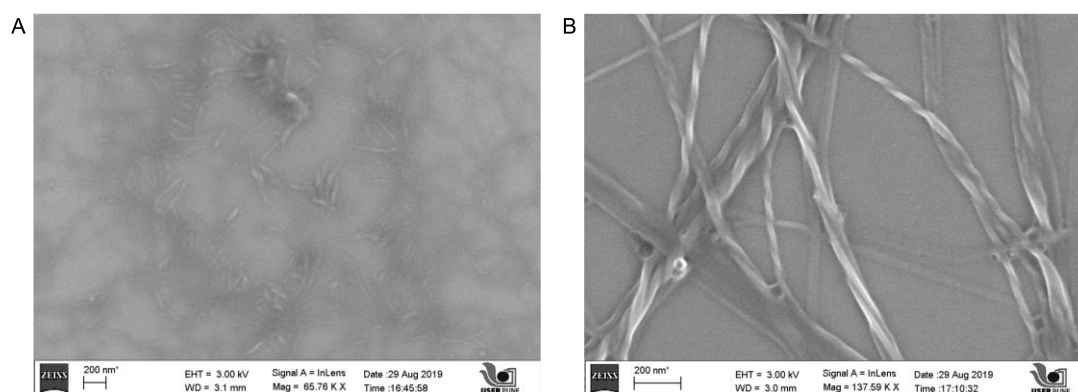


Fig. S1. The FESEM image of compound **4S** from CHCl₃ (A) and MeOH (B) solution.

4.2. FESEM Studies for Compound **4R** in CHCl₃ and MeOH

The surface morphology followed by the aggregation pattern of the compound **4R** in the solid state was examined by field emission scanning electron microscopy (FESEM) studies. For that, the compound **4R** was dissolved in CHCl₃ or moist MeOH (2% water in methanol solvent) to prepare a 150 μ M solution. The compound solution was drop-casted on a silicon wafer, dried, and then used for FESEM studies.

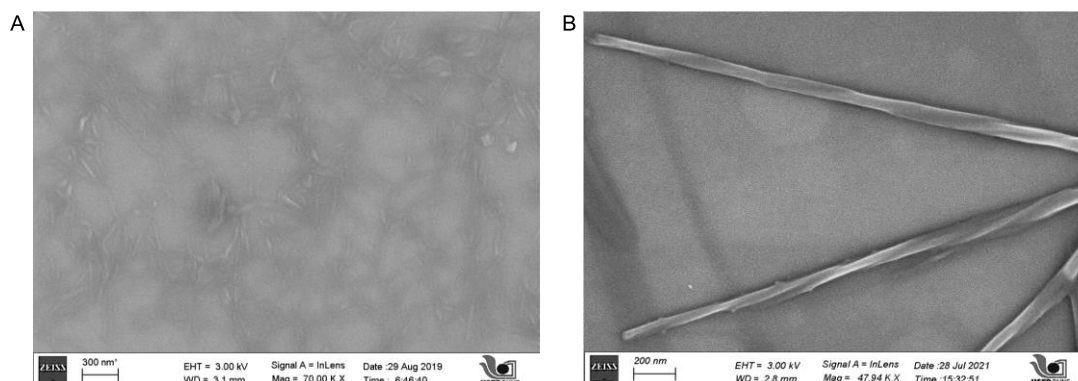


Fig. S2. The FESEM image of compound **4R** from CHCl₃ (A) and moist MeOH (B) solution.

5. High-Resolution Transmission Electron Microscopy (HRTEM)

A 100 μM solution of the compounds **4S** and **4R** were prepared in moist MeOH (2% water in methanol solvent) solvent system. The compound solution was then drop-casted on a TEM grid, dried, and then used for HRTEM imaging.

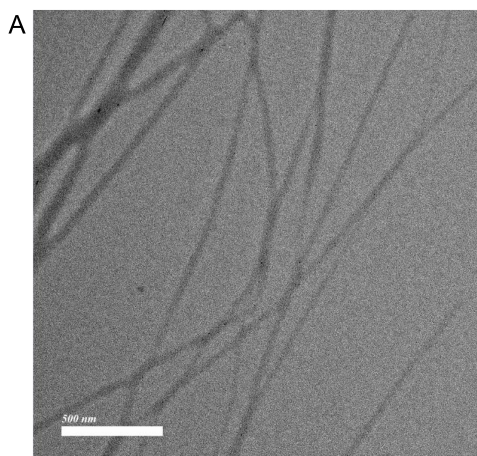


Fig. S3. The HRTEM images of free compound **4R**.

6. Crystallographic Measurement and Analysis

The single crystal suitable for X-ray analysis for **2R** was obtained by slow evaporation of moist methanol (2% water in methanol solvent) solution of the respective compounds. On the other hand, the **14R** crystallizes from nitrobenzene and acetonitrile solution (1:10) as a pale yellow crystal. The single-crystal X-ray data were collected on a Bruker Smart Apex Duo diffractometer using Mo K α radiation for both the compounds either at 100 K, and 150 K. Olex 2 graphical interface⁴ were used with ShelXT⁵ to solve the structures using intrinsic phasing and refined with ShelXL⁶ with full matrix least square minimization on F². All non-hydrogen atoms were refined anisotropically, except for those in minor disordered parts. Crystallographic parameters for compounds **2R** and **14R** are summarised in Table S1.

Table S1. Crystallographic data for complexes **2R** and **14R**

Compound	2R	14R
Temperature / K	100 K	150 K
Empirical Formula	C ₂₃ H ₄₀ N ₂ O ₅	C ₂₅ H ₄₀ N ₂ O ₄
Formula Weight [g mol ⁻¹]	424.57	432.59
crystal system	monoclinic	triclinic
space group	C 2	P-1

a [Å]	37.83(2)	9.3339(19)
b [Å]	4.929(3)	9.6044(18)
c [Å]	12.845(7)	14.535(3)
α [°]	90	81.540(6)
β [°]	97.014(13)	78.191(6)
γ [°]	90	89.814(6)
cell V [Å ³]	2377(2)	1261.1(4)
Z	4	2
reflections collected	13714	16669
independent reflections, R _{int}	4026	4296
Data/restrains/parameters	4026/1/287	4296/6/284
goodness-of-fit	0.996	1.035
final R indices [I > 2 σ (I)]: R ₁ , wR ₂	0.0668, 0.1355	0.0938, 0.2134
CCDC no.	2122390	2122389

$$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o| \text{ and } wR_2 = |\Sigma w(|F_o|^2 - |F_c|^2)|/\Sigma|w(F_o)^2|^{1/2}$$

6.1. ORTEP Diagram of Compound 2R

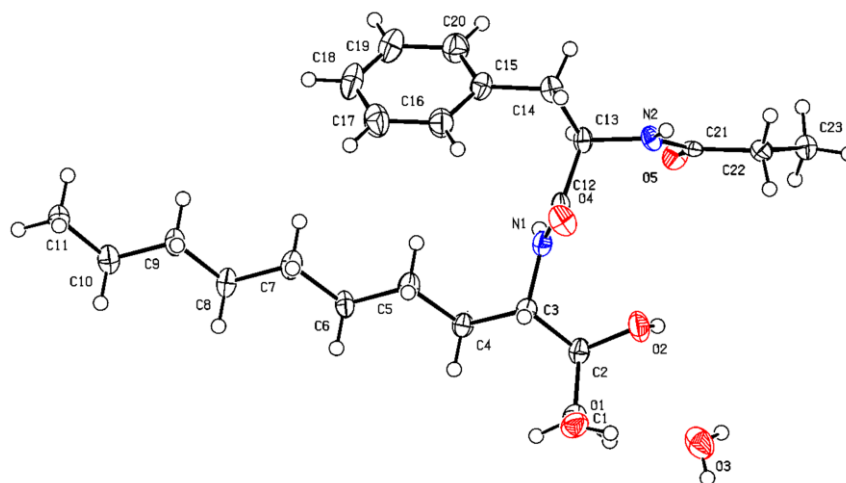


Fig. S4. The ORTEP diagram of compound **2R** where the ellipsoids are shown with 50% probability.

6.2. ORTEP Diagram of Compound 14R

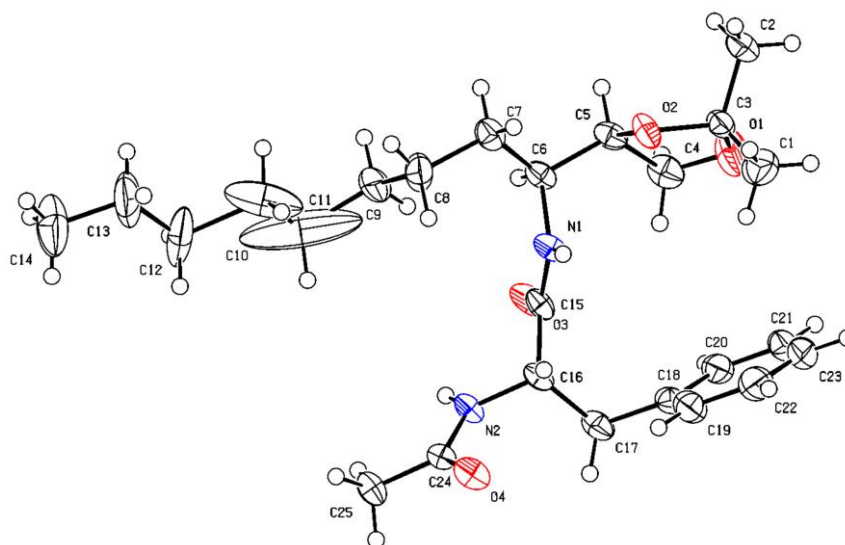


Fig. S5. The ORTEP diagram of compound **14R** where the ellipsoids are shown with 50% probability.

7. IR studies of Channel-forming Molecule in Lipid Membrane

7.1. Preparation of Vesicles for IR Spectra

In a 10 mL round-bottomed flask 0.5 mL of EYPC lipid solution (25 mg/mL in chloroform) was taken. The chloroform in the lipid solution was then evaporated by a slow stream of nitrogen gas while slowly rotating the round-bottomed flask to get a thin film of lipid inside it. Then the trace amount of chloroform present in the lipid was evaporated by drying it in a high vacuum for about 4 h. The thin film of lipid was hydrated with buffer solution (10 mM HEPES, pH 7.0), and then the vortexing was done 4-5 times (2 minutes each) over the period of 1 h. Then the hydrated vesicles suspension was subjected to 15 freeze-thaw cycles (liquid nitrogen and 55 °C temperature water bath), and extrusions were done 19 times (must be odd number) using 200 nm polycarbonate membrane. The extruded vesicles suspension (LUVs) was then collected and used for the IR sample preparation.

7.2. Preparation of Sample for IR Experiment

The channel-forming compound stock solution (20 μ L in DMSO) was added to the vesicles suspension to prepare channel incorporated vesicles. The vesicles solution was then subjected to centrifuge with 15000 rpm rotational speed for 1 h at 20 °C. Then the upper aqueous layer was removed and the process were repeated for three times using HEPES buffer

as washing solvent (10 mM HEPES, pH 7.0). The resulting compound incorporated lipid membrane (collected from the bottom of the centrifuge tube) were dried under vacuum and used for IR spectroscopic studies.

7.3. The IR Experiments

The IR spectra were recorded for lipid membrane in the presence and absence of the channel-forming molecule. The change in the IR spectrum of the vesicles in the presence of the channel-forming molecule (5 mol% with respect to lipid) compared to the free vesicles indicated the insertion of the channel-forming compound inside the bilayer membrane. Additionally, the broadening of the diol O–H peak (3268 cm^{-1}) and shifting of the amide C=O peak (1628 cm^{-1} to 1640 cm^{-1}) of the compound in the presence of lipid indicated the formation of channel inside the bilayer membrane.

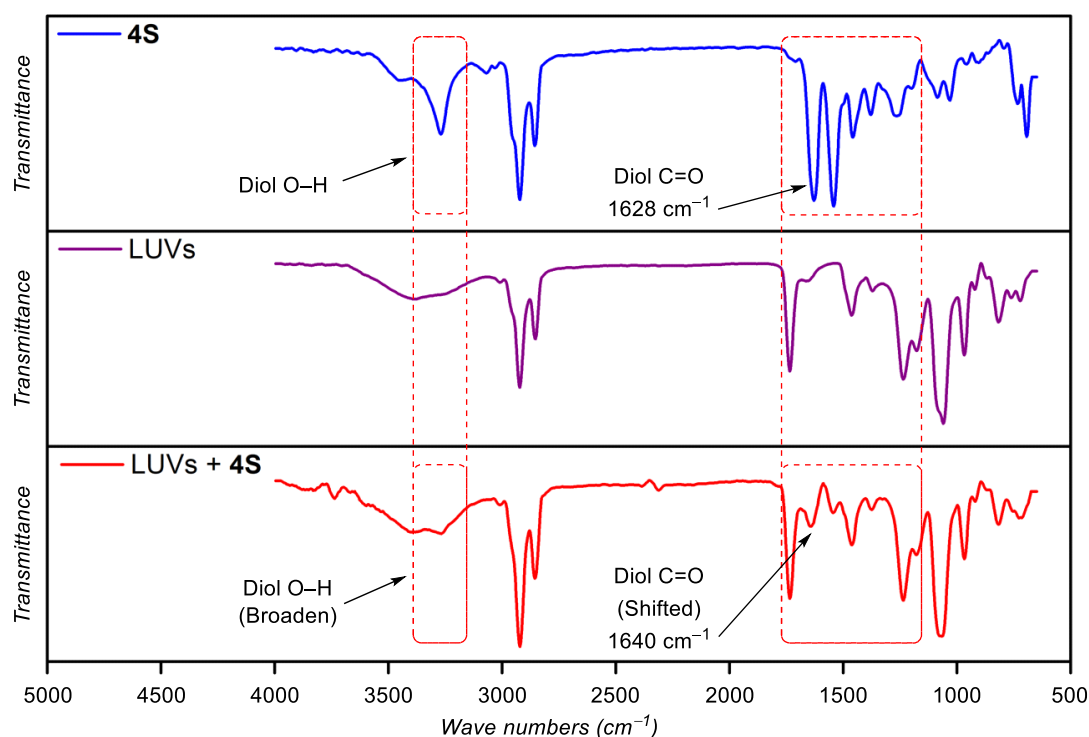


Fig. S6. The IR spectra of the channel-forming molecule, free lipid vesicles, and channel-forming molecule incorporated lipid vesicles (5 mol% with respect to lipid).

8. Water Transport Studies⁷⁻¹²

8.1. Stopped-flow experiments for checking water transport activity

8.1.1. HEPES buffer preparation for experiments

We have prepared a salt solution using autoclaved water of strength 100 mM of NaCl and 10 mM of HEPES. Initially, the pH of the solution was below 7.0, and to make the pH at around 7 required amount of NaOH (0.5 M) solution was added.

8.1.2. Vesicles preparation for permeability assay

At first, 0.25 mL of DOPC lipid solution (25 mg/mL in chloroform) was taken in a 10 mL round-bottomed flask, and into that required amount of channel-forming molecule (mol% compound with respect to lipid) in chloroform was added. The chloroform in the compound-lipid solution was then evaporated by a slow stream of nitrogen gas while slowly rotating the round-bottomed flask to get a thin film of lipid inside it. Then the trace amount of chloroform present in the compound-lipid was evaporated by drying it in a high vacuum for about 8 h. The thin film of lipid was hydrated with buffer solution (10 mM HEPES, 100 mM NaCl, pH 7.0), and then sonication followed by vortexing was done 4-5 times (2 minutes each) over the period of 1 h. Then the hydrated vesicles suspension was subjected to 15 freeze-thaw cycles (liquid nitrogen and 55 °C temperature water bath), and extrusions were done 19 times (must be odd number) using 200 nm polycarbonate membrane. The extruded vesicles were then diluted to 6 mL using the buffer as mentioned above (10 mM HEPES, 100 mM NaCl, pH 7.0) to get the desired concentration (mol% of compound with respect to lipid), assuming no loss of lipid throughout the process. The vesicles compositions, inside: 10 mM HEPES, 100 mM NaCl, pH 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH 7.0.

8.1.3. Description of stopped-flow experiments for water transport studies

The above-mentioned vesicles solution was exposed to an equal volume of 300 mM Sucrose solution (300 mM Sucrose, 10 mM HEPES, 100 mM NaCl, pH 7.0), which leads to the shrinkage of the vesicles due to the outwardly directed water transport through the water channel. The sudden shrinkage of the size of the vesicle has then directed to change in light scattering intensity with time (90° angle recorded at 600 nm wavelength), which was monitored on a stopped-flow instrument (SFM 400 and MOS 450 from Biologic Science Instruments). The light scattering intensity was plotted against time by fitting the following exponential decay equation (Eq. S1).^[6]

$$y = A.exp(-kx) + y_0 \quad \text{Eq. S1}$$

where, x is the time, y is the light scattering intensity change, and k is the exponential coefficient for the light scattering change.

This exponential coefficient value, k was used to calculate the osmotic permeability value, P_f following the below equation:

$$P_f = \frac{k}{\frac{S}{V_0} \times V_w \times \Delta_{osm}} \quad \text{Eq. S2}$$

where, P_f is the osmotic permeability, k is the exponential coefficient, S is the initial surface area of the vesicles, V_0 is the initial volume of the vesicles, V_w is the molar volume of water, Δ_{osm} is the change in osmolarity of the vesicular suspension after the addition of sucrose solution.

The corrected water permeability, P_w by the water channel molecules were calculated by subtracting the exponential coefficient value a compound from the exponential coefficient value of the blank data from the following equation:

$$P_w = (P_{f(compound)} - P_{f(blank)}) \times \frac{S}{N} \quad \text{Eq. S3}$$

where, P_w is the corrected water permeability, $P_{f(compound)}$ is the osmotic permeability of the compounds, $P_{f(blank)}$ is the osmotic permeability of the blank vesicles, S is the surface area of the vesicles, and N is the number of self-aggregated channels per vesicles and can be calculated from the Eq. S4.

Each channel aggregate consists of 16 peptide-diol molecules (2×8 layered assembly has shown optimal channel formation in MD simulation studies, discussed latter) with a cross-sectional area of nearly 2.6 nm^2 (alkyl chain attached to peptide group less significantly differ the area calculation). The lipid molecule has a cross-sectional area of 0.34 nm^2 and the lipid bilayer has a thickness of ca. 5 nm. The ‘unit area’ was calculated based on one channel aggregate. The number of ‘unit areas’ is equivalent to the number of self-aggregated channels per vesicles (N). The N can be calculated from the following equation:

$$N = \frac{SA_{total}}{SA_{unit}} = \frac{2\pi r^2 + 2\pi(r-5)^2}{A_{channel} + \left(\frac{1-x}{x}\right) \times 0.34} \quad \text{Eq. S4}$$

where, SA_{total} is the total surface area of a vesicles; SA_{unit} is the ‘unit area’; $A_{channel}$ is the cross-sectional area of a single channel aggregate; x is the corrected mCLR considering each channel aggregate is made up of 16 peptide-diol molecules. The number of single channel aggregates per liposome can then be used to calculate the single channel permeability (P_w) following Eq. S3. Please note, in our system the single channel water permeability is the sum of the water permeability of two water arrays in a single self-assembled system.

We have re-calculated the water permeability values utilizing the recently reported equation considering the intra- and extra-vesicular osmolyte concentrations correction factor.

$$P_{f(corrected)} = \frac{k}{\frac{s}{V_0} \times V_w} \times \frac{C_{in,t=0} + C_{out}}{2 \times C_{out}^2} \quad \text{Eq. S5}$$

where, $C_{in, t=0}$ is the change in osmolyte concentration inside the vesicles at $t = 0$ and C_{out} is the osmolyte concentration outside the vesicles.

The $P_{f(corrected)}$ values obtained from Eq. S5, considering the osmolyte concentration correction factor, was further used to calculate the corrected water permeability and single channel permeability for all channel forming molecules following the Eq. S3 and S4.

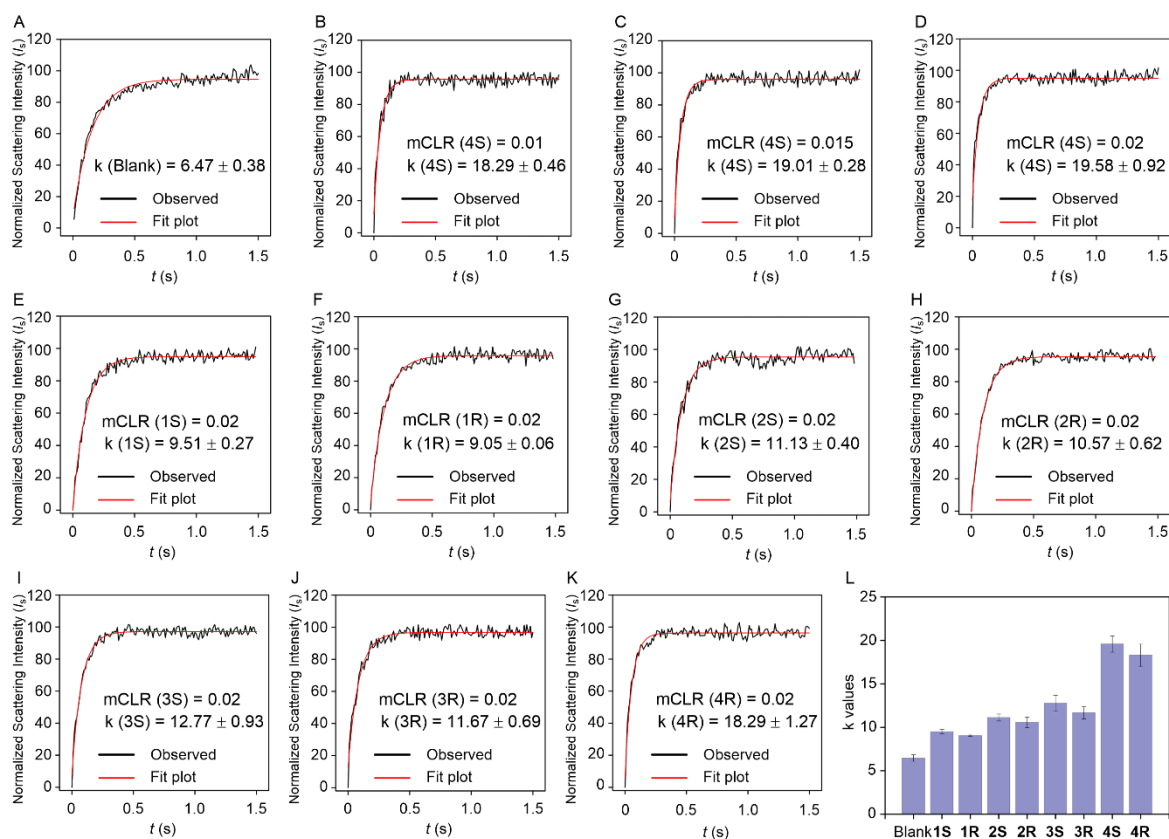


Fig. S7. Kinetic curves for water permeability measurement of blank (A), channel **4S** at different mCLRs (B, C, and D), channel **1S** (E), channel **1R** (F), channel **2S** (G), channel **2R** (H), channel **3S** (I), channel **3R** (J), and channel **4R** (K). The comparison of exponential coefficient values of blank, **1S-4S**, and **1R-4R** (L). All the data are triplicated and used to calculate rate constants with their standard deviation values for each compound.

8.1.4. Estimation of insertion efficiency⁹

The insertion efficiency of peptide 1,2-diol channels in the vesicles was measured using the UV-Vis spectroscopic technique. At first, we have prepared different sets of vesicles (without extrusion, following the process mentioned above) for each compound by inserting the channel forming molecules (mCLR 0.005 to 0.03) and measured the UV-Vis absorbance intensity. These intensity values were used to generate a calibration curve, which has been shown to follow a linear pattern. Next, we prepared a similar type of vesicles (with extrusion, following the process mentioned above) by inserting the channel-forming molecules (mCLR 0.02) and measured the UV-Vis absorbance intensity. This absorbance intensity was used to calculate the actual concentration of the compound (corrected mCLR, after the extrusion process) from the calibration curve. Following these methods, we have calculated the percentage of insertion efficiency (ratio of corrected concentration and initial concentration) for **4S** and **4R**, and the corrected concentrations for these channels were used to calculate the single-channel

permeability. The insertion efficiency of **4S** and **4R** are found to be 84% (0.0168/0.02) and 86% (0.0172/0.02), respectively.

