**Electronic Supplementary Information (ESI)**

# Micro-urchin from synthetic self-assembling molecules

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**Content**

## Materials and Methods

### General Informations

All reagents were used without further purification. All solvents employed in the reactions were distilled or dried from appropriate drying agents prior to use. Progress of reactions was monitored by thin layer chromatography (TLC). Purification of compounds was done by silica gel column chromatography. Silica gel G (Merck) was used for TLC and silica gels with 60-120 mesh, 100-200 mesh and 230-400 mesh were used for column chromatography. Melting points were recorded in a Fisher-Scientific melting point apparatus and were uncorrected. Optical rotations were measured with a Rudolph Research Analytical Autopol® V Polarimeter; where concentrations are given in gram/100 mL. IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets. $^{1}$H NMR spectra were recorded on Brucker-DPX-300 spectrometer using tetramethylsilane ($^{1}$H) as an internal standard. Coupling constants are in Hz and the $^{1}$H NMR data are reported as s (singlet), d (doublet), br (broad), t (triplet), m (multiplet). High Resolution mass spectra (HRMS) were recorded in Bruker MicrO-TOF-QII model using ESI technique.

### Preparation of gel:

Gels were prepared by dissolving the weighed amount of compound in the sample tube in appropriate polar solvent followed by the addition of non polar solvent (Table S1). Gel formation was confirmed by visual tube inversion method.

**General Procedures**:

**General method of preparation of (S-1)/(R-1)**:

To an ice-cooled and stirred solution of Boc-L-Aspartic acid/ Boc-D-Aspartic acid (1.03 g, 4.4 mmol) in 100 mL of dry $CH_2Cl_2$ was added N-hydroxysuccinimide (HOSu) (1.21 g, 10.56 mmol), DCC (2.17 g, 10.56 mmol) and stirred for 10 min. To the reaction mixture was added dry $NEt_3$ (1.47 mL, 10.56 mmol) followed by 1-hexylamine (1.40 mL, 10.56 mmol) and stirred for 24 h at RT. The reaction mixture was filtered and the filtrate was concentrated, admixed with 500 mL of ethyl acetate, washed sequentially with 0.2 N $H_2SO_4$, saturated $NaHCO_3$ solution and brine solution. The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. The crude product was chromatographed on a column of silica gel using ethyl acetate-hexane as eluent to afford the pure product **S-1**/**R-1**.

**General method for the deprotection of S-1/ R-1**:

To a stirred and homogenous ice cooled solution of **S-1**/**R-1** (0.399 g, 1 mmol) in dry dichloromethane (4.8 mL) was added trifluoroacetic acid (1.2 mL, 15 mmol) and stirred for 3 h. After completion of the reaction, trifluoroacetic acid was removed by applying vacuum. The residue obtained was dissolved in dichloromethane and washed with saturated $Na_2CO_3$ solution. The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure to obtain **S-2**/**R-2**.

**Conversion of 1,3,5-benzenetricarboxylic acid to trimethyl 1,3,5-benzene tricarboxylate**:

To a stirred, homogeneous solution of 1, 3, 5-benzenetricarboxylic acid (4.0 g, 19.0 mmol) in 75 mL of dry MeOH was added 1 mL conc. $H_2SO_4$ and the resulting solution was refluxed for 24 h at 90 ºC. Methanol was evaporated; the white residue obtained was dissolved in 75 mL of $CHCl_3$ and washed with aqueous $NaHCO_3$ solution. The organic layer was dried over $Na_2SO_4$ and evaporated to yield 4.31g of trimethyl 1,3,5-benzenetricarboxylate.

**Conversion of trimethyl 1,3,5-benzenetricarboxylate to dimethyl 1,3,5-benzenetricarboxylate**:

Trimethyl 1, 3, 5-benzenetricarboxylate (2.56 g, 10.1 mmol) was dispersed in 230 mL of dry MeOH under ice-cooled condition. To this non-homogenous solution was added aqueous NaOH (9.1 mL of 1M NaOH) portion wise in 0.5 h interval over a period of 8 h. The ice bath was removed after completion of the addition and the suspension was left stirred vigorously at RT for 14 h. Solvent was evaporated and the white solid obtained was dissolved in dichloromethane and washed with aqueous $NaHCO_3$ solution, dried over $Na_2SO_4$, concentrated to yield the unreacted starting material (0.438 g, 17 %). The aqueous layer was acidified with 2N HCl to pH 2, yielding a milky white suspension,

which was then extracted with ethyl acetate (2 x 200 mL). The ethyl acetate part was washed with 100 mL of brine solution, dried over $Na_2SO_4$ and evaporated to yield dimethyl 1,3,5-benzenetricarboxylate as white powder.

**Preparation of acid chloride of dimethyl-1,3,5-benzenetricarboxylate**:

Dimethyl 1,3,5-benzenetricarboxylate (0.238 g, 1 mmol) was dissolved in freshly distilled $SOCl_2$ (1.5 mL, 20 mmol) and refluxed at 85-90$^oC$ for 4h. The reaction mixture was subjected to high vacuum to remove excess $SOCl_2$ and the product was obtained as a white solid in quantitative yield.

**General experimental procedure for the preparation of S-3/R-3**:

**Method I**: **DCC Coupling Reaction**

To an ice cooled and well stirred solution of dimethyl 1,3,5-benzenetricarboxylate (0.238 g, 1 mmol) in 30 mL of dry dichloromethane was added N-hydroxysuccinimide (HOSu) (0.276 g, 1.2 mmol), DCC (0.495 g, 1.2 mmol) and stirred for 10 min. To this was added dry $NEt_3$ (0.695 mL, 5 mmol) followed by **S-2**/ **R-2** (0.299 g, 1 mmol) and stirred for 24 h at RT. The residue from the reaction mixture was filtered and the filtrate was concentrated, admixed with 100 mL of ethyl acetate, washed sequentially with 2N $H_2SO_4$, saturated $NaHCO_3$ solution and brine solution. The organic layer was dried over $Na_2SO_4$, concentrated under reduced pressure and the crude product was chromatographed on a column of silica gel using chloroform-methanol as eluent to afford pure product **S-3**/**R-3**.

**Method II: Acid chloride-amine coupling**:

To an ice cooled solution of **S-2**/ **R-2** (0.299 g, 1 mmol) in 30 mL of dry dichloromethane was added dry $NEt_3$ (0.835 mL, 6 mmol) and was stirred for 15 min. at 0$^oC$. The acid chloride of dimethyl 1,3,5-benzenetricarboxylate (0.256 g, 1 mmol) was dissolved in dry dichloromethane, transferred to a pressure equalizing dropping funnel and added dropwise to the reaction mixture over a duration of 30 min. The reaction mixture was stirred for overnight, admixed with 50 mL of dichloromethane, washed sequentially with 2N $H_2SO_4$, saturated $NaHCO_3$ solution and brine solution. The organic layer was dried over $Na_2SO_4$, concentrated under reduced pressure and the crude product was chromatographed on a column of silica gel using chloroform-methanol as eluent to afford of the pure product **S-3**/**R-3**.

**General Experimental Procedure for the alkaline hydrolysis of R-3** to **R-5**:

To an ice cooled and well stirred solution of **R-3** (0.519 g, 1 mmol) in 10 mL of methanol was added 10 mL of 2M NaOH and was refluxed for 3 h at 40$^{o}$C. After 3 h, solvent was evaporated, the solid obtained was treated with 50 mL of water and acidified with $NaHSO_4$ to pH = 3, extracted with ethyl acetate to obtain **R-5**.

**General method of preparation of S-4 and R-4**:

To an ice cooled and well stirred solution of **S-2**/ **R-2** (0.299 g, 1 mmol) in 30 mL of dry dichloromethane was added dry $NEt_3$ (1.1 mL, 8 mmol) and was stirred for 15 min. Benzoyl chloride (0.140 g, 1 mmol) in dry dichloromethane was added dropwise to the reaction mixture over a period of 30 min. and was stirred for 14 h. The reaction mixture was admixed with 50 mL of dichloromethane, washed sequentially with 2N $H_2SO_4$, saturated $NaHCO_3$ solution and brine solution. The organic layer was dried over $Na_2SO_4$, concentrated under reduced pressure and the crude product was chromatographed on a column of silica gel using chloroform-methanol as eluent to afford the pure product **S-4/R-4**.

**General method of preparation of 6a**:

**Method I**: **DCC Coupling Reaction of dimethyl 1, 3, 5-benzenetricarboxylate with 1-hexylamine**
To an ice cooled and well stirred solution of dimethyl 1,3,5-benzenetricarboxylate (0.71 g, 3 mmol) in 70 mL of dry dichloromethane was added N-hydroxysuccinimide (HOSu) (0.41 g, 3.6 mmol), DCC (0.74 g, 3.6 mmol) and stirred for 10 min. To the reaction mixture was added dry $NEt_3$ (0.50 mL, 3.6 mmol) followed by 1-hexylamine (0.39 mL, 3.6 mmol) and stirred for 24 h at RT. The reaction mixture was filtered and the filtrate was concentrated, admixed with 100 mL of ethyl acetate, washed sequentially with 2N $H_2SO_4$, saturated $NaHCO_3$ solution and brine solution. The organic layer was dried over $Na_2SO_4$, concentrated under reduced pressure and the crude product was chromatographed on a column of silica gel using ethyl acetate-hexane as eluent to afford the pure product **6a**.

**Method II**: **Acid chloride-amine coupling**

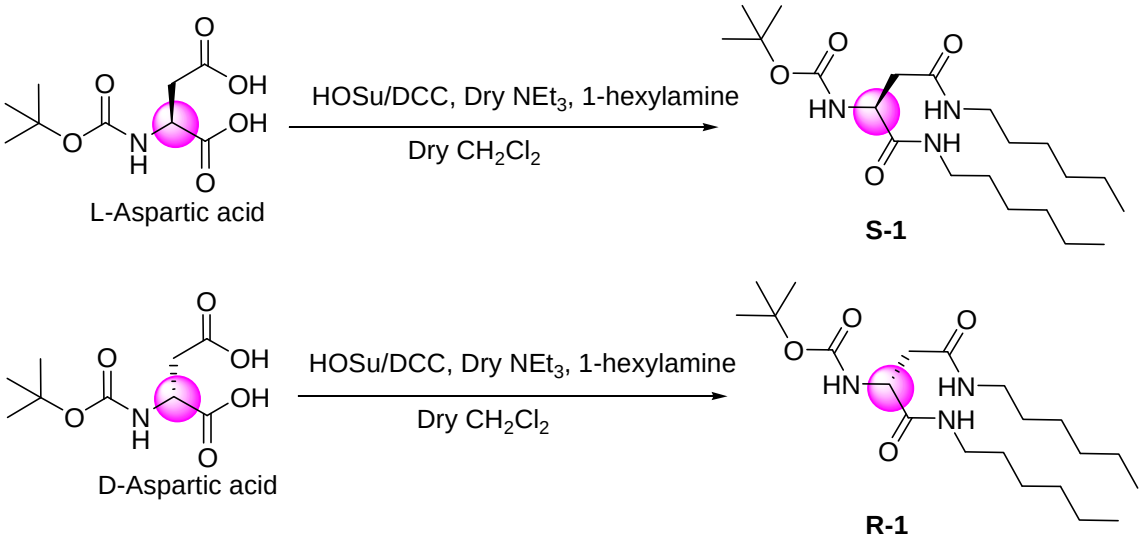
To an ice cooled and well stirred solution of 1-hexylamine (0.26 mL, 2 mmol) in 60 mL of dry dichloromethane was added dry $NEt_3$ (1.1 mL, 8 mmol) and was stirred for 15 min. The acid chloride of dimethyl 1,3,5-benzenetricarboxylate (0.513 g, 2 mmol) in dry dichloromethane was added

dropwise to the reaction mixture over a period of 30 min. and was left stirred overnight. The reaction mixture was admixed with 50 mL of dichloromethane, washed sequentially with 2N $H_2SO_4$, saturated $NaHCO_3$ solution and brine solution. The organic layer was dried over $Na_2SO_4$, concentrated under reduced pressure and the crude product was chromatographed on a column of silica gel using chloroform-methanol as eluent to afford **6a**.

