Electronic Supplementary Information

Pseudopeptosomes: Non-lipidated vesicular assemblies from bispidine appended pseudopeptides

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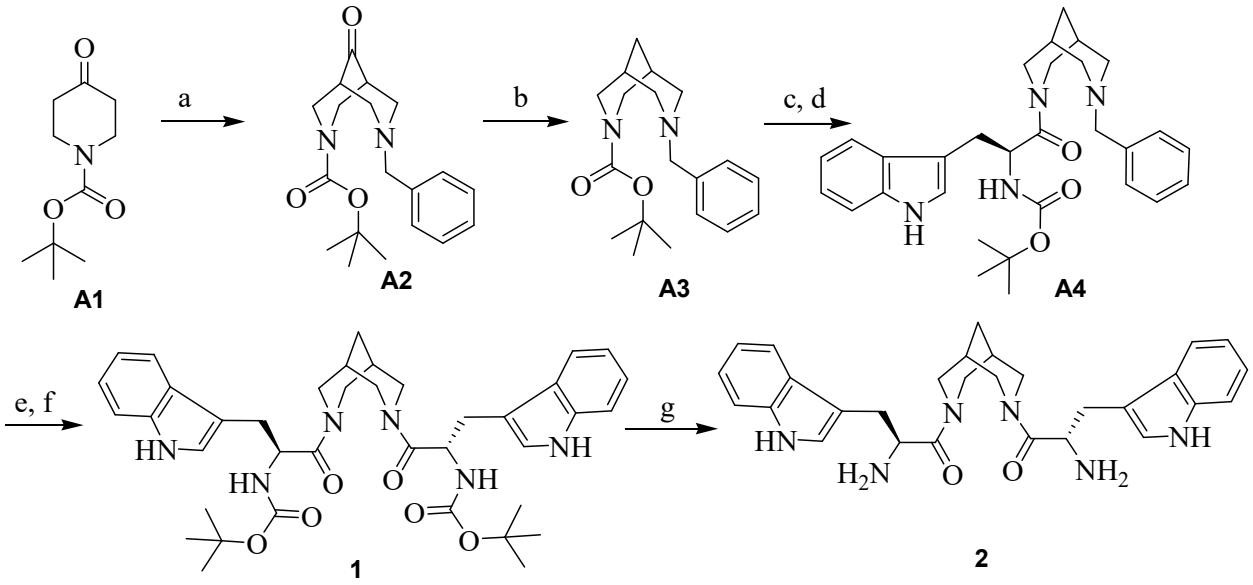
Experimental method

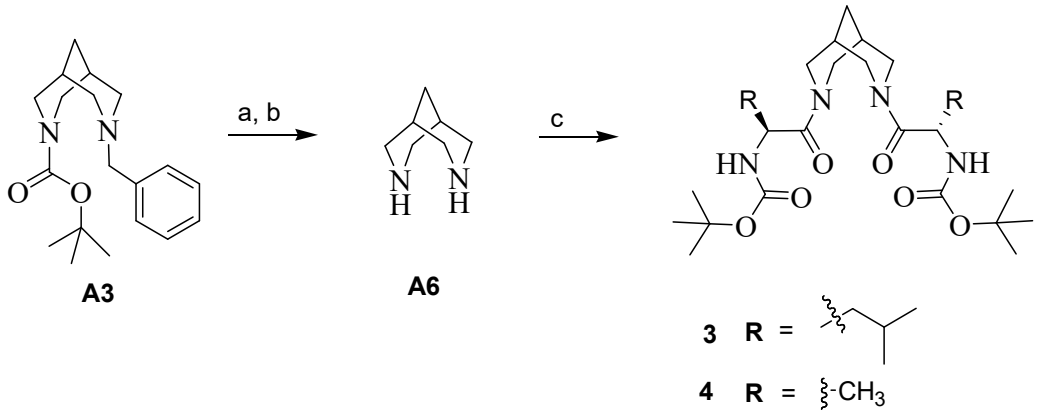
All the chemical reactions were performed in oven-dried glassware. Reagents used in our experiments were purchased from Sigma-Aldrich and Alfa Aesar (India). Standard drying methods were employed for solvents used in reactions. Silica gel thin-layer chromatography(TLC) was used to monitor all reactions. Compounds were purified by silica gel (100−200 mesh) column chromatography using ethyl acetate/hexane as the eluent. Infrared spectra were recorded as KBr pellets using a Perkin Elmer FT-IR/FIR Spectrometer Frontier. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300, 400, and 500MHz spectrometers. The NMR chemical shifts were reported relative to tetramethylsilane as the reference. Circular Dichroism (CD) spectra were recorded on AVIV model 410 spectropolarimeter. A 1mm path length of cuvette was used to record CD spectra. Mass spectra (HRMS) were recorded in a Bruker MicrO-TOF-QII model using ESI technique. Melting points were recorded on a Fisher scientific melting point apparatus. High-performance liquid chromatography (HPLC), Shimadzu (Model CBM-20A) was used. Optical rotations were measured with a Rudolph Research Analytical Autopol® V Polarimeter. The concentrations were reported in gram/100mL. X-ray diffraction analysis was carried out on a BRUKER AXS SMARTAPEX diffractometer with a CCD area detector (Mo Kα = 0.71073Å, monochromator: S3 graphite). Structure solution, refinement, and data output were carried out with the ShelXTL program. Powder X-Ray diffraction (PXRD) pattern were taken on Bruker D8 Advance diffractometer using Ni-filtered Cu Kα radiation.

Synthetic schemes



Reagents: (a) Benzyl amine, HCHO, AcOH, MeOH, 65 ºC, 10 h, 60%; (b) NH_2NH_2, Glycol, KOH, 8 h,165 ºC, 85%; (c) TFA, DCM, 4 h at RT; (d) Boc-Trp-OH, DCC, NHS, DCM, NEt_3, 24 h at RT; (e) 10% of Pd/C, MeOH, AcOH, 8 h at RT; (f) Boc-Trp-OH, DCC, NHS, DCM, NEt_3, 24 h, at RT; (g) EtOAc.HCl.

Scheme S1. Synthesis of **1**.



Reagents: (a) 10% of Pd/C, MeOH, AcOH, 8 h at RT; (b) TFA, DCM, 4 h at RT; (c) Boc-Leu-OH or Boc-Leu-OH, DCC, NHS, DCM, NEt_3, 24 h at RT.

Scheme S2. Synthesis of **3** and **4**.

Synthesis of **1**

To a well stirred solution of compound **A4**[1] (0.438 g, 0.87 mmol) in methanol was added few drops of acetic acid and 10% of Pd/C (0.043 g) and the solution was kept under positive pressure of H_2 gas. Stirred the reaction mixture for 8 h under H_2 at room temperature. The reaction mixture was filtered and the filtrate was evaporated in *vacuo*. The amine obtained was then added to the dichloromethane (DCM) solution of tert-butyloxy carbonyl (Boc) protected tryptophan (0.221 g, 0.72 mmol), N-Hydroxysuccinimide (NHS) (0.099 g, 0.86 mmol), N,N′-Dicyclohexylcarbodiimide (DCC) (0.177 g, 0.86 mmol) and triethylamine (NEt_3) (0.4 mL, 2.88 mmol). The solution was stirred for 24 h at room temperature and monitored by thin layer chromatography (TLC). After completion of the reaction, the mixture was evaporated and re-dissolved in ethyl acetate. The ethyl acetate part was washed with 0.2N H_2SO_4, saturated aq. $NaHCO_3$ and water. The organic layer was collected and dried over anhyd. Na_2SO_4 and evaporated under vacuum to obtain the crude product, which was purified by column chromatography (Ethyl acetate/Hexane in 4:6) to give 0.400 g compound **1** as a solid.

Yield: 78.8%

Mp: 172-174 ºC

$[\alpha]_D^{21}$ = -12º (c 0.05, methanol);

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (m, 2H), 1.42 (m, 2H), 1.56 (s, 18H), 1.79 (d, *J* = 12.9 Hz, 2H), 2.12 (d, *J* = 13.8 Hz, 2H), 2.92-3.28 (m, 4H), 3.47 (d, *J* = 12.9 Hz, 2H), 4.40 (d, *J* = 13.5 Hz, 2H), 4.88 (m, 2H), 5.90 (d, *J* = 7.5 Hz, 2H), 6.94 (m, 2H), 7.00 – 7.25 (m, 4H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 7.5 Hz, 2H), 8.53 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.8, 28.26, 28.35, 28.7, 30.9, 31.5, 45.3, 49.7, 51.1, 79.6, 110.8, 111.0, 119.0, 119.2, 121.6, 123.1, 127.7,

136.0, 155.7, 170.6; IR (KBr): 3418, 2976, 2927, 2863, 1696, 1635, 1496, 1456, 1392, 1366, 1250, 1235, 1169; HRMS calcd. for $C_{39}H_{50}N_6O_6Na$ m/z 721.3684, found m/z 721.3704.

Synthesis of **2**

To **1** (0.150 g, 0.21 mmol) was added ethyl acetate saturated with HCl gas (10ml) in ice cold condition. The reaction mixture was stirred for 4 h at room temperature. The reaction mixture was evaporated and washed thrice with pentane to obtain 0.107 g of **2** as white solid.

Yield: Quantitative.

Mp: 240 -242 ^oC;

$[\alpha]_D^{21}$ = + 60^o (c 0.05, methanol);

^1H NMR (500 MHz, CD_3OD) δ 1.55 (br s, 2H), 1.72 (br s, 2H), 2.24 (d, *J* = 13 Hz, 2H), 2.33 (d, *J* = 13.5 Hz, 2H), 3.20 (dd, *J* = 9.5, 9 Hz, 4H), 3.35 (m, 2H), 3.76 (d, *J* = 13.5 Hz, 2H), 4.33 (d, *J* = 14 Hz, 2H), 4.88 (m, 2H), 7.04 (t, *J* = 7.5 Hz, 2H), 7.13 (t, *J* = 7.5 Hz, 2H), 7.19 (m, 2H), 7.38 (d, *J* = 8 Hz, 2H), 7.42 (d, *J* = 8 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 27.0, 29.2, 31.0, 45.4, 50.8, 50.9, 106.2, 111.2, 117.4, 118.9, 121.5, 124.1, 126.9, 136.6, 168.8; IR (KBr): 3402, 2922, 2866, 1634, 1491, 1457, 1343, 1243, 1100; HRMS calcd. for $C_{29}H_{35}N_6O_6$ m/z 499.2816, found m/z 499.2800.

Synthesis of **3**

To a well stirred solution of Boc and benzyl protected bispidine compound **A3** (0.295 g, 0.935 mmol) in methanol (10 mL) was added few drops of acetic acid and 10% of Pd/C (0.029 g) and kept under positive pressure of H_2 gas. Stirred the reaction mixture for 8 h at room temperature. The reaction mixture was filtered and evaporated in *vacuo* to obtain the amine. The obtained amine

was dissolved in DCM and trifluoroacetic acid (0.715 mL, 9.35 mmol), stirred for 2 h at room temperature. The reaction mixture was evaporated and used as such for further reaction. The obtained amine was added to the DCM solution containing tert-butyloxy carbonyl (Boc) protected leucine (0.432 g, 1.87 mmol), NHS (0.258 g, 2.24 mmol), DCC (0.460 g, 2.24 mmol) and NEt_3 (0.31 mL, 2.24 mmol). The resultant solution was stirred for 24 h at room temperature. After completion of reaction, the reaction mixture was evaporated and re-dissolved in ethyl acetate and the filtrate was washed with 0.2N H_2SO_4, saturated aq. $NaHCO_3$ and water. The organic part was dried over anhyd. Na_2SO_4 and evaporated under vacuum to obtain the crude product, which was purified by column chromatography (Ethyl acetate/Hexane in 4:6) to give 0.450 g compound **3** as a solid.

In the ^{1}H NMR spectrum in $CDCl_3$, compound **3** showed signals for major and minor conformers. We have mentioned the signals for minor conformer, wherever possible.^{13}C NMR also showed signal for minor conformer, which is also included in the data which leads to increased number of signals.

Yield: 87%. Mp: 156 -158 ^{o}C;

$[\alpha]_D^{21}$ = -88^o (c 0.05, methanol);

^{1}H NMR ($CDCl_3$, 500MHz): δ 0.88 (d, *J* = 6.5 Hz, minor), 0.93 (d, *J* = 7 Hz, 6H), 0.95 (d, *J* = 6.5 Hz, minor), 1.02 (d, *J* = 6.5 Hz, 6H), 1.16 (m, minor), 1.28 (m, 4H), 1.40 (s, 18H), 1.50 (s, minor), 1.63 (m, minor), 1.69 (m, 2H), 1.87 (br s, 2H), 1.90 (s, minor), 1.99 (s, minor), 2.12 (br s, 2H), 2.84 (d, *J* = 13.5 Hz, minor), 2.96 (d, *J* = 14 Hz, 2H), 3.26 (d, *J* = 13 Hz, minor), 3.41 (d, *J* = 12.5 Hz, 2H), 3.81 (d, *J* = 13 Hz, 2H), 4.06 (d, *J* = 13 Hz, minor), 4.51 (m, 4H), 4.58 (m, minor), 4.68 (d, *J* = 14 Hz, minor), 5.21 (d, *J* = 9 Hz, 2H), 5.59 (d, *J* = 8 Hz, minor); ^{13}C NMR (75 MHz, $CDCl_3$)

δ: 21.3, 22.2, 23.4, 23.5, 24.4, 24.7, 27.4, 28.3, 28.5, 29.9, 43.0, 43.6, 45.9, 46.7, 48.5, 48.7, 49.6, 49.8, 79.2, 79.3, 155.7, 171.5, 173.0; IR (KBr): 3403, 3326, 2961, 2931, 2868, 2860, 1710, 1658, 1628, 1531, 1452, 1436, 1390, 1366, 1244, 1172, 1047; HRMS calcd. for $C_{29}H_{52}N_4NaO_6$ m/z 575.3779, found m/z 575.3796.