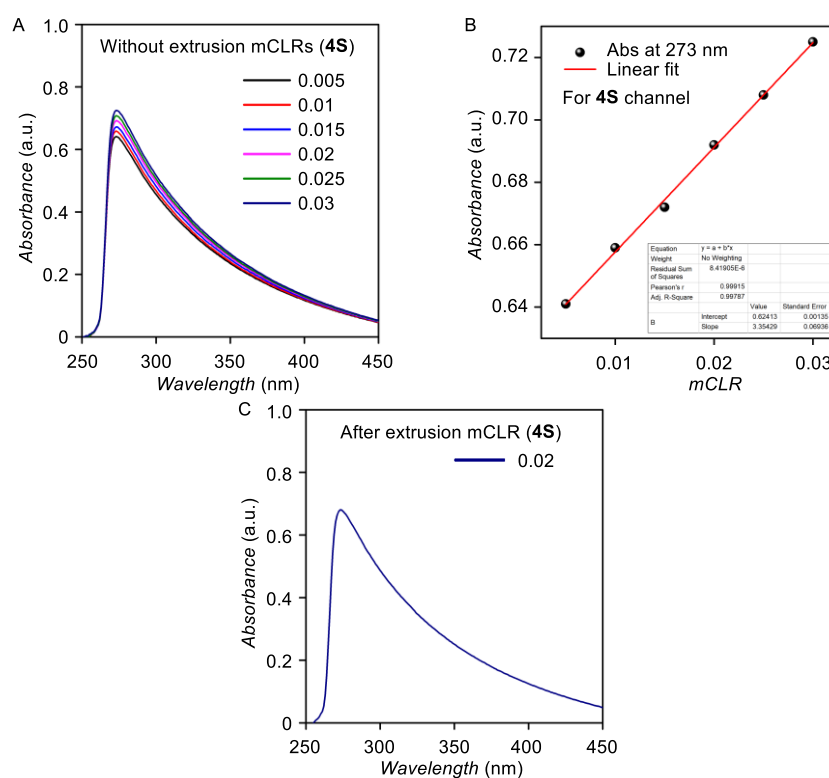


Fig. S8. Determination of actual loading of channel-forming compounds in vesicles using UV-Vis spectroscopy. The UV-Vis absorbance intensity of vesicles (without extrusion) with increasing concentration of **4S** (A), Calibration plot (B), and the absorbance intensity of vesicles (with extrusion) with **4S** (mCLR 0.02, C).

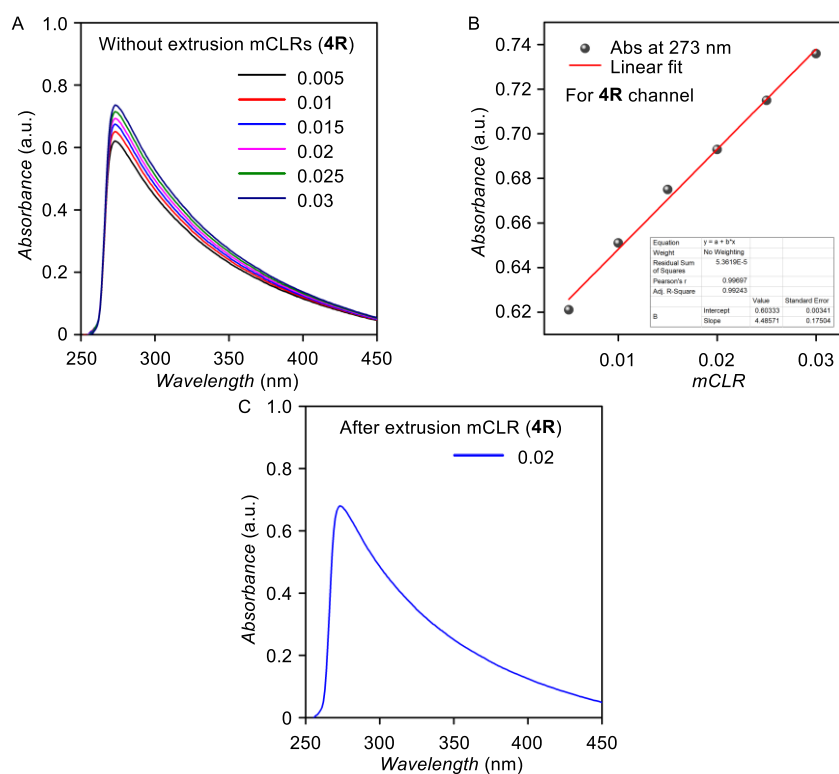


Fig. S9. Determination of actual loading of channel-forming compounds in vesicles using UV-Vis spectroscopy. The UV-Vis absorbance intensity of vesicles (without extrusion) with increasing concentration of **4R** (A), Calibration plot (B), and the absorbance intensity of vesicles (with extrusion) with **4R** (mCLR 0.02, C).

Table S2. Water permeability calculation tables for compounds **1S-4S**, and **1R-4R** with corrected mCLRs and without considering the osmolyte concentration correction factor.

Channels	Exponential coefficient (k)	Water permeability, (P_f , $\mu\text{m/s}$)	Corrected water permeability, (P_f , $\mu\text{m/s}$)	Water permeability (P_w , cm^3/s)	Water permeability/channel (water molecules/s)
Blank	6.47	54.28	0.00	-	-
1S	9.51	85.71	31.43	-	-
1R	9.05	81.54	27.26	-	-
2S	11.13	100.32	46.04	-	-
2R	10.57	95.29	41.01	-	-
3S	12.77	115.06	60.78	-	-
3R	11.67	105.20	50.92	-	-
4S	19.58	176.47	122.19	4.47×10^{-14}	1.49×10^9
4R	18.29	164.85	110.57	3.95×10^{-14}	1.32×10^9

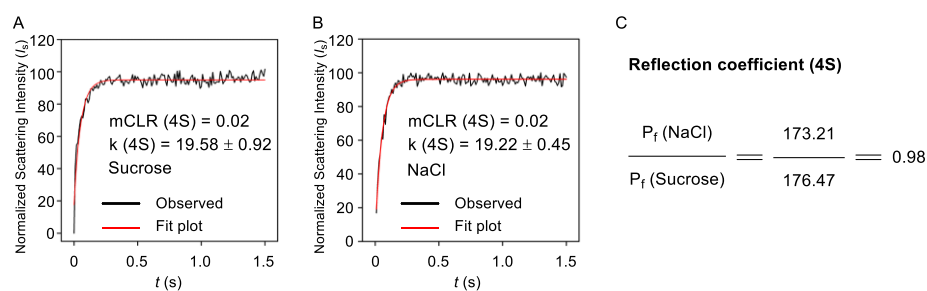


Fig. S10. Comparison of water permeability of **4S** in 300 mM sucrose (A) and 250 mM NaCl solution (B). The reflection coefficient value of **4S** (C).

Table S3. Water permeability calculation tables for compounds **1S-4S**, and **1R-4R** with corrected mCLRs and considering the osmolyte concentration correction factor.

Channels	Exponential coefficient (k)	Water permeability, (P_f , $\mu\text{m/s}$)	Corrected water permeability, (P_f , $\mu\text{m/s}$)	Water permeability (P_w , cm^3/s)	Water permeability/channel (water molecules/s)
Blank	6.47	19.48	0.00	-	-
1S	9.51	30.24	10.75	-	-
1R	9.05	28.77	9.28	-	-
2S	11.13	35.39	15.91	-	-
2R	10.57	33.62	14.13	-	-
3S	12.77	40.60	21.11	-	-
3R	11.67	37.12	17.63	-	-
4S	19.58	62.27	42.78	1.51×10^{-14}	5.05×10^8
4R	18.29	58.17	38.68	1.34×10^{-14}	4.48×10^8

9. Ion Transport Studies⁸

9.1. Cation transport studies in HPTS assay

9.1.1. HEPES buffer, HPTS solution, and stock solution preparation for assay

We have prepared a buffer solution using autoclaved water of 10 mM HEPES (pH 7.0) and this buffer was used to prepare a salt solution of strength 200 mM of M_2SO_4 ($\text{M}^+ = \text{Na}^+$ and K^+). Initially, the pH of the buffer solution was below 7 and to make the pH at around 7, the required amount of MOH (0.5 Molar, $\text{M}^+ = \text{Na}^+$ and K^+) solution was added. Then HPTS solution of 1 mM was prepared from solid HPTS using the above-mentioned HEPES buffer solution

(without salt). The stock solution of the transporter for the HPTS assay was prepared using HPLC grade DMSO.

9.1.2. Vesicles preparation for cation transport assay

At first, 1.0 mL of EYPC lipid solution (25 mg/mL in chloroform) was taken in a 10 mL round-bottomed flask. The chloroform present in the lipid solution was then evaporated by a slow stream of nitrogen gas while rotating the round-bottomed flask to get a thin film of lipid inside it. Then the trace amount of chloroform present in the lipid was evaporated by drying it in a high vacuum for about 8 h. The thin film of lipid was hydrated with HPTS solution (1.0 mM HPTS, 10 mM HEPES, pH 7.0) while vortexing 4-5 times over the period of 1 h. Then the hydrated vesicles suspension was subjected to 15 freeze-thaw cycles, and extrusions were done 19 times (must be an odd number) using a 100 nm polycarbonate membrane. The extravesicular dye was separated from vesicles by size exclusion column chromatography (using Sephadex G-50 gel) eluting with buffer solution (10 mM HEPES, pH 7). After collecting, the vesicles from the column were diluted to 6 mL using buffer (10 mM HEPES, pH 7.0) to get the concentration of ~ 5.5 mM of EYPC-LUVs $\Rightarrow$ HPTS, assuming no loss of lipid throughout the process. The vesicles compositions, inside: 1 mM HPTS, 10 mM HEPES, pH 7.0 and outside: 10 mM HEPES, pH 7.0.

9.1.3. Description of cation transport study

In a clean cuvette, 1975 μ L of buffer solution (10 mM HEPES, 200 mM of M_2SO_4 ($M^+ = Na^+$ and K^+), pH 7.0), 25 μ L of HPTS trapped vesicles solution were taken and placed in a fluorescence instrument equipped with a magnetic stirrer. The fluorescence emission intensity of the HPTS dye, I_t was measured at $\lambda_{em} = 510$ nm (where, $\lambda_{ex} = 450$ nm) for 350 s. For each reading, the start time of the instrument was considered as $t = 0$ s. Then at $t = 20$ s, 20 μ L of 0.5 M NaOH solution was added to the same cuvette to generate a pH gradient ($\Delta pH = 0.8$) between intra and extra vesicular medium. Then 20 μ L solution of transporters in DMSO of different concentrations were added at $t = 100$ s. At $t = 300$ s, 10% triton X-100 (25 μ L) was added to destroy all the vesicles to destruct the pH gradient.

The fractional emission intensity (in percentage), I_F (Fig. S11B) was calculated after normalizing all the data using the following equation (Eq. S6).

$$\% \text{ of } I_F = (I_t - I_0) / (I_\infty - I_0) \times 100 \quad \text{Eq. S6}$$

Where, I_0 is the initial fluorescence intensity *i.e.* before the addition of the transporter compound, I_t is the fluorescence intensity at time t and I_∞ is the final fluorescence intensity *i.e.* after the addition of Triton X-100.

Before plotting the data, the time axis was normalized using the following equation:

$$t = t - 100 \quad \text{Eq. S7}$$

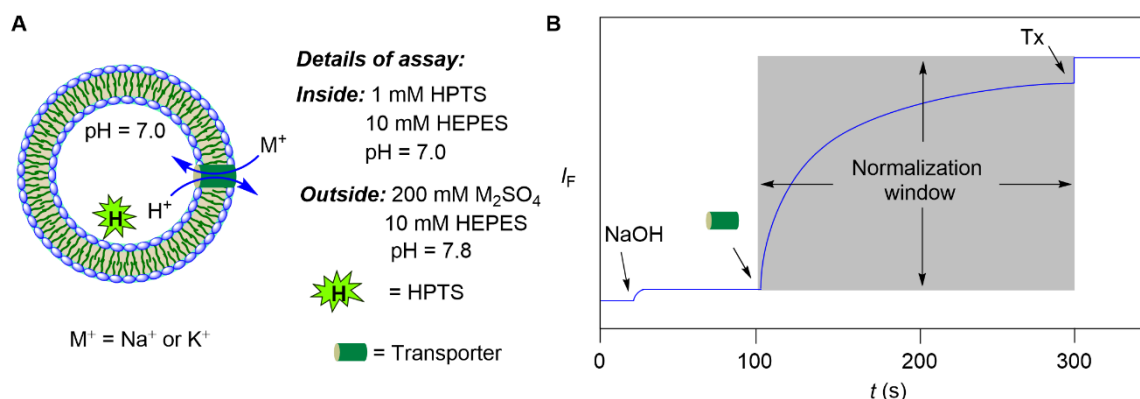


Fig. S11. Schematic representation of fluorescence kinetics assay for checking cation transport activity across EYPC-LUVs>HPTS (A), and normalized working window for the same experiment (B).

9.2. Proton and anion transport studies in HPTS assay

9.2.1. HEPES buffer, HPTS solution and stock solution preparation for assay:

We have prepared a buffer solution using autoclaved water of 10 mM HEPES (pH 7.0) and this buffer was used to prepare a salt solution of strength 100 mM of NaCl and 66 mM of Na_2SO_4 . Initially the pH of the buffer solution was below 7.0, and to make the pH at around 7.0, the required amount of NaOH (0.5 M) solution was added. Then HPTS solution of 1.0 mM was prepared from solid HPTS using the above-mentioned HEPES buffer solution (100 mM of NaCl). The stock solution of the transporter for the HPTS assay was prepared using HPLC grade DMSO.

9.2.2. Vesicles preparation for proton and anion transport assay

At first, 1 mL of EYPC lipid solution (25 mg/mL in chloroform) was taken in a 10 mL round-bottomed flask. The chloroform present in the lipid solution was then evaporated by a slow stream of nitrogen gas while rotating the round-bottomed flask to get a thin film of lipid inside it. Then the trace amount of chloroform present in the lipid was evaporated by drying it in a high vacuum for about 8 h. The thin film of lipid was hydrated with HPTS solution (1.0 mM

HPTS, 100 mM NaCl, 10 mM HEPES, pH 7.0) while vortexing 4-5 times over the period of 1 h. Then the hydrated vesicles suspension was subjected to 15 freeze-thaw cycles, and extrusions were done 19 times (must be an odd number) using a 100 nm polycarbonate membrane. The extravesicular dye was separated from vesicles by size exclusion column chromatography (using Sephadex G-50 gel) eluting with buffer solution (10 mM HEPES, 100 mM NaCl, pH 7.0). After collecting, the vesicles from the column were diluted to 6 mL using the buffer as mentioned above (10 mM HEPES, 100 mM NaCl, pH 7.0) to get the concentration of ~ 5.5 mM of EYPC-LUVs $\supset$ HPTS, assuming no loss of lipid throughout the process. The vesicles compositions, inside: 1.0 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH 7.0.

9.2.3. Description of proton and anion transport study

In a clean cuvette, 1975 μ L of buffer solution (10 mM HEPES, 66 mM of Na₂SO₄, pH 7.0), 25 μ L of HPTS trapped vesicles solution were taken and placed in a fluorescence instrument equipped with a magnetic stirrer. The fluorescence emission intensity of the HPTS dye, I_t was measured at $\lambda_{em} = 510$ nm (where $\lambda_{ex} = 450$ nm) for 350 s. For each reading, the start time of the instrument was considered as $t = 0$ s. Then at $t = 20$ s, 20 μ L of 0.5 M NaOH solution was added to the same cuvette to generate a pH gradient (Δ pH = 0.8) between intra and extra vesicular medium. Then 20 μ L solution of transporters in DMSO of different concentrations were added at $t = 100$ s. At $t = 300$ s, 10% Triton X-100 (25 μ L) was added to destroy all the vesicles to destruct the pH gradient.

The fractional emission intensity (in percentage), I_F (Fig. S12B) was calculated after normalizing all the data using the following equation (Eq. S6).

$$\% \text{ of } I_F = (I_t - I_0) / (I_\infty - I_0) \times 100 \quad \text{Eq. S6}$$

Where, I_0 is the initial fluorescence intensity *i.e.*, before the addition of the transporter compound, I_t is the fluorescence intensity at time t , and I_∞ is the final fluorescence intensity *i.e.*, after the addition of Triton X-100.

Before plotting the data, the time axis was normalized using the following equation:

$$t = t - 100 \quad \text{Eq. S7}$$

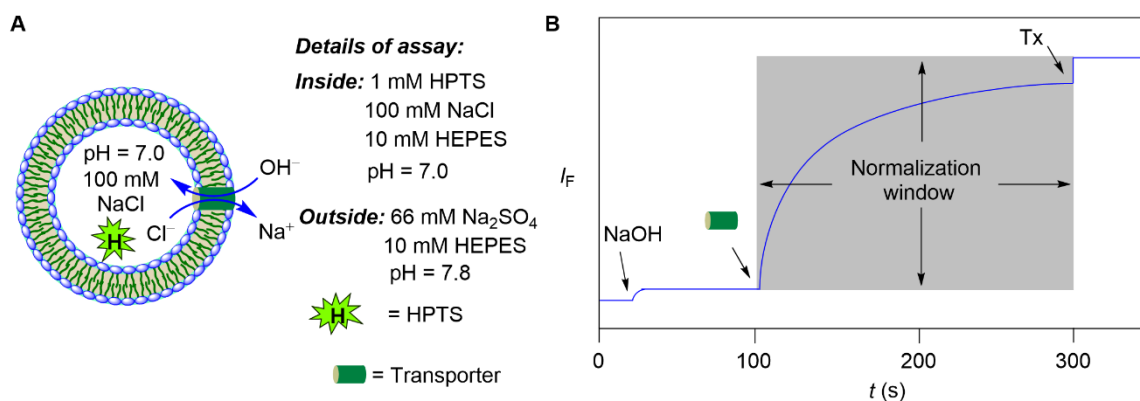


Fig. S12. Schematic representation of fluorescence kinetics assay for checking proton and anion transport activity across EYPC-LUVs⊃HPTS (A), and normalized working window for the same experiment (B).

9.3. Lucigenin assay for checking Cl⁻ transport activity¹³

9.3.1. Salt and Stock solution preparation for Lucigenin assay

We have prepared a 225 mM NaNO₃ salt solution using autoclaved water. Then 1.0 mM Lucigenin solution was prepared using the 225 mM NaNO₃ solution. The stock solution of the transporter for the Lucigenin assay was prepared from a solid compound by using HPLC grade CH₃CN : CH₃OH (10 : 1) solution.

9.3.2. Vesicles preparation procedure for Lucigenin assay

At first, 1.0 mL of EYPC lipid solution (25 mg/mL in chloroform) was taken in a 10 mL round-bottomed flask. The chloroform present in the lipid solution was then evaporated by a slow stream of nitrogen gas while rotating the round-bottomed flask to get a thin film of lipid inside it. Then the last trace amount of chloroform present in the lipid was evaporated by drying it in a high vacuum for about 8 h. The thin film of lipid was hydrated with 1 mL of Lucigenin solution (1.0 mM Lucigenin, 225 mM NaNO₃) while vortexing 4-5 times for 2 min with a time interval of 10 min. Then the hydrated vesicles suspension was subjected to 15 freeze-thaw cycles, and extrusions were done 19 times (must be an odd number) using a 200 nm polycarbonate membrane. The extravesicular dye was separated from vesicles by size exclusion column chromatography (using Sephadex G-50 gel) eluting with NaNO₃ solution (225 mM). After collecting, the vesicles from the column were diluted to 4 mL using the buffer as mentioned above (225 mM NaNO₃) to get the concentration of ~8 mM of EYPC-LUVs⊃Lucigenin, assuming no loss of lipid throughout the process. The vesicles compositions, inside: 1.0 mM Lucigenin, 225 mM NaNO₃ and outside: 225 mM NaNO₃.

9.3.3. Description of lucigenin assay

In a clean cuvette, 1950 μL of salt solution (225 mM NaNO_3), 50 μL of lucigenin trapped vesicles solution were taken and placed in a fluorescence instrument equipped with a magnetic stirrer. The fluorescence emission intensity of the Lucigenin dye, I_t was measured at $\lambda_{\text{em}} = 535$ nm (where $\lambda_{\text{ex}} = 455$ nm) for 350 s. For each reading, the start time of the instrument was considered as $t = 0$ s. Then at $t = 50$ s, 33 μL of 2N NaCl solution was added to the cuvette for generating a Cl^- concentration gradient across the lipid bilayer. Then 20 μL solution of transporters in ACN : MeOH (6 : 1) solvent of different concentrations were added at $t = 100$ s. At $t = 300$ s, 10% Triton X-100 (25 μL) was added to destroy all the vesicles to destruct the Cl^- concentration gradient.

The fractional emission intensity (in percentage), I_F (Fig. S13B) was calculated after normalizing all the data using the following equation (Eq. S8).

$$\% \text{ of } I_F = (I_t - I_0) / (I_\infty - I_0) \times (-100) \quad \text{Eq. S8}$$

Where, I_0 is the initial fluorescence intensity, *i.e.*, just before the addition of the transporter solution, I_t is the fluorescence intensity at time t , and I_∞ is the final fluorescence intensity, *i.e.*, after the addition of Triton X-100.

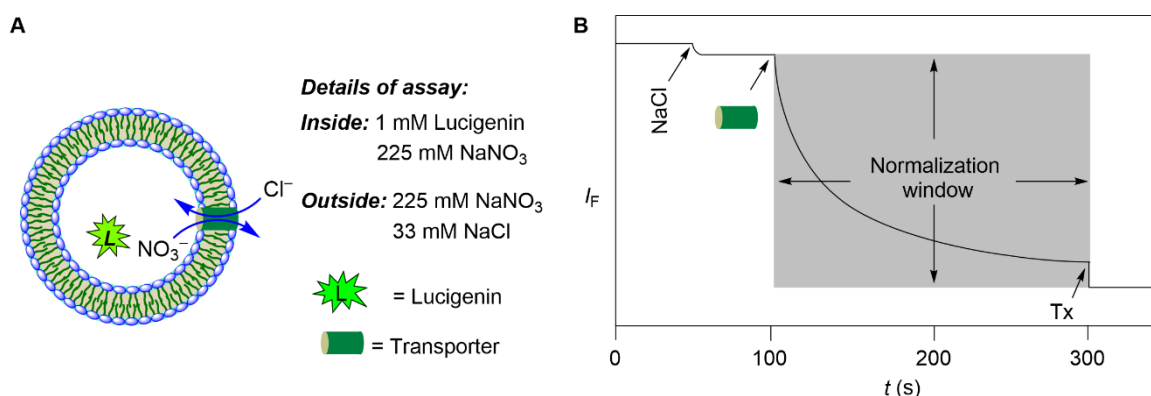


Fig. S13. Schematic representation of fluorescence kinetics assay for checking Cl^- ion transport selectivity across EYPC-LUVs $\Rightarrow$ Lucigenin (A), and normalized working window for the same experiment (B).

Before plotting the data, the time axis was normalized using the Eq. S7,

9.4. Standard HPTS assay for checking ion transport activity with iso-osmolar intra- and extra-vesicular solutions

9.4.1. HEPES buffer, HPTS solution, and stock solution preparation for assay

We have prepared a buffer solution using autoclaved water of 10 mM HEPES (pH 7.0) and this buffer was used to prepare a NaCl solution of strength 100 mM. Initially, the pH of the buffer solution was below 7 and to make the pH at around 7, the required amount of NaOH (0.5 Molar) solution was added. Then HPTS solution of 1.0 mM was prepared from solid HPTS using the above-mentioned HEPES buffer solution. The stock solution of the transporter for the HPTS assay was prepared using HPLC grade DMSO.

9.4.2. Vesicles preparation for ion transport assay

At first, 1.0 mL of EYPC lipid solution (25 mg/mL in chloroform) was taken in a 10 mL round-bottomed flask. The chloroform present in the lipid solution was then evaporated by a slow stream of nitrogen gas while rotating the round-bottomed flask to get a thin film of lipid inside it. Then the trace amount of chloroform present in the lipid was evaporated by drying it in a high vacuum for about 8 h. The thin film of lipid was hydrated with HPTS solution (1.0 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH 7.0) while vortexing 4-5 times over the period of 1 h. Then the hydrated vesicles suspension was subjected to 15 freeze-thaw cycles, and extrusions were done 19 times (must be an odd number) using a 200 nm polycarbonate membrane. The extravesicular dye was separated from vesicles by size exclusion column chromatography (using Sephadex G-50 gel) eluting with buffer solution (10 mM HEPES, 100 mM NaCl, pH 7.0). After collecting, the vesicles from the column were diluted to 6 mL using buffer (10 mM HEPES, 100 mM NaCl, pH 7.0) to get the concentration of ~ 5.5 mM of EYPC-LUVs $\supset$ HPTS, assuming no loss of lipid throughout the process. The vesicles compositions, inside: 1.0 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH 7.0.

9.4.3. Description of ion transport study

In a clean cuvette, 1975 μ L of buffer solution (10 mM HEPES, 100 mM NaCl, pH 7.0), 25 μ L of HPTS trapped vesicles solution were taken and placed in a fluorescence instrument equipped with a magnetic stirrer. The fluorescence emission intensity of the HPTS dye, I_t was measured at $\lambda_{em} = 510$ nm (where, $\lambda_{ex} = 450$ nm) for 350 s. For each reading, the start time of the instrument was considered as $t = 0$ s. Then at $t = 20$ s, 20 μ L of 0.5 M NaOH solution was

added to the same cuvette to generate a pH gradient ($\Delta\text{pH} = 0.8$) between intra and extra vesicular medium. Then 20 μL solution of transporters in DMSO of different concentrations were added at $t = 100$ s. At $t = 300$ s, 10% triton X-100 (25 μL) was added to destroy all the vesicles to destruct the pH gradient.

The fractional emission intensity (in percentage), I_F was calculated after normalizing all the data using the Eq. S6.