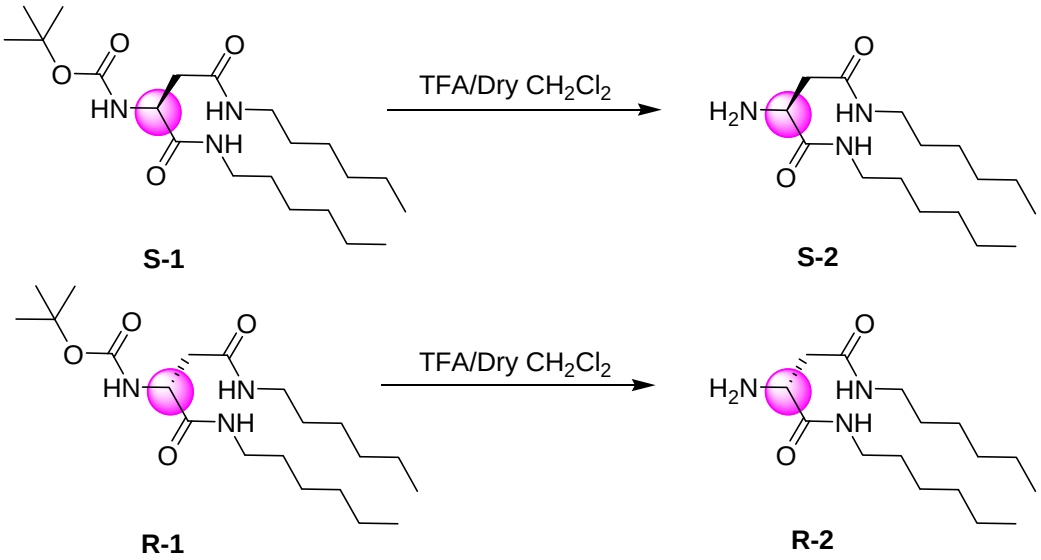
**Experimental procedure for hydrolysis of 6a to 6b**:

To an ice cooled and well stirred solution of **6a** (0.321 g, 1 mmol) in 5.2 mL of methanol was added 5.2 mL of 2M aqueous KOH and refluxed for 2 h at 90-100$^oC$. After completion of the reaction, solvent was removed under vacuum, water was added to the solid obtained and was acidified with 2N HCl to pH = 2, followed by extraction with ethyl acetate. Organic layer was dried over $Na_2SO_4$, concentrated to yield **6b**.

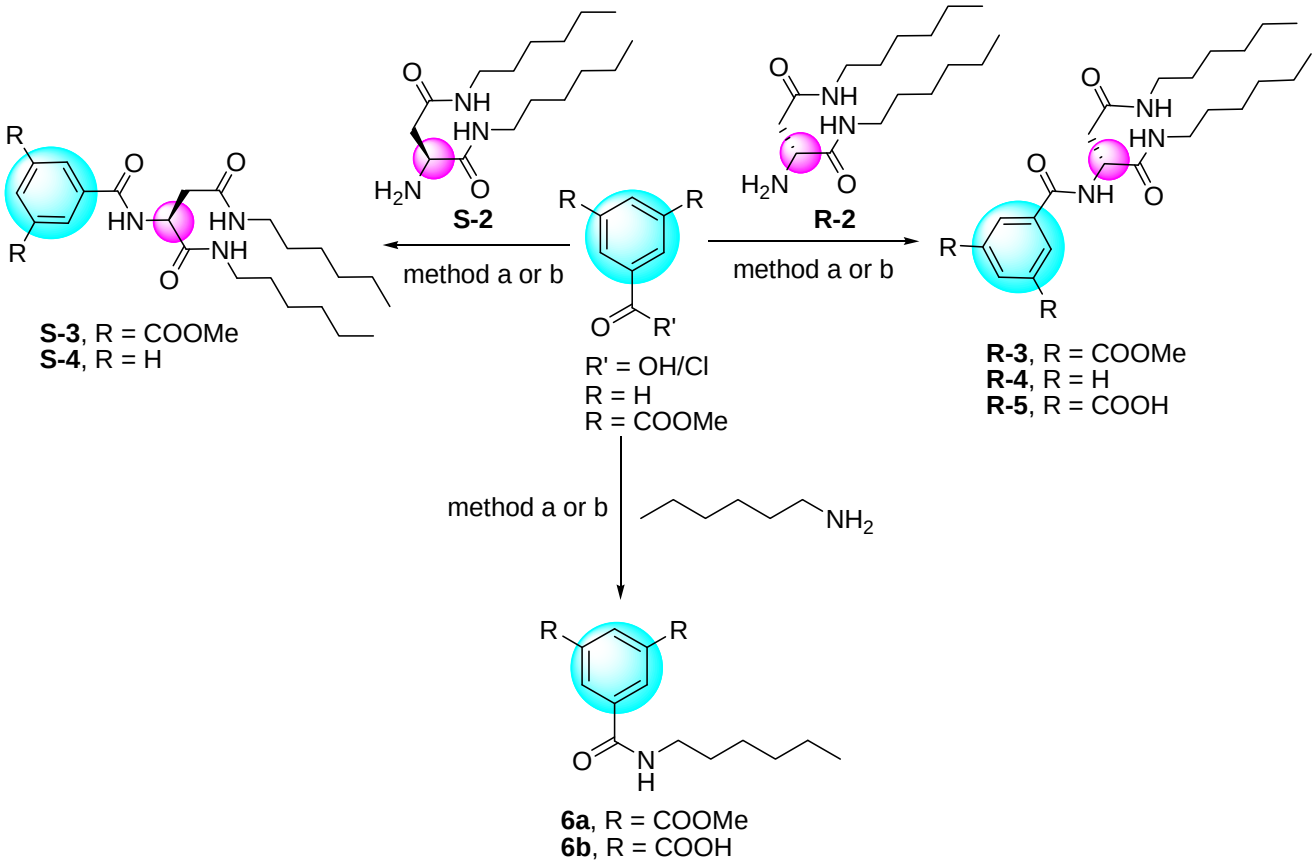
**Schemes**



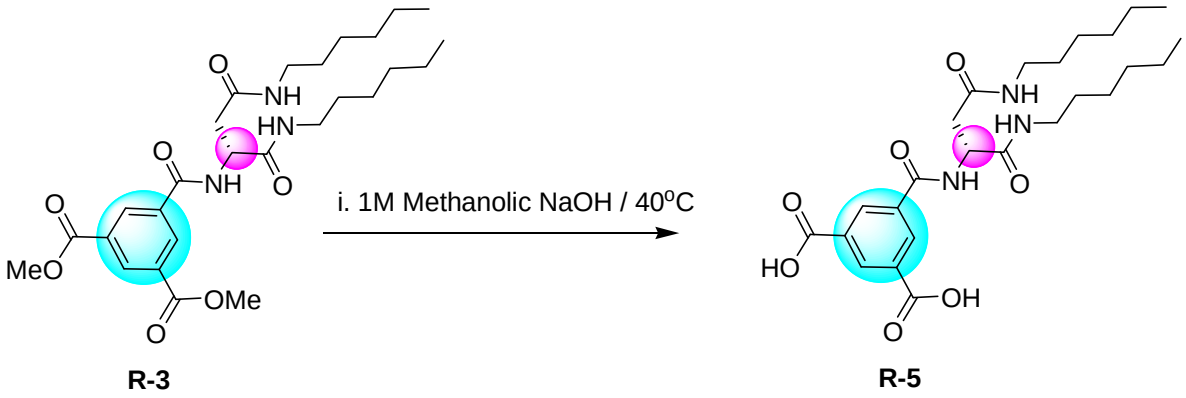

*Scheme S1*: Synthesis of **S-1** and **R-1**



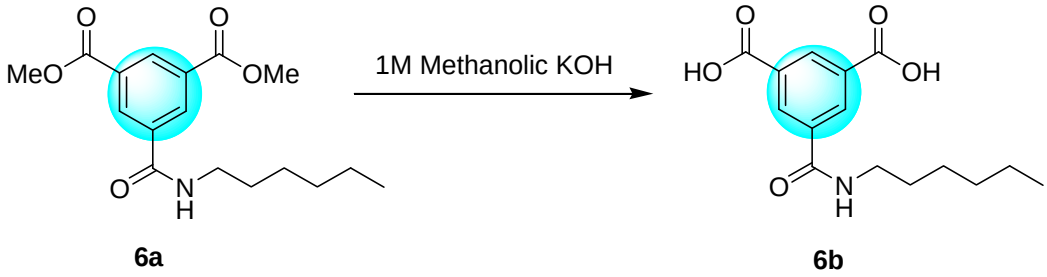

*Scheme S2*: Deprotection of **S-1** to **S-2** and **R-1** to **R-2**




*Scheme S3*: Synthesis of various achiral and chiral aspartic acid-based self-assembling molecules; **method a** (R'= OH): Dry $CH_2Cl_2$/HOSu/DCC/Dry $NEt_3$; **method b** (R'= Cl): Dry $CH_2Cl_2$/Dry $NEt_3$




*Scheme S4*: Alkaline hydrolysis of **R-3** to **R-5**




*Scheme S5*: Alkaline hydrolysis of **6a** to **6b**

**Table S1**: Screening of solvents for gelation of **S-1**, **R-1**, **S-2**, **R-2**, **S-3**, **R-3**, **S-4, R-4, R-5, 6a** and **6b**.

| **Solvent** | **S-1** | **R-1** | **S-2** | **R-2** | **S-3** | **R-3** | **S-4** | **R-4** | **R-5** | **6a** | **6b** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hexane | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| Ethyl acetate | NG | NG | NG | NG | NS | NS | NS | NS | NS | NG | NS |
| Chloroform | NG | NG | NG | NG | NG | NG | NG | NG | PS | NG | PS |
| Dichloromethane | NG | NG | NG | NG | NG | NG | NG | NG | PS | NG | NS |
| Methanol | NG | NG | NG | NG | PS | PS | PS | PS | NG | NG | NG |
| Ethyl acetate + Hexane | G | G | NG | NG | NS | NS | NS | NS | NS | G | NS |
| Chloroform + Hexane | G | G | NG | NG | G | G | G | G | Ppt | G | Ppt |
| Dichloromethane + Hexane | G | G | NG | NG | G | G | G | G | Ppt | G | NS |
| Chlorofom + Methanol | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |
| Dichloromethane + Methanol | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG | NG |

NS (not soluble), PS (partially soluble), G (gelating), NG (Not gelating) Ppt (Precipitate)

**Table S2: Chemical shift values of R-3 in different concentrations**

| Conc. | δ value of α–CH | δ value of β–CONH(hexyl) | δ value of α–CONH(hexyl) | δ value of Asp NH |
|---|---|---|---|---|
| 1 mM | 4.843 | 5.938 | 7.480 | 8.428 |
| 10 mM | 4.853 | 6.017 | 7.480 | 8.431 |
| 20 mM | 4.869 | 6.13 | 7.497 | 8.462 |
| 30 mM | 4.876 | 6.226 | 7.513 | 8.473 |
| 40 mM | 4.905 | 6.328 | 7.534 | 8.506 |
| 50 mM | 4.907 | 6.407 | 7.552 | 8.515 |
| 55 mM | 4.921 | 6.448 | 7.559 | 8.523 |

**Table S3: Difference in chemical shift values of R-3 with increase in concentration**

| Conc. | Δδ (CH) | Δδ (β–CONH-hexyl) | Δδ (α–CONH-hexyl) | Δδ (AspNH) |
|---|---|---|---|---|
| 1 mM | 0 | 0 | 0 | 0 |
| 10 mM | 0.01 | 0.079 | 0 | 0.003 |
| 20 mM | 0.026 | 0.192 | 0.017 | 0.034 |
| 30 mM | 0.033 | 0.288 | 0.033 | 0.045 |
| 40 mM | 0.062 | 0.39 | 0.054 | 0.078 |
| 50 mM | 0.064 | 0.469 | 0.072 | 0.087 |
| 55 mM | 0.078 | 0.51 | 0.079 | 0.095 |

## Microscopy Methods

**Optical Microscopy**:

Optical micrographs were obtained from Nikon Eclipse TS100. Samples were viewed by mounting the sample on glass slide.

**Transmission Electron Microscopy (TEM)**:

1 mM solution of the sample was used for TEM. Methanol or chloroform-hexane (1:1) were used as the solvents. The solution of the sample was filtered using nylon syringe filter (13 mm / 0.2 μm). About 2 μl aliquot of the sample solution was placed on a 200 mesh copper grid and stained with 2 % wt. phosphotungstate in water for 2 min and the excess fluid was removed. Samples were viewed using a Philips CM 12 electron microscope.