Synthesis of **4**

To a well stirred solution of Boc and benzyl protected bispidine compound **A3** (0.295 g, 0.935 mmol) in methanol (10 mL) was added few drops of acetic acid and 10% of Pd/C (0.029 g) and kept under positive pressure of H_2 gas. Stirred the reaction mixture for 8h at room temperature. The reaction mixture was filtered and evaporated in *vacuo* to obtain the amine. The obtained amine was dissolved in DCM and trifluoroacetic acid (0.715 mL, 9.35 mmol), stirred for 2 h at room temperature. The reaction mixture was evaporated and used as such for further reaction. The obtained amine was added to the DCM solution containing tert-butyloxy carbonyl (Boc) protected Ala (0.353 g, 1.87 mmol), NHS (0.258 g, 2.24 mmol), DCC (0.460 g, 2.24 mmol) and NEt_3 (0.31 mL, 2.24 mmol). The resultant solution was stirred for 24 h at room temperature. After completion of reaction, the reaction mixture was evaporated and re-dissolved in ethyl acetate and the filtrate was washed with 0.2N H_2SO_4, saturated aq. $NaHCO_3$ and water. The organic layer was collected and dried over anhyd. Na_2SO_4 and evaporated under vacuum to obtain the crude product, which was purified by column chromatography (Ethyl acetate/Hexane in 4:6) to give 0.390 g compound **4** as a solid.

In the ^1H NMR spectrum in $CDCl_3$, compound **4** showed signals for major and minor conformers. We have mentioned the signals for minor conformer wherever possible.^{13}C NMR also showed

signal for minor conformer, therefore more ^{13}C peaks are observed in the ^{13}C spectrum of compound **4**.

Yield: 89%. Mp: 166 -168 ºC;

$[\alpha]_D^{21}$ = -77º (c 0.05, methanol);

^1H NMR (400 MHz, $CDCl_3$) δ: 1.16 – 1.27 (d, *J* = 7.2 Hz, major + minor, 6H), 1.41 (s, 18H), 1.50 (minor), 1.90 (m, 2H), 1.93 (minor), 1.99 (minor), 2.12 (m, 2H), 2.86 (minor d, *J* = 13.6 Hz), 2.94 (d, *J* = 13.6 Hz, 2H), 3.27 (minor d, *J* = 13.2 Hz), 3.40 (d, *J* = 12.8 Hz, 2H), 3.86 (d, *J* = 12.8 Hz, 2H), 4.04 (minor d, *J* = 12.8 Hz), 4.50 (m, 2H), 4.58 (d, *J* = 13.6 Hz, 2H), 4.72 (minor d, *J* = 13.6 Hz), 5.38 (d, *J* = 8 Hz, 2H), 5.74 (minor d, *J* = 8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 18.8, 19.7, 27.6, 28.3, 28.6, 30.4, 32.2, 45.8, 46.2, 46.6, 49.8, 50.0, 79.37, 79.43, 155.1, 155.4, 171.4, 172.4; IR (KBr): 3423, 3327, 2981, 2931, 2868, 1688, 1640, 1533, 1368, 1296, 1174, 1057; HRMS calcd. for $C_{23}H_{40}N_4NaO_6$ m/z 491.2840, found m/z 491.2824.

Methods: -

1. **Scanning electron microscopy:**

Sample solution for the imaging was made by dissolving the 1 mg of the compound in 1 ml of the solvent. A drop of the sample solution was placed on the glass slide allowed to dry in the air and coated with 10nm of gold. All the samples were analyzed using scanning electron microscope ZEISS EVO 50.

2. **Atomic force microscopy:**

A solution of compound (1mg/ml) was prepared in the solvent of choice. A drop of the sample solution was placed on the silicon wafer and allowed to dry in the air. All the sample were analyzed using Bruker Dimension Icon atomic force microscope. Nanoscope 5.31r software was used to analyze the data.

3. **Transmission Electron Microscopy (TEM):**

Samples for TEM were prepared by dissolving the compound in methanol. About 5µl aliquot of the sample solution was placed on a 200 mesh copper grid and allowed dry. Imaging was done using a TECHNAI G2 (20S-TWIN) electron microscope.

4. **Confocal Microscopy:**

Rhodamine was mixed with the sample prepared in methanol and vortexed for 30 seconds. About 20µl of the sample was drop cast on 20*60 mm slides and allowed to dry. Excess dye was removed by washing the slides with MilliQ water thrice. The slide was allowed to dry and used for imaging. Epifluorescence imaging was done in UV filter using 1.40 UPlanSApo 100x objective in Olympus IX51 epifluorescence microscope model. Slides were prepared similarly for confocal microscopy. Confocal images were captured using Olympus FV1200 microscope at UPlanSApo 100xo objective (λ at 540nm for Rhodamine B stained vesicles and λ at 515 nm for Nile Red stained vesicles). The step size in z-stacking was kept at 100 nm. Images were captured at 12.5µs/pixel frame rate. Cellsens software was used to construct 3D view of the z-stack of vesicles.

5. **Fluorescence spectrometer:**

All the steady-state fluorescence experiments were carried out on the Horiba Scientific Fluoromax-4 Spectrofluorometer. Fluorescence measurements were carried out by using 1 cm quartz cuvettes and the temperature was maintained at 25 ^{o}C.

6. **Cytotoxicity Assay in RAW264.7 cells:**

The resazurin assay was used to analyze the peptide's cytotoxicity profile. Stock concentration: 0.15 mg/ml, Working concentration: 0.03 mg/ml. After 24 hours of compound treatment media was removed and 20µl of 0.15mg/ml resazurin was added to 80 µl serum free media. This was incubated at 37^{0}C in 5% CO_2 for 20 minutes. And the reading was taken at 560/590 nm in MultiSkan Go.

7. **DOSY experiments:**

The experiments were performed on a 500 MHz Bruker Avance III HD spectrometer with a BBFO probe. A standard method with stimulated echo and LED using bipolar gradient pulses is utilized here to calculate the diffusion constant. The length of the gradient pulse(p30) is calibrated (1.2 ms) to obtain a 98% decay in the signal on increasing the gradient strength(gpz6) using 1D DOSY pulse sequence. The value of diffusion time (d20) allowed during the pulse sequence is 0.06 s. 32 transients in t1-dimension were recorded with linear increase in the gradient strength using 2D DOSY pulse sequence. The diffusion constants were fitted using Bruker standard Relaxation analysis tool.

8. **Cellular uptake of Rhodamine mixed with 2:**

Rhodamine (5µg/ml) labeled compound was used to treat RAW264.7 cells (on coverslip) for 24 hours. Washed twice with 1X PBS. Prepared slides and imaged in Olympus Confocal FV1200 microscope at 60x magnification (LUCPLFLN lens). Fluorescence of Rhodamine was observed using a 543 nm laser.

9. **Cellular uptake of FITC-tagged peptide (CorTS1(LRRLRLRRNRL-NH_2) - Corneal Targeting Sequence 1)[2]:**

Seeded ~25000 RAW264.7 cells in 35 mm Petri dishes containing 19 mm coverslips and allowed cells to grow for 24 hours at 37^{0}C in 5% CO_2 in DMEM containing 10% FBS and 1% Penstrep. After 24 hours, washed once in 1X PBS and treated the petri dishes as follows for 24 hours:

i. CONTROL – No compound, No CorTS1, No rhodamine in serum-free DMEM
ii. DYE CONTROL – No compound, no CorTS1, rhodamine dye (5µg/ml) in serum-free DMEM
iii. PEPTIDE CONTROL – No compound, CorTS1 (10µM), No dye in serum-free DMEM
iv. PEPTIDE DYE CONTROL – No Compound, CorTS1 (10µM), Rhodamine dye (5µg/ml) in serum-free DMEM
v. 200 µg/ml compound, CorTS1 (10µM), Rhodamine dye (5µg/ml) in serum-free DMEM

After 24 hours washed with 1X PBS twice, prepared slides, and captured images in Olympus Confocal FV1200 microscope at 60x magnification using 473 nm, and 543 nm laser.

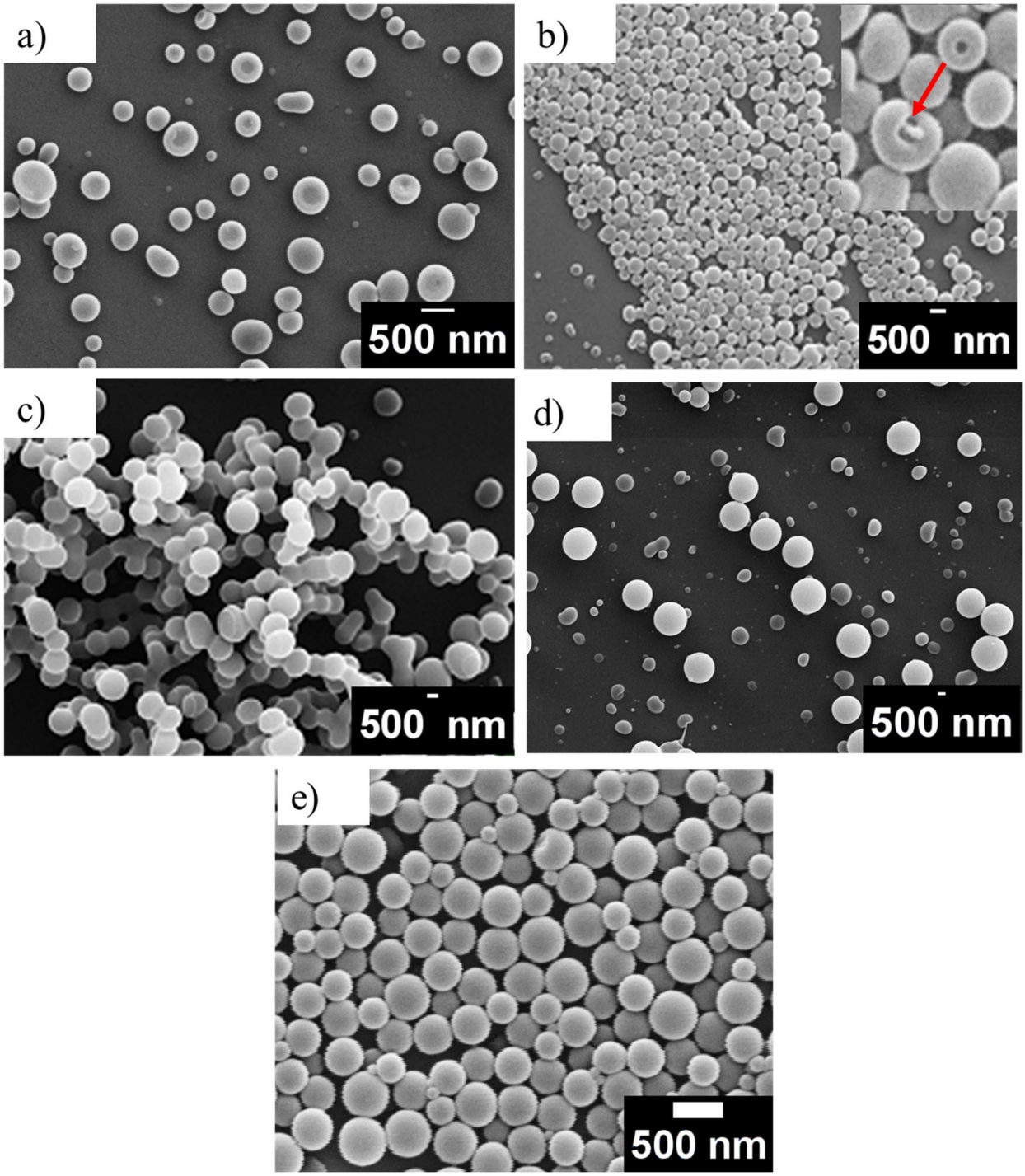


Figure S1. (a) SEM image of **1** (concentration: 0.476 mM) (b) SEM image of **1** (concentration: 0.715 mM) (c) SEM image of **1** (concentration: 2.14 mM) (d) SEM image of (**1** + 0.02 equivalents of Rhodamine B dye) compound **1** with 1.43 mM concentration was used. (e) SEM image of **1** (concentration: 1.43 mM) in 1:1 (water: ethanol) mixture showing vesicular self-assembly.