Before plotting the data, the time axis was normalized using the Eq. S7

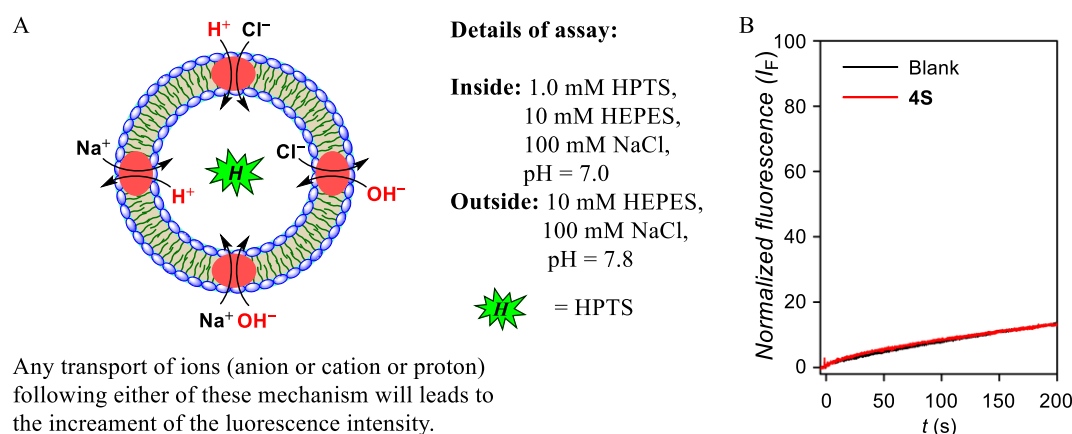


Fig. S14. Schematic representation of fluorescence kinetics assay for checking ion transport activity across EYPC-LUVs $\Rightarrow$ HPTS (**A**), and normalized working window for the same experiment (**B**).

9.5. Carboxyfluorescein (CF) leakage assay¹⁴

9.5.1. Vesicles preparation for CF leakage assay

At first, 0.5 mL of EYPC lipid solution (25 mg/mL in chloroform) was taken in a 10 mL round-bottomed flask. The chloroform present in the lipid solution was then evaporated by a slow stream of nitrogen gas while rotating the round-bottomed flask to get a thin film of lipid inside it. Then the trace amount of chloroform present in the lipid was evaporated by drying it in a high vacuum for about 8 h. The thin film of lipid was hydrated with CF solution (50 mM CF, 10 mM NaCl, 10 mM HEPES, pH 7.0) while vortexing 4-5 times over the period of 1 h. Then the hydrated vesicles suspension was subjected to 15 freeze-thaw cycles and extrusions were done 19 times (must be an odd number) using a 200 nm polycarbonate membrane. The extravesicular dye was separated from vesicles by size exclusion column chromatography (using Sephadex G-50 gel) eluting with buffer solution (10 mM HEPES, 100 mM NaCl, pH

7). After collecting, the vesicles from the column were diluted to 3 mL using buffer (10 mM HEPES, 100 mM NaCl, pH 7.0) to get the concentration of ~ 5.5 mM of EYPC-LUVs $\supset$ CF, assuming no loss of lipid throughout the process. The vesicles compositions, inside: 50 mM CF, 10 mM HEPES, 10 mM NaCl, pH 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH 7.0.

9.5.2. Description of CF leakage assay

In a clean cuvette, 1975 μ L of buffer solution (10 mM HEPES, 100 mM NaCl, pH 7.0), 25 μ L of CF trapped vesicles solution were taken and placed in a fluorescence instrument equipped with a magnetic stirrer. The fluorescence emission intensity of the CF dye, I_t was measured at $\lambda_{em} = 517$ nm (where, $\lambda_{ex} = 492$ nm) for 350 s. For each reading, the start time of the instrument was considered as $t = 0$ s. Then at $t = 50$ s, 20 μ L of channel molecule in DMSO of different concentrations were added at $t = 100$ s. At $t = 250$ s, 10% Triton X-100 (25 μ L) was added to destroy all the vesicles to destruct the concentration gradient.

The fractional emission intensity (in percentage), I_F was calculated after normalizing all the data using the following equation (Eq. S9).

$$\% \text{ of } I_F = (I_t - I_0) / (I_\infty - I_0) \times 100 \quad \text{Eq. S9}$$

Where, I_0 is the initial fluorescence intensity, *i.e.*, before the addition of the transporter compound, I_t is the fluorescence intensity at time t and I_∞ is the final fluorescence intensity, *i.e.*, after the addition of Triton X-100.

10. MD Simulation Setup

The current experiments obtained the **4R** 2×8 layered synthetic channel atomistic coordinates obtained from the crystal structure of **2R** with water. The coordinates of the 8-layered channel was initially minimized using MAESTRO (<https://www.schrodinger.com/products/maestro>), which was subsequently used for computer simulation of the membrane-constituted channel. We used the CHARMM-GUI Membrane builder¹⁵ (<https://www.charmmgui.org/?doc=input/membrane-.bilayer>), an online webserver for assembling the channel inside a DOPC (1,2-Dioleoyl-sn-glycero-3-phosphocholine) phospholipid bilayer for subsequent MD simulation. The synthetic channel was modelled using the general AMBER force field (GAFF)^{16, 17} and the DOPC lipid parameters were obtained from the CHARMM36m force field.¹⁸⁻²⁰ Total 144 DOPC lipids were used to construct the bilayer by keeping 72 lipids in each of the upper and lower membrane leaflets. The salt concentration was maintained at 0.10 M by adding 12 atoms of Na⁺ and 12 atoms of Cl⁻ ions. The whole assembly was solvated by introducing a total of 7247 water molecules on both sides of the lipid bilayer. The water was modelled by TIP3P-charmm²¹ water molecules. The total system size was 42853 particles and a rectangular box was used with dimensions $7.14 \times 7.14 \times 8.01$ nm³. The assembled system was minimized using the steepest-decent algorithm under restraints. Then the system was further subjected to restrained MD simulations in multiple stages and in each consecutive stage, the restraints were gradually reduced in each step. The temperature was set to 298.15 K and a Berendsen thermostat²² was used during the equilibration. Initial random velocities were assigned to each atom, generated according to Maxwell-Boltzmann distribution of velocities at that temperature.

Finally, the production MD simulations were performed in an NPT ensemble using the leapfrog integrator with a 2-fs time step. The Verlet cutoff scheme²³ was employed throughout the simulation with the Lennard-Jones potential extending to 1.2 nm with dispersion corrections. The electrostatic interactions were implemented with a short-range electrostatic cutoff at 1.2 nm, and long-range interactions were treated by Particle Mesh Ewald summation²⁴ with cubic interpolation and in a Fourier-grid space of 0.16 nm. The neighbor lists were updated every 20 steps. All bond lengths involving hydrogen atoms of the lipids and the ligands were constrained using the LINCS algorithm²⁵ and water molecules were kept rigid using the SETTLE²⁶ approach. The average temperature is maintained at 298.15 K by coupling solute, membrane, and solvent separately to the Nose-Hoover thermostat²⁷ with a relaxation constant of 1.0 ps. The pressure was controlled at 1 bar by Parrinello-Rahman barostat²⁸ with a time

constant of 5 ps and compressibility of 4.5×10^{-5} bar with semi-isotropic coupling (XY and Z directions coupled separately) to achieve tensionless bilayer. The system was periodic in all three directions. Two independent trajectories each of duration 0.9 microseconds were produced using GROMACS 2019 version.^{29, 30} The trajectory was saved at every 100 ps for the analysis.

The stability of the synthetic channel was ascertained by computing the Radius of gyration of the assembled structure and Root-mean-squared deviation (RMSD) from the crystal structure. The simulation trajectory was also analyzed via investigating the dynamical nature of water leakage and permeation event inside the membrane. Towards, we used an algorithm by Carlos et al.³¹ For this purpose, the time profile of z-coordinate of each water molecule was individually tracked. A water molecule was considered to be ‘leaked’ if it has moved at least till the center of the bilayer. Similarly, a successful permeation event of the water molecule is ascertained if the water molecule has completed a full pass from one of the leaflets to the other through the bilayer.

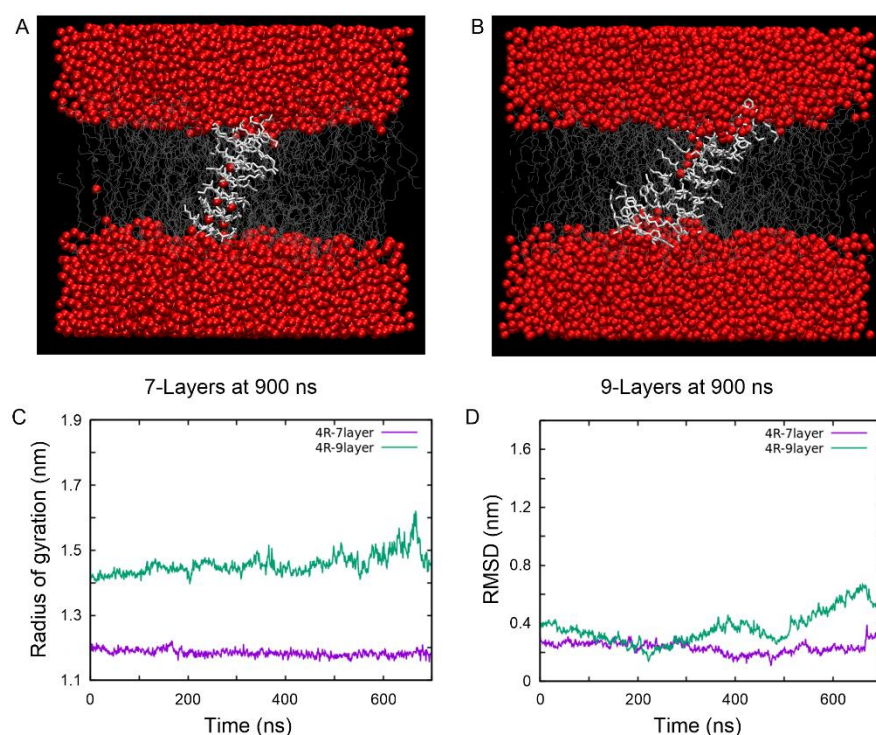


Fig. S15. Assessing the optimal number of layer: Simulated Snapshot of superstructure with 2×7 layers (A) and 2×8 layers (B) after 900 ns of MD simulation. Same colour code as used Fig. 4 has been used. Time profile of radius of gyration (C) and RMSD (D) for both these superstructures are also shown.

From the crystal structure of **2R** with water (Fig. 2D–2F), it is evident that the hydroxyl and amide groups of the channel forming molecules interact with the water molecule while facing inside, whereas the octyl chain and phenyl groups are oriented outside the channel. In the hydrophobic lipid bilayer membrane, the solid-state representation of the water channel will preferably be preserved because the polar hydroxyl groups will repel with the hydrophobic tail of lipid molecules and subsequently be facing inside for the formation of the water channel to minimize the repulsion. In contrast, the octyl chain will face outside, which will interact with the hydrophobic lipid tails. In this representation, there will be less chance for the deformation of the channel structure while increasing the peptide attached alkyl chain length from methyl (for **2R**) to pentyl (for **4R**), as the alkyl chains are exposed outside the channel structure. The formation of stable channel structure by our **4R** molecules with water was observed, where the channel structure is stable without applying any lateral pressure on the membrane.

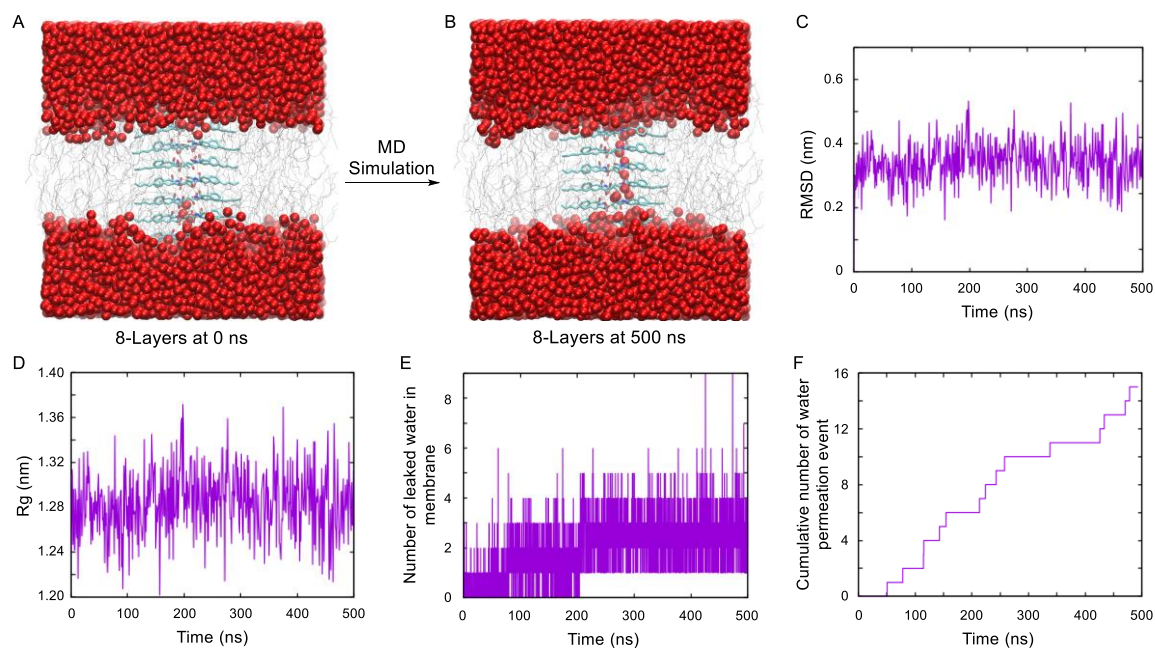


Fig. S16. The snapshots from MD simulation of 2×8 layered **2R** synthetic channel at initial point i.e., 0 ns (A) and at the completion of the simulation i.e. 500 ns (B). The RMSD (C) and radius of gyration (D) of 2×8 layered **2R** channel. Water permeation analysis across 2×8 layered **2R** superstructure: Time profile of number of leaked waters inside the membrane (E) and the cumulative time profiles of water permeation events (F).

The **4R** channel has shown efficient experimental water permeability. Therefore, we have chosen this derivative for MD simulation studies as mentioned above, where the initial coordinates were generated from the solid structure of the **2R** channel by elongating the alkyl chain length. However, similar MD simulation studies (Fig. S16A and S16B) were also

performed for the **2R** channel as well, where the initial channel structure was generated directly from the crystal structure. Here, we have also observed the maintenance of a stable channel structure with a slight deviation of RMSD (Fig. S16C) and R_g values (Fig. S16D) without applying any larger magnitude lateral pressures or restraining forces. Moreover, the salt-exclusion water permeation events were observed from these experiments (Fig. S16E and S16F), which indicated that for a particular configuration product, the elongation of alkyl chain length (facing outside) does not make a difference in the channel-forming strategy.

In the process of searching the channel structure of **4S**, we have generated the 2×8 layered **4S** channel by inverting the stereochemistry of the chiral phenylalanine centre and elongating the peptide attached methyl group to pentyl on each molecule of the 2×8 layered **2R** channel, and subsequently the MD simulation studies were performed. However, in this case, stable channel formation was not observed applying a similar simulation condition (Fig. S17). Maybe some other type of channel assembly is formed in the case of the **4S** channel inside the membrane for the transportation of water.

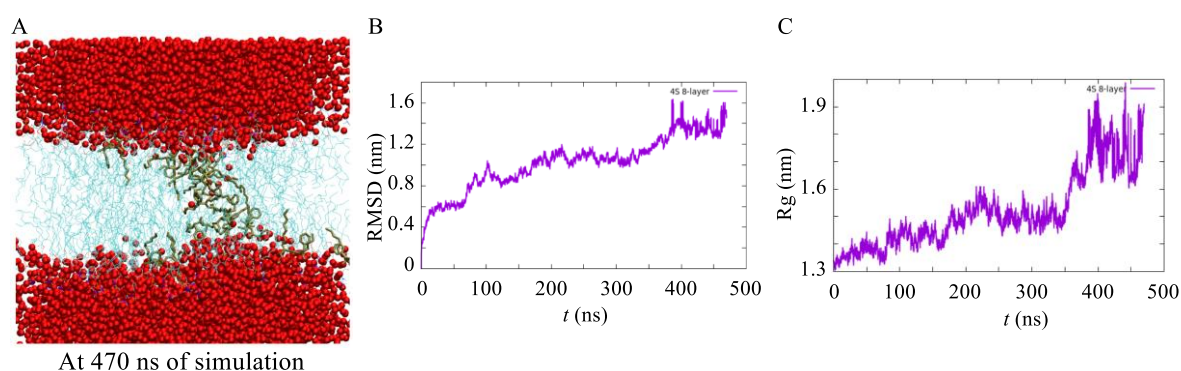


Fig. S17. Snapshot from MD simulation of 2×8 layered **4S** synthetic channel at 470 ns (A). The RMSD (B) and radius of gyration (C) of 2×8 layered **4S** channel.

11. NMR Spectra of Compounds

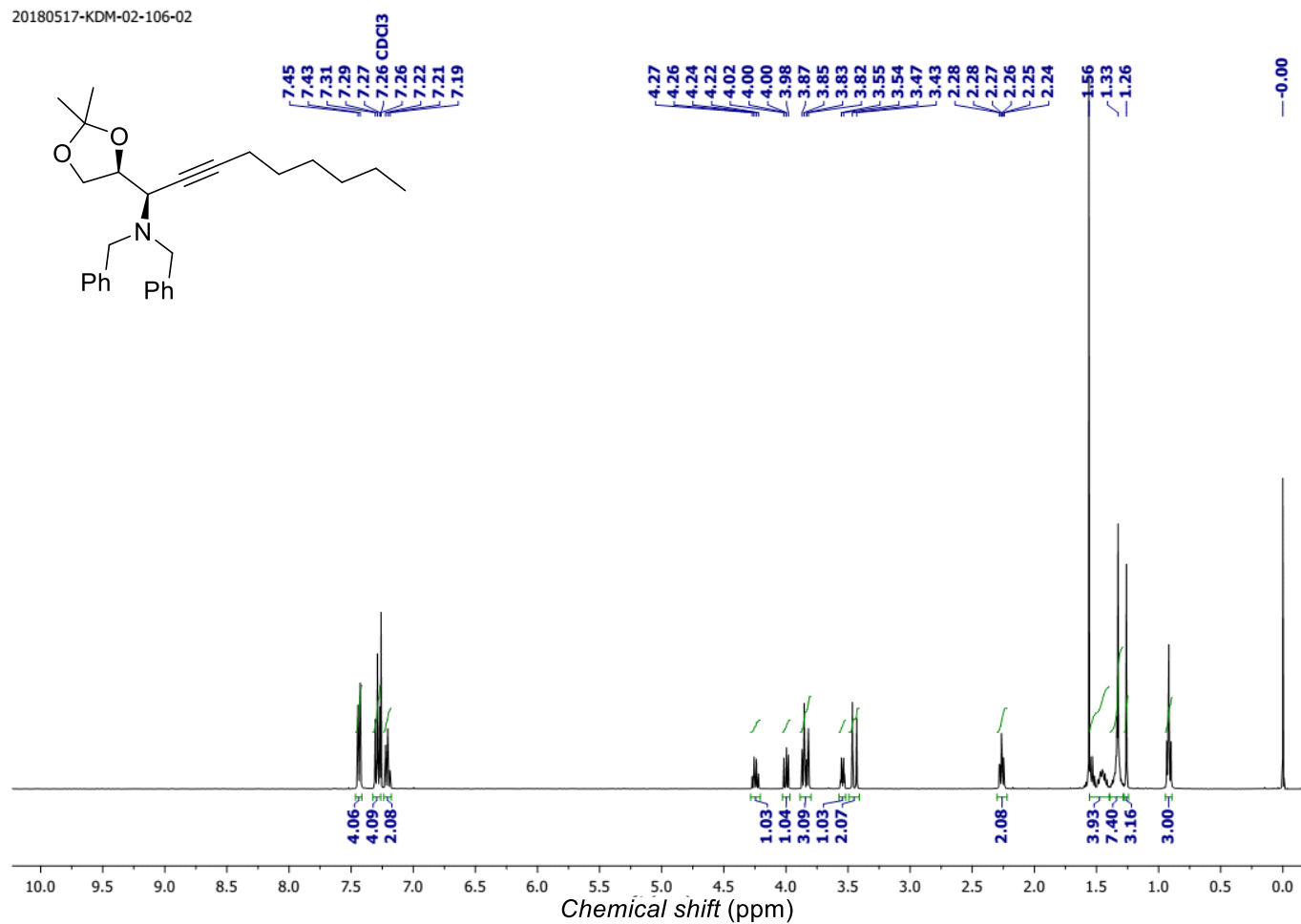


Fig. S18. ¹H NMR spectrum of compound **12** in CDCl₃.

20180219-KDM-02-R3

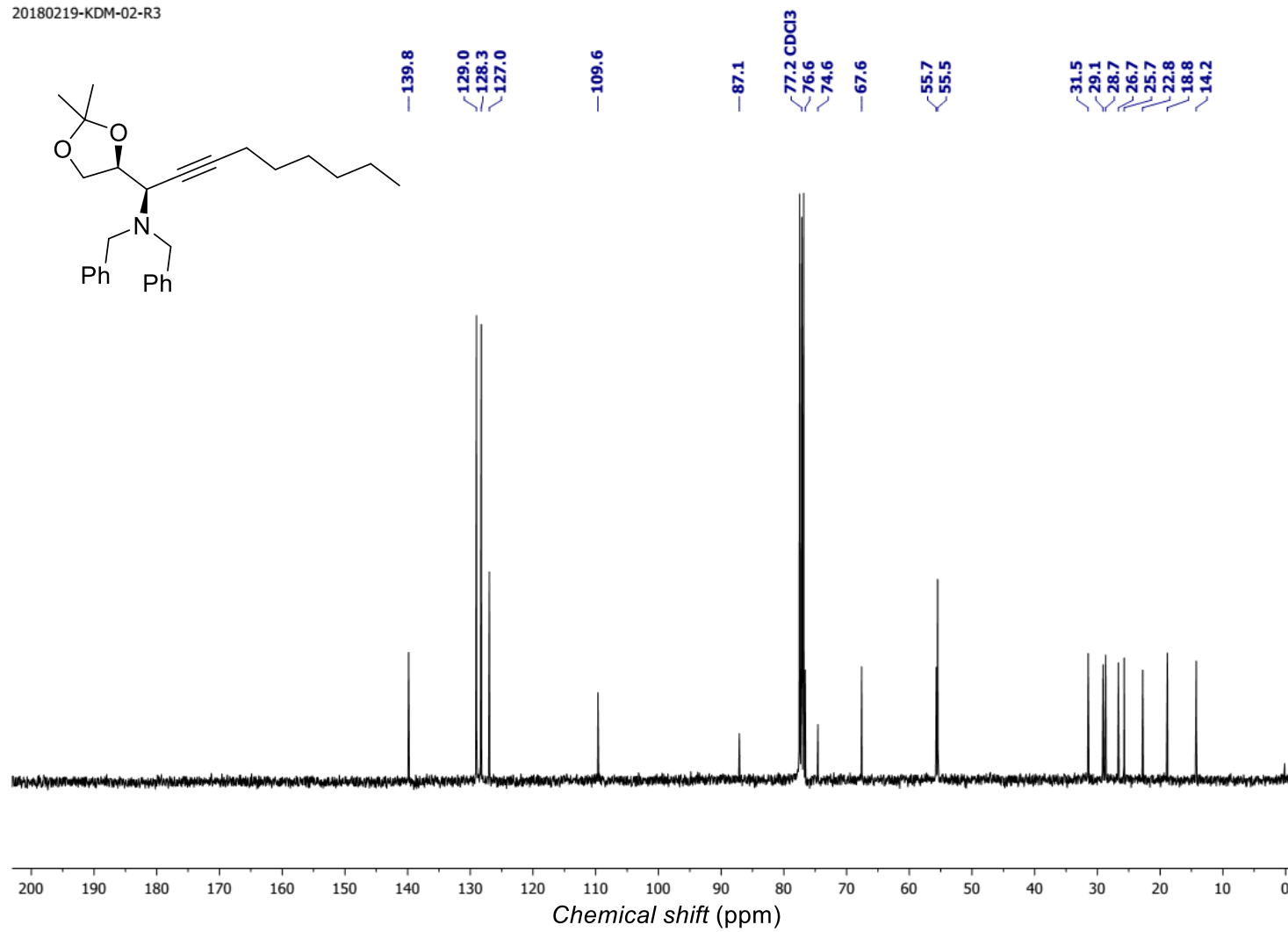


Fig. S19. ¹³C NMR spectrum of compound 12 in CDCl₃.