**Scanning Electron Microscopy (SEM)/ Field Emission Scanning Electron Microscopy (FESEM)**:

For viewing the gel under the scanning electron microscope, gel piece/ supernatant was mounted on carbon tape pasted on a stub, dried at room temperature and coated with ~ 10 nm of gold. In addition, gel was also retained in the tube for air-drying at room temperature for one week to several weeks.

Dried gel piece was mounted on the carbon tape pasted on a stub and coated with ~ 10 nm of gold. Analysis of the gel was done using scanning electron microscope ZEISS EVO 50 SEM and field emission scanning electron microscope (FESEM) Quanta 3D FEG (FEI).

**Dynamic Light Scattering (DLS)**:

Solution with various concentrations in chloroform was prepared, filtered by Nylon syringe filter (13 mm / 0.2 μm) and was used for the measurement. The variation in average size distribution with concentration was measured in a Malvern Zetasizer ZS90 DLS spectrometer with 633 nm CW laser.

**Supplementary Figures**

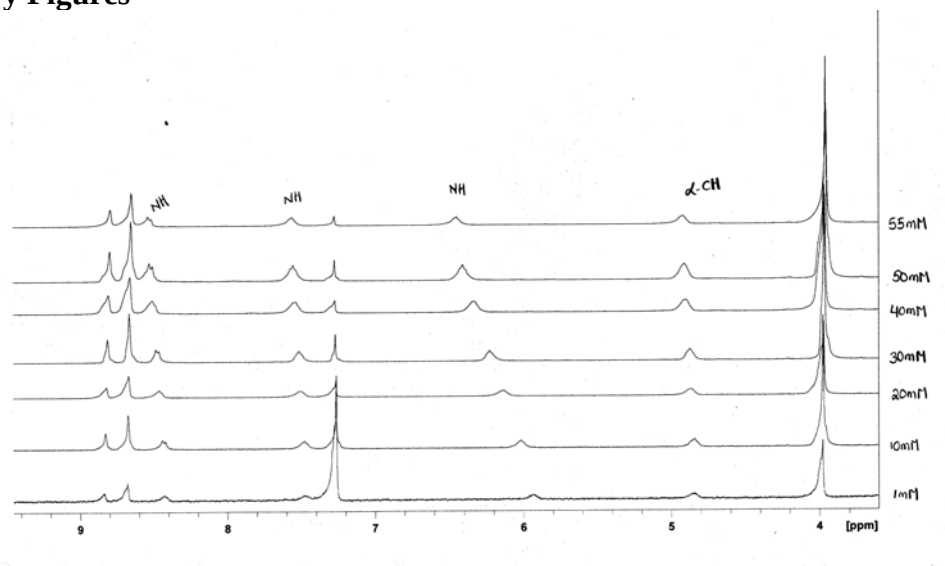



*Figure S1*: Overlay $^{1}$H NMR spectra of **R-3** in different concentrations showing shift in δ value of NH and CH protons

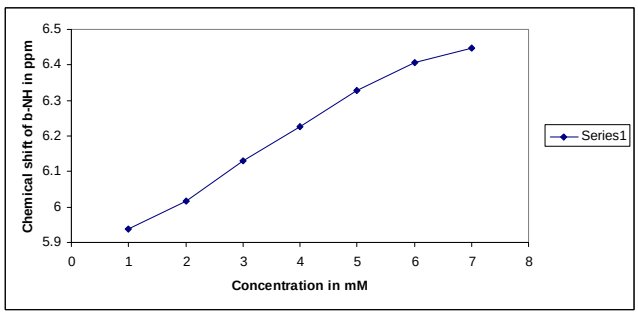
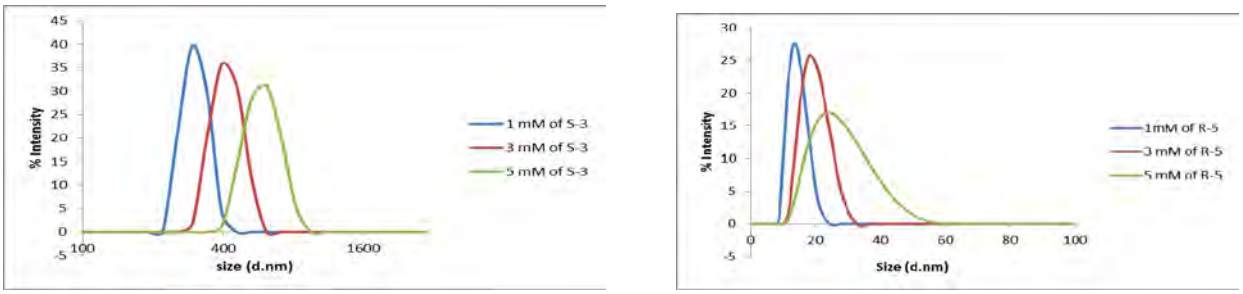


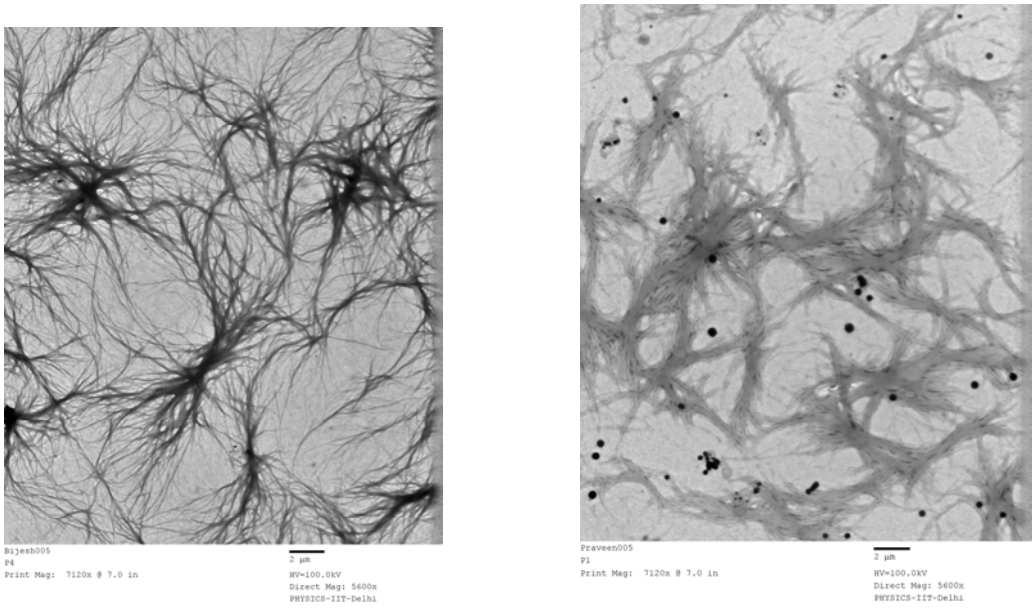

*Figure S2*: Plot showing chemical shift of β-NH Vs Conc




(a) (b)

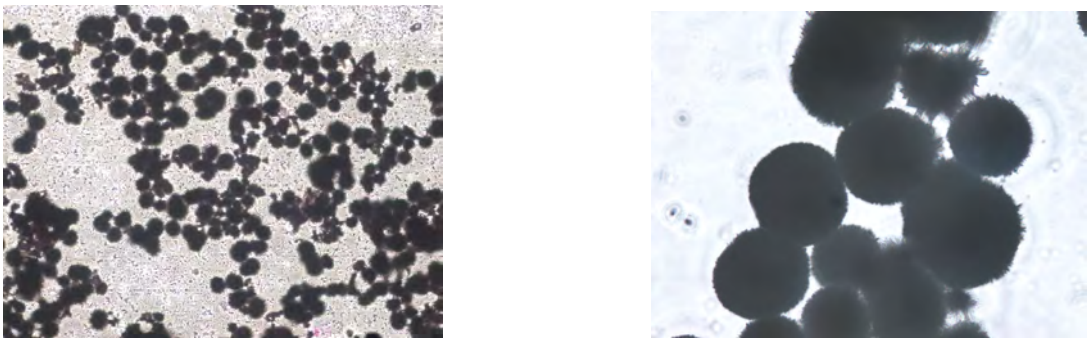
*Figure S3*: DLS of (a) **S-3** and (b) **R-4** showing the average size distribution at different concentrations in chloroform

**Microscopy Images**



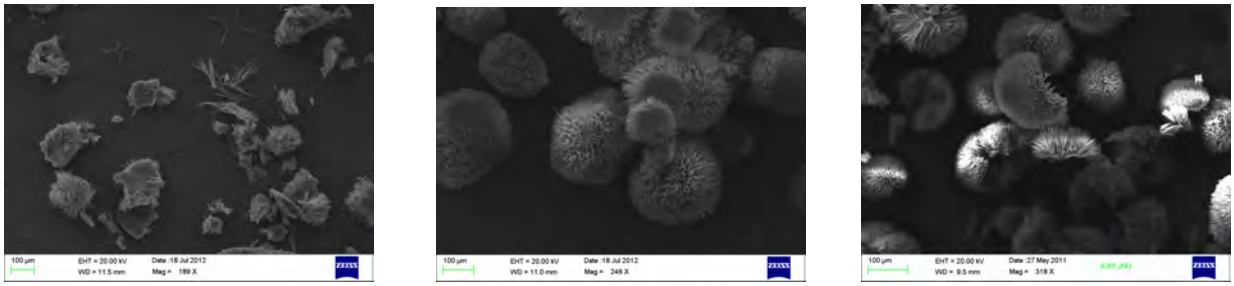
(a) (b)

*Figure S4*: (a) TEM image of 1 mM solution of **S-3** in chloroform-hexane (1:1) (b) TEM image of 1 mM solution of **R-3** in chloroform-hexane (1:1)



(a) (b)

*Figure S5*: Optical micrographs of (a) 55 mM solution (gel) of **R-3** in 1:1 chloroform-hexane (b) 55 mM solution (gel) of **R-3** + **S-3** (1:1)



(a) (b) (c)

*Figure S6*: SEM image of **R-3** in (a) 22 mM solution in 1:1 $CHCl_3$: Hexane (partial gel) (b) 44 mM solution in 1:1 $CHCl_3$: Hexane (gel) (c) 55 mM solution in 1:1 $CHCl_3$: Hexane (gel)

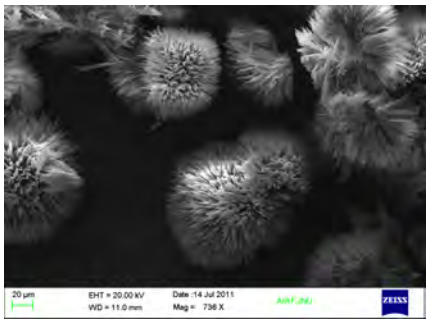
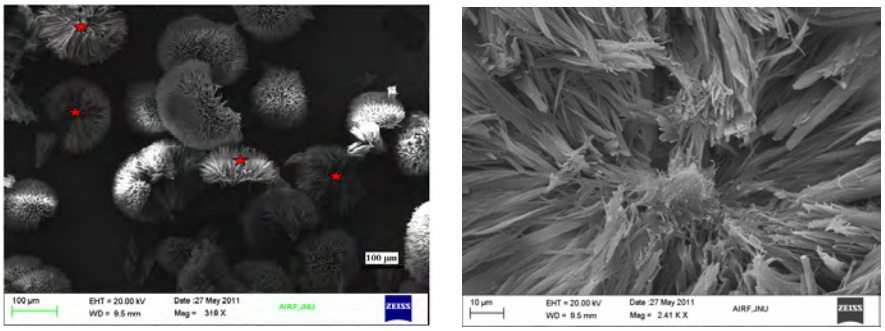


*Figure S7*: SEM image of 40 days old **R-3** gel (55 mM)



(a) (b)

*Figure S8*: SEM image of **R-3** gel (55 mM); (a) stars indicate the damaged urchin like-structures observed in SEM (b) interior part of urchin supporting the absence of a core shell.