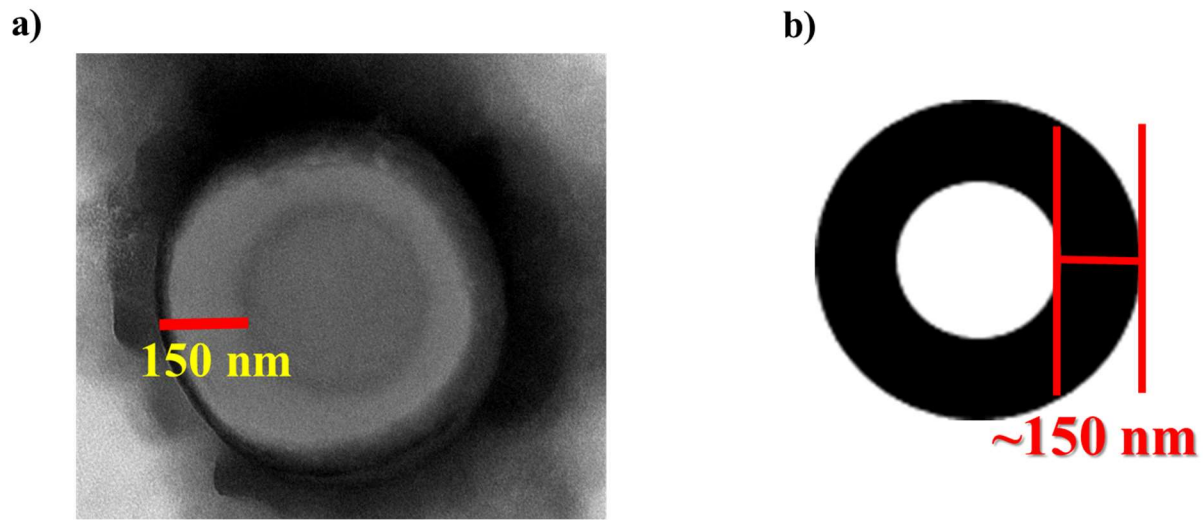


Figure S2. (a) TEM image of **1** in 1mg/ml. The scale bars indicate the wall thickness of vesicles. (b) Cartoon representation of vesicle indicating thickness of wall. The sample was stained with a negative stain by using phosphotungstic acid for 1 minute.

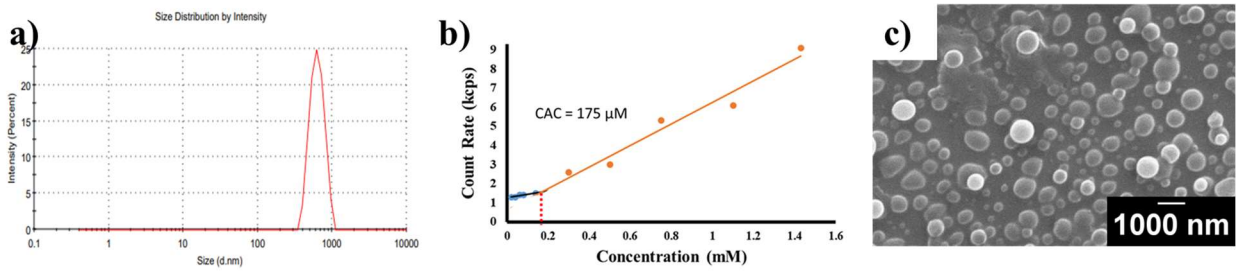


Figure S3. (a) Dynamic light scattering of **1** in 1mg/ml (b) CAC of **1** calculated from DLS (c) SEM image of **2** in 10 % methanol and water.

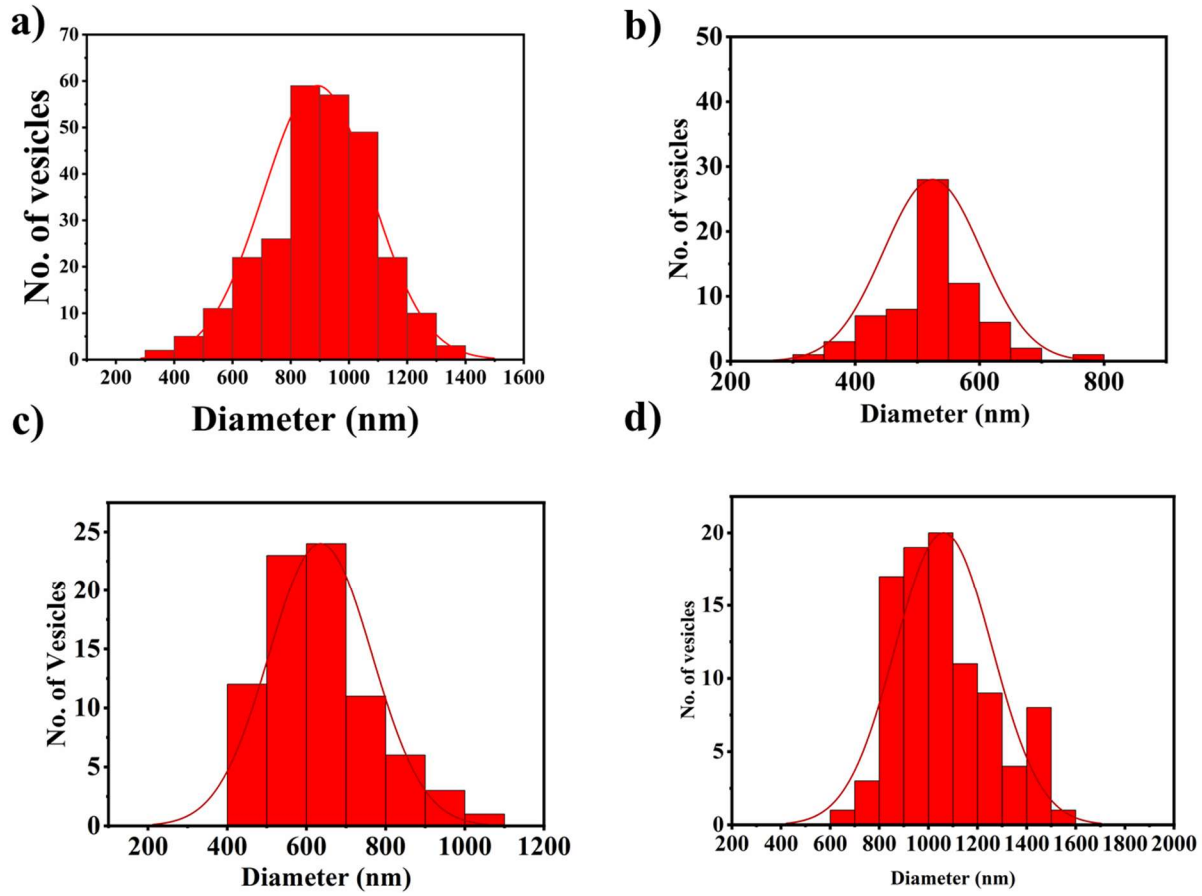


Figure S4. Histogram based on SEM images (a) Size distributions of diameters of vesicles at 1 mg/ml of **1** (b) Size distribution of diameters of vesicles at 1 mg/ml of **2** in 10 % of methanol and water. (c) Size distributions in diameters of vesicles at 1 mg/ml of **3** (c) Size distribution of diameters of vesicles at 1 mg/ml of **4**.

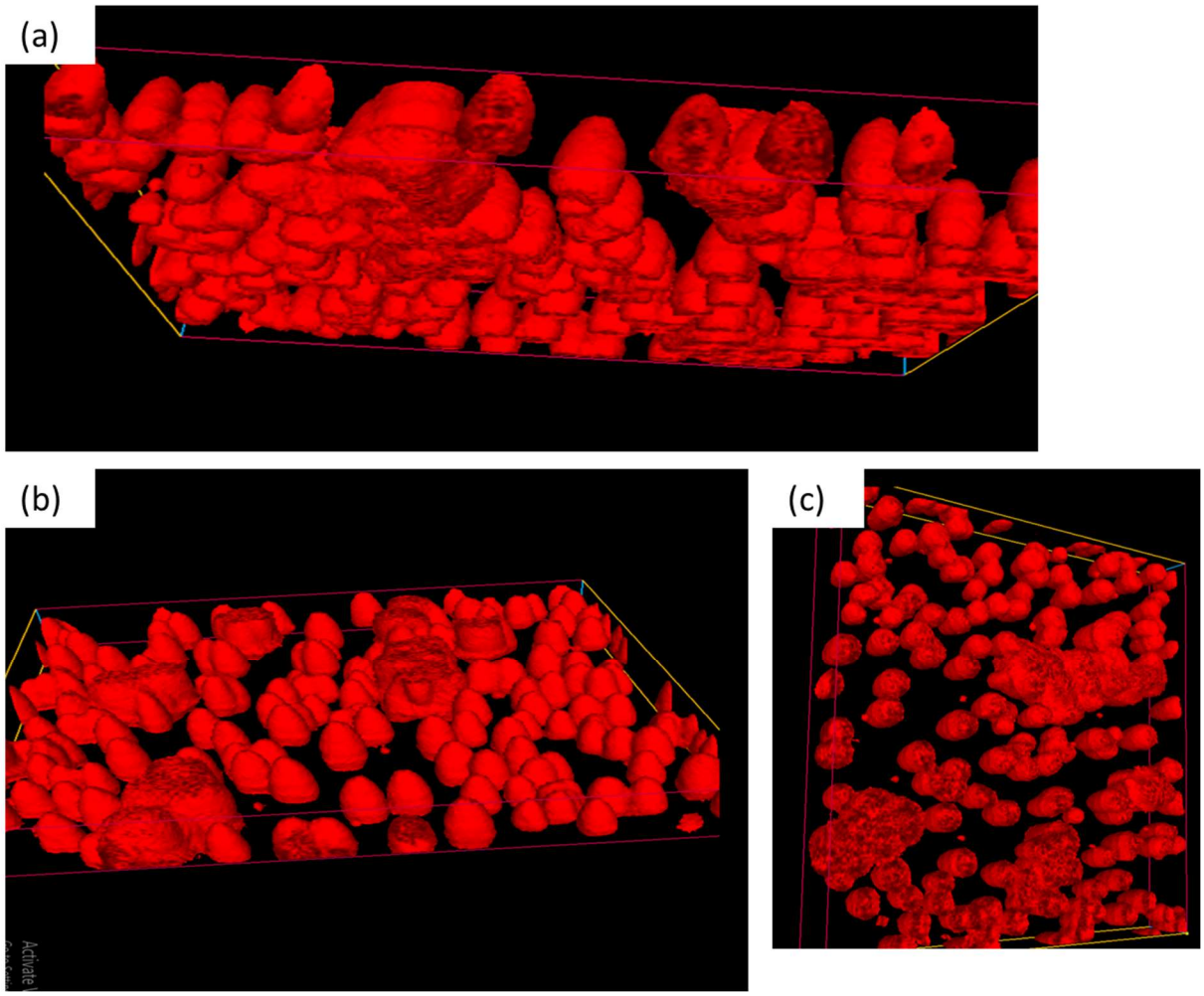


Figure S5. Side view of 3D confocal microscopy at 100x Objective (5x zoomed image) of **1** + 0.02 equivalents of Rhodamine B dye at λ_{ex} at 540 nm (a) side view (b) Top view (c) Bottom view constructed using Olympus Cellsens software. Step size=100nm, Z ~ 4µm.

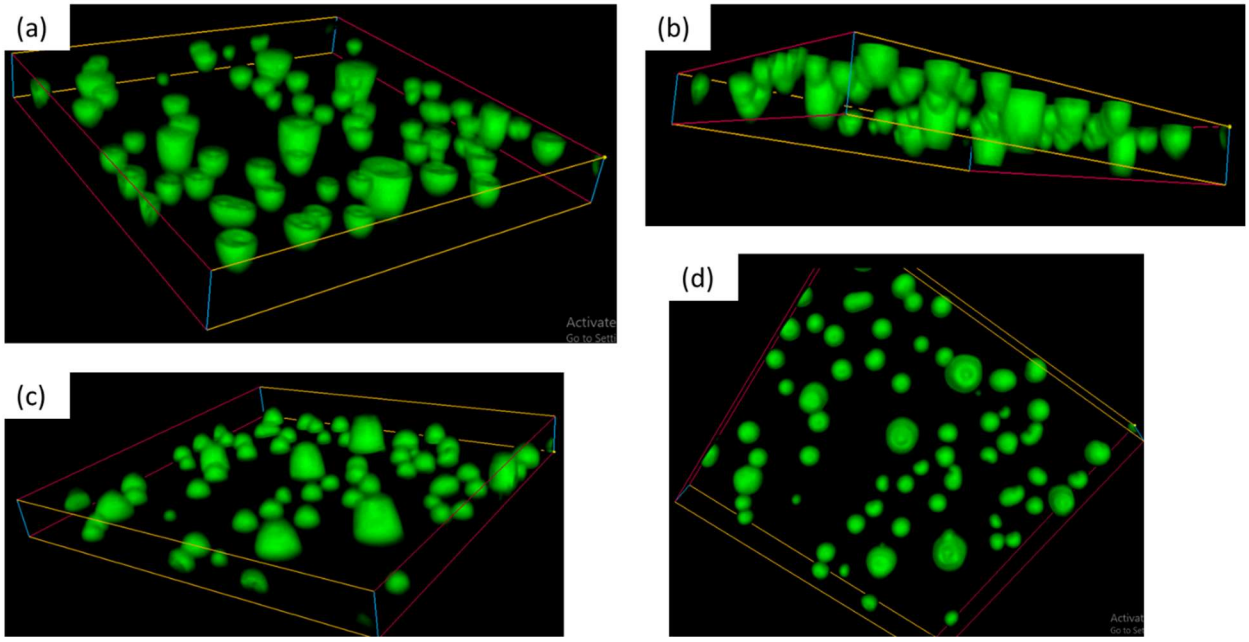


Figure S6. (a) Bottom face view of 3D confocal microscopy at 100x objective (5x zoomed image) of **1** + 0.02 equivalents of Nile Red dye at λ_{ex} at 515 nm (b) Side view (c) Top view (d) Top side view constructed using Olympus Cellsens software. Step size=100nm, Z ~ 4µm.