20180310-KDM-02-106

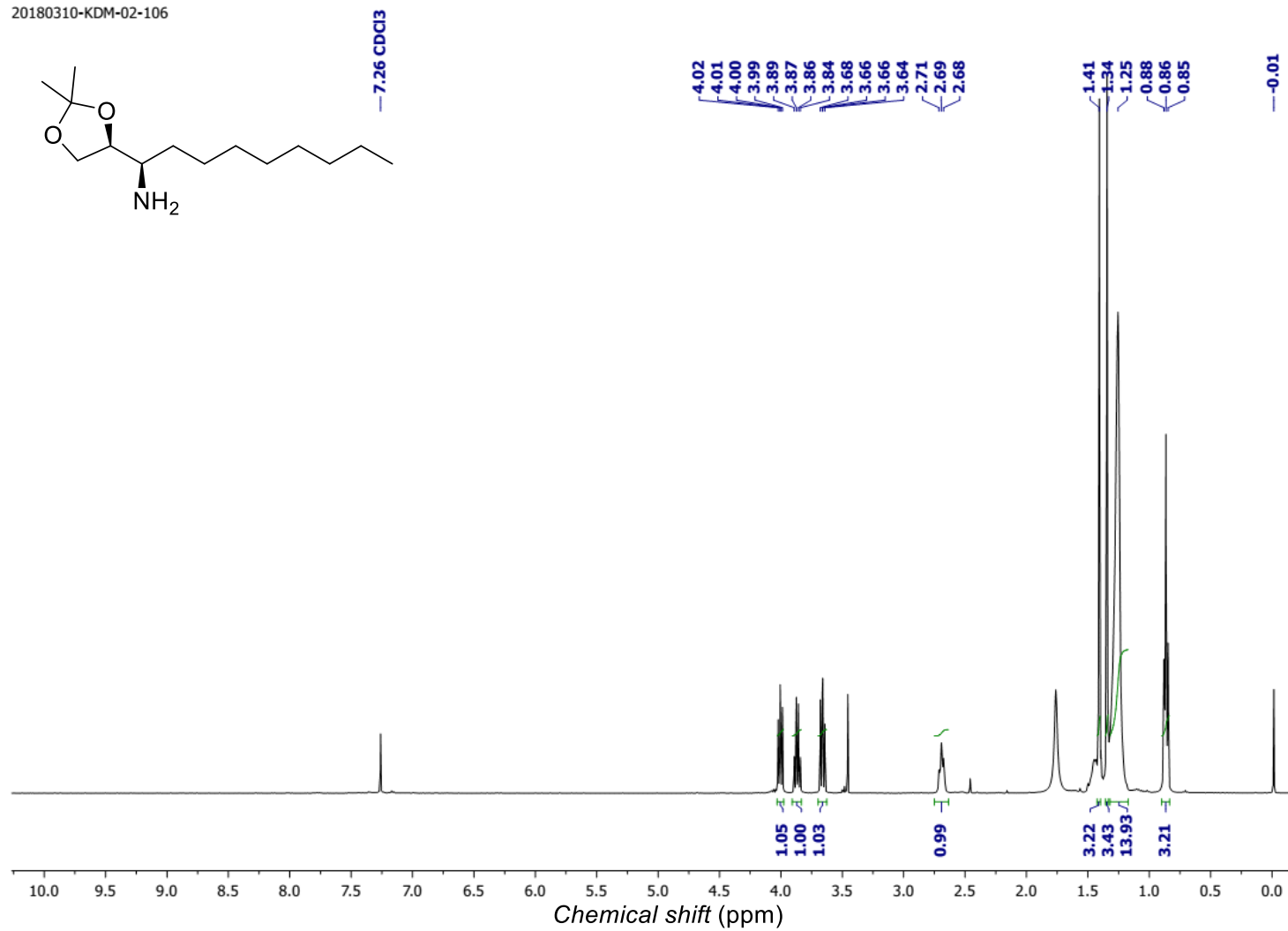


Fig. S20. ^1H NMR spectrum of compound **13** in CDCl_3 .

20180312-KDM-02-106_CARBON
KDM-02-106

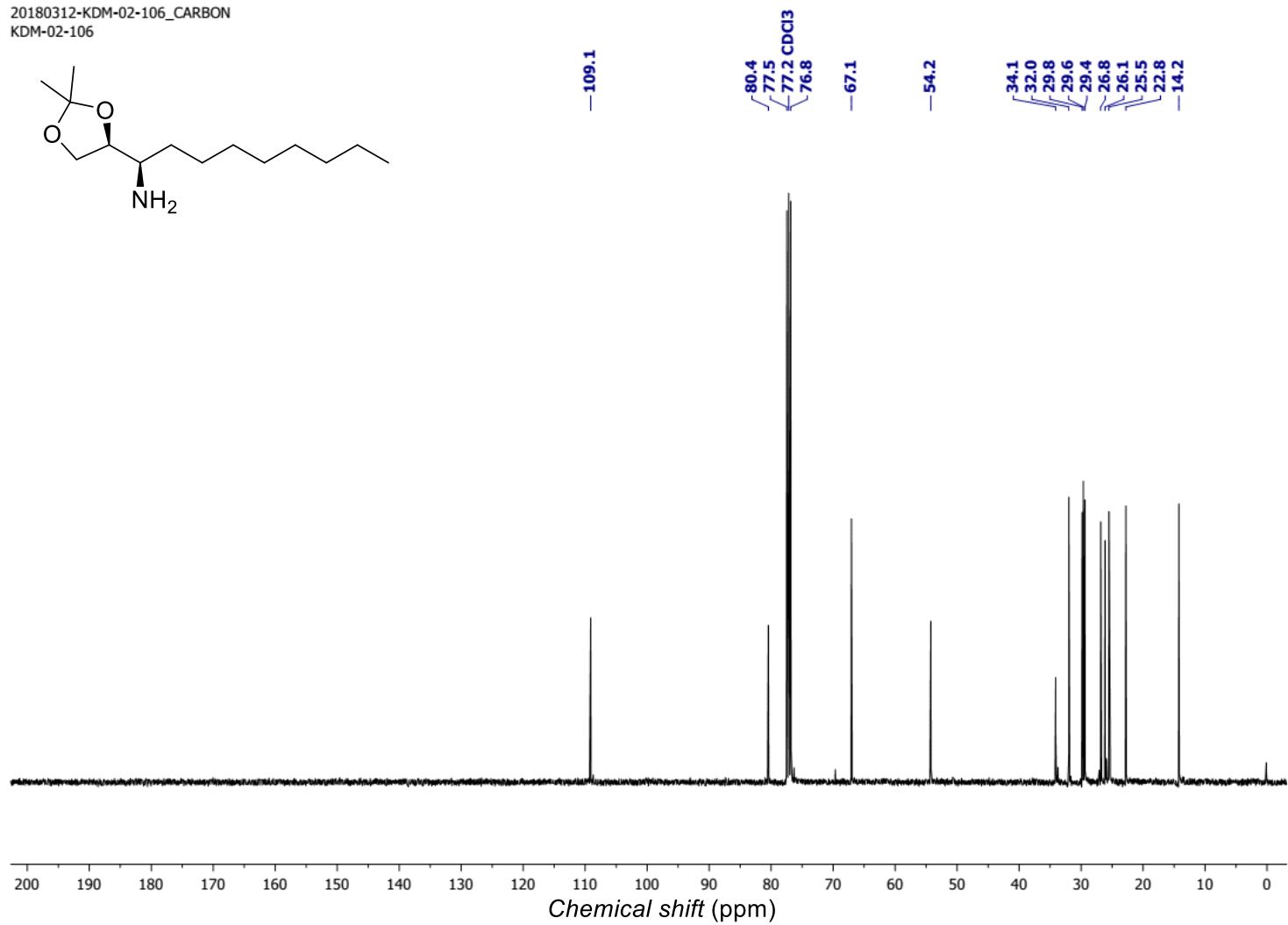


Fig. S21. ¹³C NMR spectrum of compound **13** in CDCl₃.

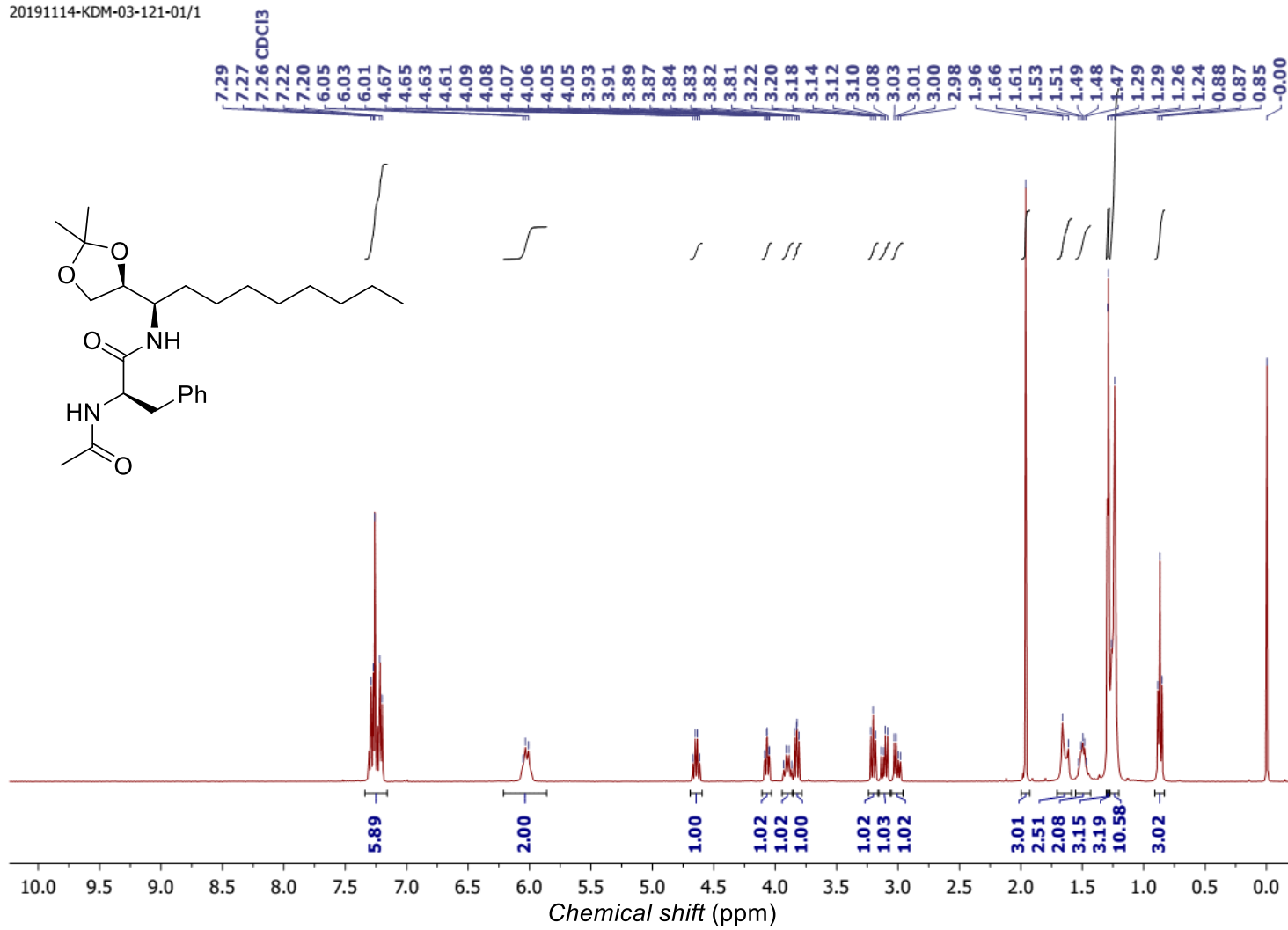


Fig. S22. ^1H NMR spectrum of compound **14R** in CDCl_3 .

S45

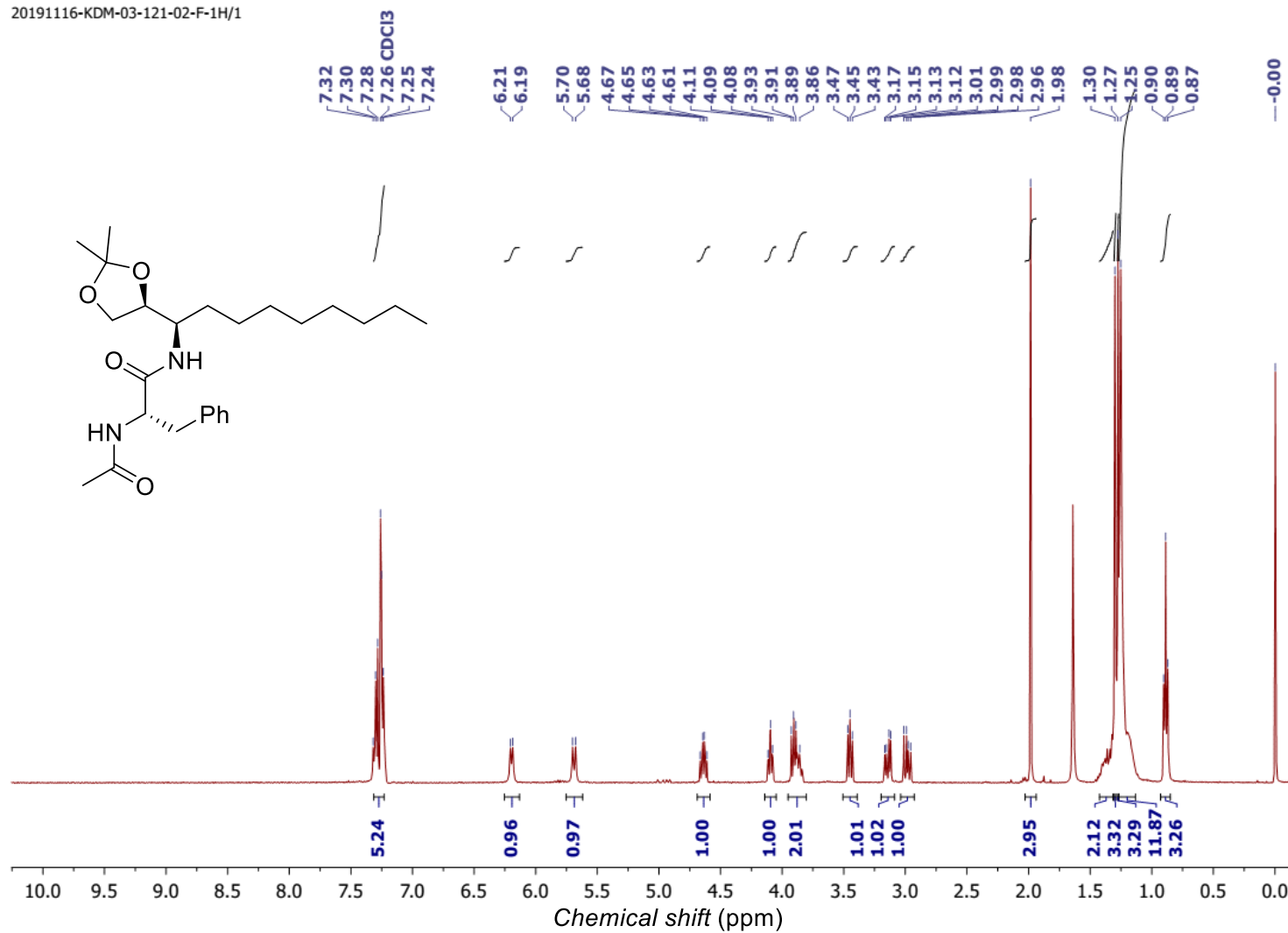


Fig. S24. ¹H NMR spectrum of compound 14S in CDCl₃.

20191128-KDM-03-121-02-F-13C/1

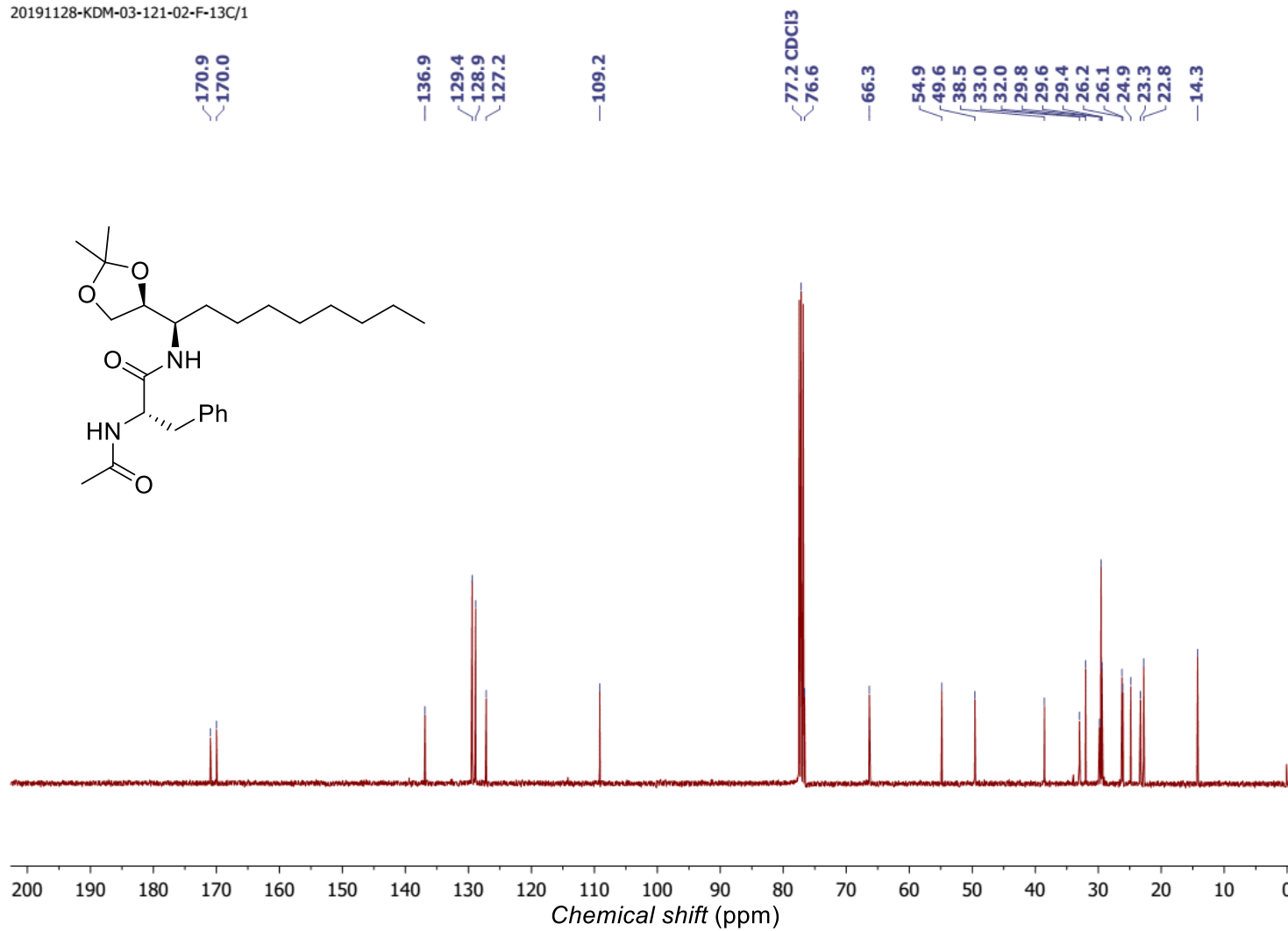


Fig. S25. ^{13}C NMR spectrum of compound **14S** in CDCl₃.

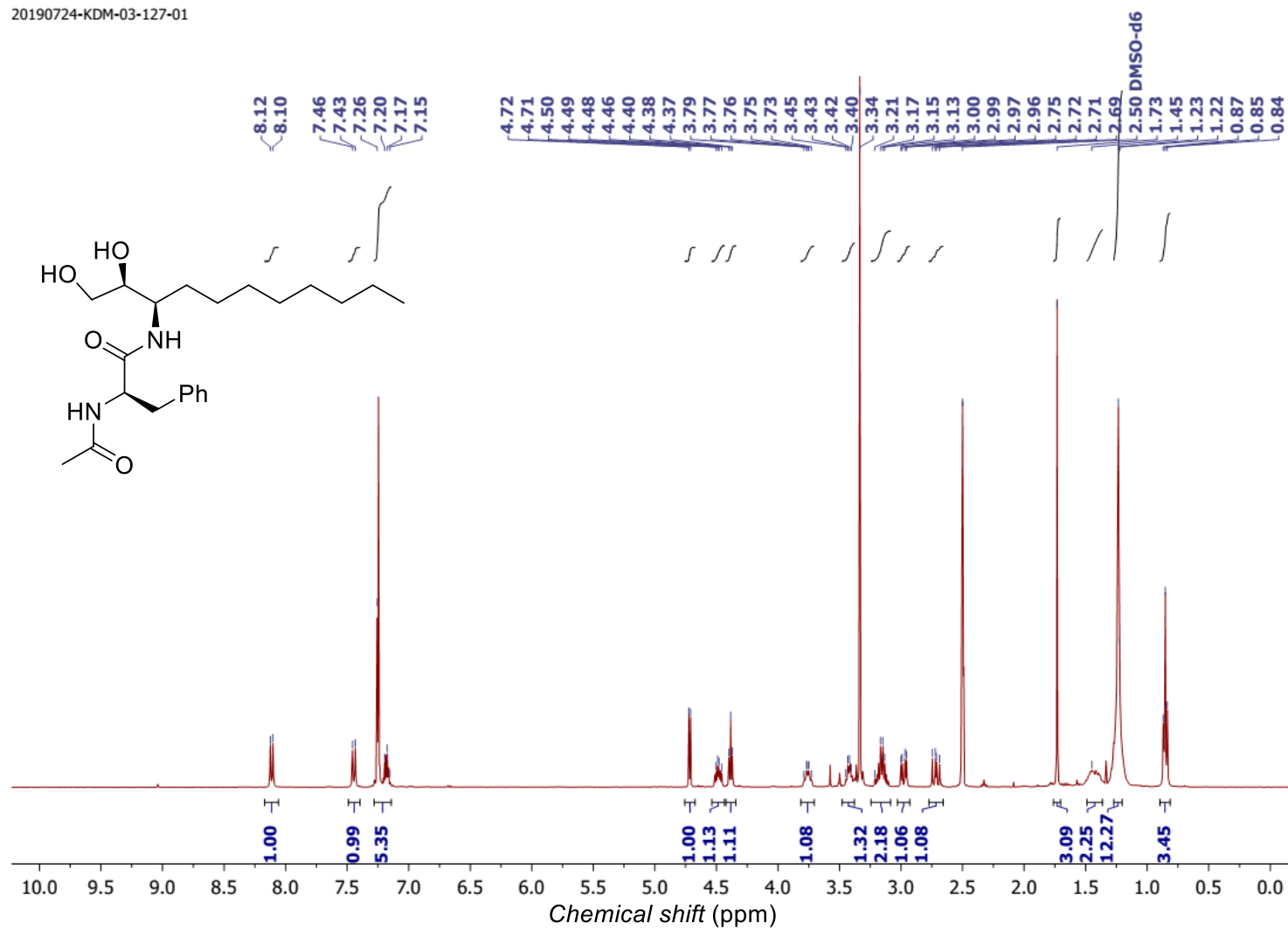


Fig. S26. ^1H NMR spectrum of compound **1R** in DMSO-d_6 .

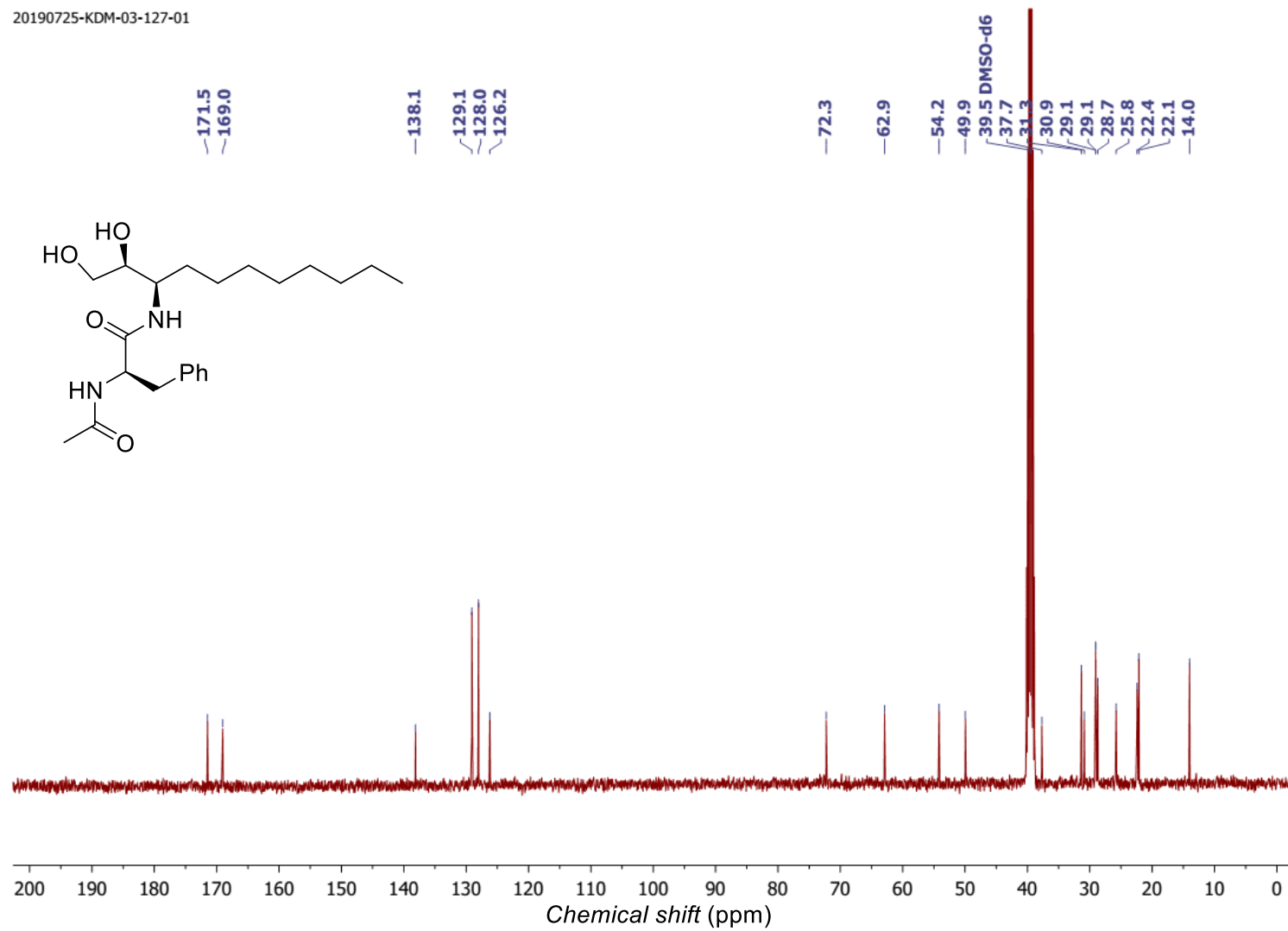


Fig. S27. ^{13}C NMR spectrum of compound **1R** in $\text{DMSO}-d_6$.