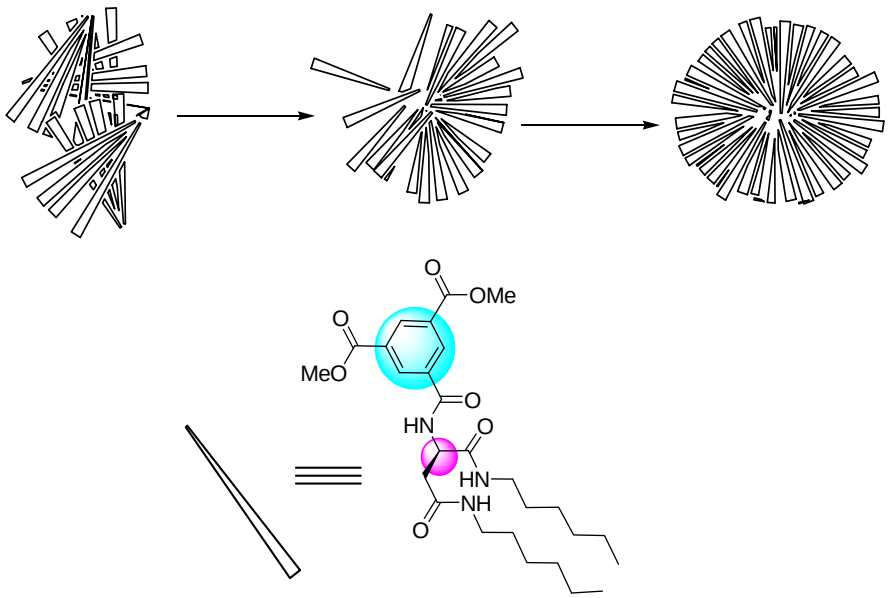
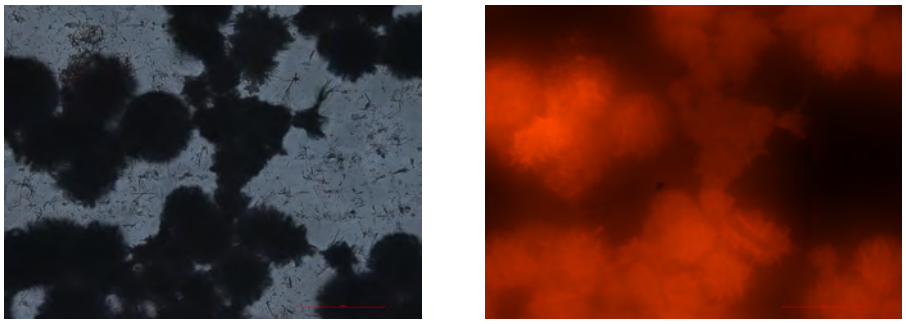
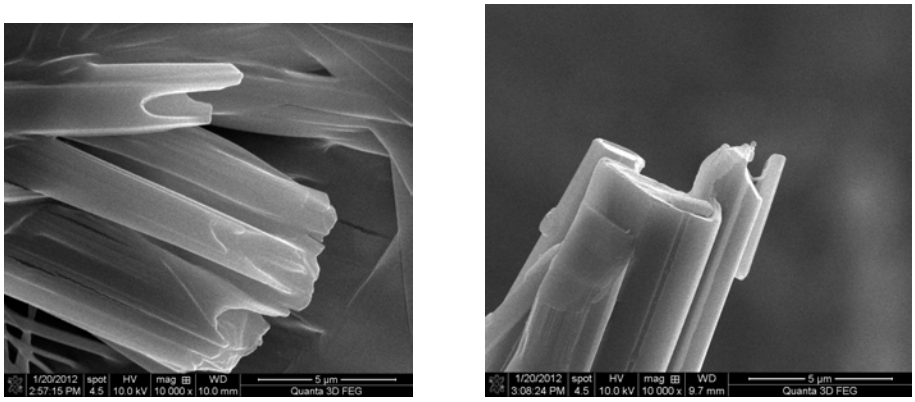



*Figure S9*: The probable mechanism of self-assembly of **S-3**/**R-3** to micro-urchin



(a) (b)

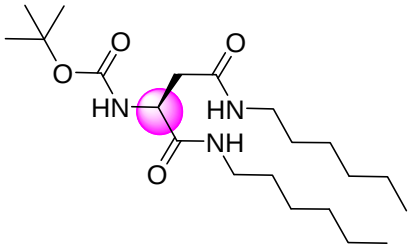
*Figure S10*: (a) Optical micrographs of 55 mM solution (gel) of **R-3** (b) Optical micrographs of 55 mM solution (gel) of **R-3**+ rhodamine -B dye



(a) (b)

*Figure S11*: Layered structure of **6a** under FESEM (a) Freshly prepared gel in ethyl acetate-hexane (1:3) (b) 25 days old gel from ethyl acetate-hexane (1:3)

## Spectral Data



**S-1**

**Nature**: White solid

**M. Pt.**: 120 -122$^{o}$C

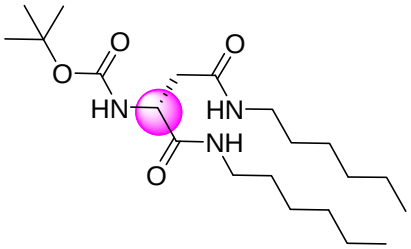
**Yield**: 68%

**$[\alpha]_D^{29}$** + 11.8 (c 0.27 g, $CHCl_3$)

**IR(KBr)**: 3328, 2927, 2858, 1686, 1654, 1530, 1459, 1369, 1308, 1248, 1173, 1049 $cm^{-1}$.

**$^1$H NMR (300 MHz, $CDCl_3$)** δ: 0.86 (t, *J* = 6.6Hz, 6H, 2x-C$\underline{H}_3$), 1.28 (brs, 12H, 2x(-C$\underline{H}_2$)$_3$C$H_3$), 1.45 (brs, 9H+4H, -OC(C$\underline{H}_3$)$_3$ + (2x-NHCH$_2$C$\underline{H}_2$), 2.50 (dd, $J_1$ = 14.7Hz, $J_2$ = 6.3Hz, 1H, Asp(C$\underline{H}_2$)), 2.83 (dd, $J_1$ = 14.7Hz, $J_2$ = 6.3Hz, 1H, Asp(C$\underline{H}_2$)), 3.15-3.22 (brm, 4H, (2x-NHC$\underline{H}_2$CH$_2$)), 4.40 (brs, 1H, Asp(C$\underline{H}$), 6.23-6.25 (brs, 2H, -N$\underline{H}$), 7.00 (brs, 1H, -N$\underline{H}$); **$^{13}$C NMR (75 MHz, $CDCl_3$)** δ: 14.0 (C$\underline{H}_3$), 22.5 (C$\underline{H}_2$), 26.5 (C$\underline{H}_2$), 28.2 C(C$\underline{H}_3$)$_3$), 29.3 (C$\underline{H}_2$), 31.4 (C$\underline{H}_2$), 37.7 (Asp (C$\underline{H}_2$)), 39.6 (-NH(C$\underline{H}_2$)), 51.5 (Asp (C$\underline{H}$)), 80.0 (-C(CH$_3$)$_3$), 156.0 (CO), 171.0 (CO), 171.2 (CO).

**HRMS** calcd for $C_{21}H_{41}N_3O_4Na$ m/z 422.2995, found m/z 422.3031.



**R-1**

**Nature**: White solid

**M. Pt.**: 120-122$^{o}$C

**Yield**: 80%

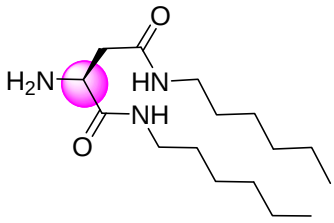
**$[\alpha]_D^{29}$** -11.8 (c 0.27g, $CHCl_3$)

**IR (KBr)**: 3329, 2928, 2858, 1686, 1654, 1530, 1459, 1369, 1308, 1248, 1173, 1049 $cm^{-1}$.

**$^1$H NMR (300 MHz, $CDCl_3$)** δ: 0.87 (t, *J* = 6.3Hz, 6H, 2x-C$\underline{H}_3$), 1.28 (s, 12H, 2x(-C$\underline{H}_2$)$_3$C$H_3$), 1.44 (brs, 9H+4H, -OC(C$\underline{H}_3$)$_3$ + (2x-NHCH$_2$C$\underline{H}_2$), 2.52 (dd, $J_1$ = 14.4Hz, $J_2$ = 6.3Hz, 1H, Asp(C$\underline{H}_2$)), 2.83

(dd, $J_1$ = 14.7Hz, $J_2$ = 6.3Hz, 1H, Asp(C<u>H</u>$_2$)), 3.20-3.22 (m, 4H, (2x-NHC<u>H</u>$_2$CH$_2$)), 4.42 (brs, 1H, Asp(C<u>H</u>)), 6.36 (brs, 1H, -N<u>H</u>), 6.42 (brs, 1H, -N<u>H</u>), 7.00 (brs, 1H, -N<u>H</u>); **$^{13}$C NMR (75 MHz, CDCl$_3$)** δ: 14.0 (<u>C</u>H$_3$), 22.6 (<u>C</u>H$_2$), 26.5 (<u>C</u>H$_2$), 28.3 C(<u>C</u>H$_3$)$_3$), 29.4 (<u>C</u>H$_2$), 31.5 (<u>C</u>H$_2$), 37.8 (Asp <u>C</u>H$_2$), 39.7 (NH<u>C</u>H$_2$), 51.6 (Asp <u>C</u>H), 80.1 (-<u>C</u>(CH$_3$)$_3$), 156.0 (CO), 171.0 (CO), 171.3 (CO).

**HRMS** calcd for $C_{21}H_{41}N_3O_4Na$ m/z 422.2995, found m/z 422.2989.




**S-2**

**Nature**: White solid

**M. Pt.**: 127-130$^o$C

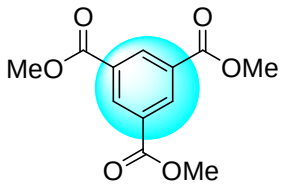
**Yield**: Quantitative

**IR (KBr)**: 3288, 3094, 2925, 2856, 1635, 1557, 1460, 1317, 1219 cm$^{-1}$.

**$^1$H NMR (300 MHz, CDCl$_3$)**: δ 0.89 (s, 6H, 2x-CH$_2$C<u>H</u>$_3$), 1.28 (brs, 12H, 2x(-C<u>H</u>$_2$)$_3$CH$_3$), 1.49 (brs, , 4H, (2x-NHCH$_2$C<u>H</u>$_2$)), 2.92 (s, 2H, Asp(C<u>H</u>$_2$)), 3.22 (m, 4H, (2x-NHC<u>H</u>$_2$CH$_2$)), 4.53 (brs, 1H, Asp(C<u>H</u>)), 7.14 (brs, 1H, -N<u>H</u>), 7.81 (brs, 1H, -N<u>H</u>); **$^{13}$C NMR (75 MHz, CDCl$_3$)**: δ 14.0 (<u>C</u>H$_3$), 22.6 (<u>C</u>H$_2$), 26.6 (<u>C</u>H$_2$), 29.5 (CH$_2$), 31.5 (<u>C</u>H$_2$), 33.9 (Asp <u>C</u>H$_2$), 39.4 (<u>C</u>H$_2$), 52.8 (Asp <u>C</u>H), 171.0 (CO), 173.8 (CO).

**HRMS** calcd for $C_{16}H_{33}N_3O_2Na$ m/z 322.2465, found m/z 322.2455.

.




**Nature**: White solid

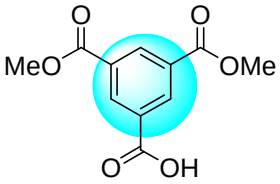
**M. Pt.**: 140$^o$C-142$^o$C

**Yield**: 90%

**IR (KBr)**: 3093, 2956, 1731, 1441, 1252 cm$^{-1}$.

**$^1$H NMR (CDCl$_3$, 300 MHz)**: δ 3.92 (s, 9H), 8.80 (s, 3H).

**HRMS** calcd for $C_{12}H_{13}O_6$ m/z 253.0712, found m/z 253.0702




**Nature**: White solid

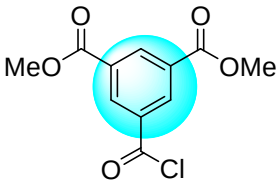
**M.Pt**: 146 -148$^{o}$C

**Yield**: 94%

**IR (KBr)**: 3318, 3009, 2958, 2658, 2582, 1735, 1697, 1607, 1445, 1381, 1277, 1192, 1148, 1106, 994 $cm^{-1}$.

**$^{1}$H NMR (300 MHz, DMSO-$d_6$)** δ: 3.93 (s, 6H, -COOC<u>H</u>$_3$), 8.62-8.68 (brs, 3H, Ar<u>H</u>).

**HRMS** calcd for $C_{11}H_{10}O_6K$ m/z 277.0114, found m/z 277.0120.

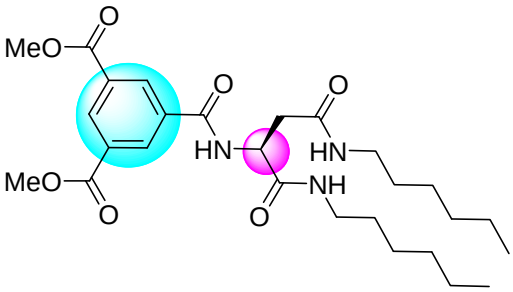



**Nature**: White solid

**M. Pt.**: 140 -142$^{o}$C

**Yield**: Quantitative

$^{1}$H NMR (300MHz, $CDCl_3$) δ: 4.01 (s, 6H, -COOC$H_3$), 8.92 (s, 2H, ArH), 8.96 (s, 1H, ArH) ppm.