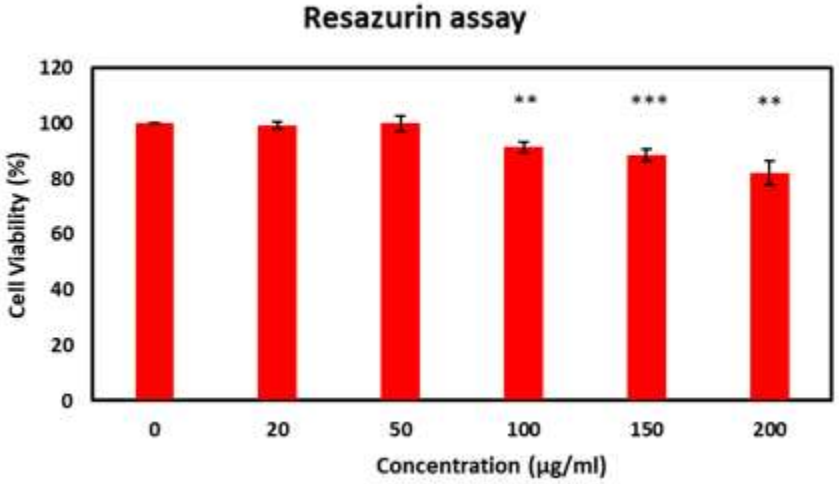


Figure S7. Cell Viability profile of **2** for RAW264.7 cells. Cells were incubated with various concentrations (0, 20, 50, 100, 150, and 200µg/ml) of the compound for 24 h followed by 20-minute incubation with Resazurin (in serum-free media). Untreated cells act as control (n=3±std dev) 2-tailed unpaired t-test was conducted to check the significance of the difference between control and compound treatments.

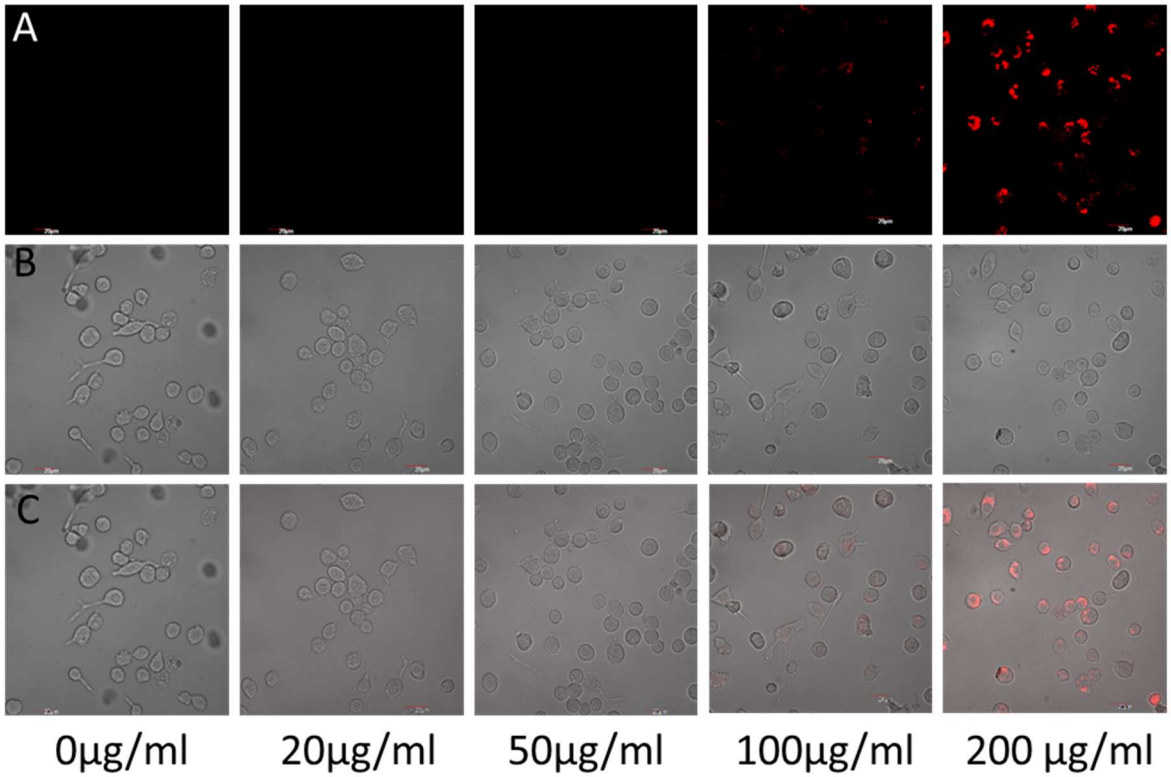


Figure S8. Representative confocal microscopy images of RAW264.7 cells treated with the Rhodamine B along with **2**. **A** Fluorescent image, **B** DIC image, and **C** DIC-Fluorescence merge image. Cells were incubated with various concentrations (0, 20, 50, 100 and 200 µg/ml) of the rhodamine along with **2** for 24 h followed by PBS washing and Imaging in Olympus Confocal FV1200 microscope at 60 x magnification (scale bar = 20 µm).

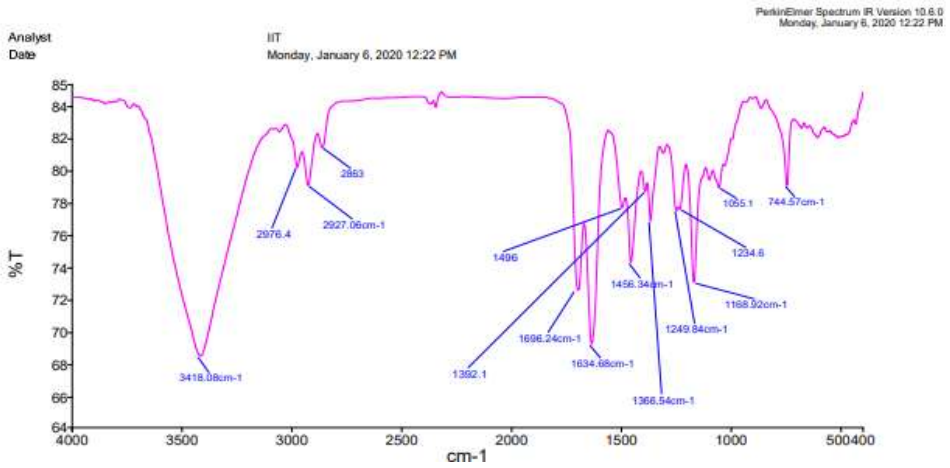


Figure S9. The FT-IR spectrum of **1** showed strong bands at 3418 cm^{-1} and 1634 cm^{-1} (amide I), and 1696 cm^{-1} indicating β-strand arrangement.

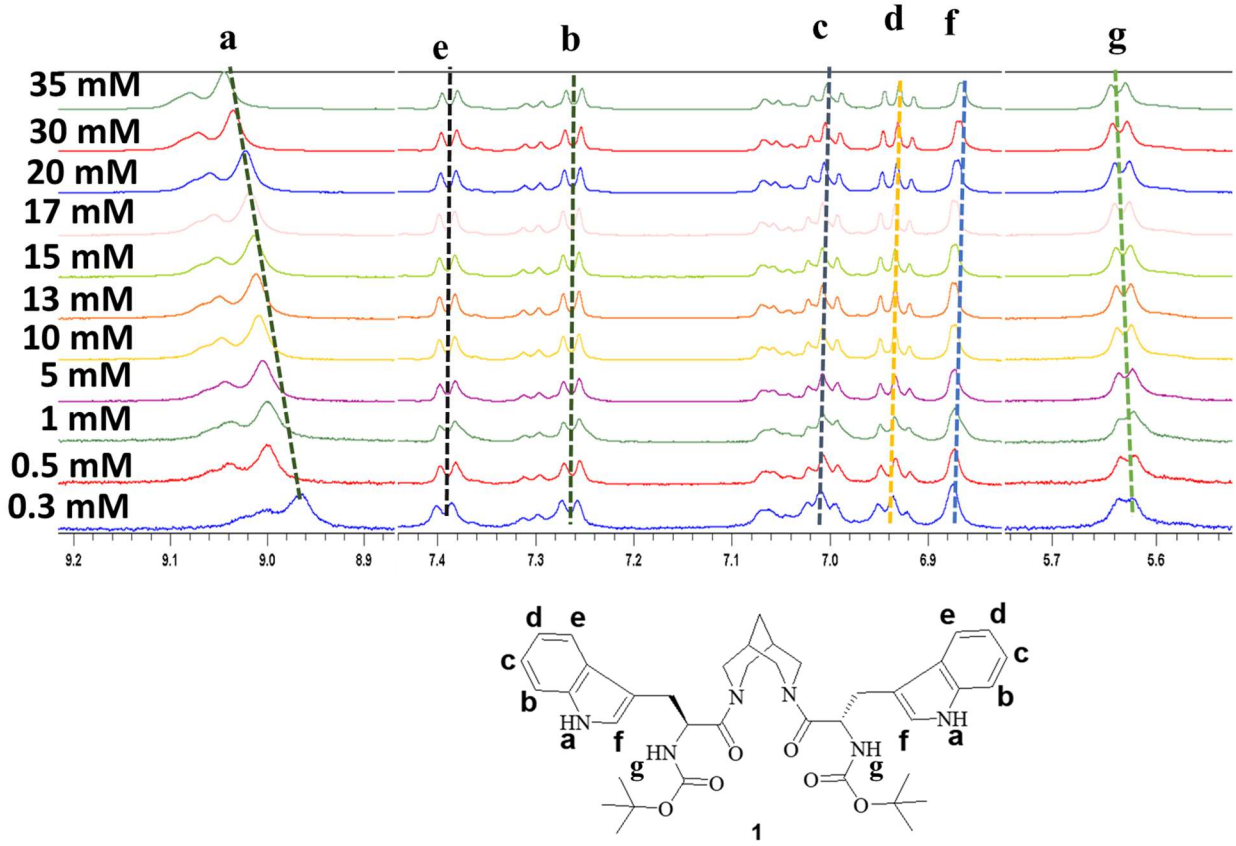
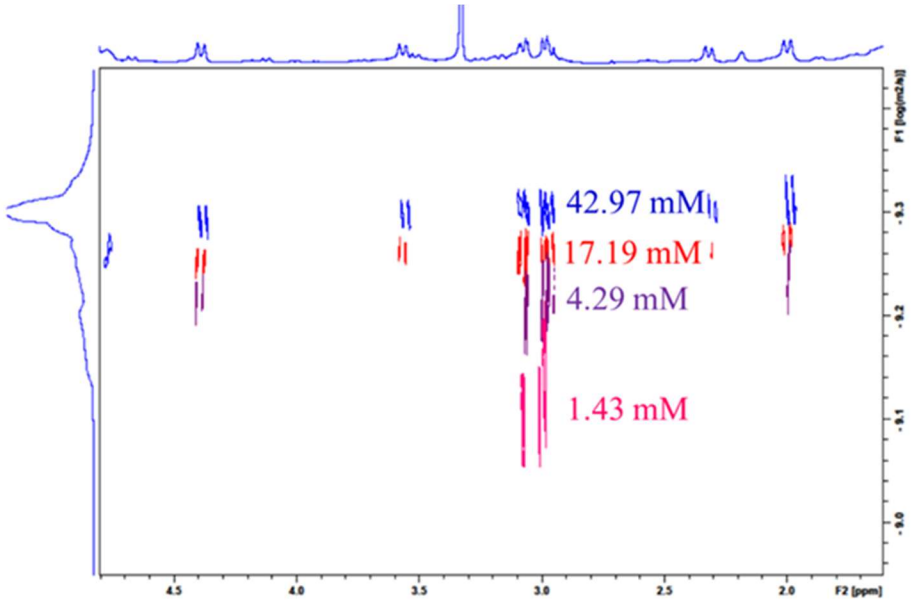
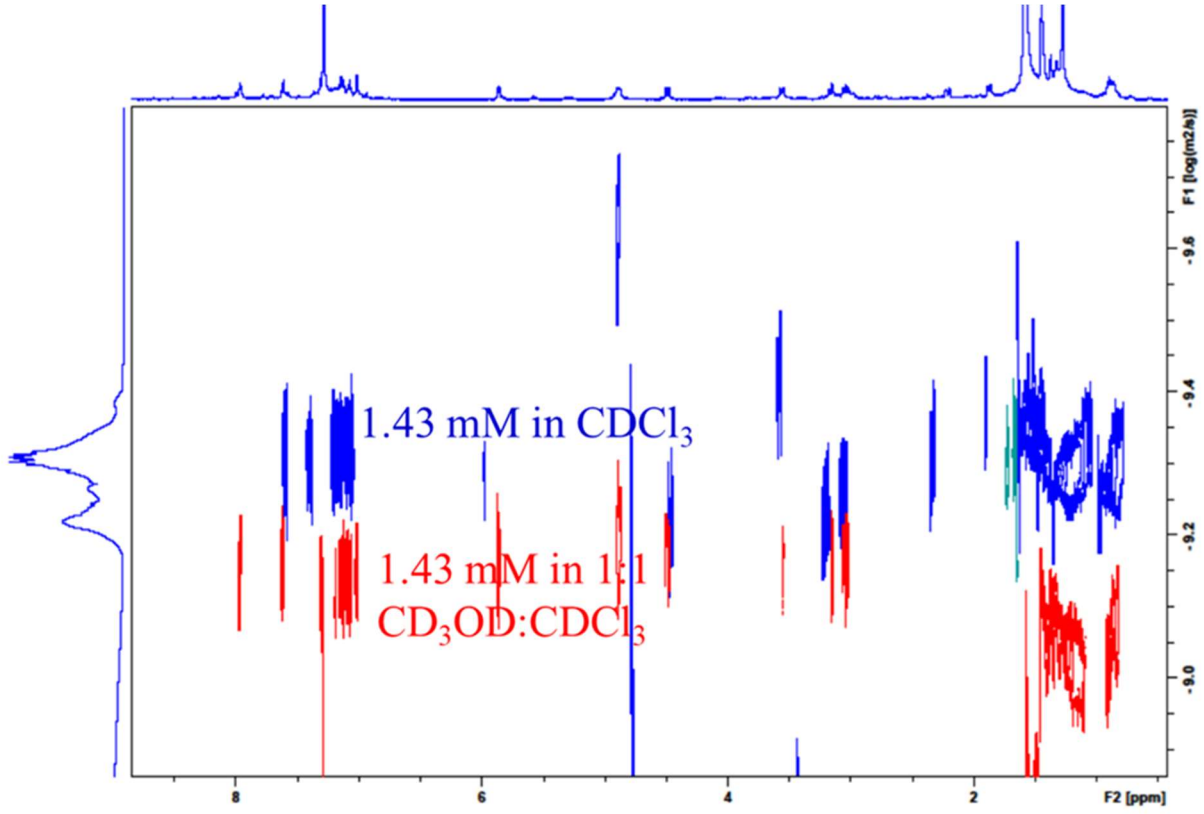