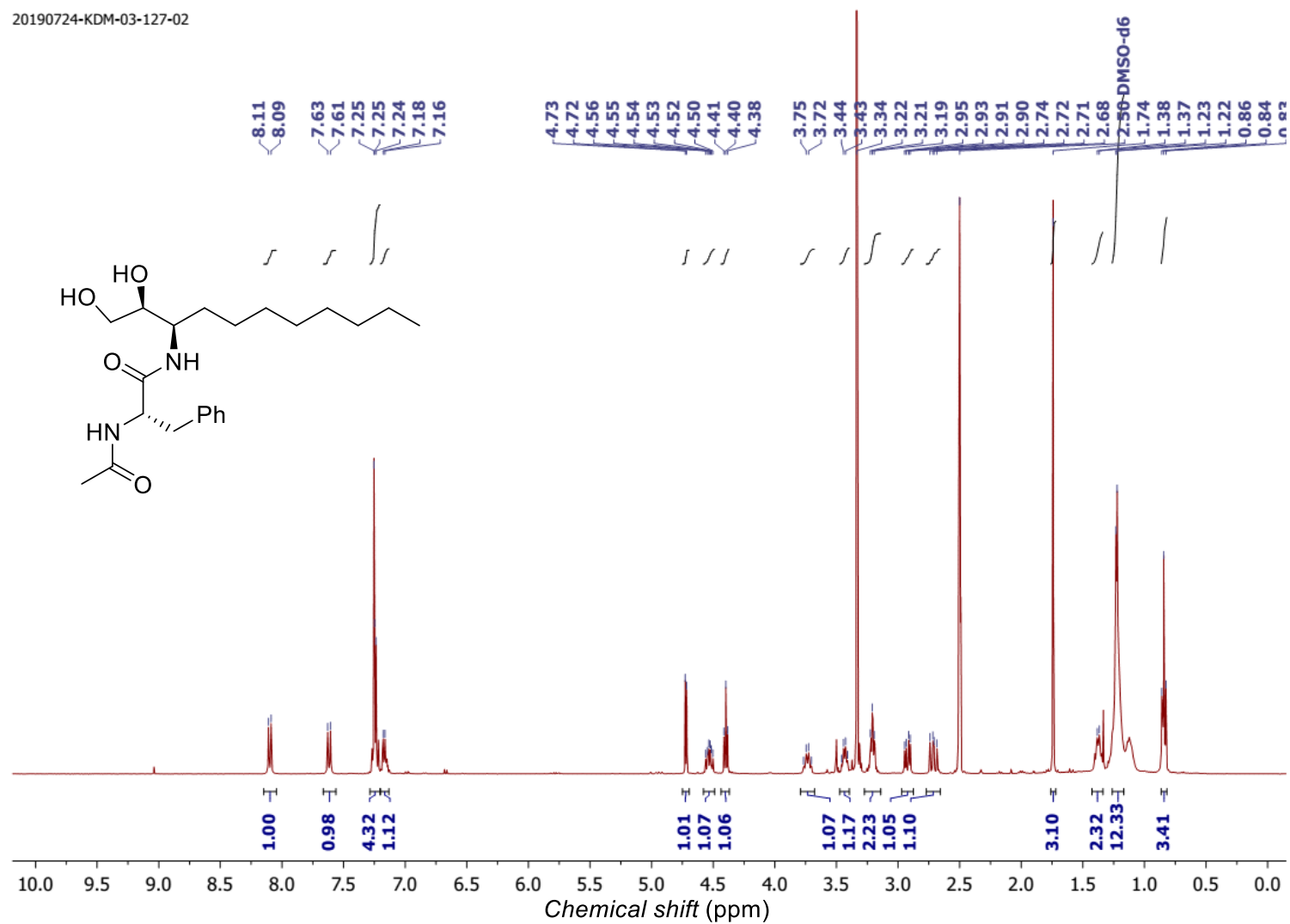


Fig. S28. ^1H NMR spectrum of compound 1S in $\text{DMSO}-d_6$.

20190725-KDM-03-127-02

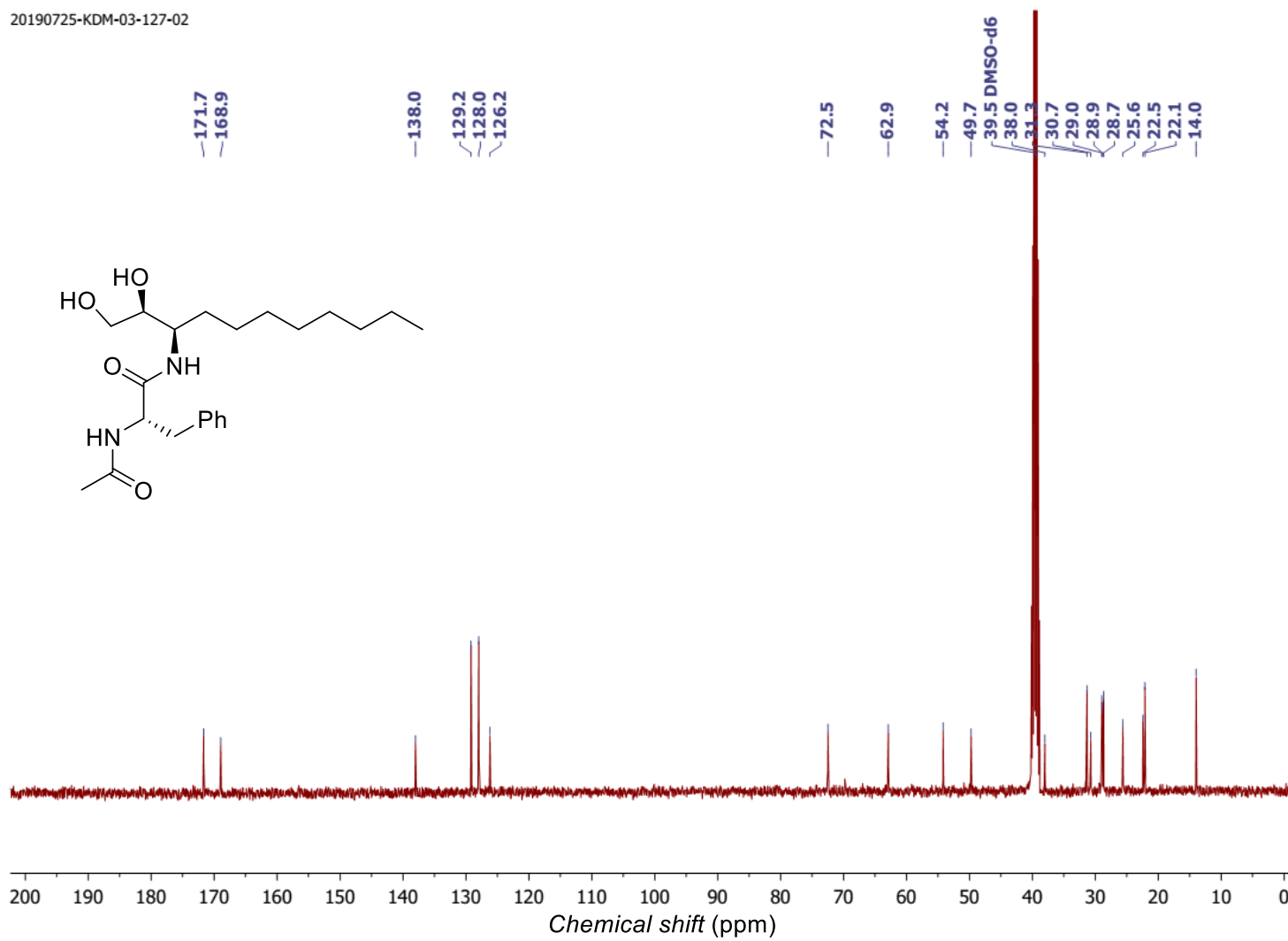


Fig. S29. ¹³C NMR spectrum of compound **1S** in DMSO-*d*₆.

20180830-KDM-02-193-02/1
1H

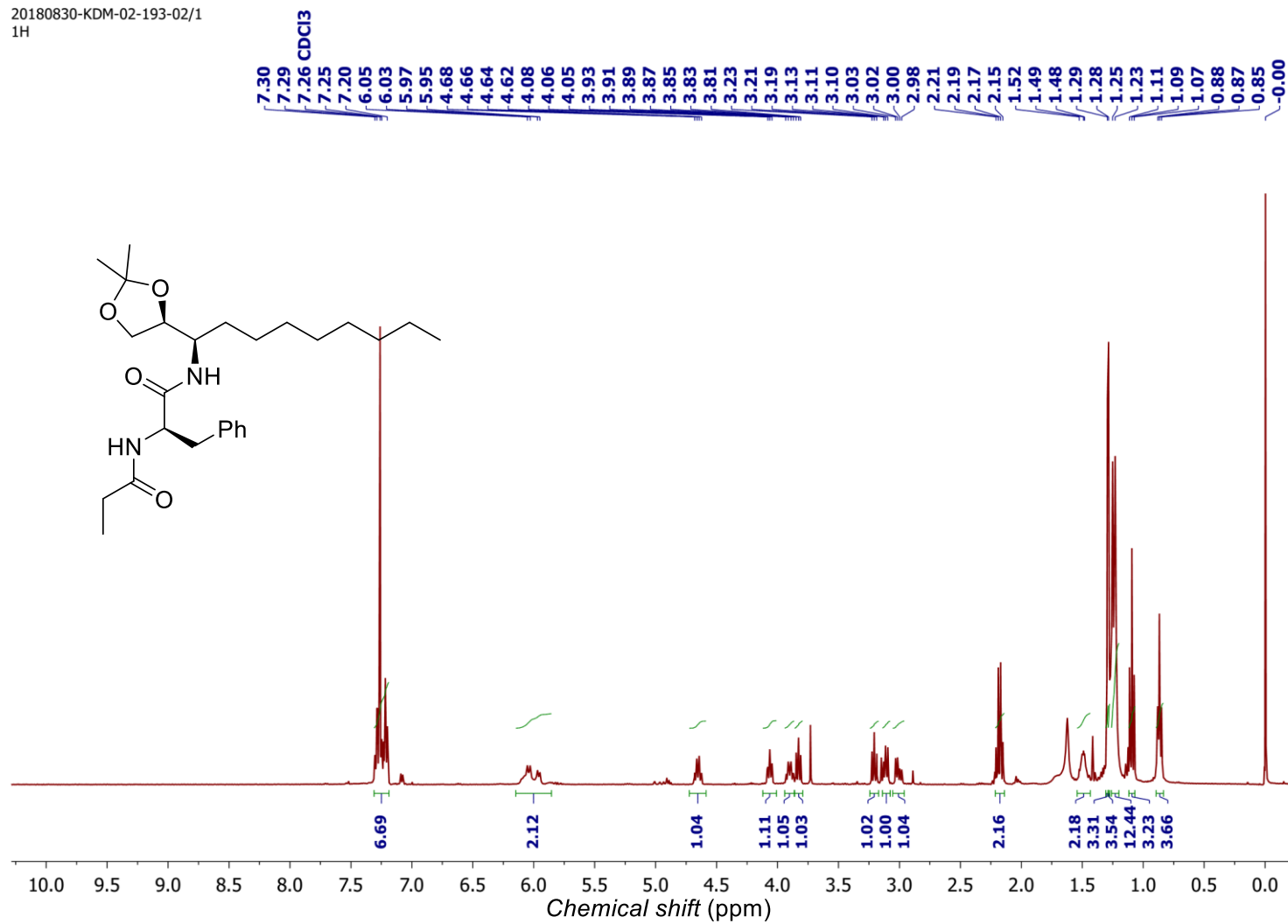


Fig. S30. ^1H NMR spectrum of compound **15R** in CDCl_3 .

20210420-KDM-02-193-011

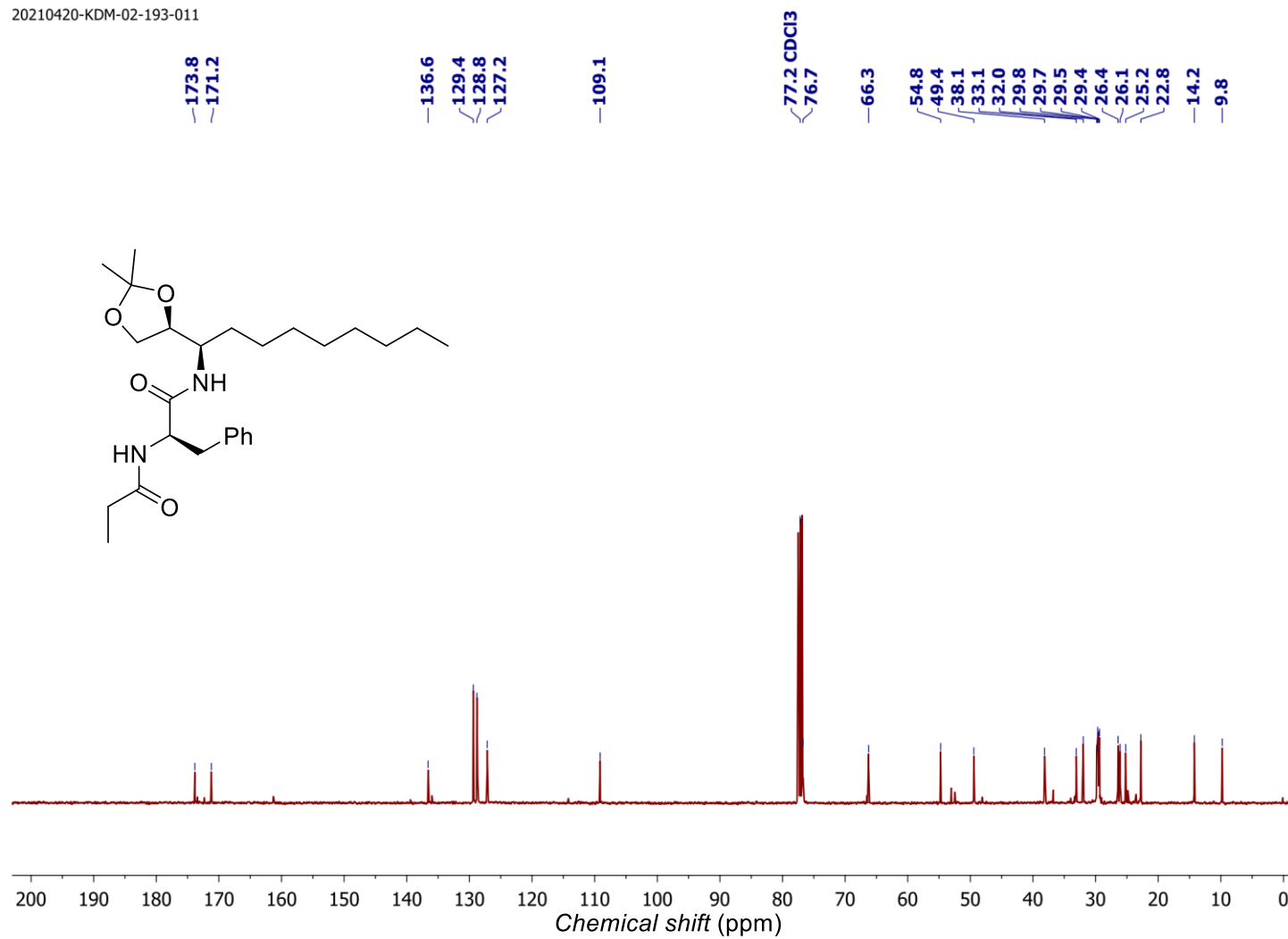


Fig. S31. ^{13}C NMR spectrum of compound **15R** in CDCl_3 .

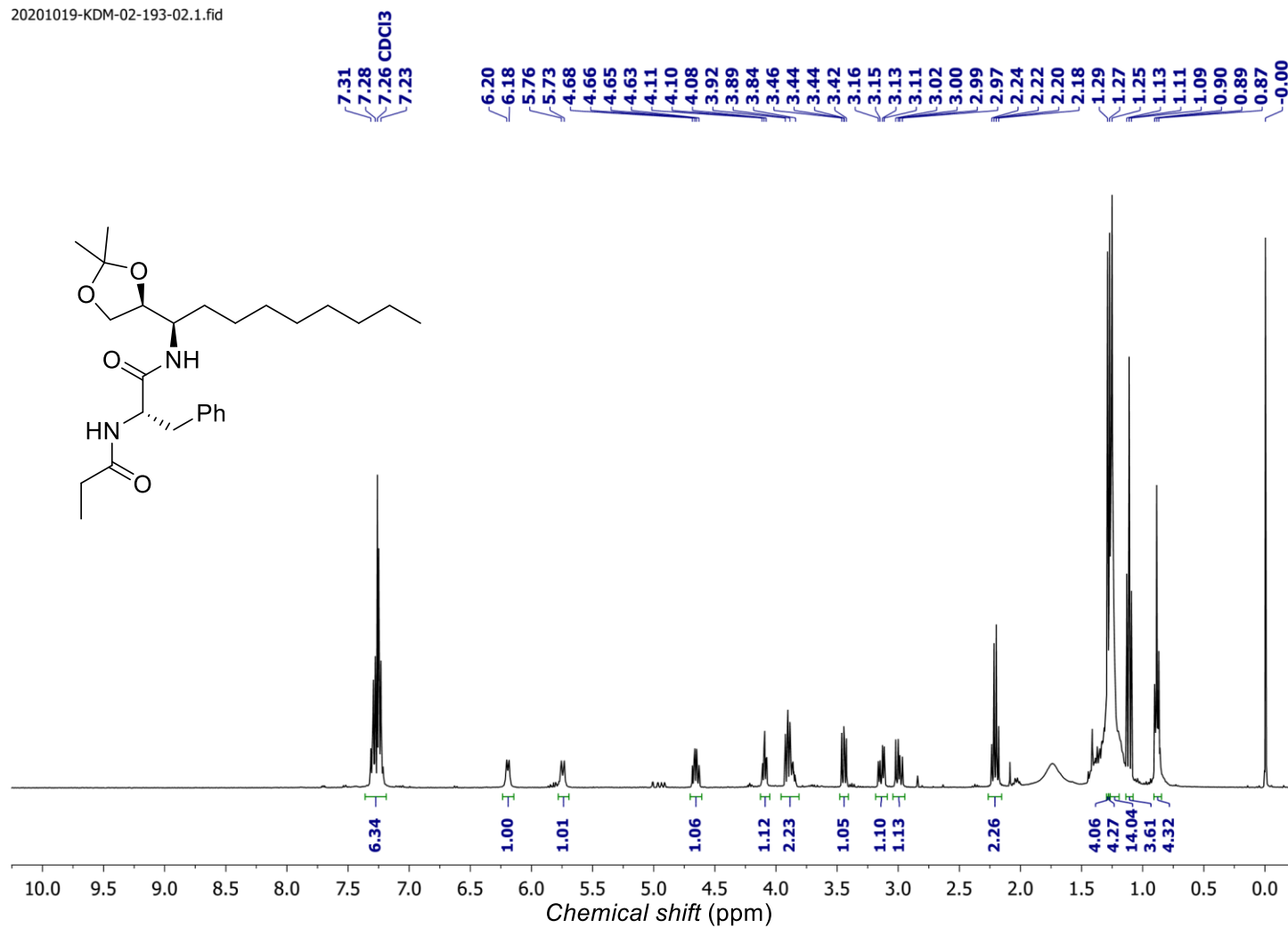


Fig. S32. ¹H NMR spectrum of compound 15S in CDCl₃.

20201019-KDM-02-193-02.2.fid

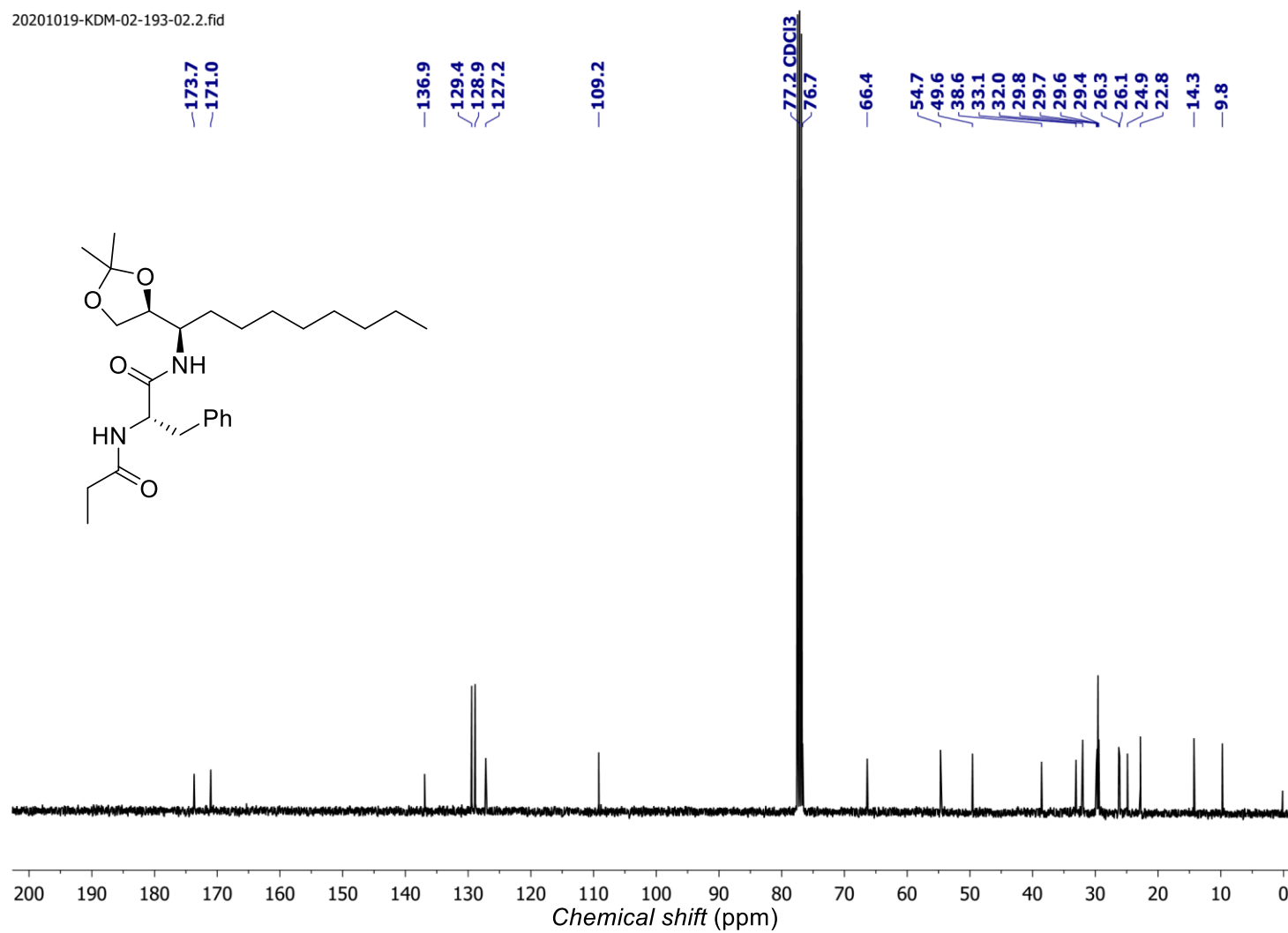


Fig. S33. ^{13}C NMR spectrum of compound **15S** in CDCl_3 .