**S-3**

**Nature**: White solid

**M. Pt.**: 205 -207$^{o}$C

**$R_f$**: 0.42 ($CHCl_3$: MeOH:: 97:3)

**Yield**: 43%

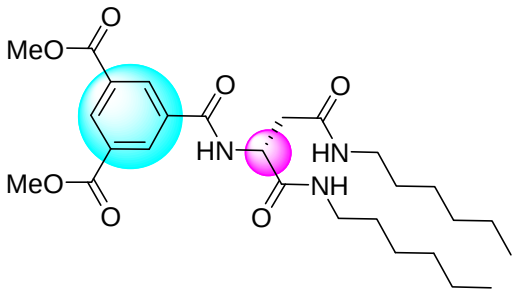
**$[\alpha]_D^{28}$** +23.3 (c 0.21 g, $CHCl_3$)

**IR (KBr)**: 3293, 3092, 2929, 2860, 1736, 1640, 1548, 1441, 1370, 1246 $cm^{-1}$.

**$^1$H NMR (300 MHz, $CDCl_3$)** δ**:** 0.86 (s, 6H, 2x-C<u>H</u>$_3$), 1.27 (brs, 12H, 2x(-C<u>H</u>$_2$)$_3$CH$_3$), 1.48-1.50 (brm, 4H, -NHCH$_2$C<u>H</u>$_2$), 2.62 (dd, $J_1$ = 15.0Hz, $J_2$ = 7.5Hz, 1H, Asp (C<u>H</u>$_2$)), 2.92 (dd, $J_1$ = 15.0Hz, $J_2$ = 3.0Hz, 1H, Asp (C<u>H</u>$_2$)), 3.22-3.28 (brm, 4H, 2x-NHC<u>H</u>$_2$CH$_2$-), 3.97 (s, 6H, 2x-COOC<u>H</u>$_3$), 4.88 (brs, 1H, (AspC<u>H</u>)), 6.30 (brs, 1H, -N<u>H</u>), 7.51 (brs, 1H, -N<u>H</u>), 8.49 (d, $J$ = 6.3Hz, 1H, -N<u>H</u>), 8.66 (s, 2H, Ar<u>H</u>), 8.80 (s, 1H, Ar<u>H</u>) ppm; **$^{13}$C NMR (75 MHz, $CDCl_3$):** δ 14.0 (<u>C</u>H$_3$), 22.5 (<u>C</u>H$_2$), 26.5 (<u>C</u>H$_2$), 29.4 (CH$_2$), 31.4 (<u>C</u>H$_2$), 37.8 (Asp <u>C</u>H$_2$), 39.8 (-NH<u>C</u>H$_2$), 50.8 (Asp <u>C</u>H), 52.5 (COO<u>C</u>H$_3$), 131.1 (ArC), 132.2 (ArC), 133.5 (ArC), 134.5 (ArC), 165.3 (amide CO+ester CO), 170.7 (CO), 171.0 (CO).

**HRMS** calcd for $C_{27}H_{42}N_3O_7$ m/z 520.3023, found m/z 520.3026

**HRMS** calcd for $C_{27}H_{41}N_3O_7Na$ m/z 542.2842, found m/z 542.2851.




**R-3**

**Nature**: White solid

**M. Pt.**: 204 -207$^o$C

**$R_f$**: 0.42 ($CHCl_3$: MeOH :: 97:3)

**Yield:** 60%

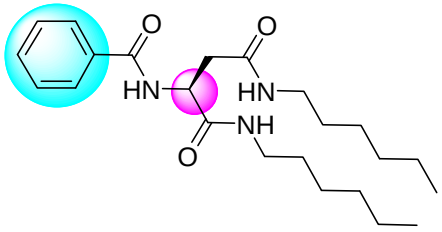
**$[\alpha]_D$** -23.3 (c 0.21 g, $CHCl_3$)

**IR (KBr)**: 3292, 3093, 2929, 2859, 1736, 1640, 1546, 1440, 1370, 1246, 1108, 1000 $cm^{-1}$.

**$^1$H NMR (300 MHz, $CDCl_3$)** δ**:** 0.87 (s, 6H, 2xCH$_2$C<u>H</u>$_3$), 1.28 (brs, 12H, 2x(C<u>H</u>$_2$)$_3$CH$_3$), 1.44-1.51 (brm, 4H, 2x-NHCH$_2$C<u>H</u>$_2$-), 2.61 (dd, $J_1$ = 15.0Hz, $J_2$ = 7.5Hz, 1H, Asp (C<u>H</u>$_2$)), 2.91 (dd, $J_1$ = 15.0Hz, $J_2$ = 3.0Hz, 1H, Asp (C<u>H</u>$_2$)), 3.24-3.27 (brm, 4H, 2x-NHC<u>H</u>$_2$-), 3.97 (s, 6H, 2xCOOC<u>H</u>$_3$), 4.86 (brs, 1H, Asp(C<u>H</u>)), 6.16 (brs, 1H, -N<u>H</u>), 7.50 (brs, 1H, -N<u>H</u>), 8.47 (d, $J$ = 6.3Hz, 1H, -N<u>H</u>), 8.66 (s, 2H, Ar<u>H</u>), 8.81 (s, 1H, Ar<u>H</u>); **$^{13}$C NMR (75 MHz, $CDCl_3$)**: δ 14.0 (<u>C</u>H$_3$), 22.5 (<u>C</u>H$_2$), 26.5 (<u>C</u>H$_2$), 29.3

($CH_2$), 31.4 ($\underline{C}H_2$), 37.7 (Asp $\underline{C}H_2$), 39.8 (-NH$\underline{C}H_2$), 50.6 (Asp $\underline{C}$H), 52.6 (COO$\underline{C}H_3$), 131.3 (ArC), 132.2 (ArC), 133.6 (ArC), 134.5 (ArC), 165.4 (amide CO + ester CO), 170.3 (CO), 171.4 (CO).

HRMS calcd for $C_{27}H_{41}N_3O_7Na$ m/z 542.2842, found m/z 542.2830.




**S-4**

**Nature**: White solid

**M. Pt.**: 206-208$^oC$

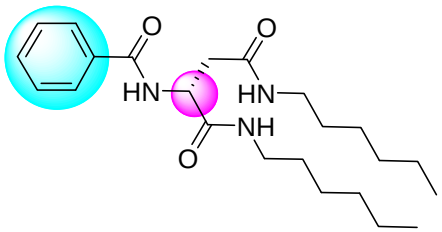
**Yield**: 74%

**$[\alpha]_D^{29}$**+ 23.0 (c 0.13 g, $CHCl_3$)

**IR (KBr)**: 3311, 2926, 2858, 1642, 1536, 1459, 1373, 1327, 1253, 1157, 681 $cm^{-1}$.

**$^1$H NMR (300 MHz, $CDCl_3$)** δ**:** 0.86 (brt, *J* = 6.9Hz, 6H, 2x-$CH_2C\underline{H}_3$), 1.26-1.29 (brm, 12H, -$(C\underline{H}_2)_3CH_3$), 1.47-1.52 (brm, 4H, -$NHCH_2C\underline{H}_2$-), 2.53 (dd, $J_1$ = 14.7Hz, $J_2$ = 6.6Hz, 1H, Asp($C\underline{H}_2$), 2.93 (dd, $J_1$ = 14.7Hz, $J_2$ = 3.0Hz, 1H, Asp$C\underline{H}_2$), 3.20-3.33 (brm, 4H, -NHC$\underline{H}_2$), 4.82-4.86 (brm, 1H, Asp(C$\underline{H}$)), 6.12 (brs, 1H, -N$\underline{H}$), 7.30 (brs, 1H, -N$\underline{H}$) 7.43-7.56 (brm, 3H, Ar$\underline{H}$), 7.87 (d, *J* = 6.9Hz, 2H, Ar$\underline{H}$), 8.50 (d, *J* = 6.0Hz, 1H, -N$\underline{H}$); **$^{13}$C NMR (75 MHz, $CDCl_3$)** δ: 14.0 ($\underline{C}H_3$), 22.5 ($\underline{C}H_2$), 26.5 ($\underline{C}H_2$), 29.3 ($CH_2$), 31.4 ($\underline{C}H_2$), 37.1 (Asp $\underline{C}H_2$), 39.7 (-NH$\underline{C}H_2$), 50.7 (Asp $\underline{C}$H), 127.2 (ArC), 128.5 (ArC), 132.0 (ArC), 133.2 (ArC), 167.3 (CO), 171.0 (CO), 171.5 (CO).

HRMS calcd for $C_{23}H_{37}N_3O_3Na$ m/z 427.2733, found m/z 426.2728.




**R-4**

**Nature**: White solid

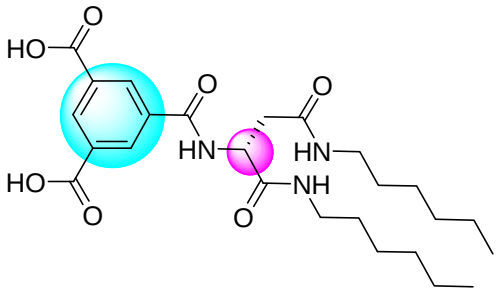
**M. Pt.**: 177 -179$^oC$

**Yield**: 62 %

**$[\alpha]_D^{29}$** -23.0 (c 0.13 g, $CHCl_3$)

**IR (KBr)**: 3287, 3074, 2926, 2858, 1637, 1540, 1461, 1432, 1369, 1302, 1199, 711 $cm^{-1}$.

**$^1$H NMR (300 MHz, $CDCl_3$)** δ: 0.86 (t, *J* = 6.9Hz, 6H, 2x-$CH_2CH_3$), 1.28 (s, 12H, 2x-$(CH_2)_3CH_3$) 1.47-1.52 (brm, 4H, 2x-$NHCH_2CH_2$-), 2.53 (dd, $J_1$ = 14.7Hz, $J_2$ = 6.6Hz, 1H, Asp($CH_2$)), 2.93 (dd, $J_1$ = 14.7Hz, $J_2$ = 3.0Hz, 1H, Asp($CH_2$)), 3.20-3.31 (brm, 4H, 2x-$NHCH_2$-), 4.81-4.87 (brs, 1H, Asp(CH)), 6.03 (brs, 1H, -NH), 7.30 (brs, 1H, -NH), 7.43-7.53 (brm, 3H, ArH), 7.87 (d, *J* = 6.3Hz, 2H, ArH) 8.50 (d, *J* = 6.3Hz, 1H, -NH); **$^{13}$C NMR (75 MHz, $CDCl_3$)** δ: 14.0 ($CH_3$), 22.5 ($CH_2$), 26.5 ($CH_2$), 29.3 ($CH_2$), 31.4 ($CH_2$), 37.0 (Asp $CH_2$), 39.7 (-$NHCH_2$), 50.6 (Asp CH), 127.2 (ArC), 128.6 (ArC), 132.0 (ArC), 133.2 (ArC), 167.3 (CO), 170.6 (CO), 171.8 (CO).
HRMS calcd for $C_{23}H_{37}N_3O_3Na$ m/z 427.2733, found m/z 426.2733.