Figure S10. Concentration dependent study of **1**. Concentration is mark on the left side of each spectrum. Selected protons are marked on the structure and spectra. All spectra were recorded on 500 MHz NMR at 25 ºC in CD_3CN.



Region (PPM)	1.43 mM	4.29 mM	17.19 mM	42.97 mM
	Unit: m^2/s			
3.028-2.945	7.79×10^{-10}	6.33×10^{-10}	5.55×10^{-10}	4.96×10^{-10}

Figure S11. Diffusion constant from DOSY of **1** at different concentration in CD_3OD.



Region	1:1 MeOD:$CDCl_3$ (Unit: m^2/s)	$CDCl_3$ (Unit: m^2/s)
7.080-7.059	4.77×10^{-10}	7.19×10^{-10}

Figure S12. Comparison between the diffusion constant of compound **1** in 1:1 CD_3OD: $CDCl_3$ and only $CDCl_3$ at 1.43 mM concentration from DOSY experiment.

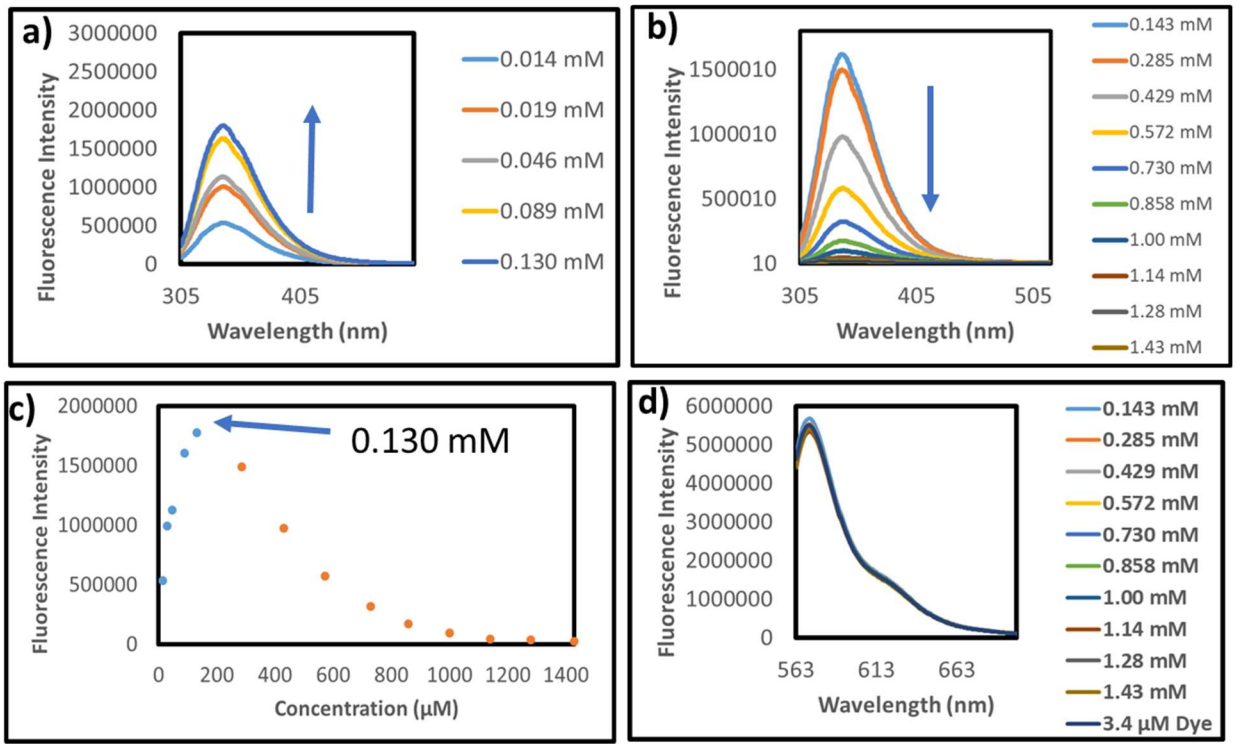


Figure S13. a) Concentration-dependent fluorescence spectra of **1** in concentration range of 14 - 130 µM in methanol (λ_{ex} at 295 nm). (b) Concentration-dependent fluorescence spectra of **1** in concentration range of 143 -1430 µM in methanol (λ_{ex} at 295 nm). (c) Plot of fluorescence emission intensity at 343 nm vs concentration of **1** (d) Fluorescence spectra of Rhodamine B dye alone and with addition of different concentrations of **1** in methanol (λ_{ex} at 553 nm).

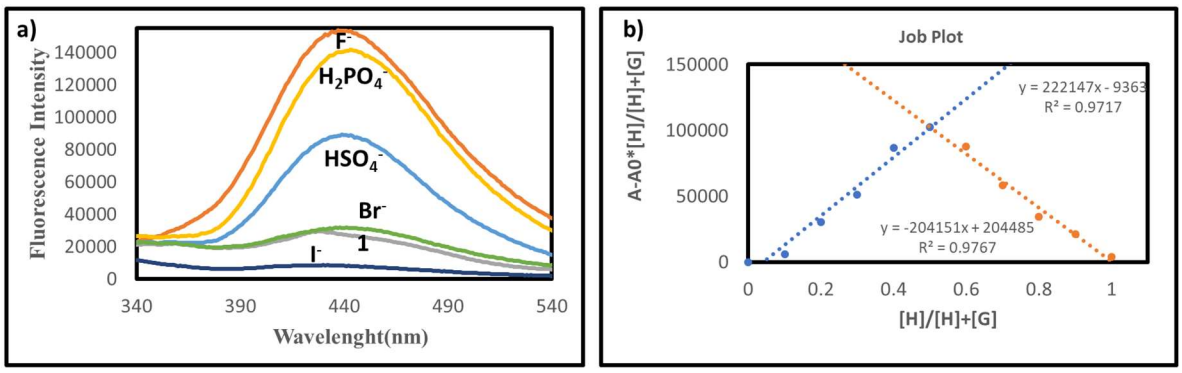


Figure S14. (a)Binding study of anions with **1** (b) Job Plot of $H_2PO_4^-$ titration against **1**.

Table S1. Chemical shift deviations of **1-4.**

Compound	Proton	δ (obs)	δ (random coil)	Chemical shift deviation i.e. Δδ(CHα) = δ(CHα) (obs) – δ (random coil)
1	Trp (CHα)	4.88 ppm	4.70 ppm	0.18 ppm
2	Trp (CHα)	4.89 ppm	4.70 ppm	0.18 ppm
3	Leu (CHα)	4.52 ppm	4.17 ppm	0.35 ppm
4	Ala (CHα)	4.50 ppm	4.35 ppm	0.15 ppm

Table S2. Crystal data and structure refinement for 1 (CCDC 2172117).

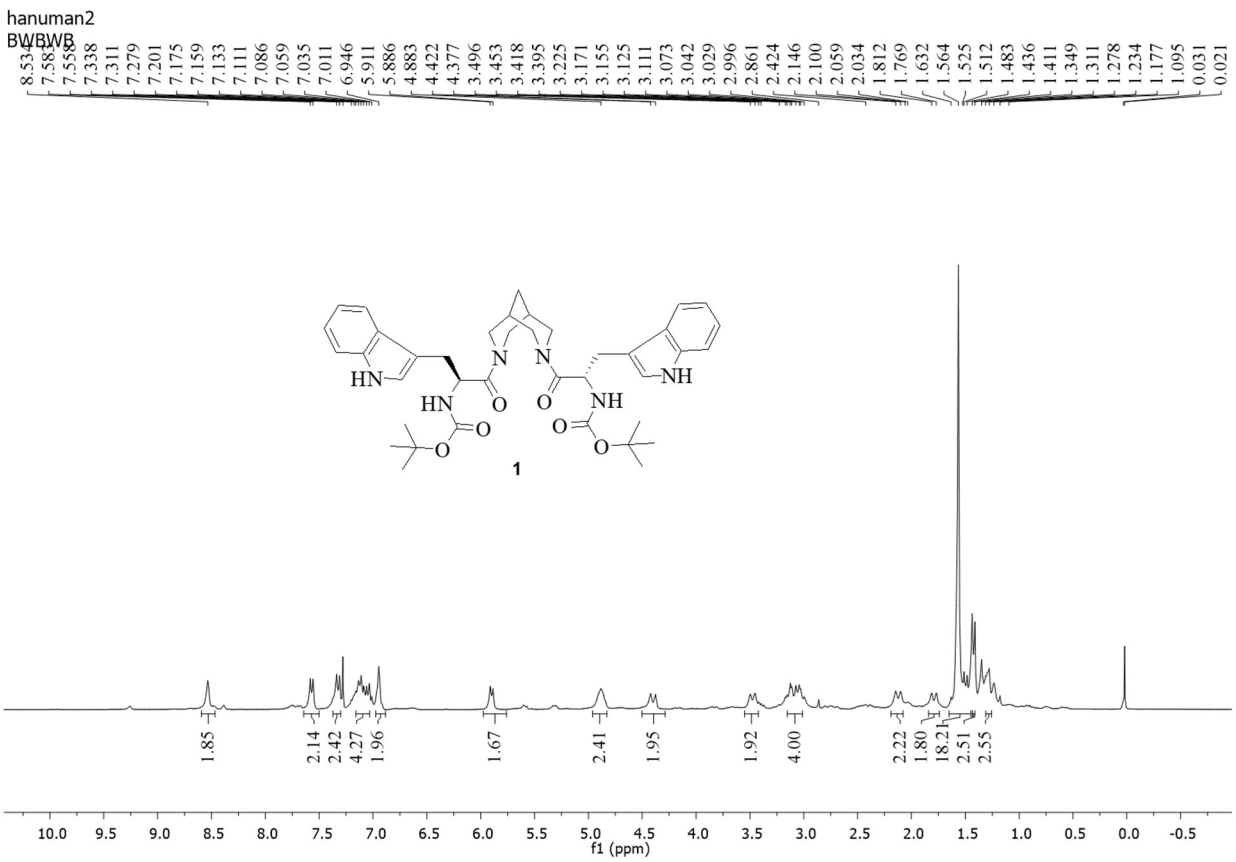
Identification code	BW2
Empirical formula	$C_{39}H_{52}N_6O_7$
Formula weight	716.884
Temperature/K	273.15
Crystal system	monoclinic
Space group	$P2_1$
a/Å	13.650(13)
b/Å	9.887(10)
c/Å	15.827(16)
α/°	90
β/°	112.53(3)
γ/°	90
Volume/Å^3	1973(3)
Z	2
ρ_{calc}g/cm^3	1.207
μ/mm^{-1}	0.084
F(000)	768.4
Crystal size/mm^3	0.25 × 0.08 × 0.10
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	3.36 to 50
Index ranges	$-14 \leq h \leq 18$, $-11 \leq k \leq 13$, $-19 \leq l \leq 17$
Reflections collected	11416
Independent reflections	5912 [R_{int} = 0.0544, R_{sigma} = 0.1647]
Data/restraints/parameters	5912/31/470
Goodness-of-fit on F^2	1.040
Final R indexes [I>=2σ (I)]	R_1 = 0.0736, wR_2 = 0.1509

Final R indexes [all data]	$R_1 = 0.1539$, $wR_2 = 0.1957$
Largest diff. peak/hole / e Å^{-3}	0.29/-0.30
Flack parameter	-2.3(18)

Table S3. Crystal data and structure refinement for 3 (CCDC 2172115).