20180904-KDM-02-195-01/1
1H

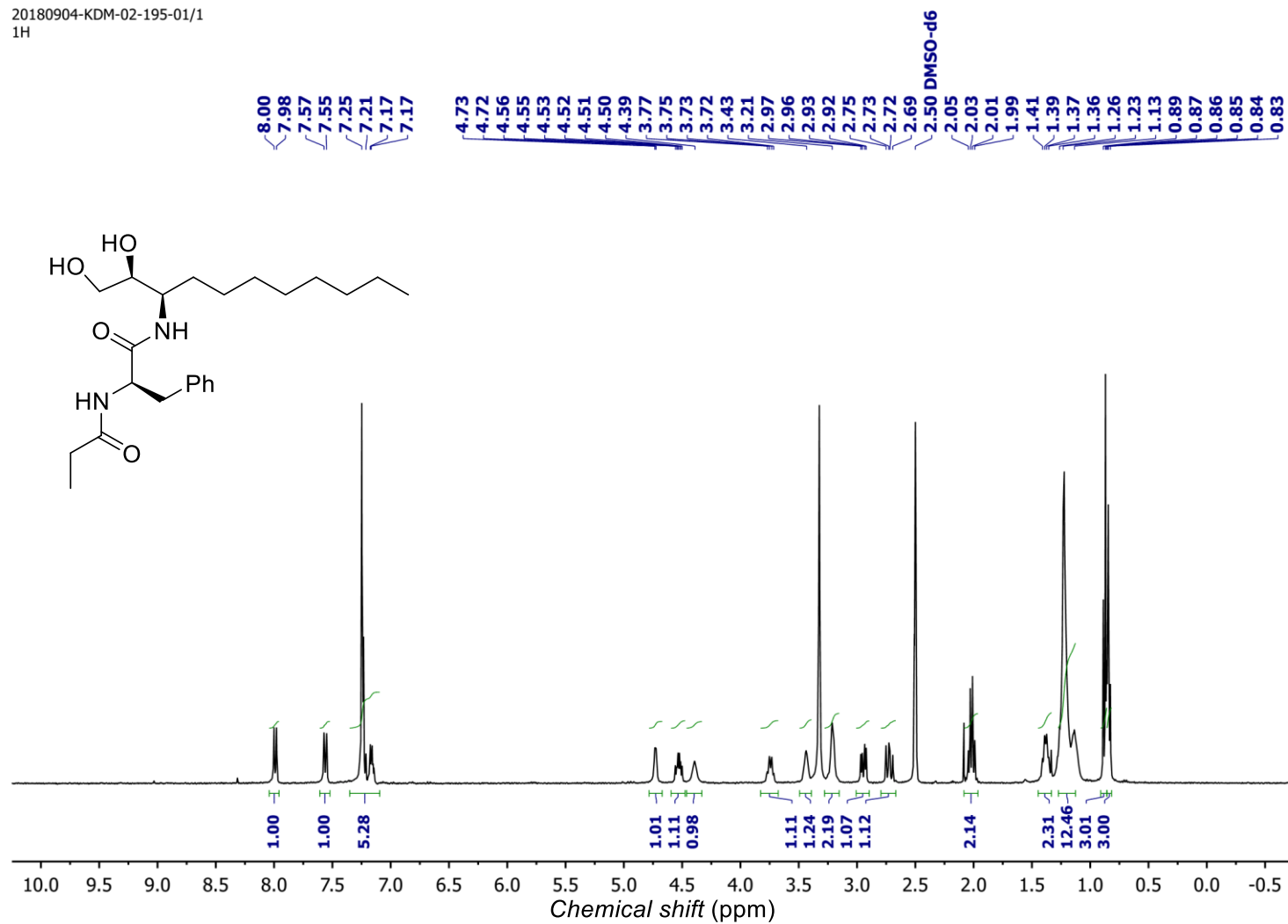


Fig. S34. ¹H NMR spectrum of compound 2R in DMSO-d₆.

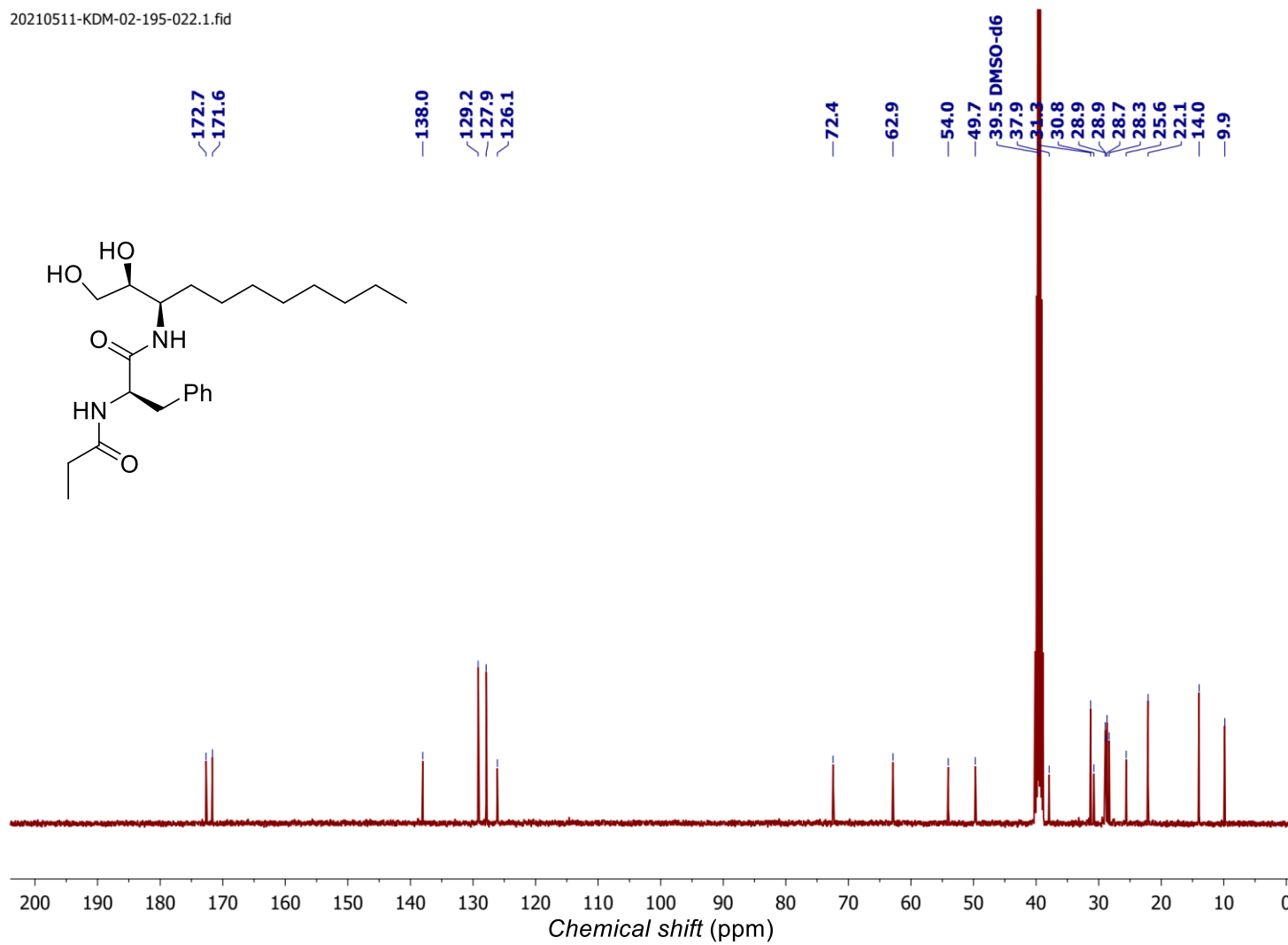


Fig. S35. ^{13}C NMR spectrum of compound **2R** in $\text{DMSO-}d_6$.

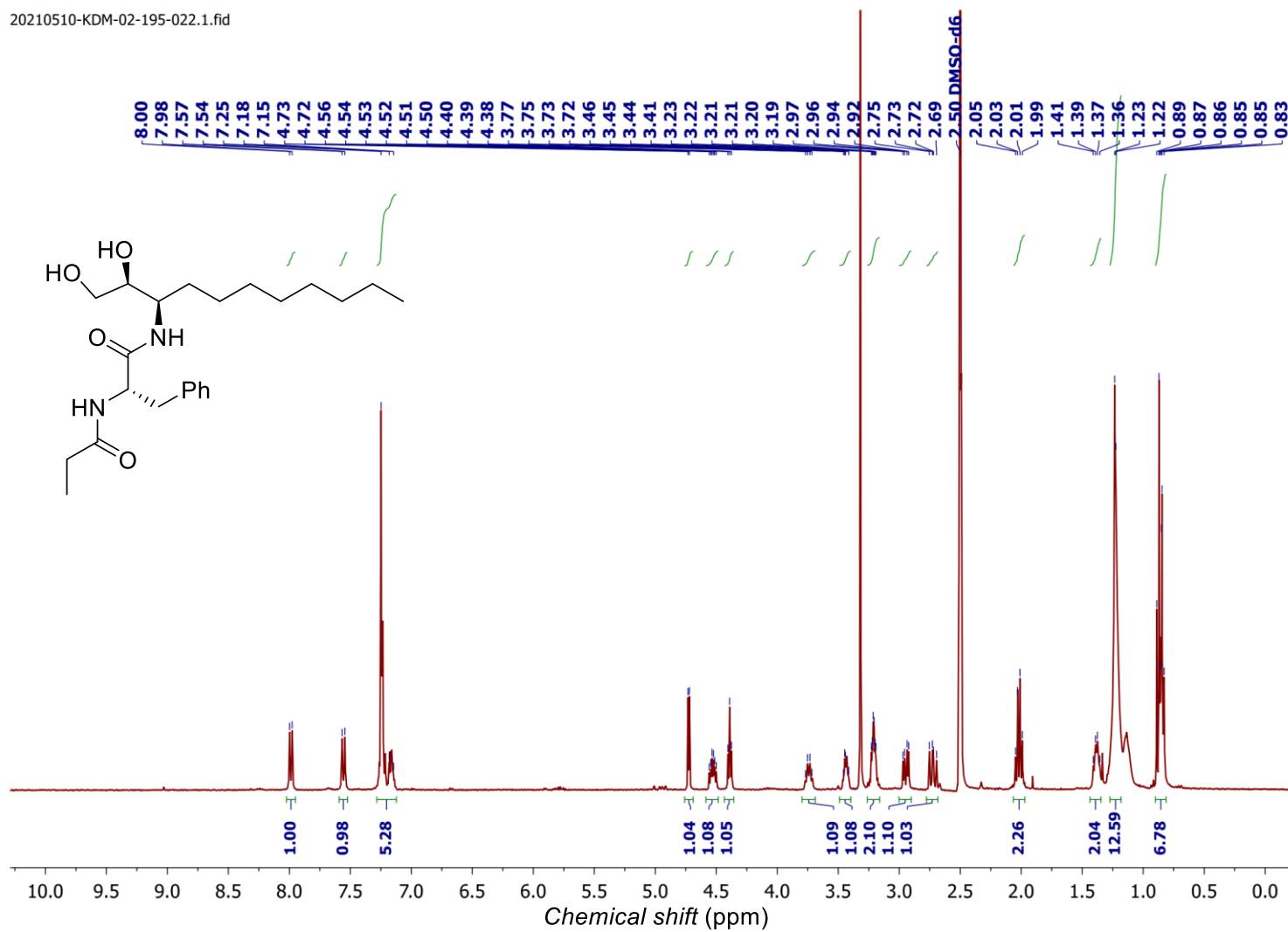


Fig. S36. ¹H NMR spectrum of compound 2S in DMSO-d₆.

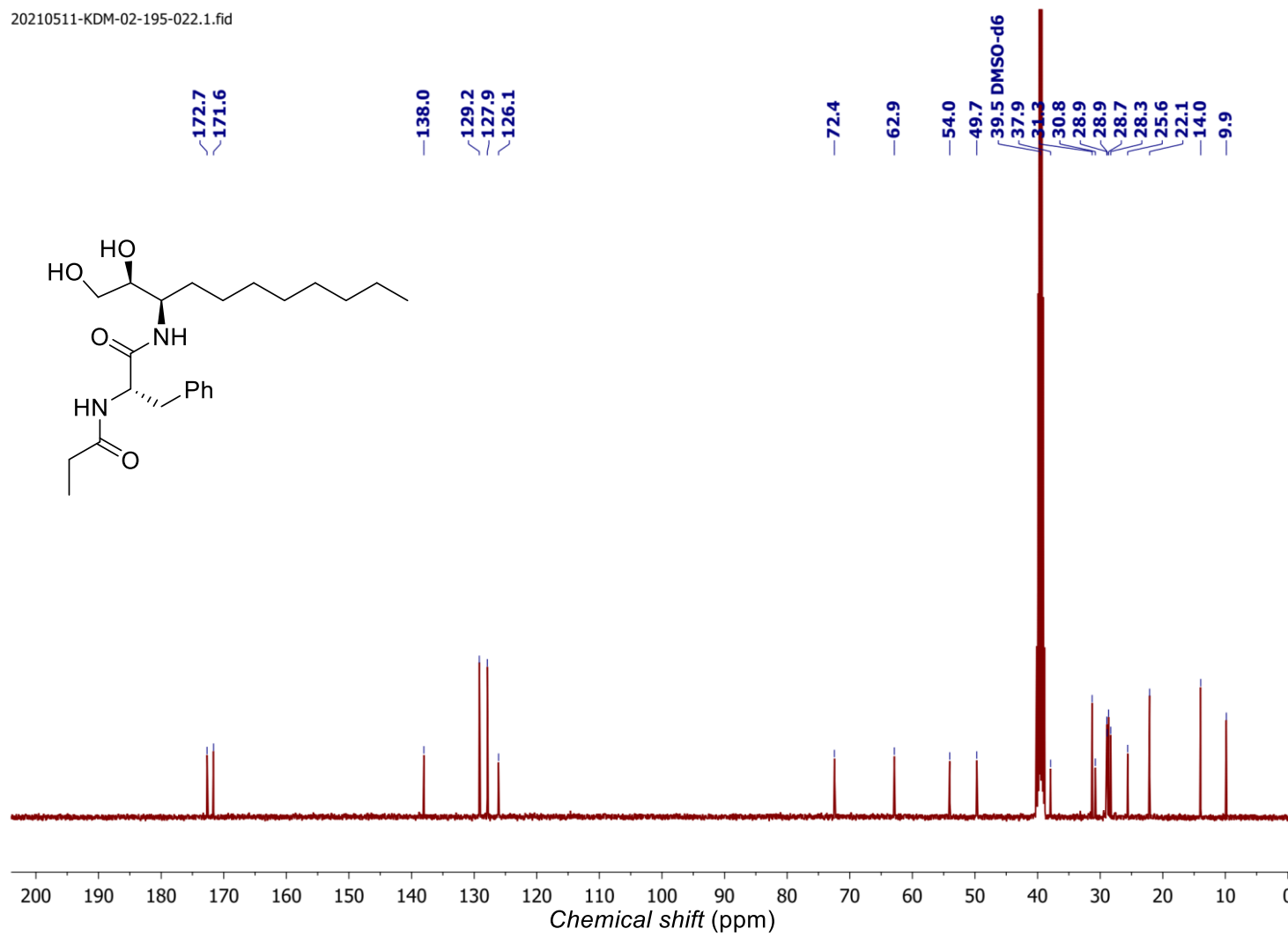


Fig. S37. ¹³C NMR spectrum of compound 2S in DMSO-*d*₆.

20190624-KDM-03-114-01_1H/1

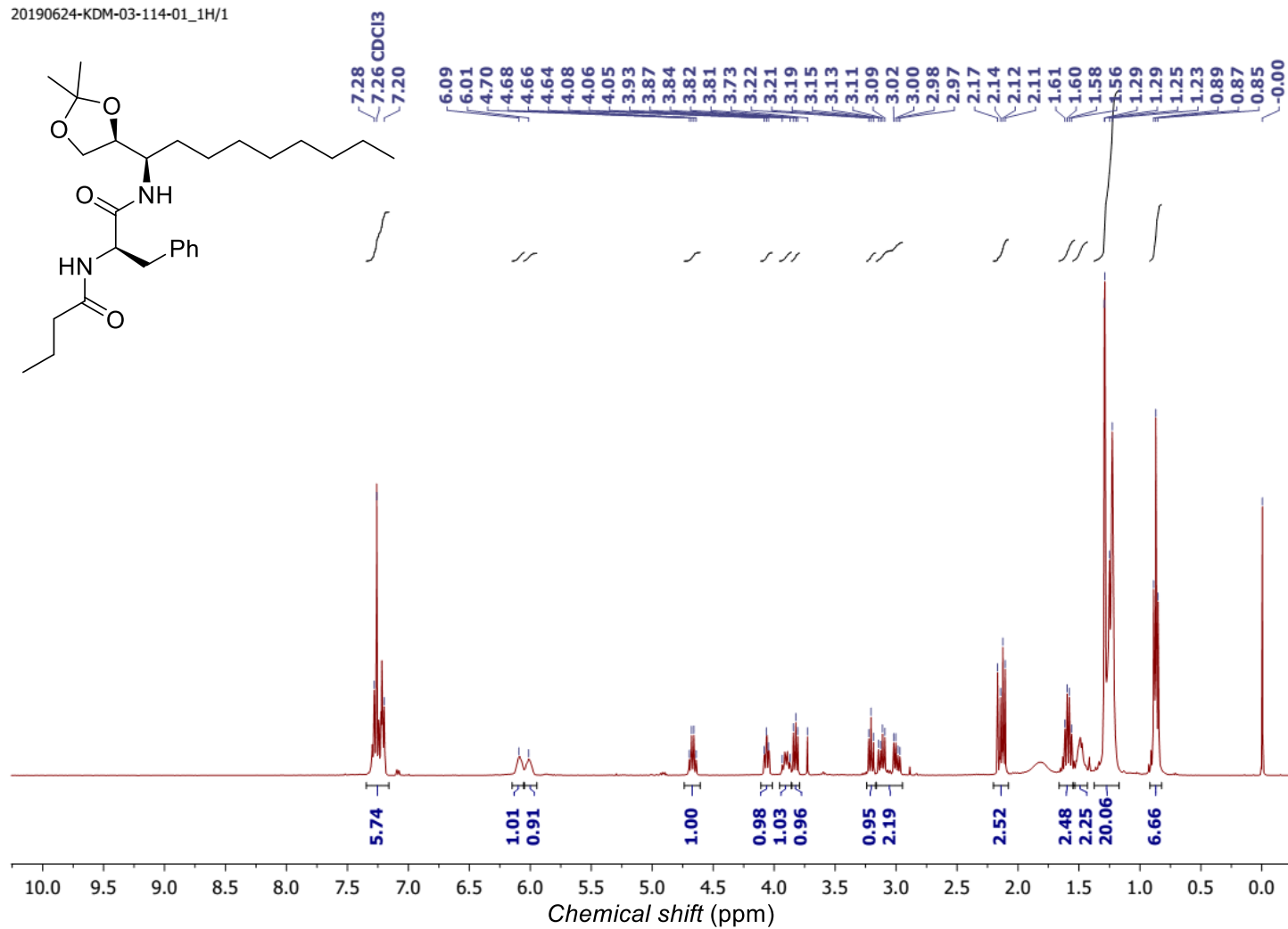


Fig. S38. ^1H NMR spectrum of compound **16R** in CDCl_3 .

20190624-KDM-03-114-01_13C/2

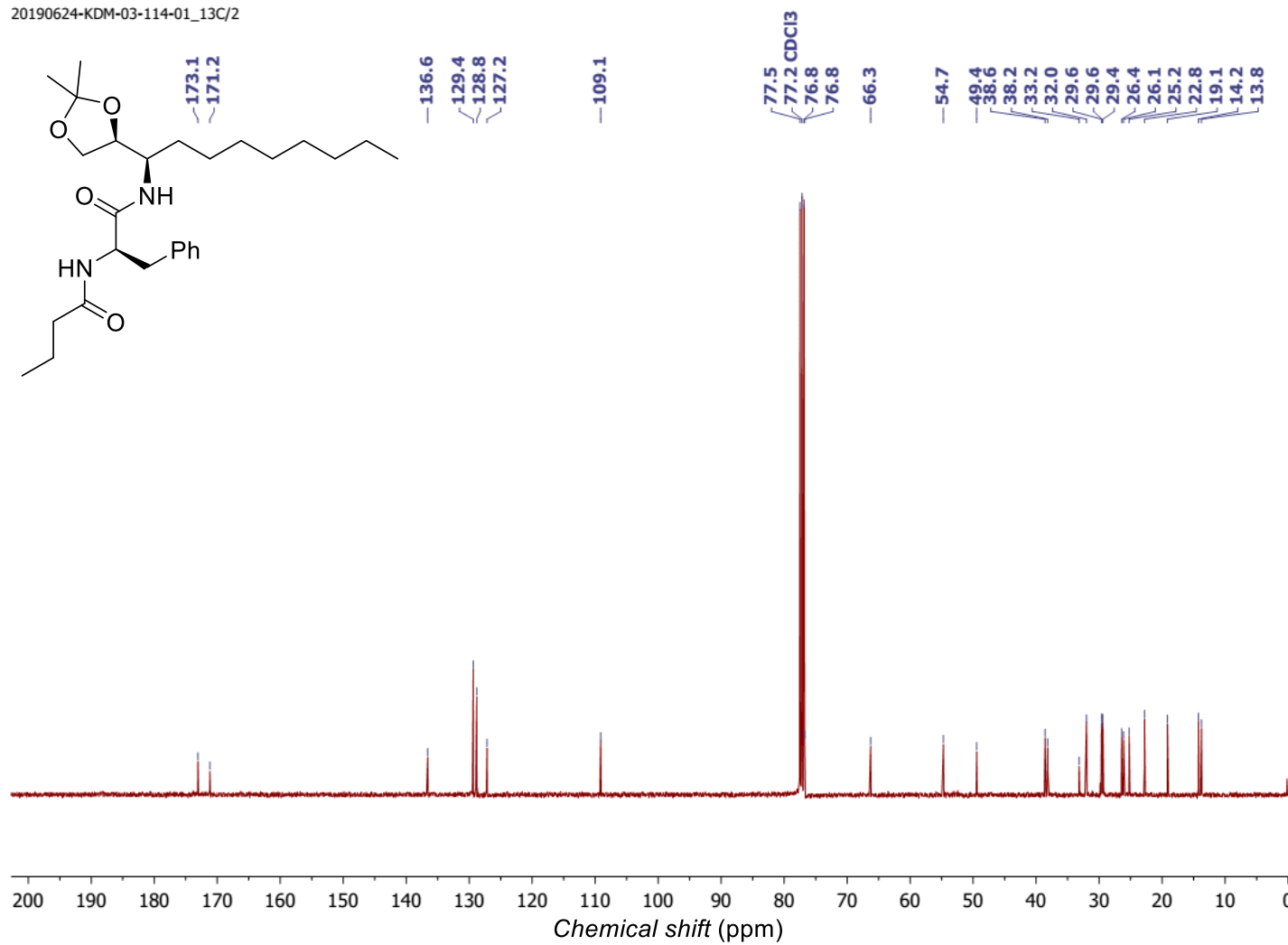


Fig. S39. ¹³C NMR spectrum of compound **16R** in CDCl₃.

20190622-KDM-03-114-02

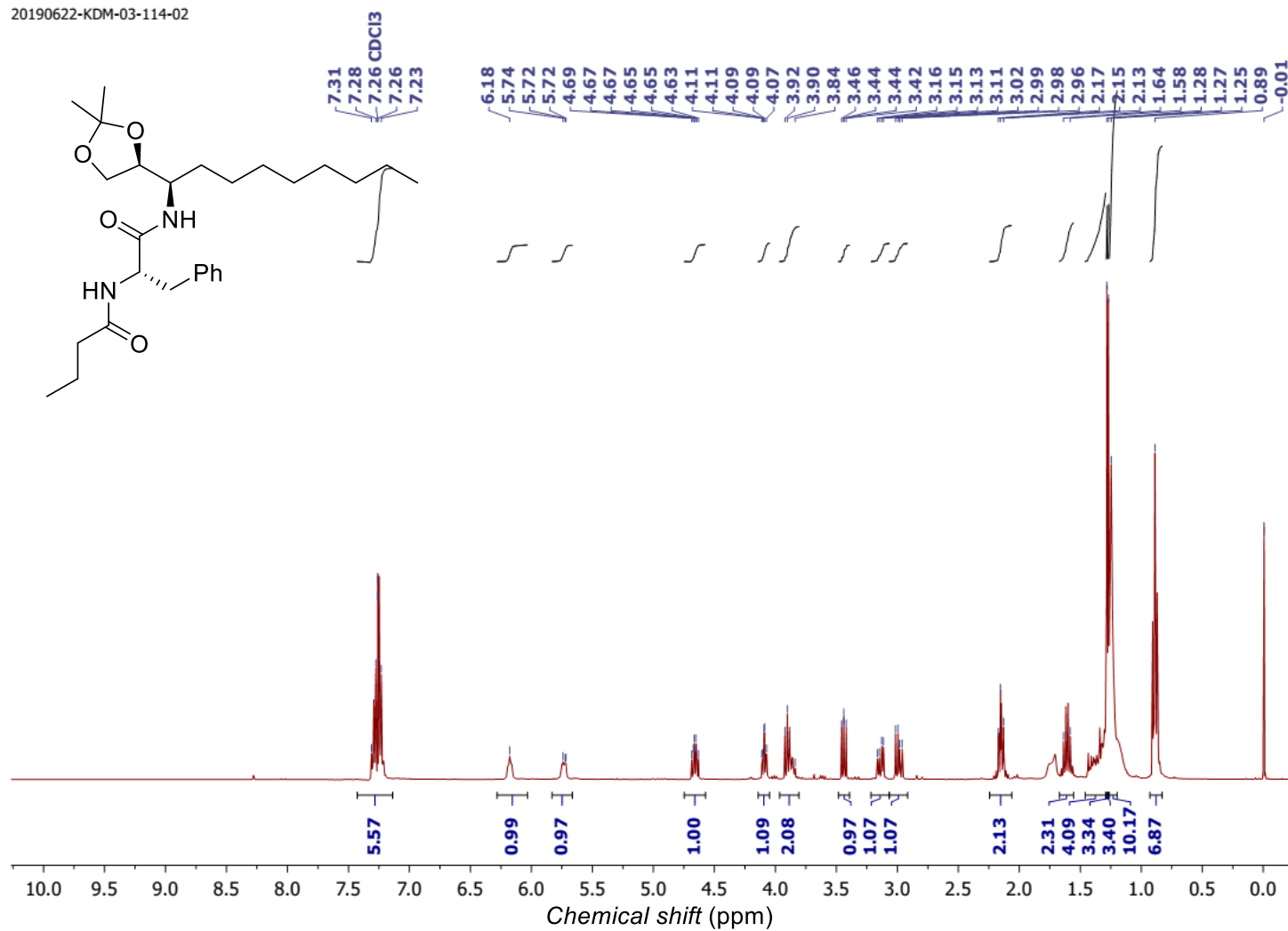


Fig. S40. ¹H NMR spectrum of compound **16S** in CDCl₃.