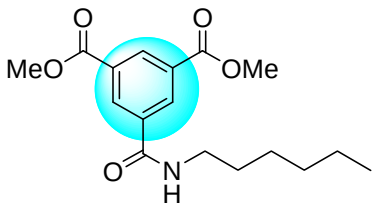

**R-5**

**Nature**: Pink solid

**M. Pt.**: 180-183$^o$C

**Yield**: Quantitative

**IR (KBr)**: 3368, 2927, 1713, 1641, 1545, 1449, 1230, 1107 $cm^{-1}$.
**$^1$H NMR (300 MHz, acetone-$d_6$):** δ 0.69 (s, 6H, -$CH_2CH_3$), 1.09-1.16 (m, 12H, 2x-$(CH_2)_3CH_3$-), 1.33 (m, 4H, 2x-$NHCH_2CH_2$-), 2.79- 2.81 (m, 2H, Asp ($CH_2$)), 3.07 (brs, 4H, 2x-$NHCH_2$-), 4.88 (brs, 1H, Asp(CH)), 7.48 (brs, 1H, -NH), 8.46-8.48 (m, 2H, -NH), 8.63-8.65 (m, 2H, ArH), 8.72 (s, 1H, ArH).
**HRMS** calcd for $C_{25}H_{37}N_3O_7Na$ m/z 514.2529, found m/z 514.2529.




**6a**

**Nature**: White solid

**M. Pt.**: 107-109$^{o}$C

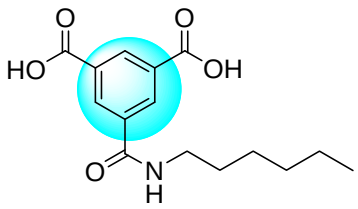
**Yield**: 84%

**IR (KBr)**: 3301, 3056, 2929, 2867, 1730, 1638, 1538, 1437, 1381, 1250, 1160, 1110, 996 cm$^{-1}$.

**$^{1}$H NMR (300 MHz, $CDCl_3$)** δ: 0.90 (t, *J* = 5.1Hz, 3H, C$\underline{H}_3$), 1.33 (brs, 6H, (-C$\underline{H}_2$)$_3$CH$_3$), 1.65 (t, *J* = 6.6Hz, 2H, -NHCH$_2$C$\underline{H}_2$), 3.49 (q, *J* = 6.3Hz, 2H, -NHC$\underline{H}_2$CH$_2$), 3.95 (s, 6H, 2x-COOC$\underline{H}_3$), 6.70 (s, 1H, N$\underline{H}$), 8.59 (s, 2H, Ar$\underline{H}$), 8.72 (s, 1H, Ar$\underline{H}$); **$^{13}$C NMR (75 MHz, $CDCl_3$)** δ: 14.0 (C$\underline{H}_3$), 22.5 (C$\underline{H}_2$), 26.7 (C$\underline{H}_2$), 29.5 (C$\underline{H}_2$), 31.5 (C$\underline{H}_2$), 40.4 (-NHC$\underline{H}_2$), 52.6 (COOC$\underline{H}_3$), 131.0 (ArC), 132.1 (ArC), 132.9 (ArC), 135.7 (ArC), 165.5 (amide CO + ester CO).

**HRMS** calcd for $C_{17}H_{23}NO_5Na$ m/z 344.1474, found m/z 344.1468.

**$^{1}$H NMR (300 MHz, DMSO-*$d_6$*)** δ: 0.87 (brs, 3H, -C$\underline{H}_3$), 1.29 (brs, 6H, (-C$\underline{H}_2$)$_3$CH$_3$), 1.53 (t, *J* = 6.3Hz, 2H, -NHCH$_2$C$\underline{H}_2$), 3.28 (q, *J* = 6.9 Hz, 2H, -NHC$\underline{H}_2$CH$_2$), 3.93 (s, 6H, 2x-COOC$\underline{H}_3$), 8.57 (brs, 1H, Ar$\underline{H}$), 8.67 (s, 2H, Ar$\underline{H}$), 8.93 (m, 1H, N$\underline{H}$).




**6b**

**Nature:** White solid

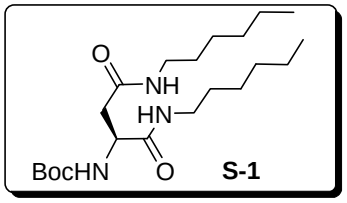
**M. Pt.**: At 280$^{o}$C, it decomposed

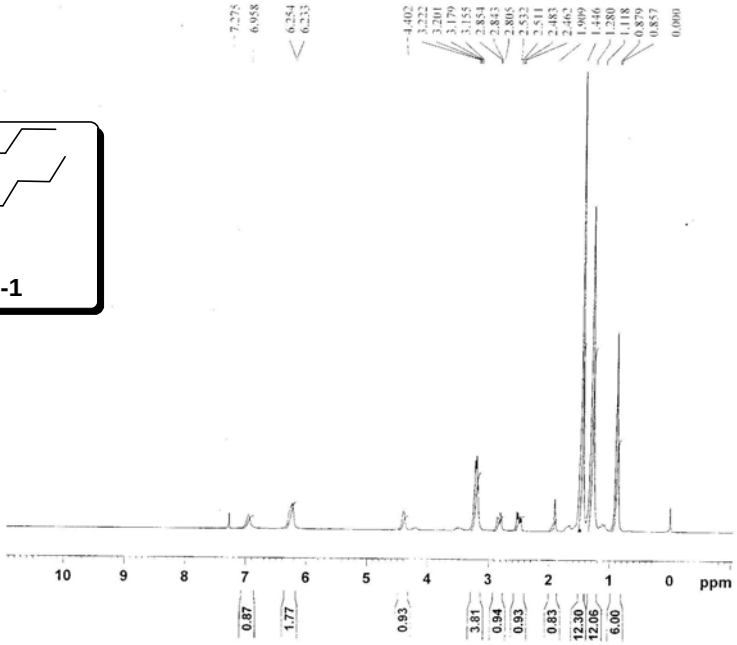
**IR (KBr)**: 3294, 2927, 2856, 2666, 2538, 1696, 1639, 1550, 1453, 1406, 1280, 1109, 928, 692 cm$^{-1}$.

**$^{1}$H NMR (300MHz, DMSO-*$d_6$*)** δ: 0.87 (s, 3H, -C$\underline{H}_3$), 1.29 (s, 6H, (-C$\underline{H}_2$)$_3$CH$_3$)), 1.54 (brs, 2H, -NHCH$_2$C$\underline{H}_2$-), 3.27 (m, 2H, -NHC$\underline{H}_2$), 8.57 (s, 1H, Ar$\underline{H}$), 8.63 (s, 2H, Ar$\underline{H}$), 8.89 (brs, 1H, -N$\underline{H}$), 13.54 (brs, 2H, -COO$\underline{H}$).

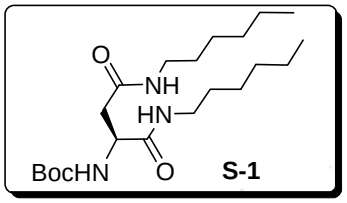
**$^{1}$H NMR (300MHz, acetone-*$d_6$*)** δ: 0.76 (t, *J* = 6.6 Hz, 3H, -C$\underline{H}_3$), 1.21-1.27 (brs, 6H, (-C$\underline{H}_2$)$_3$CH$_3$), 1.48-1.55 (m, 2H, -NHCH$_2$C$\underline{H}_2$-), 3.33 (m, 2H, -NHC$\underline{H}_2$), 8.15 (brs, 1H, -N$\underline{H}$), 8.60 (s, 2H, Ar$\underline{H}$), 8.62 (s, 1H, Ar$\underline{H}$).

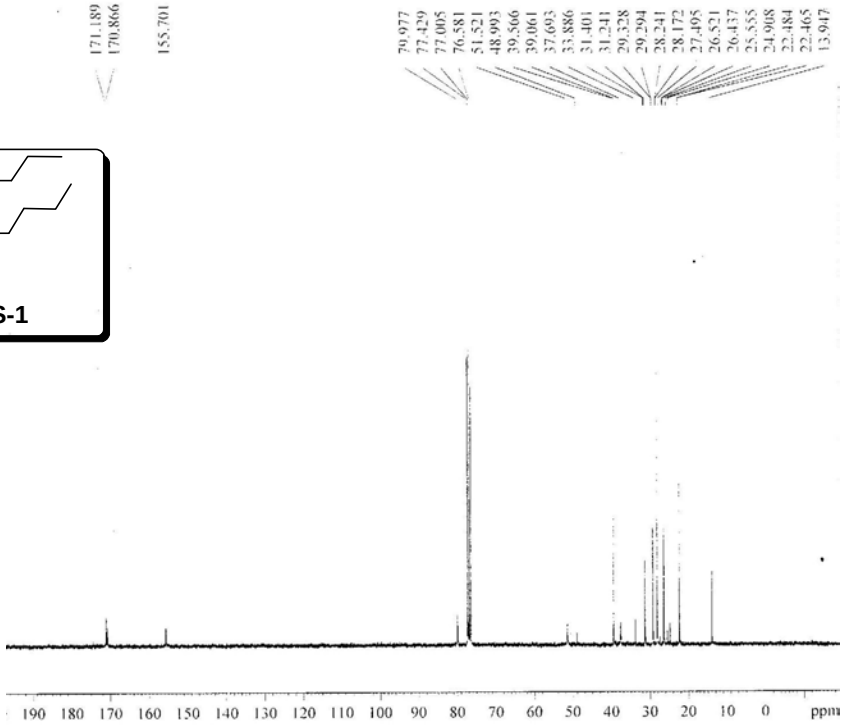
$^{1}$H NMR of **S-1** ($CDCl_3$, 300 MHz)



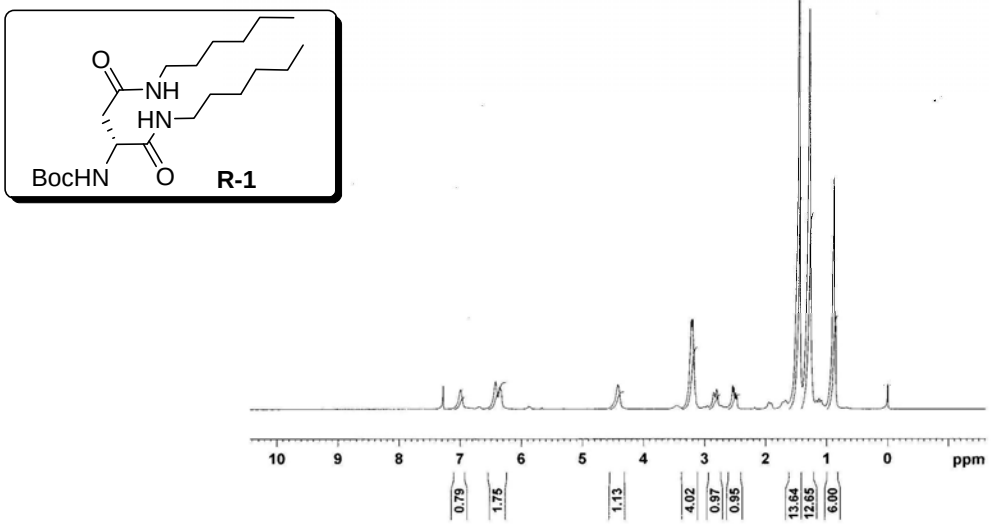




$^{13}$C NMR of **S-1** ($CDCl_3$, 300 MHz)

