SUPPLEMENTARY INFORMATION

Photodegradation of amyloid β and reduction of its cytotoxicity to PC12 cells using porphyrin derivatives

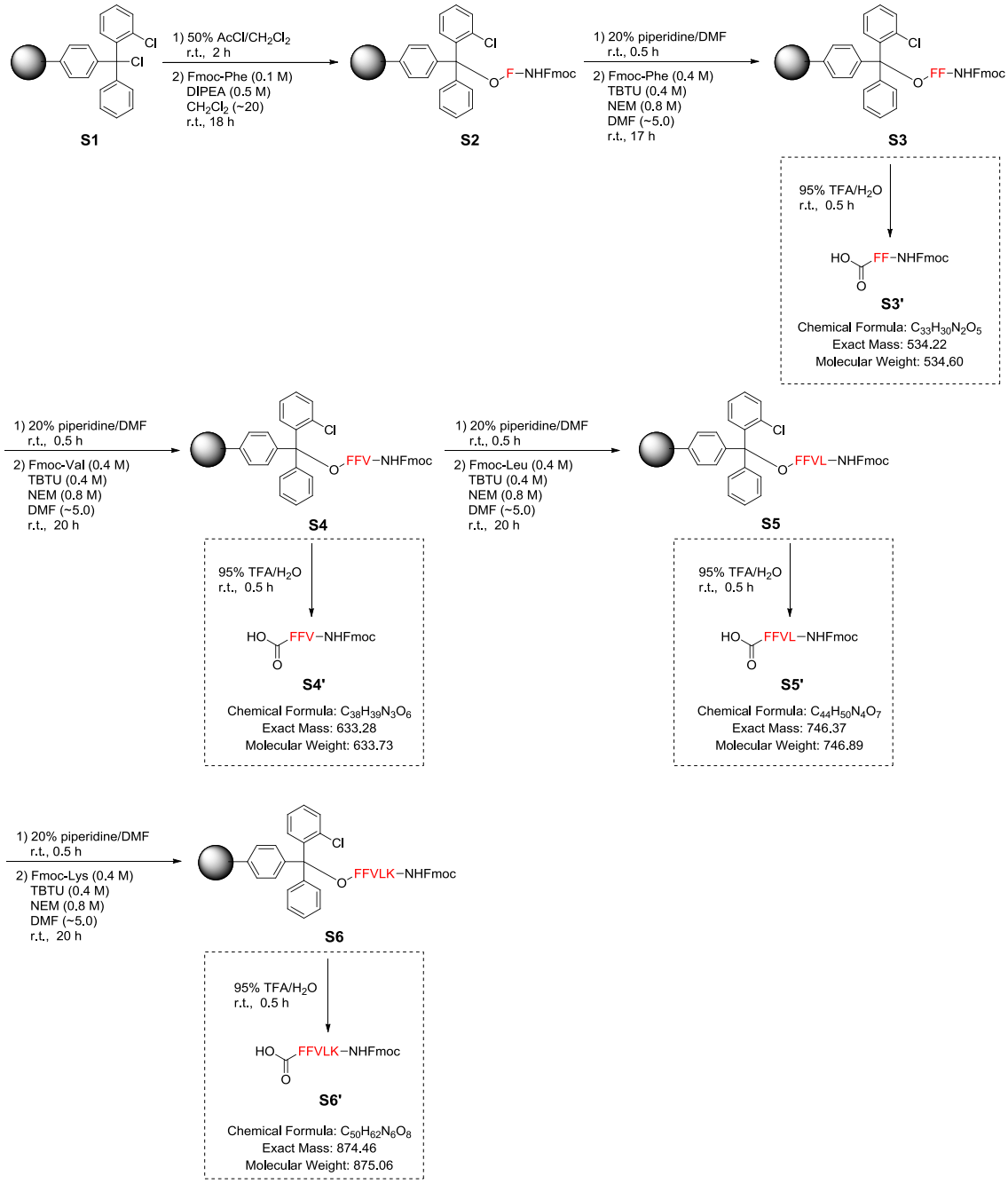
Ayumi Hirabayashi,[a] Yutaka Shindo,[b] Kotaro Oka,[b] Daisuke Takahashi,[a] and Kazunobu Toshima[a]*

[a]Department of Applied Chemistry and [b]Department of Bioscience and Informatics, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan.
E-mail: toshima@applc.keio.ac.jp; Fax: +81 45-566-1576.