20190622-KDM-03-114-02

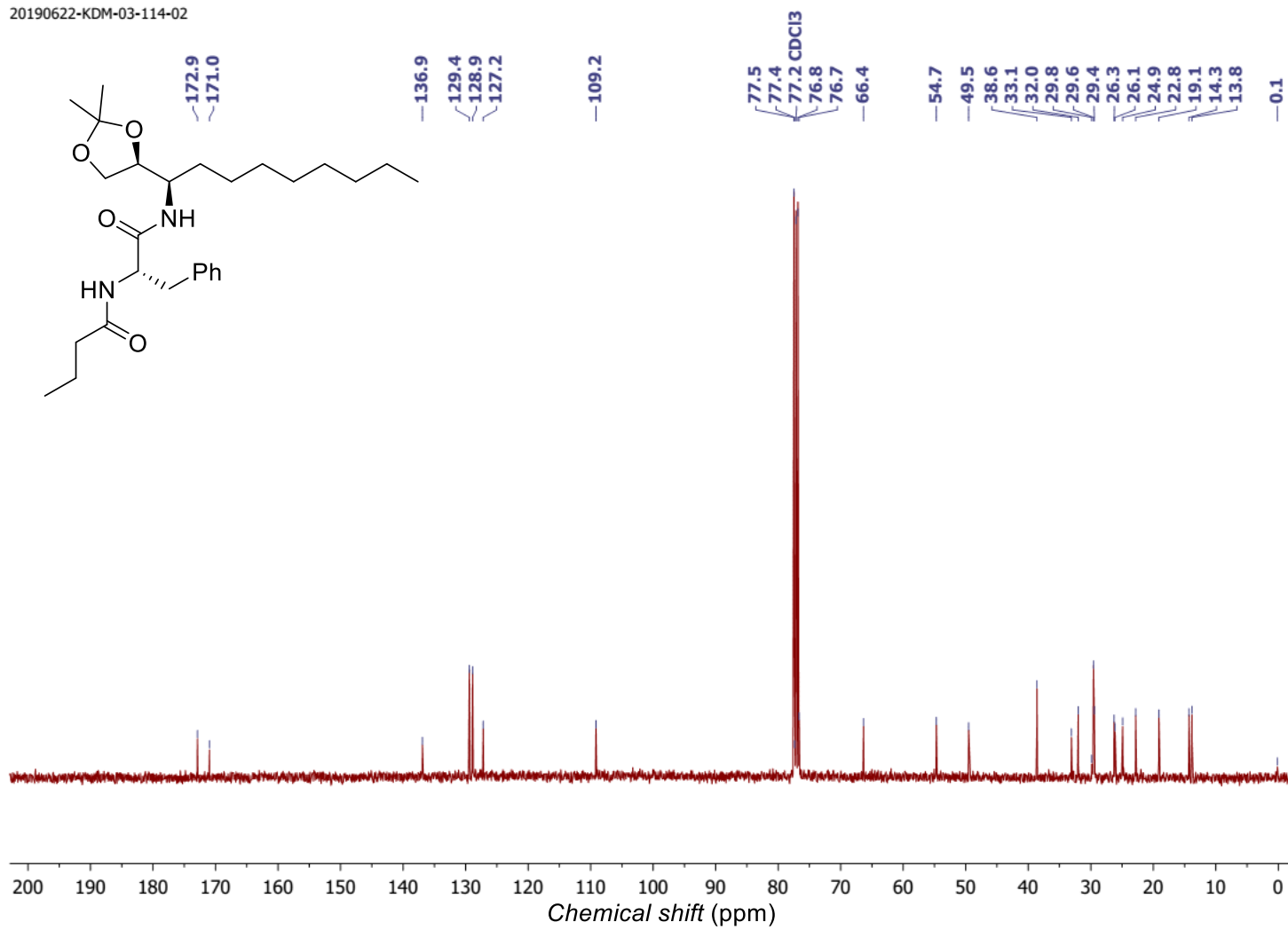


Fig. S41. ^{13}C NMR spectrum of compound **16S** in CDCl_3 .

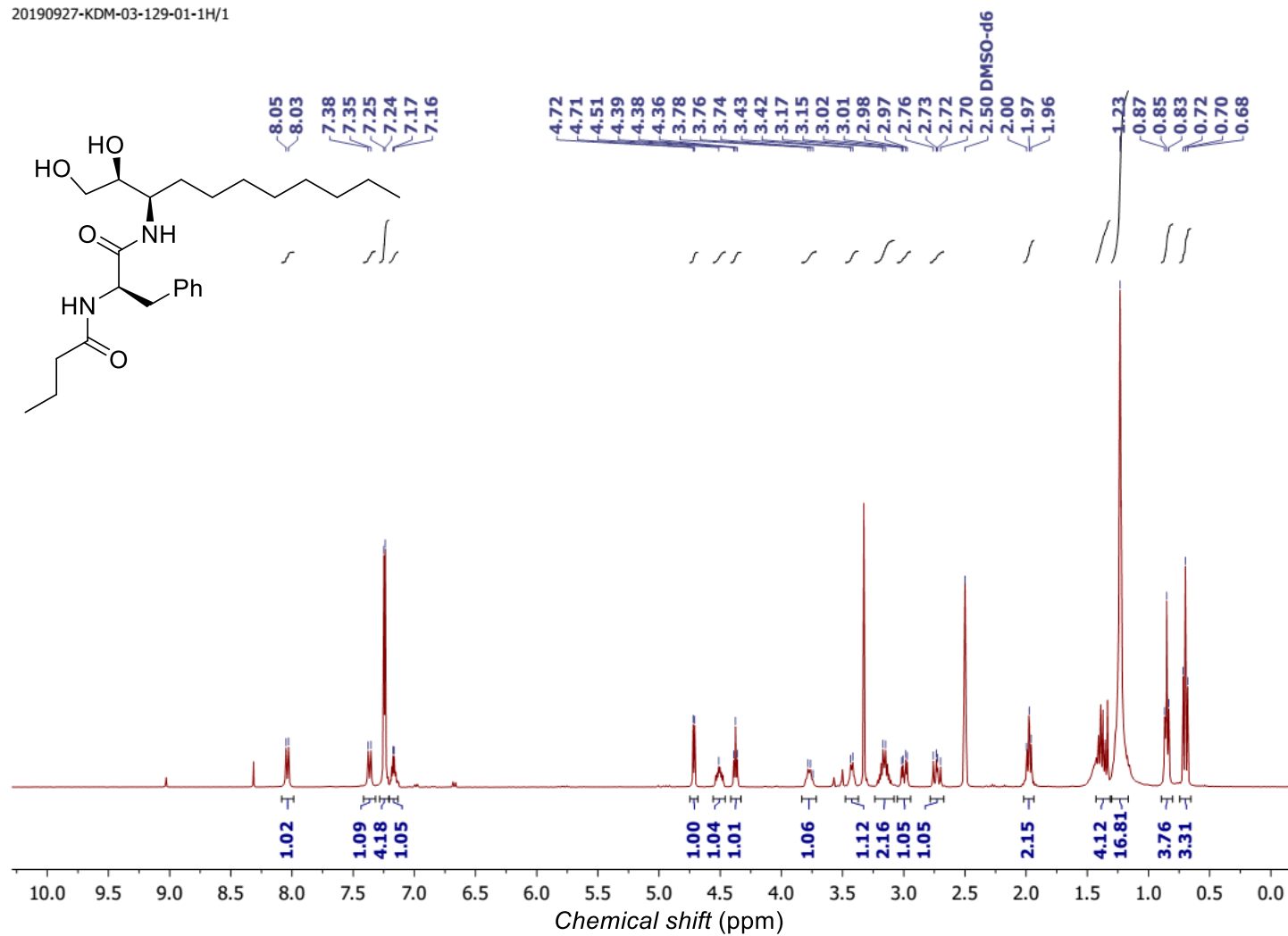


Fig. S42. ^1H NMR spectrum of compound **3R** in DMSO- d_6 .

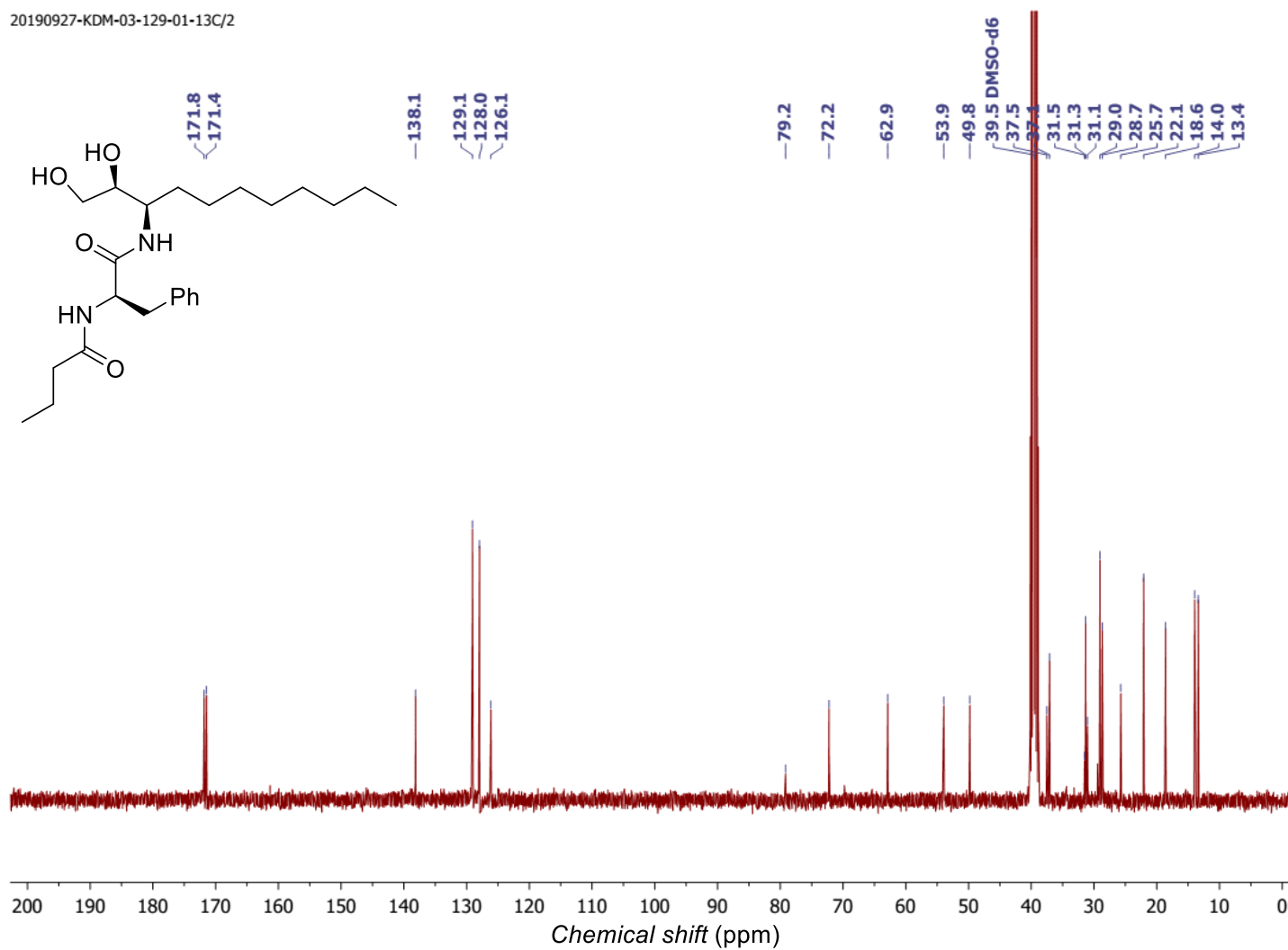


Fig. S43. ^{13}C NMR spectrum of compound **3R** in DMSO- d_6 .

20190927-KDM-03-129-02-1H/1

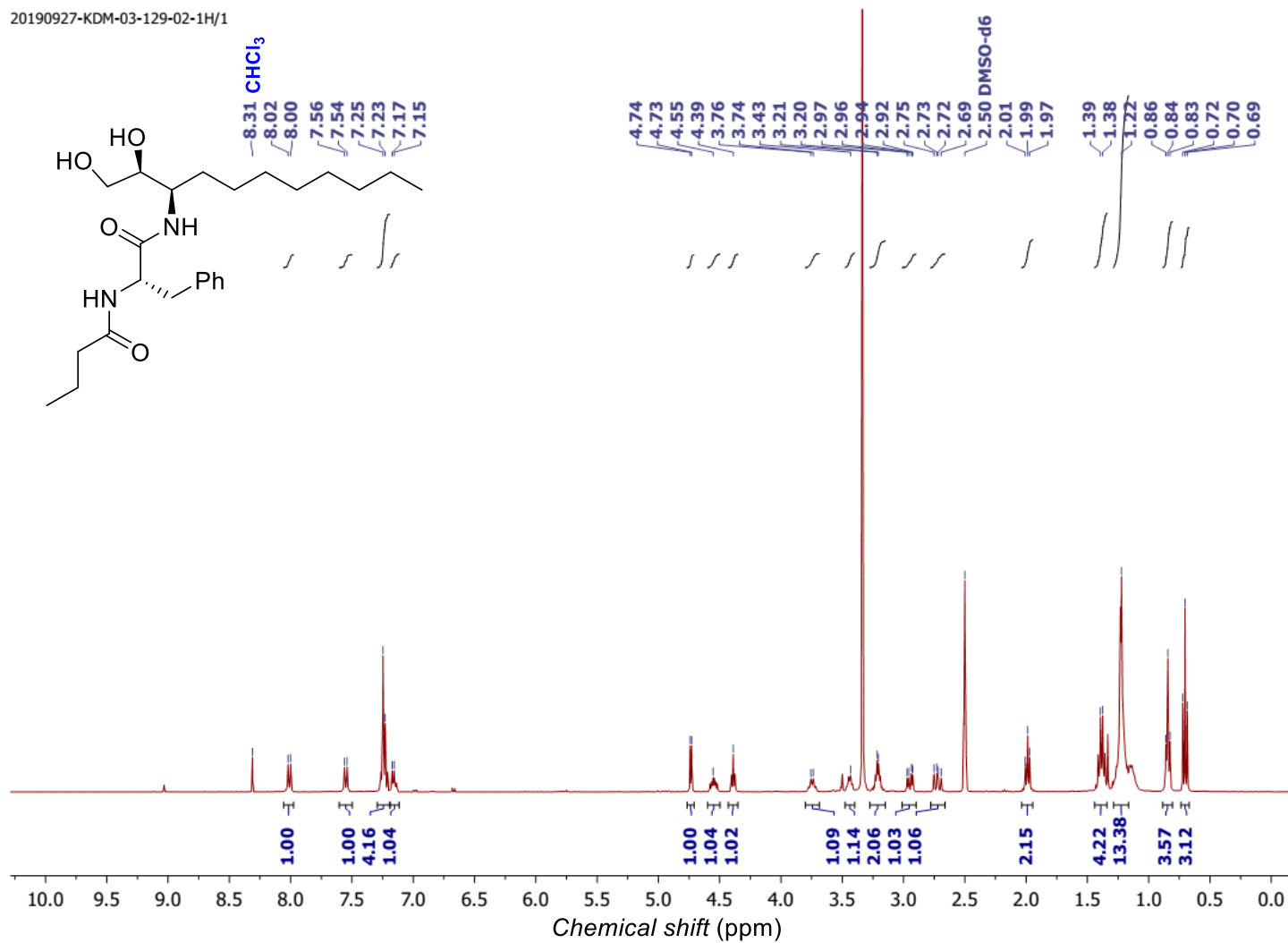


Fig. S44. ^1H NMR spectrum of compound 3S in $\text{DMSO}-d_6$.

20190927-KDM-03-129-02-13C/2

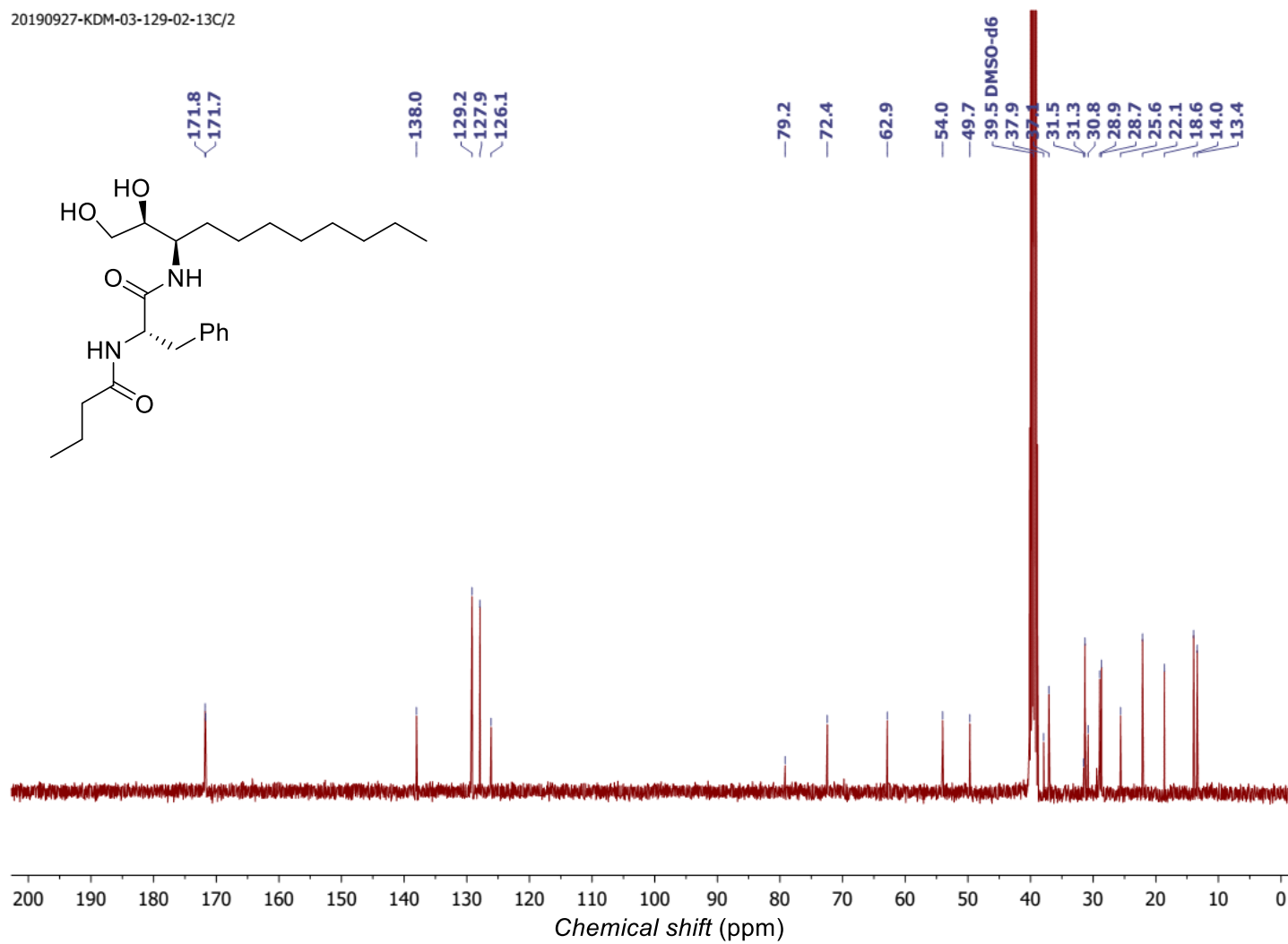


Fig. S45. ^{13}C NMR spectrum of compound 3S in $\text{DMSO}-d_6$.

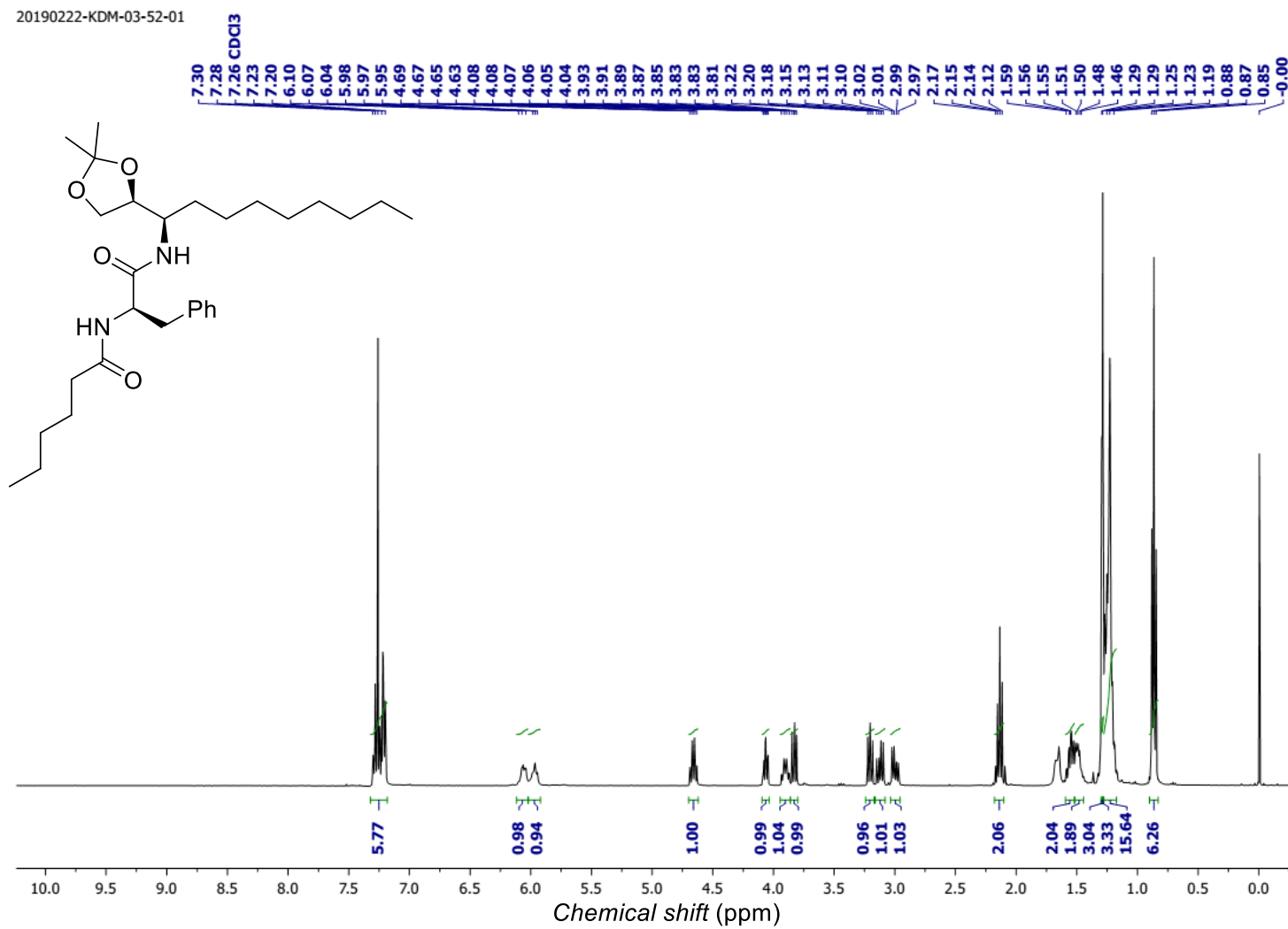


Fig. S46. ¹H NMR spectrum of compound **17R** in CDCl₃.

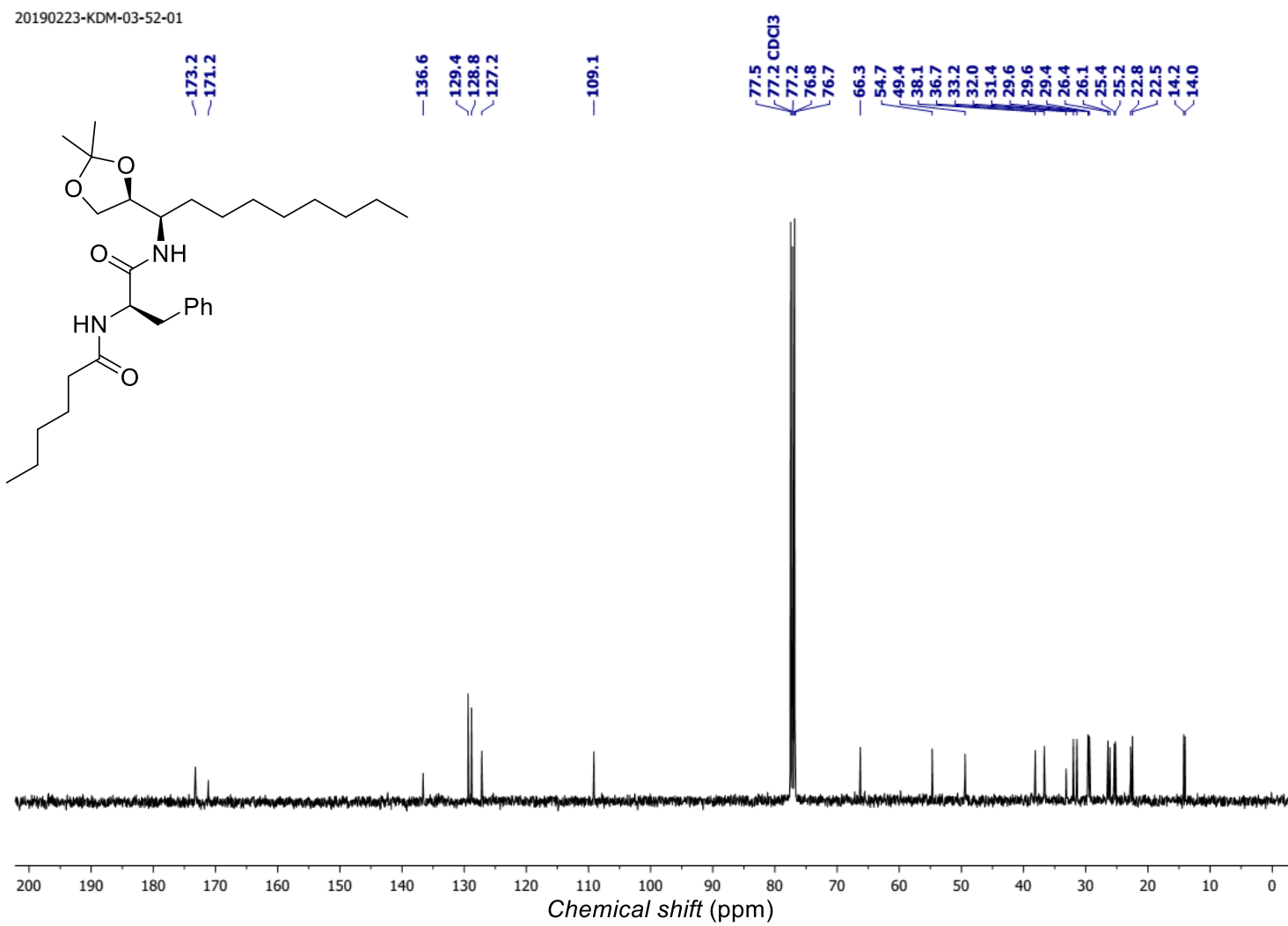


Fig. S47. ^{13}C NMR spectrum of compound **17R** in CDCl_3 .