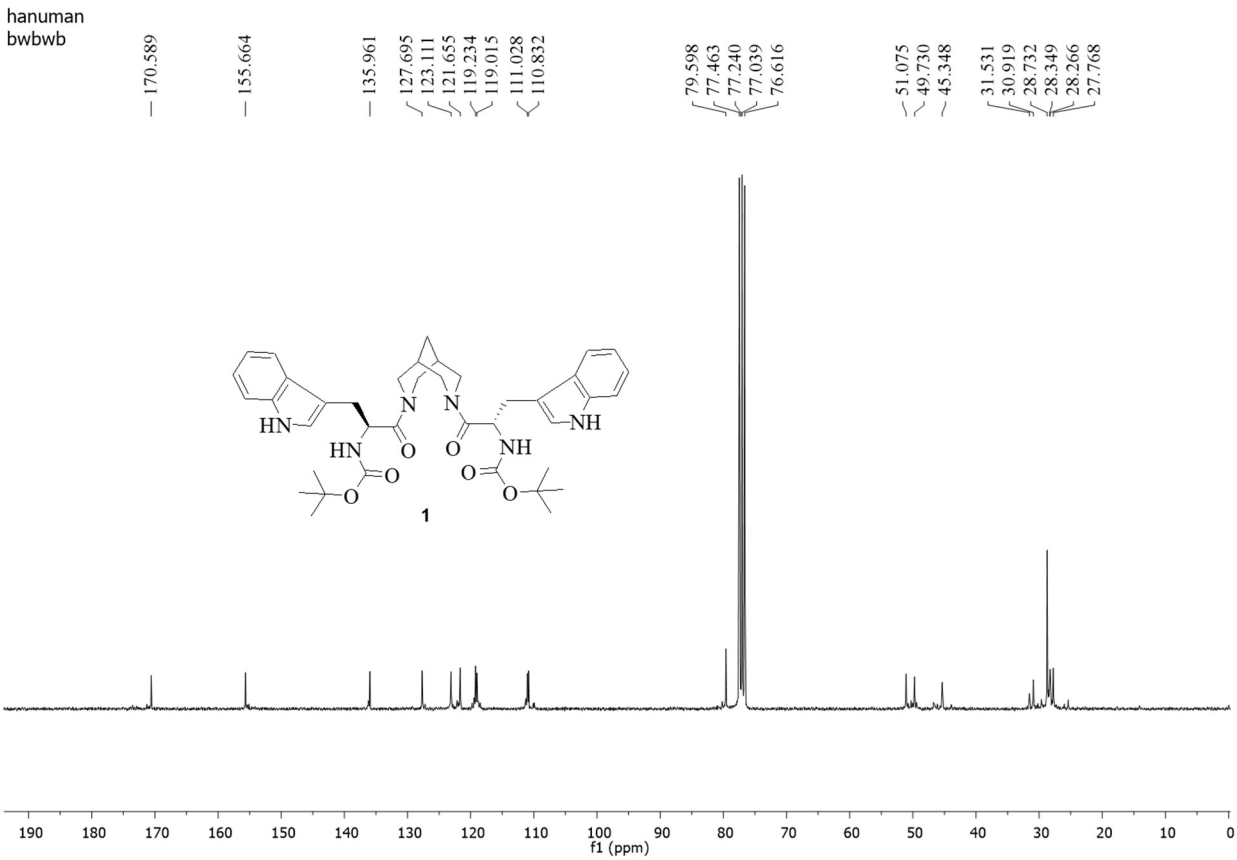
Identification code	BL2
Empirical formula	$C_{29}H_{52}N_4O_6$
Formula weight	552.760
Temperature/K	273.15
Crystal system	monoclinic
Space group	$P2_1$
a/Å	6.6858(4)
b/Å	26.2740(17)
c/Å	11.3518(7)
α/°	90
β/°	102.219(3)
γ/°	90
Volume/Å^3	1948.9(2)
Z	2
ρ_{calc}g/cm^3	0.942
μ/mm^{-1}	0.066
F(000)	604.3
Crystal size/mm^3	0.22 × 0.09 × 0.13
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	3.1 to 56.64
Index ranges	$-8 \leq h \leq 8$, $-35 \leq k \leq 35$, $-15 \leq l \leq 15$
Reflections collected	64410

Independent reflections	9644 [R_{int} = 0.0739, R_{sigma} = 0.0494]
Data/restraints/parameters	9644/37/363
Goodness-of-fit on F^2	0.989
Final R indexes [I>=2σ (I)]	R_1 = 0.0505, wR_2 = 0.1282
Final R indexes [all data]	R_1 = 0.1138, wR_2 = 0.1644
Largest diff. peak/hole / e $Å^{-3}$	0.16/-0.18
Flack parameter	-0.8(4)



1

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



^{13}C NMR ($CDCl_3$, 75MHz) of **1**

Mass Spectrum SmartFormula Report

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Sample Name	tm 1:100	Instrument / Ser#	micrOTOF-Q 10262
Comment			

Acquisition Parameter

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Focus	Active	Set Capillary	4500 V	Set Dry Heater	330 °C
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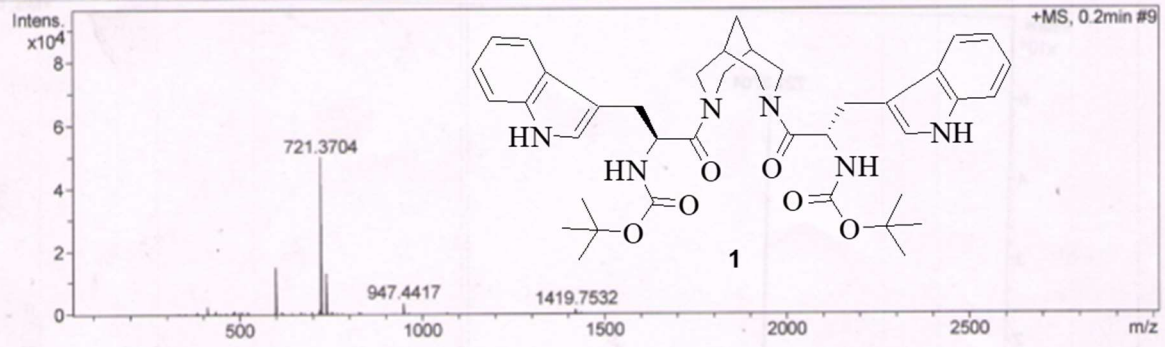
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



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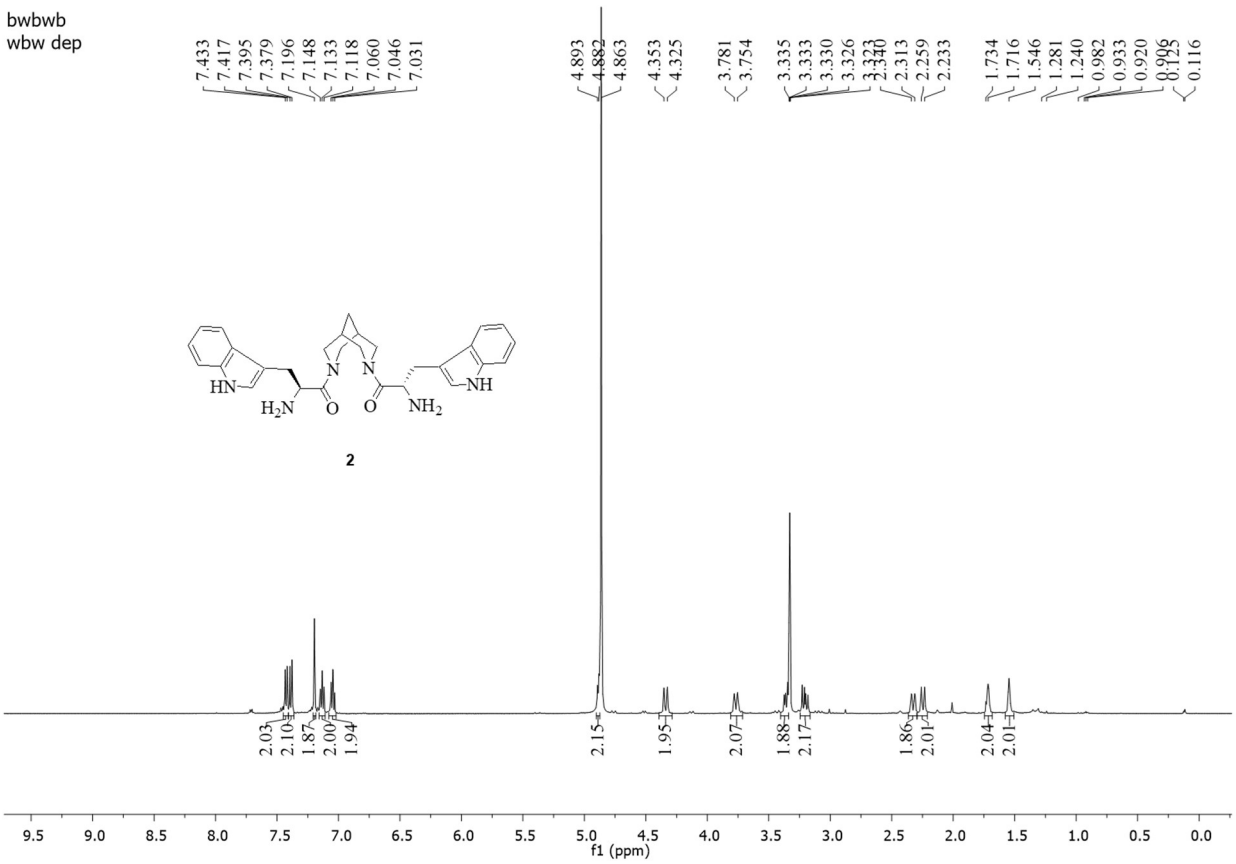
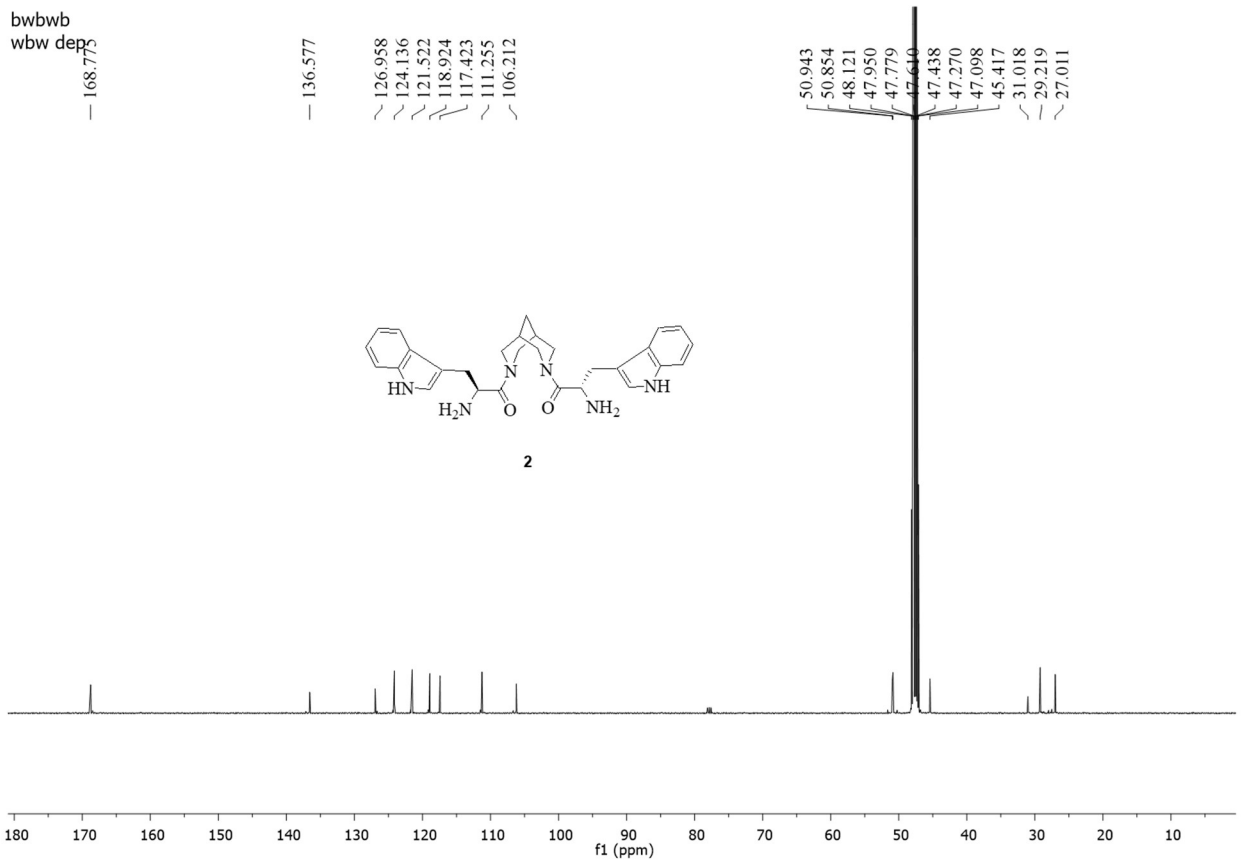
HRMS of **1**