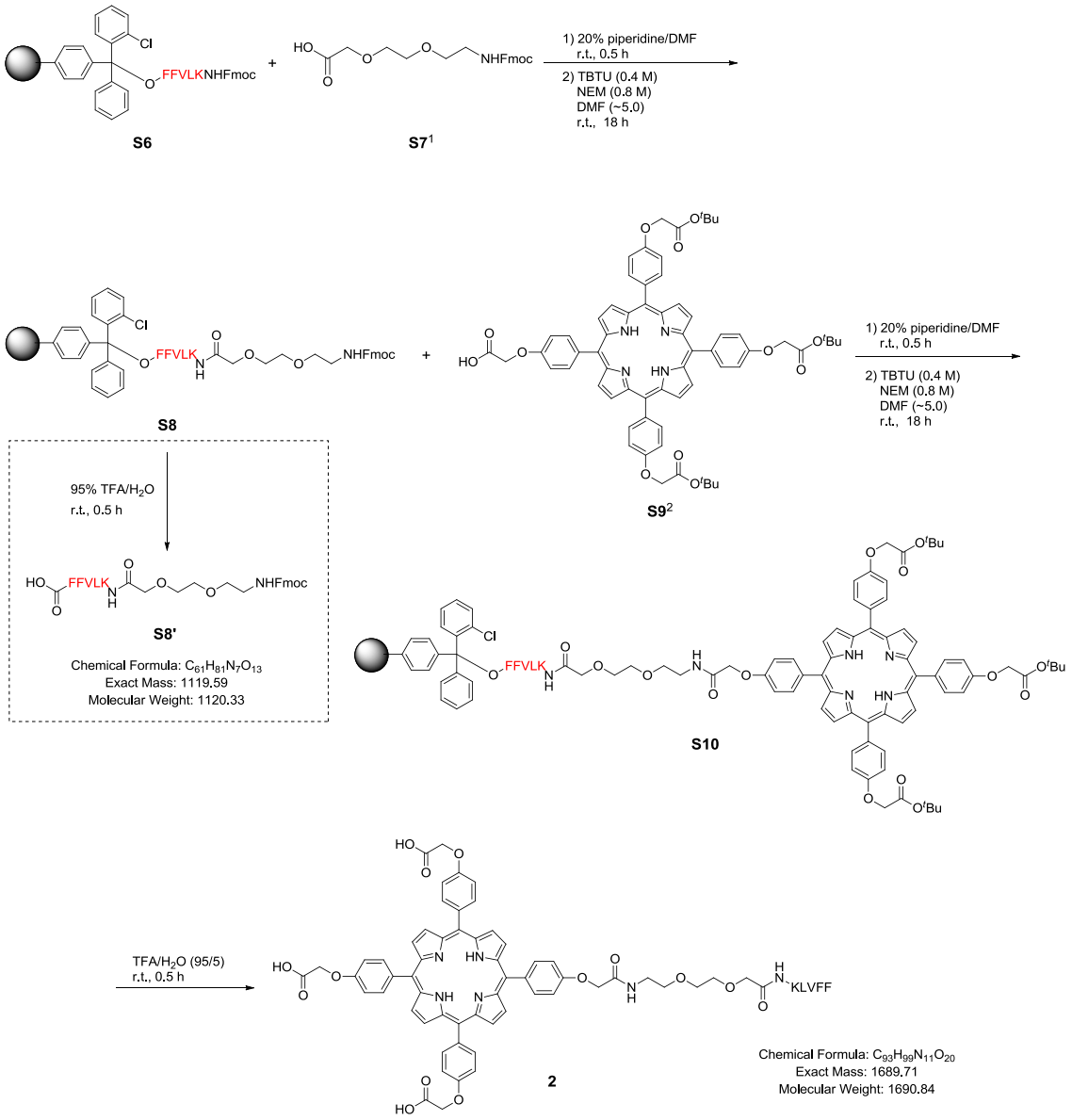
General methods for chemical synthesis.

NMR spectra were recorded on a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer in the indicated solvent. Melting points were determined on a micro hot-stage (Yanako MP-S3) and were uncorrected. ESI-TOF Mass spectra were measured on a Waters LCT premier XE. MALDI TOF MS spectra were measured on a Bruker Ultra flex. The reactions were monitored by thin layer chromatography carried out on Merck TLC 60F-254 (0.25 mm) using UV light as developing agent. Column chromatography separations were performed using silica gel 60 N (spherical, neutral) (Kanto Chemical Co., Inc.). Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon using oven-dried glass ware. Analytical and preparative reverse-phase high performance liquid chromatographies (RP-HPLC) were performed on a JASCO apparatus HPLC using analytical Mightysil RP-18 GP® (5 μm, 4.6 × 150 mm, (Kanto Chemical Co., Inc.)) and Develosil® (ODS-HG-5, 20 × 250 mm, (Nomura Chemical Co., Ltd.), respectively, with flow rate of 1.00 mL/min. Detection of products was made by UV detector (JASCO, UV-2075 Plus). All RP-HPLC procedure was carried out with a liner gradient using H_2O and MeCN with 0.1wt% TFA. Analytical ultra performance liquid chromatography (UPLC) was performed on a ACQUITY UPLC® (BEH C18, 1.7 μm, 2.1 × 50 mm, (Waters)); 20 °C; detection by UV (254 nm); 100:0 to 0:100 MeCN/H_2O, 15 min; flow rate 0.2 mL/min.

Solid-phase synthesis of porphyrin-peptide hybrid 2 (Scheme S1).



Scheme S1



Scheme S1 (continued)

General procedure for loading of *N*-Fmoc protected amino acid on 2-chlorotrityl resin.