20190222-KDM-03-54-03

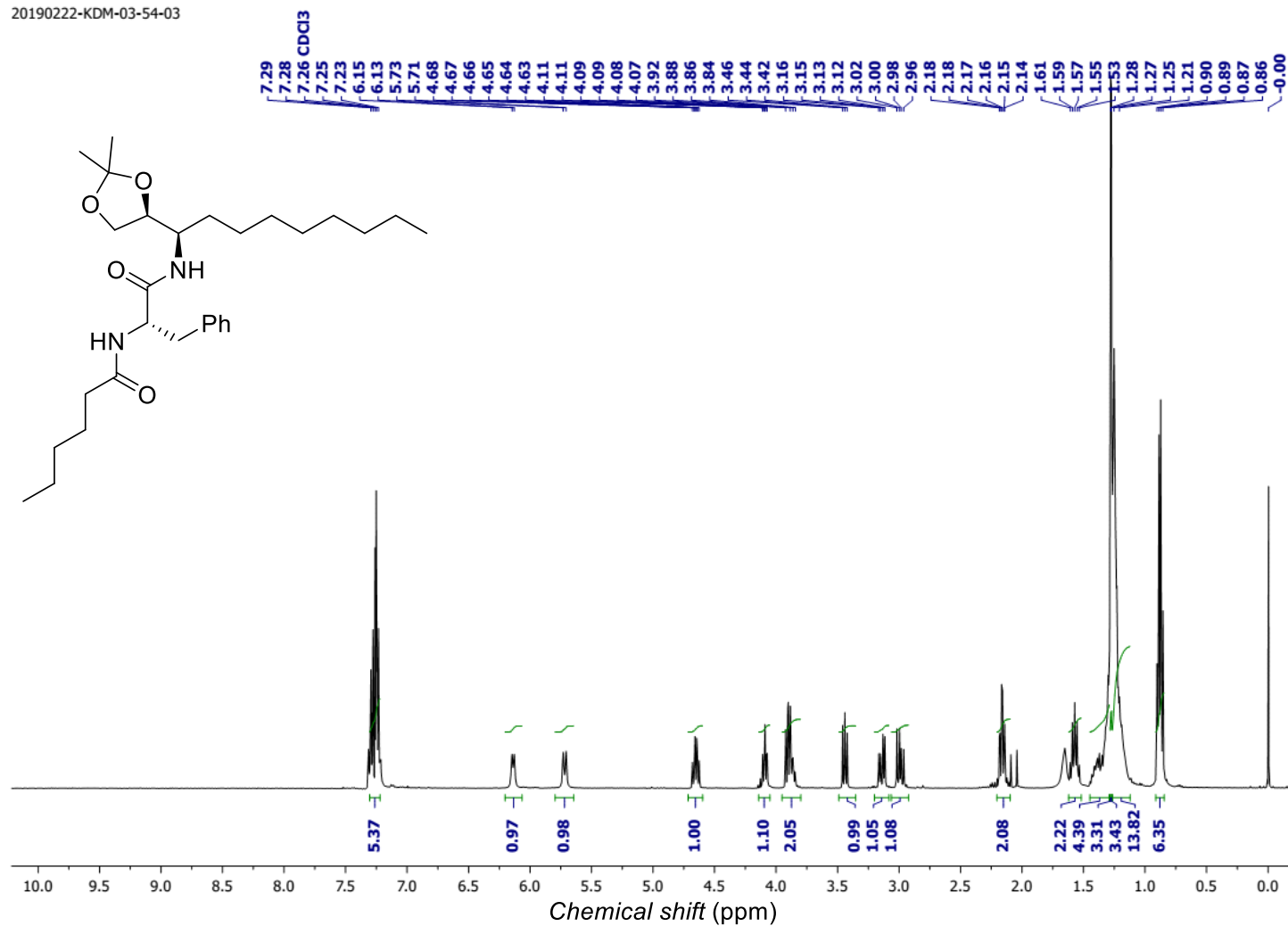


Fig. S48. ^1H NMR spectrum of compound 17S in CDCl_3 .

20190225-KDM-03-54-03/1
KDM-03-54-03

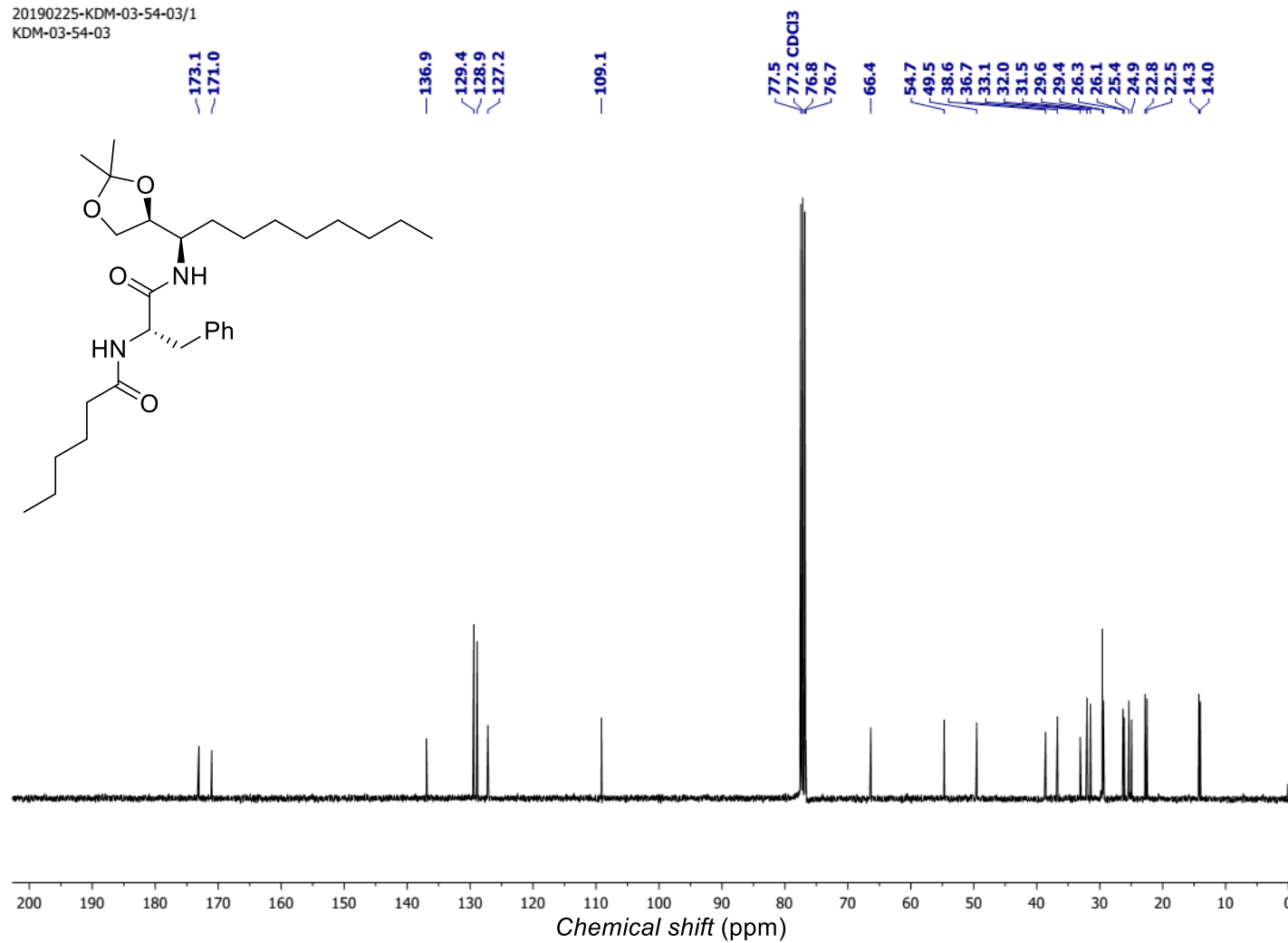


Fig. S49. ^{13}C NMR spectrum of compound **17S** in CDCl_3 .

20190301-KDM-03-48-01/1
1H

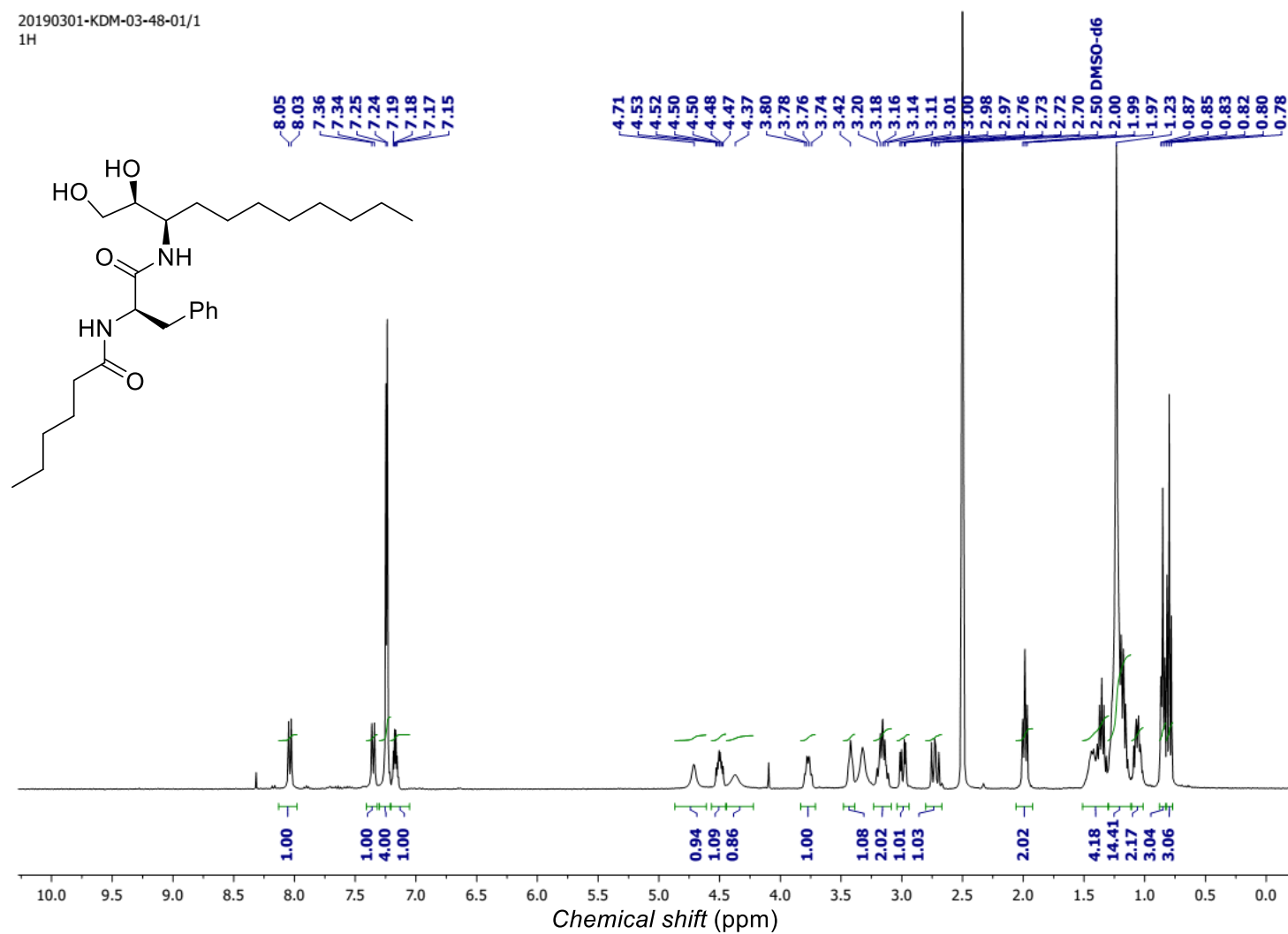


Fig. S50. ^1H NMR spectrum of compound **4R** in $\text{DMSO}-d_6$.

20190306-KDM-03-48-01

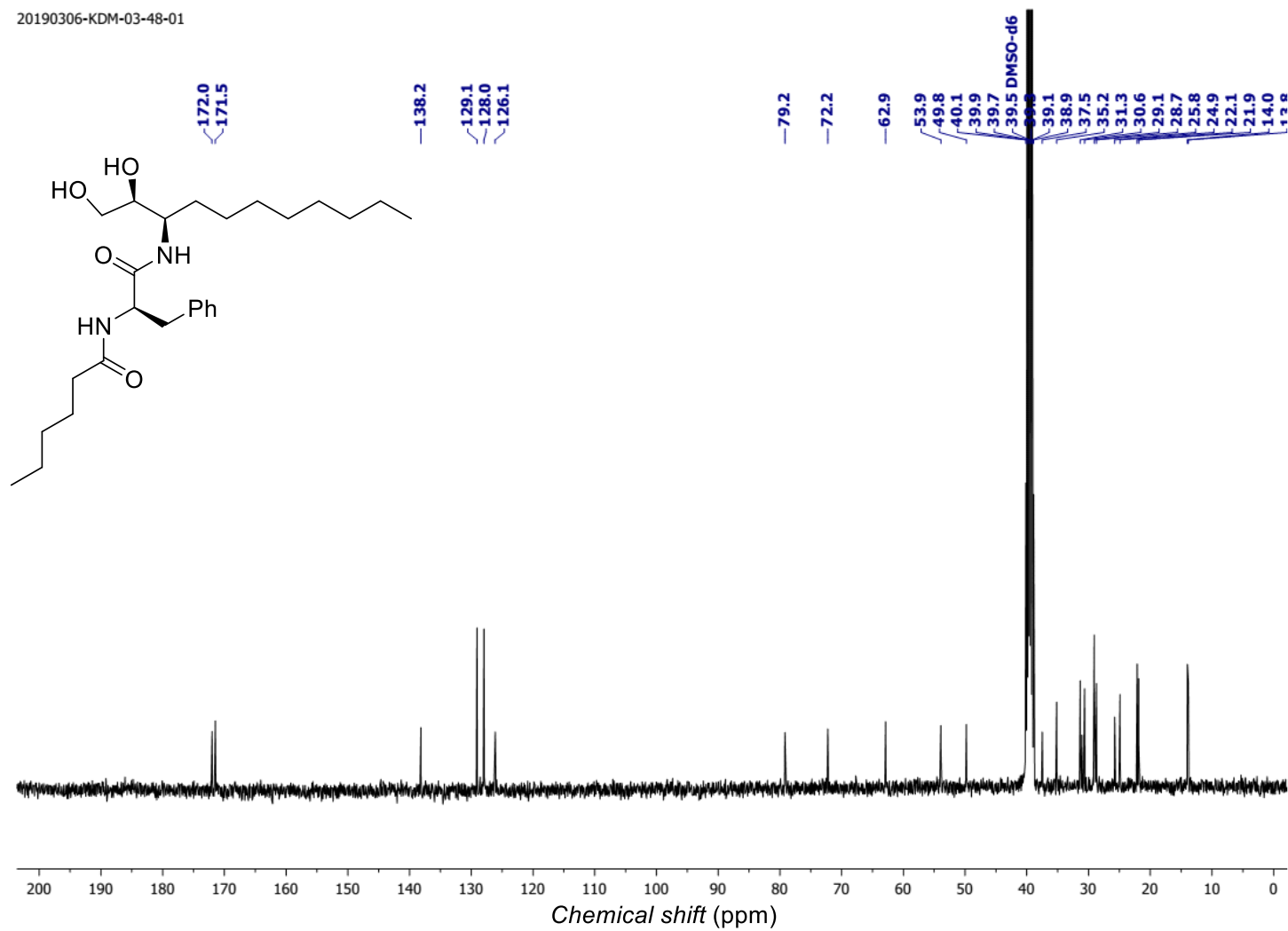


Fig. S51. ^{13}C NMR spectrum of compound **4R** in $\text{DMSO-}d_6$.

20190301-KDM-03-48-02/1
1H

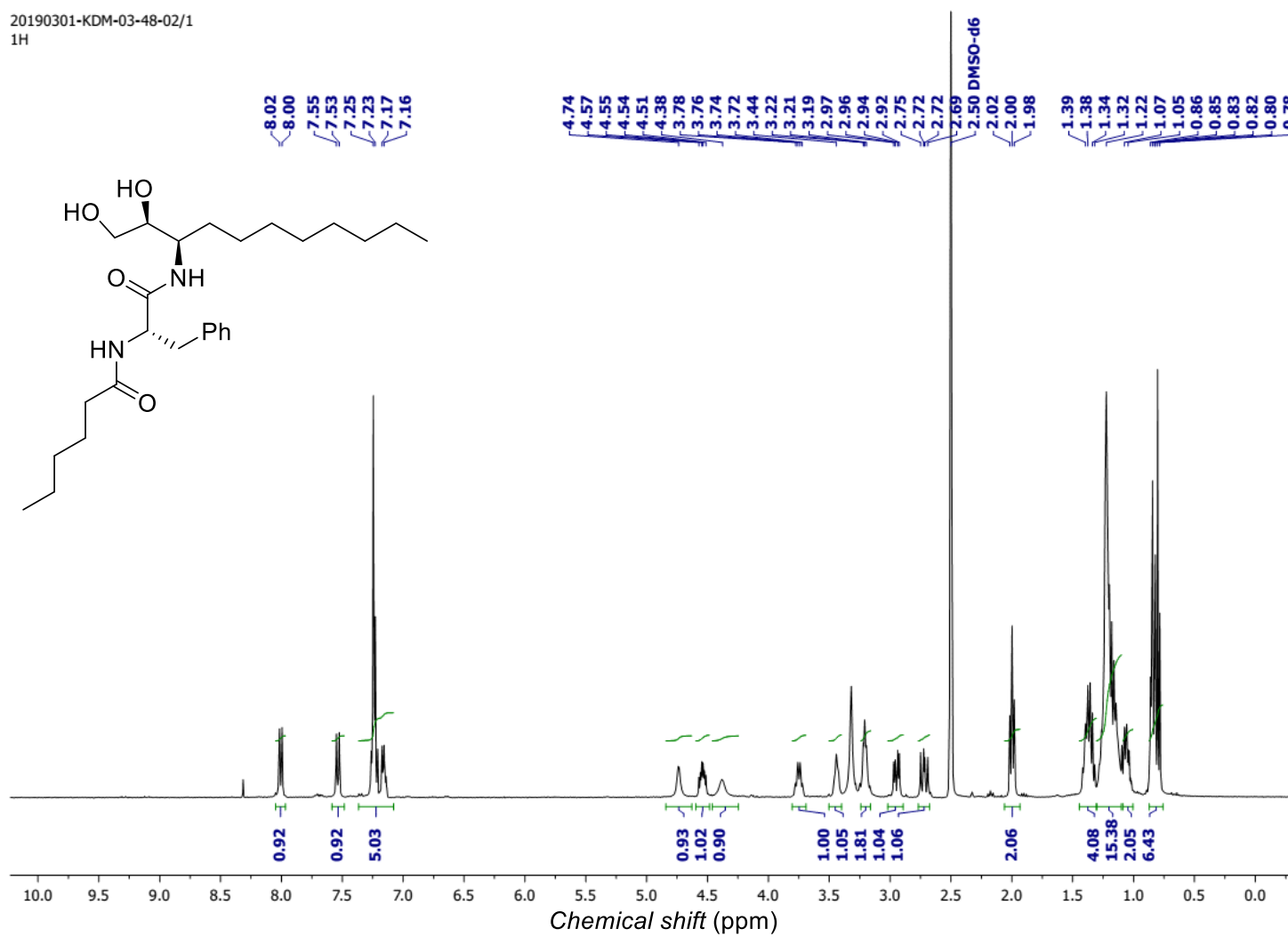


Fig. S52. ^1H NMR spectrum of compound 4S in $\text{DMSO}-d_6$.

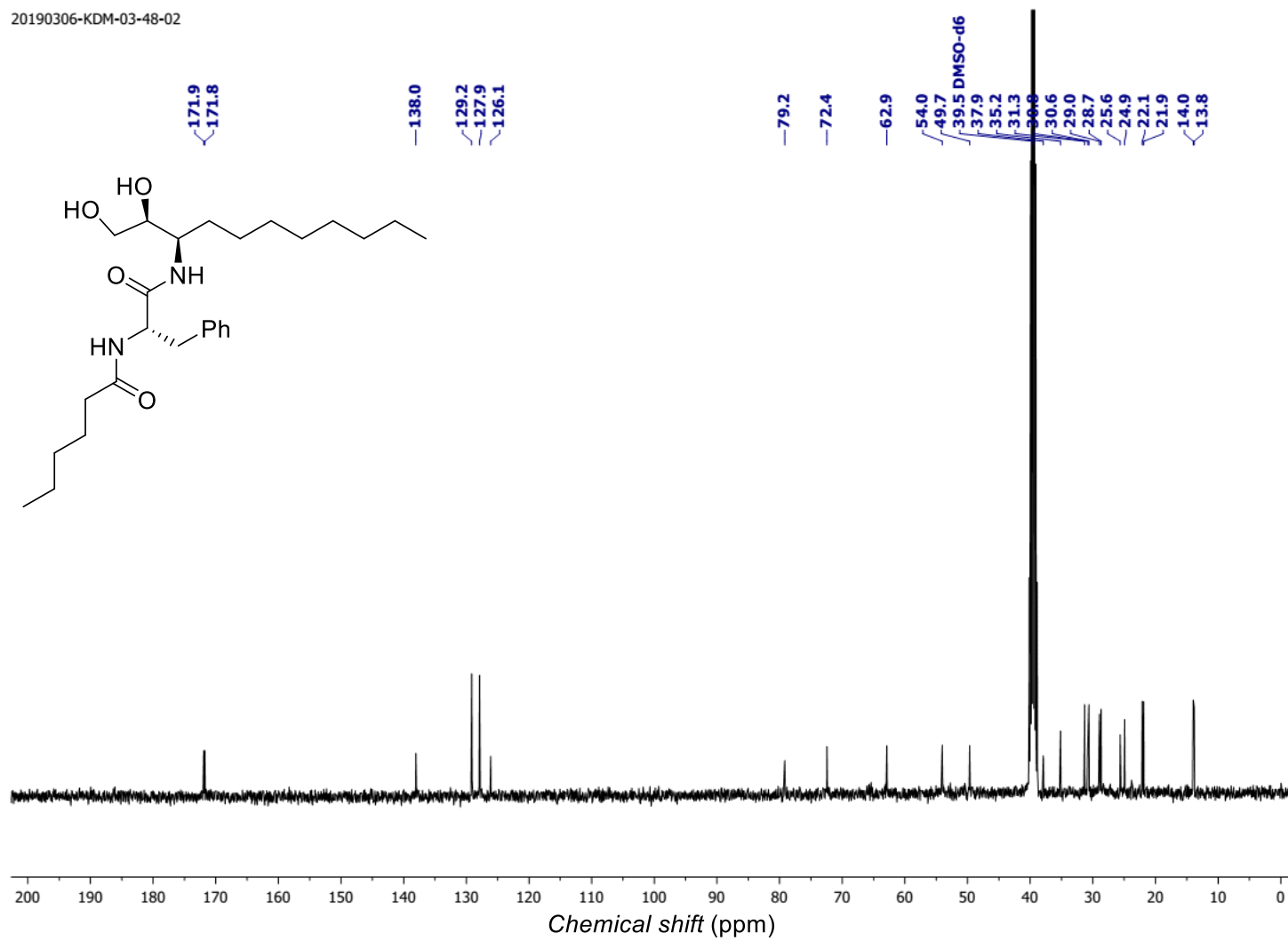


Fig. S53. ^{13}C NMR spectrum of compound 4S in $\text{DMSO}-d_6$.

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