2

^{1} H NMR (CD_3OD, 500 MHz) of **2**



^{13}C NMR (CD_3OD, 125 MHz) of **2**

Mass Spectrum SmartFormula Report

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Sample Name	tm 1 100	Instrument	micrOTOF-Q 228888.10262
Comment			

Acquisition Parameter

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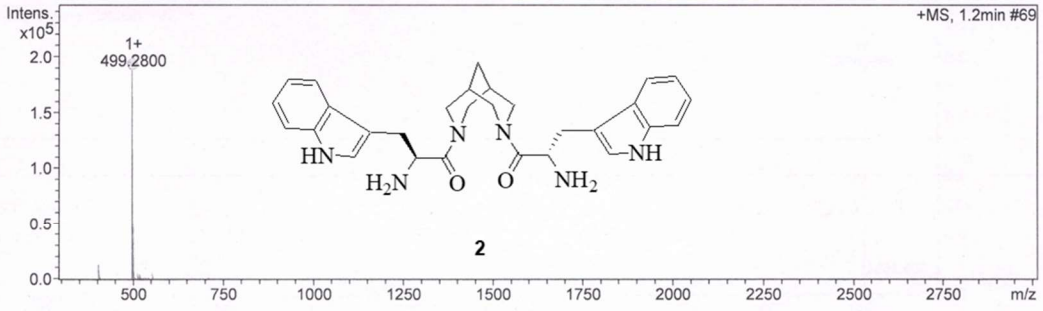
Scan End	3000 m/z	Set Collision Cell RF	700.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
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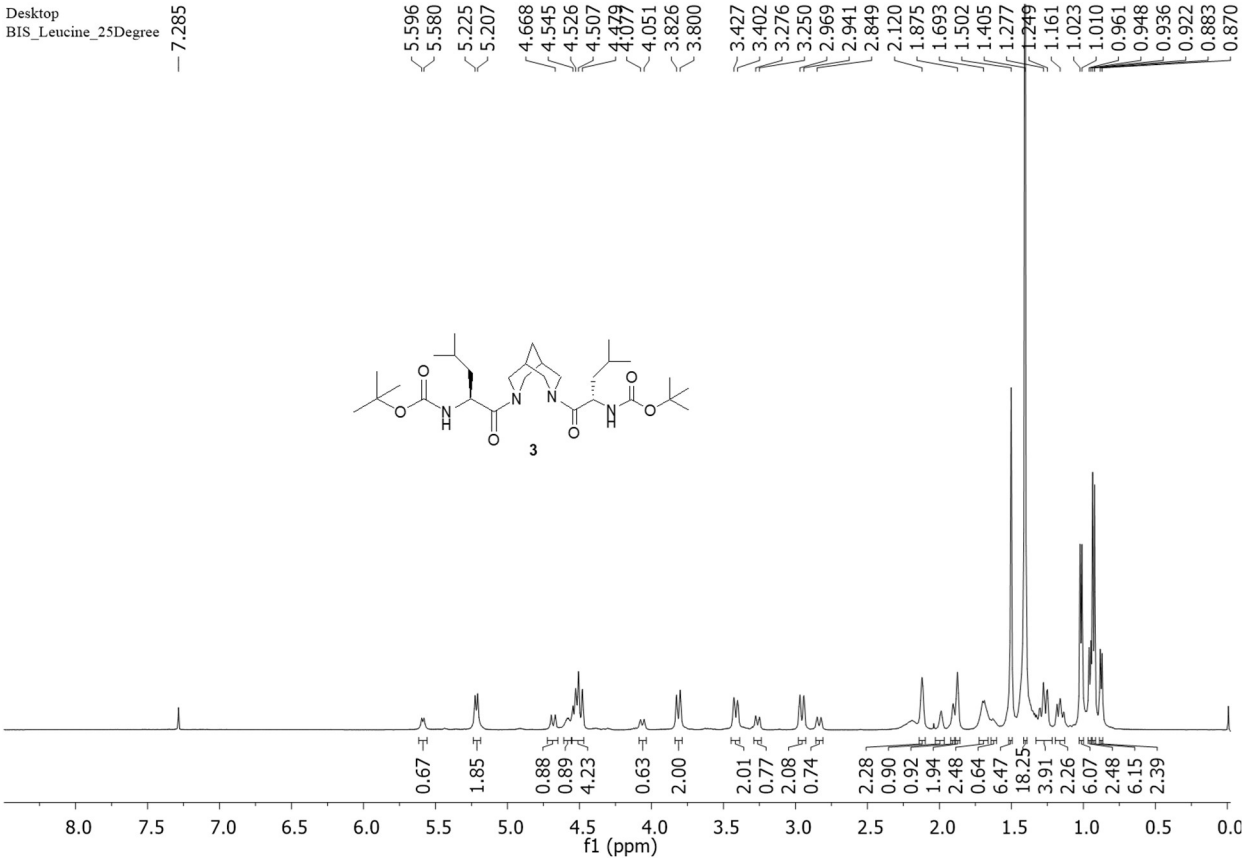
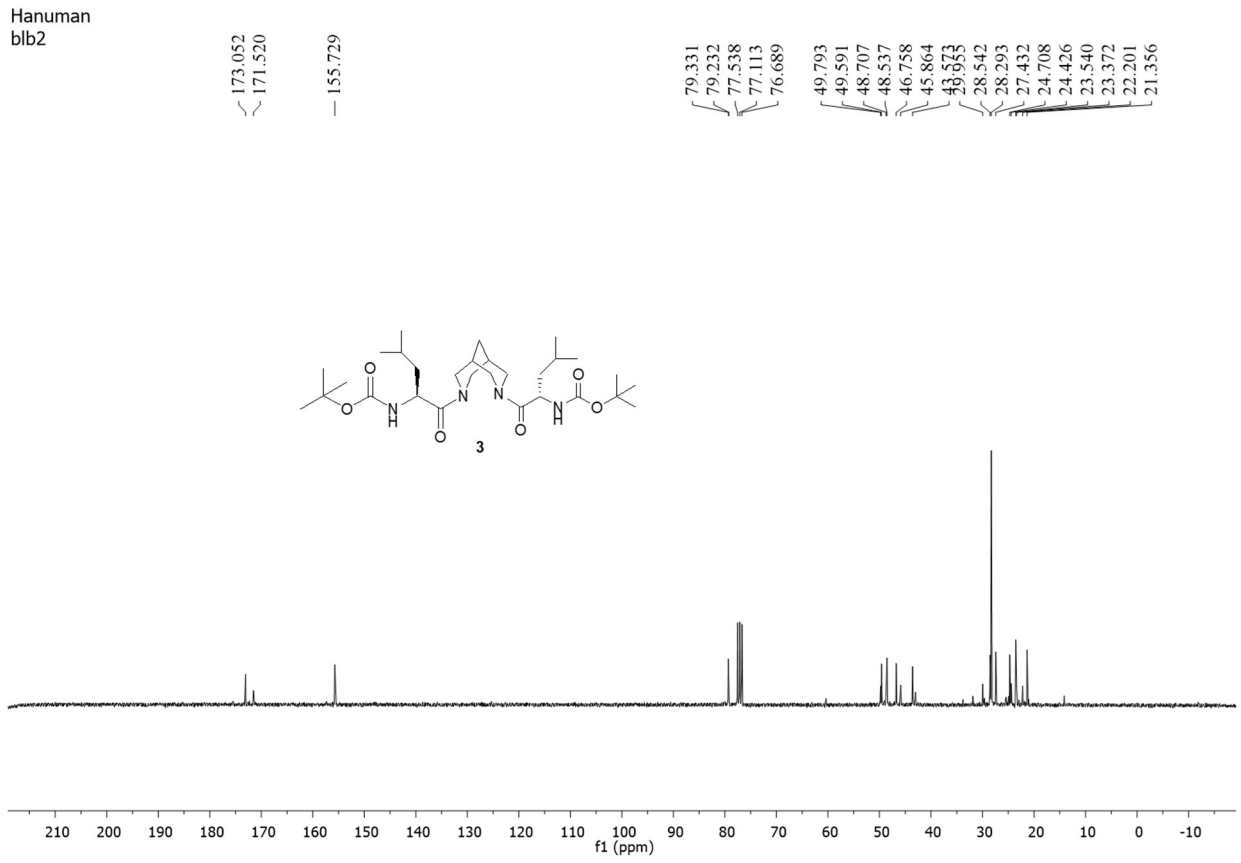
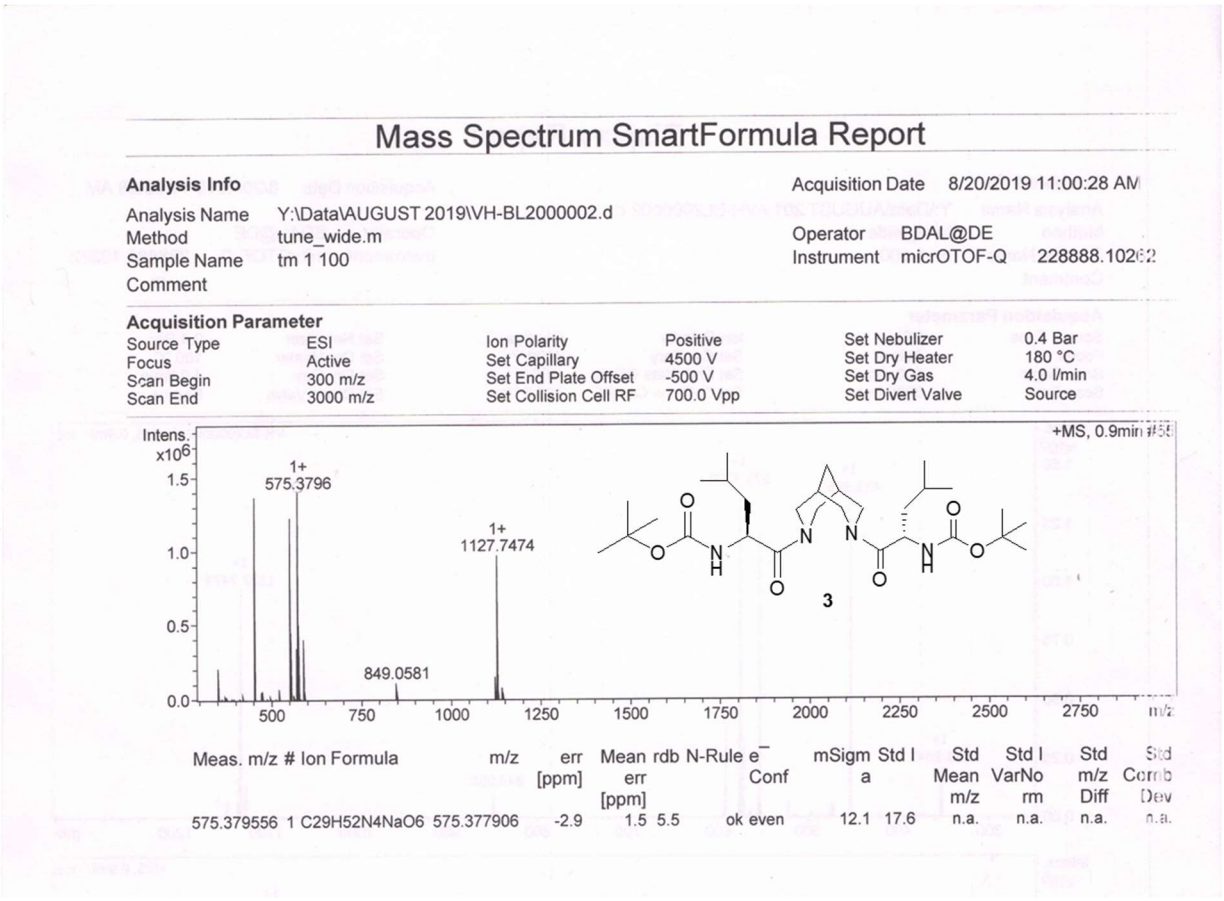
HRMS of **2**