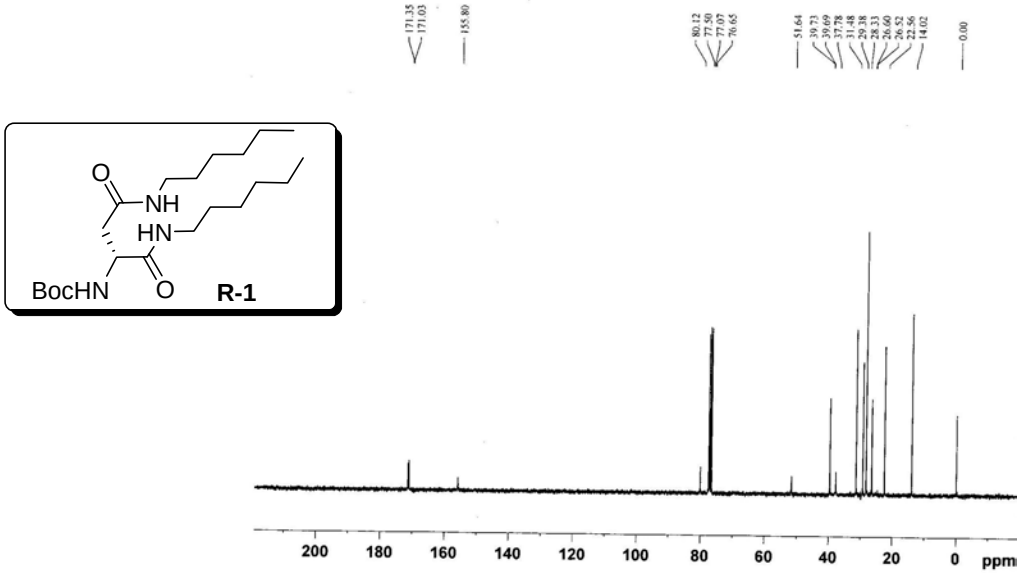


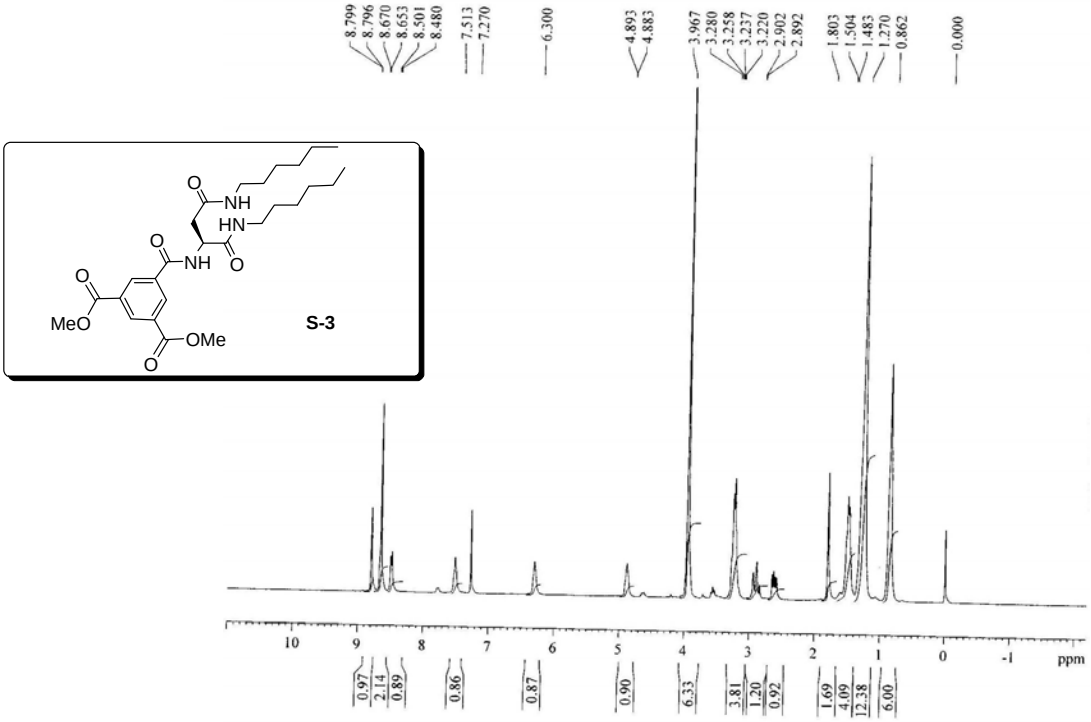
$^{1}$H NMR of **R-1** ($CDCl_3$, 300 MHz)



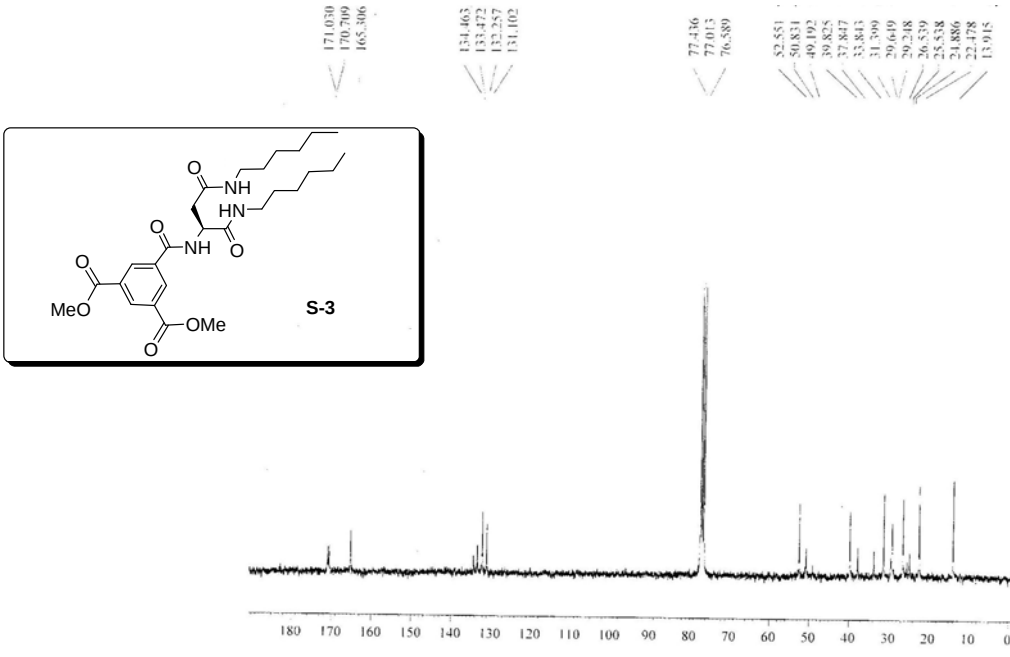

$^{13}$C NMR of **R-1** ($CDCl_3$, 300 MHz)



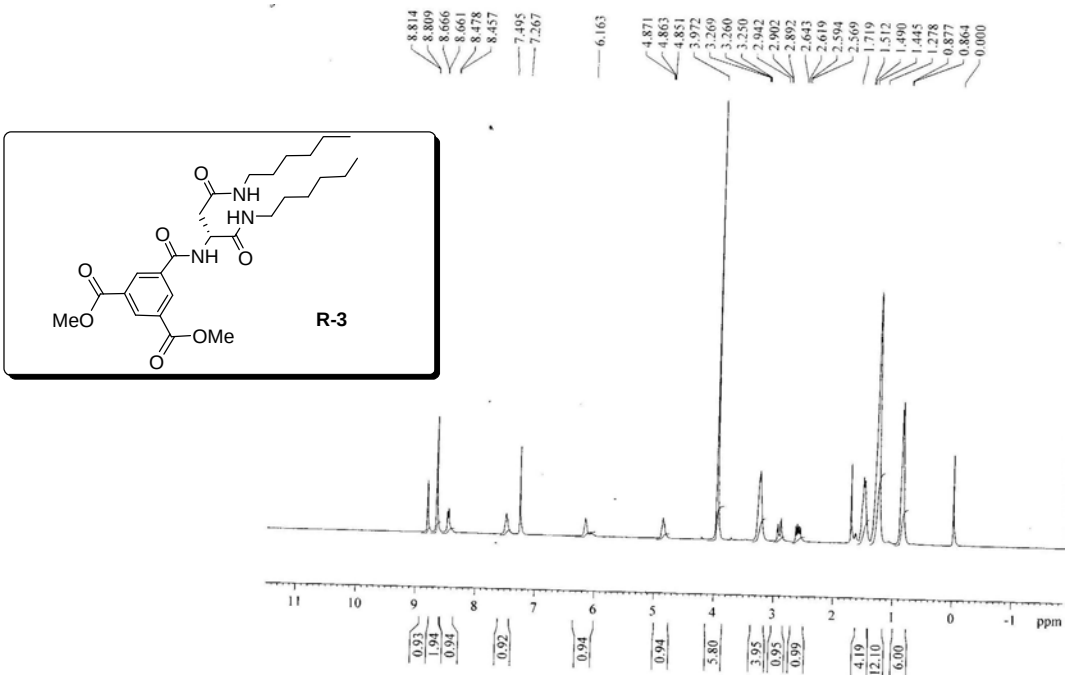
$^{1}$H NMR of **S-3** ($CDCl_3$, 300 MHz)



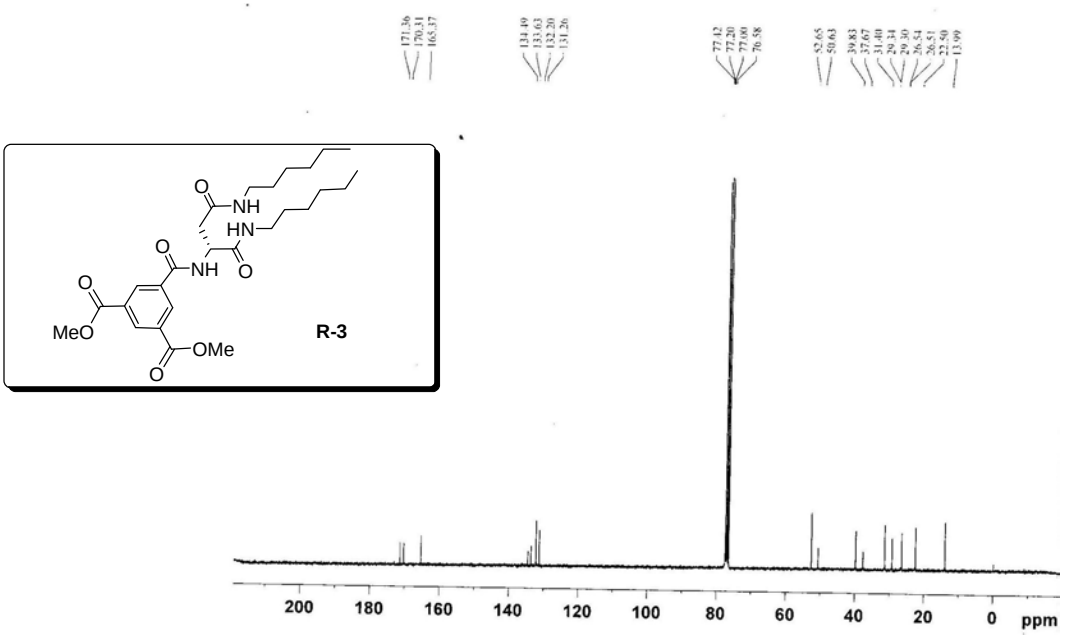

$^{13}$C NMR of **S-3** ($CDCl_3$, 300 MHz)



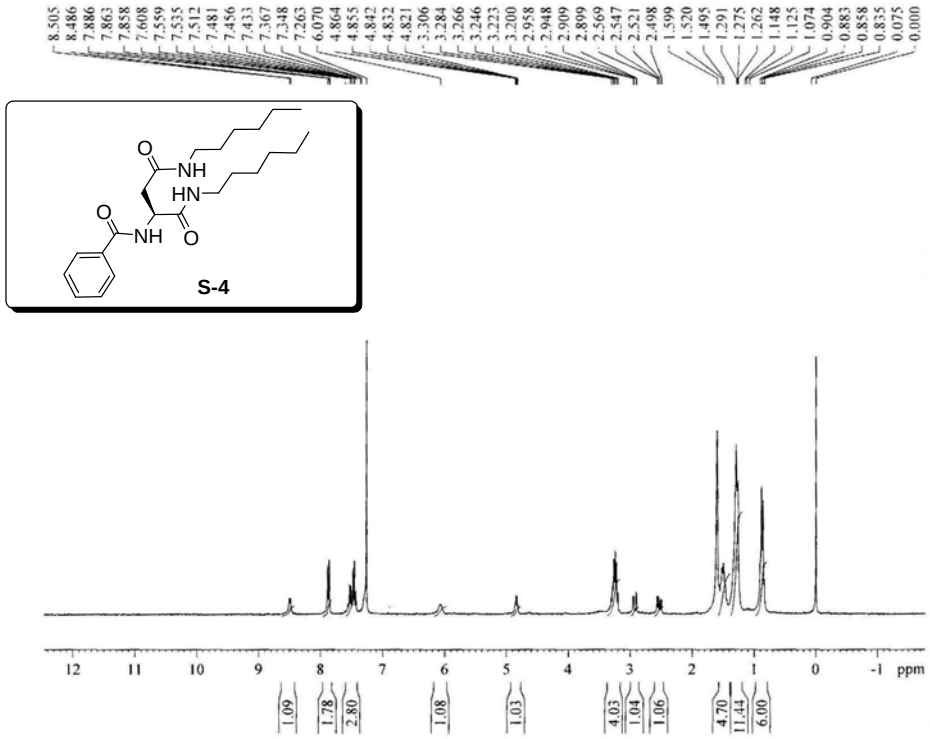
$^{1}$H NMR of R-**3** ($CDCl_3$, 300 MHz)