^{1} H NMR ($CDCl_3$, 500 MHz) of **3**



^{13}C NMR ($CDCl_3$, 75MHz) of **3**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Acquisition Parameter
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Focus Active
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Scan End 3000 m/z
Ion Polarity Positive
Set Capillary 4500 V
Set End Plate Offset -500 V
Set Collision Cell RF 700.0 Vpp
Set Nebulizer 0.4 Bar
Set Dry Heater 180 °C
Set Dry Gas 4.0 l/min
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1.0
0.5
0.0
1+
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1+
1127.7474
849.0581
+MS, 0.9min
500
750
1000
1250
1500
1750
2000
2250
2500
2750
m/z
3
Meas. m/z # Ion Formula m/z err [ppm] Mean err [ppm] rdb N-Rule e⁻ Conf mSigma Std I Std Mean m/z Std I VarNorm Std m/z Diff Std Comb Dev
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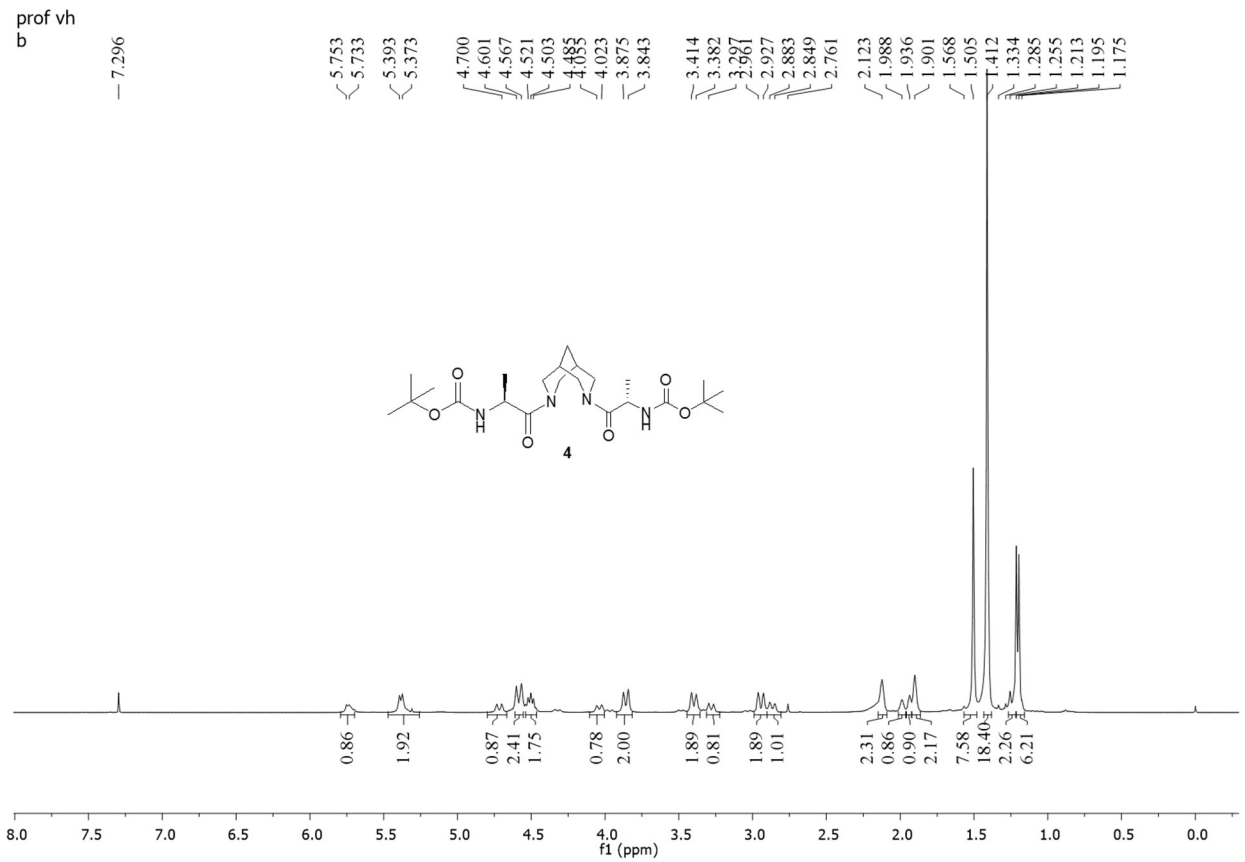
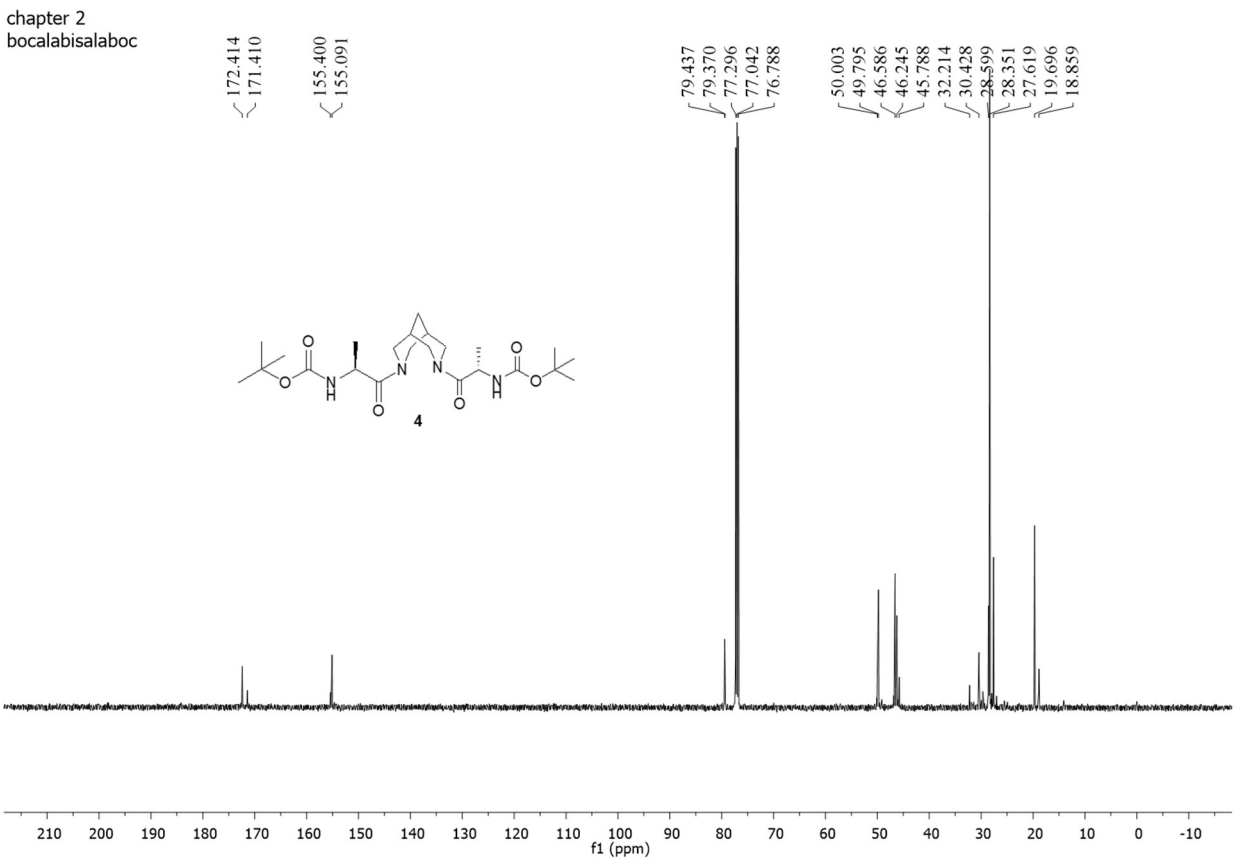
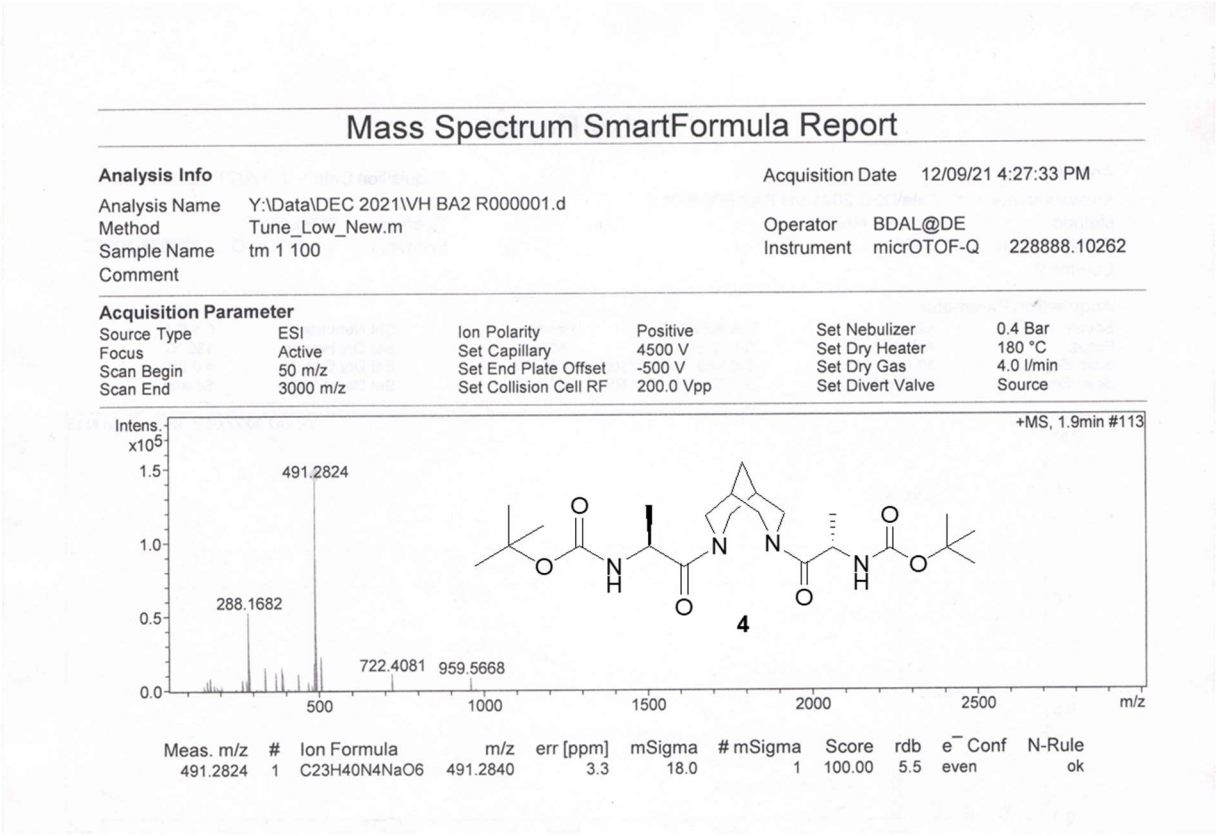
HRMS of **3**



^{1} H NMR ($CDCl_3$, 400 MHz) of **4**



^{13}C NMR ($CDCl_3$, 100MHz) of **4**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Sample Name tm 1 100
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Acquisition Parameter
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Focus Active
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Scan End 3000 m/z
Ion Polarity Positive
Set Capillary 4500 V
Set End Plate Offset -500 V
Set Collision Cell RF 200.0 Vpp
Set Nebulizer 0.4 Bar
Set Dry Heater 180 °C
Set Dry Gas 4.0 l/min
Set Divert Valve Source
+MS, 1.9min #113
Intens.
x10^5
1.5
1.0
0.5
0.0
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288.1682
722.4081
959.5668
500
1000
1500
2000
2500
m/z
4
Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e⁻ Conf N-Rule
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HRMS of **4**

==== Shimadzu LCsolution Analysis Report ====

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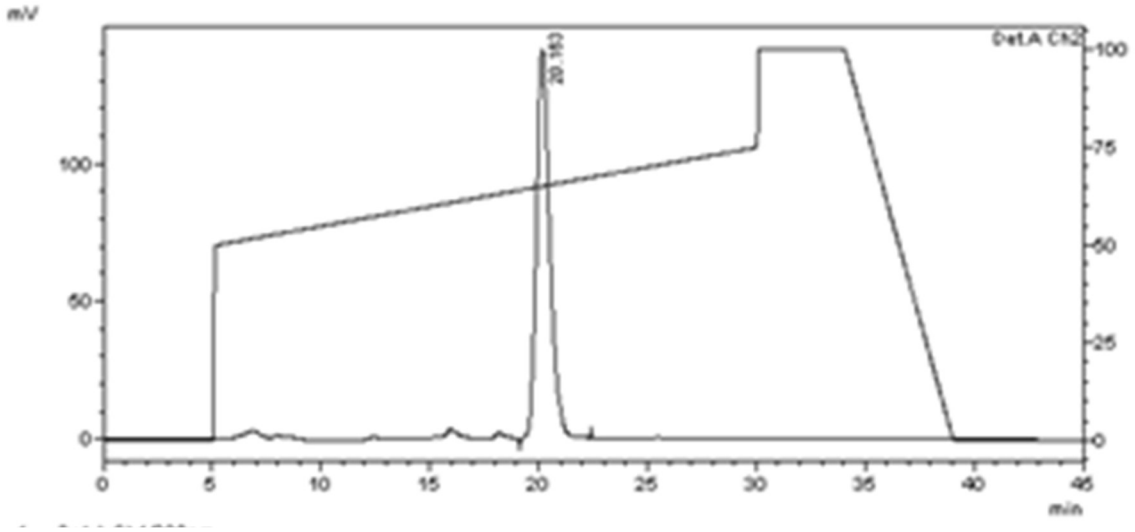

HPLC chromatogram of **1** in Solvent system ACN+TFA (0.1%) and H_2O+TFA (0.1%).

Reference

1. S. Juneja, H. Singh, S. Palui, S. Trivedi, S. S. Singh, V. Haridas and S. Pandey, *J. Phys. Chem. B*, 2019, **123**, 3112–3117.
2. S. Shankar, S. G. Shah, S. Yadav and A. Chugh, *European Journal of Pharmaceutics and Biopharmaceutics*, 2021, **166**, 216–226.