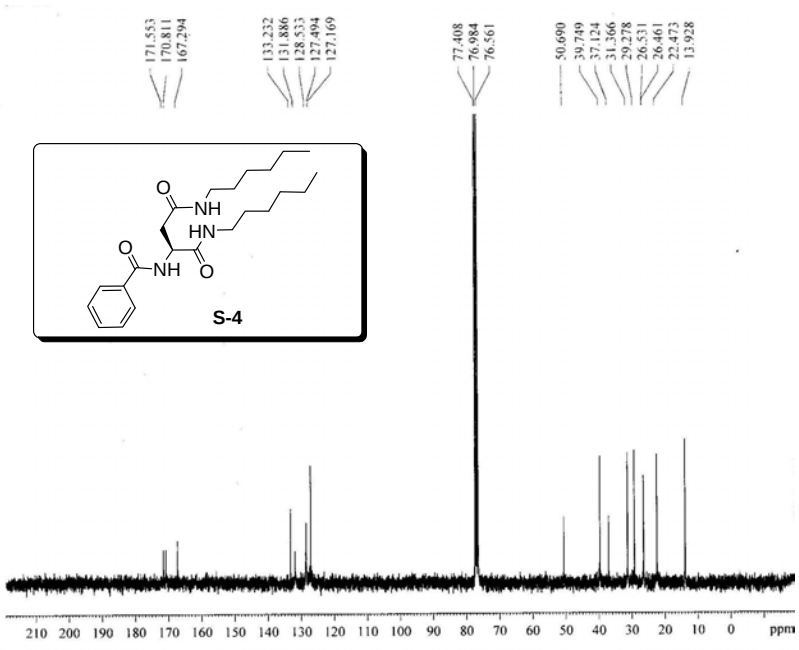

$^{13}$C NMR of **R-3** ($CDCl_3$, 300 MHz)



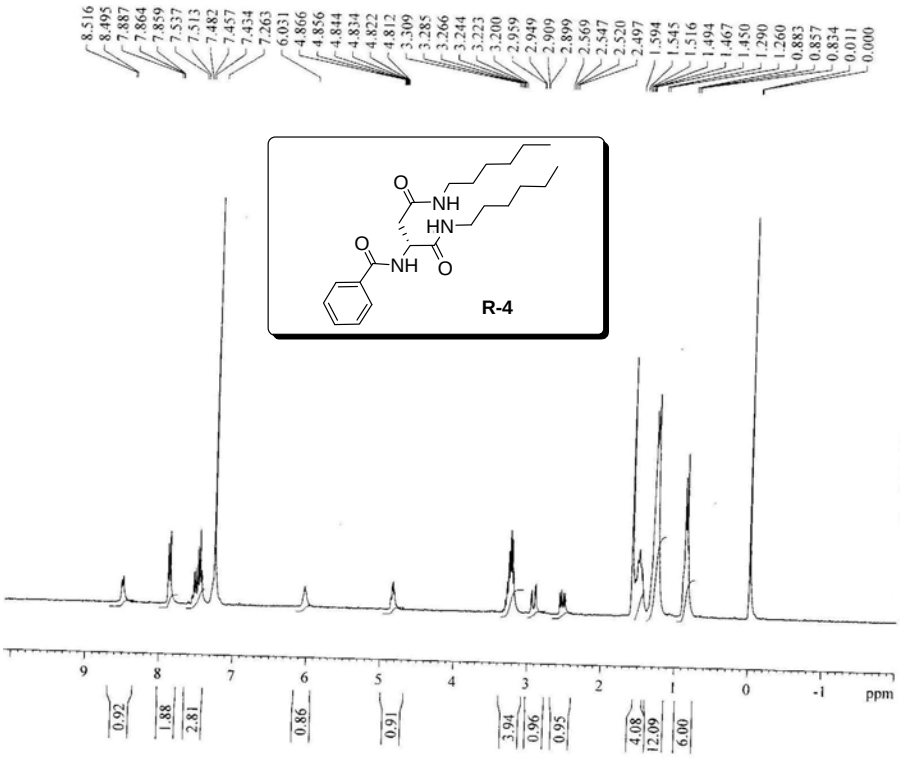
$^{1}$H NMR of **S-4** ($CDCl_3$, 300 MHz)



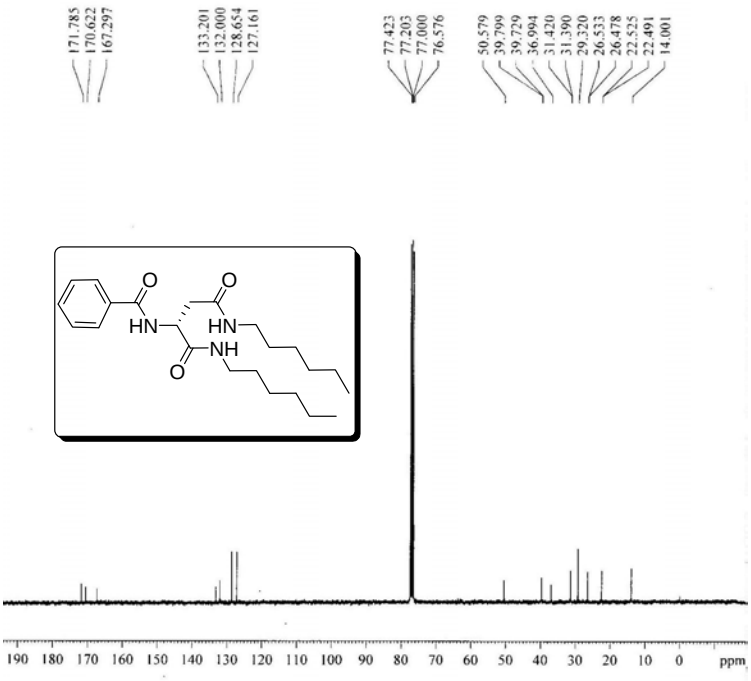

$^{13}$C NMR of **S-4** ($CDCl_3$, 300 MHz)



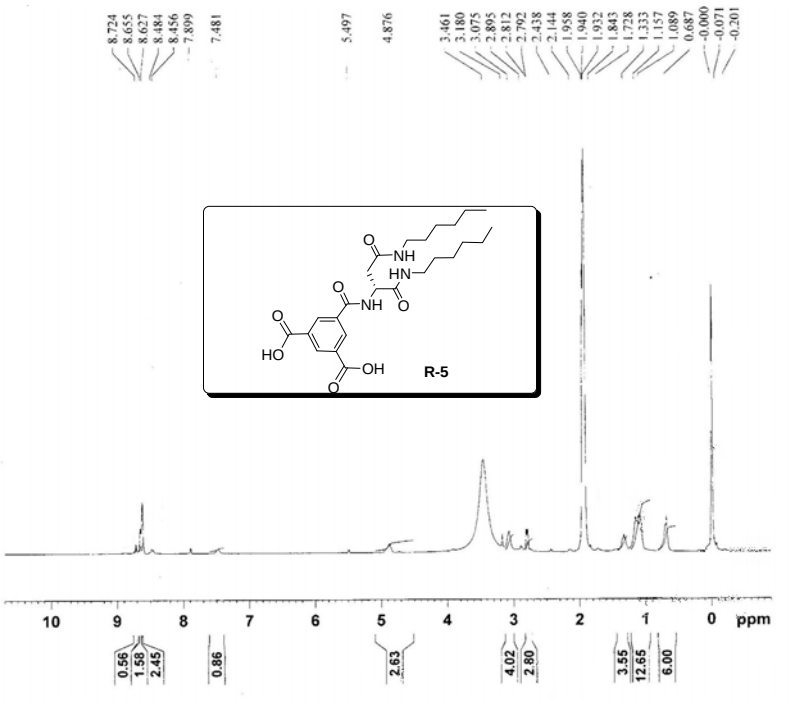
$^{1}$H NMR of **R-4** ($CDCl_3$, 300 MHz)



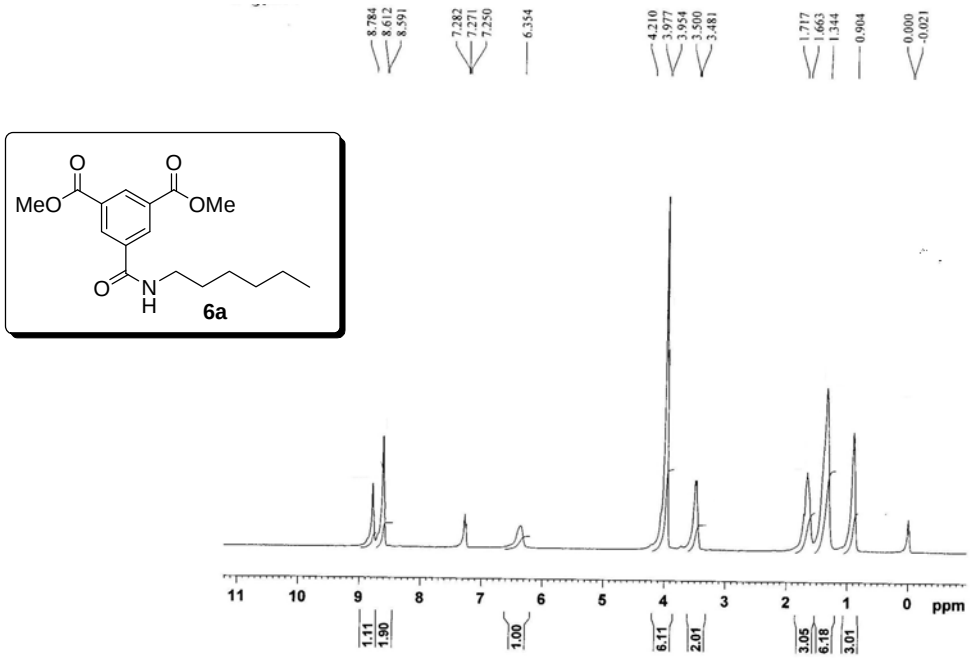

$^{13}$C NMR of **R-4** ($CDCl_3$, 300 MHz)



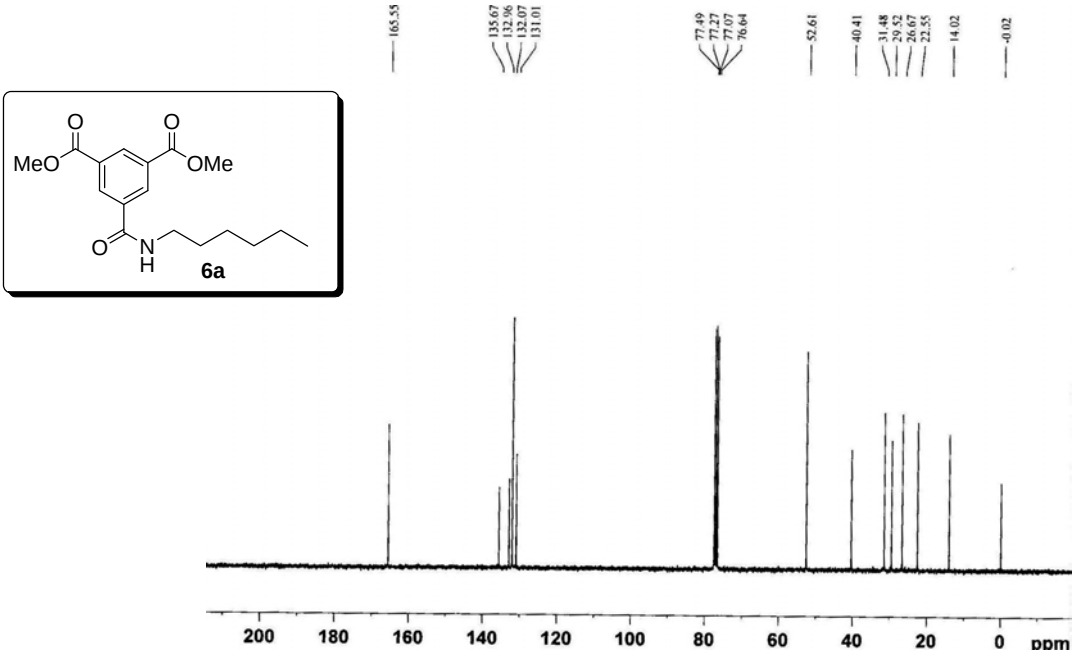
$^1$H NMR of **R-5** (*acetone-$d_6$*, 300 MHz)




$^1$H NMR of **6a** ($CDCl_3$, 300 MHz)



$^{13}$C NMR of **6a** ($CDCl_3$, 300 MHz)




$^{1}$H NMR of **6b** (DMSO-$d_6$, 300 MHz)