The 2-chlorotrityl resin (459 mg, 1.52 mmol/g, 200-400 mesh) was treated with acetyl chloride/CH_2Cl_2 (1:1 v/v) at room temperature for 2 h. After filtration, the resin was washed five times with CH_2Cl_2. The resin was treated with a solution of *N*-Fmoc protected amino acid (0.100 M) and DIPEA (0.500 M) in CH_2Cl_2 and gently shaken at room temperature for 18 h. After filtration, the resin was washed MeOH, three times with CH_2Cl_2, three times with 20% THF/CH_2Cl_2, three times with Et_2O, and dried in *vacuo* to give Fmoc amino acid linked resin.

General procedure for deprotection of Fmoc group.

The Fmoc protected peptide linked resin was treated with a solution of 20% piperidine in DMF at room temperature for 30 min. After filtration, the resin was washed three times with DMF (5 min per wash) and three times with MeOH, and dried in *vacuo* to give deprotected peptide linked resin.

General procedure for coupling of Fmoc amino acid and deprotected peptide on resin.

The deprotected resin was treated with a solution of Fmoc amino acid (0.400 M) and coupling reagent (amino acid: TBTU: NEM= 0.400 M: 0.440M: 0.800 M) in DMF and gently shaken at room temperature for 17 h. After filtration, the resin was washed three times with CH_2Cl_2, three times with DMF, three times with 20% THF/CH_2Cl_2, three times with MeOH, and three times with Et_2O, and dried in *vacuo* to give peptide linked resin.

General procedure for cleavage of peptide from resin.

The peptide linked resin was treated with a solution of 95% TFA/CH_2Cl_2 at room temperature for 30 min. After filtration, the resin was washed three times with CH_2Cl_2, three times with TFA, and three times with CH_2Cl_2, and dried in *vacuo* to give crude peptide.

Data for Fmoc-Phe-Phe-OH (S3'):

Purity 86%; UPLC (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm (Waters); 20 °C; detection by UV (254 nm); 100:0 to 0:100 MeCN/H_2O; 15 min; flow rate 0.2 mL/min); Rt 7.71 min; LRMS (ESI-TOF) *m*/*z* 533.21 (533.2076 calcd. for $C_{33}H_{29}N_2O_5$, $[M-H]^-$).

Data for Fmoc-Val-Phe-Phe-OH (S4'):

Purity 92%; UPLC (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm (Waters); 20 °C; detection by UV (254 nm); 100:0 to 0:100 MeCN/H_2O; 15 min; flow rate 0.2 mL/min); Rt 7.83 min; LRMS (ESI-TOF) *m*/*z* 632.27 (632.2761 calcd. for $C_{38}H_{38}N_3O_6$, $[M-H]^-$).

Data for Fmoc-Leu-Val-Phe-Phe-OH (S5'):

Purity 77 %; UPLC (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm (Waters); 20 °C; detection by UV (254 nm); 100:0 to 0:100 MeCN/H_2O; 15 min; flow rate 0.2 mL/min); Rt 8.29 min; LRMS (ESI-TOF) *m*/*z* 745.35 (745.3601 calcd. for $C_{44}H_{49}N_4O_7$, $[M-H]^-$).

Data for Fmoc-Lys-Leu-Val-Phe-Phe-OH (S6'):

Purity 79%; UPLC (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm (Waters); 20 °C; detection by UV (254 nm); 100:0 to 0:100 MeCN/H_2O; 15 min; flow rate 0.2

mL/min); Rt 5.93 min; LRMS (ESI-TOF) *m*/*z* 873.46 (873.4551 calcd. for $C_{50}H_{61}N_6O_8$, $[M-H]^-$).

Data for S8':

Purity 71%; UPLC (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm (Waters); 20 °C; detection by UV (254 nm); 100:0 to 0:100 MeCN/H_2O; 15 min; flow rate 0.2 mL/min); Rt 5.89 min; LRMS (ESI-TOF) *m*/*z* 1018.54 (1018.5290 calcd. for $C_{56}H_{72}N_7O_{11}$ $[M-H]^-$).

Data for 2:

Purity 80%; UPLC (ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm (Waters); 20 °C; detection by UV (254 nm); 100:0 to 0:100 MeCN/H_2O; 15 min; flow rate 0.2 mL/min); Rt 5.57 min. Isolated overall yield 19% (purity ≥91%); preparative RP-HPLC (Develosil ODS-HG-5, 20 × 250 mm, (Nomura Chemical Co., Ltd); 20 °C, detection by UV (254 nm); 20:80 to 55:45 MeCN/H_2O containing 0.1 wt% TFA; 15 min; flow rate 1.0 mL/min); Rt 5.25 min; m.p. >300 °C; HRMS (ESI+TOF) *m*/*z* 1690.7112 (1690.7146 calcd. for $C_{93}H_{100}N_{11}O_{20}$ $[M-H]^+$).^{1}H-NMR (500 MHz, DMSO-d_6, TMS) δ 8.85 (8H, s), 8.36 (1H, t, *J*=11.6 Hz), 8.22 (1H, d, *J*=8.1 Hz), 8.14 (10H, dt, *J*=8.3 and 6.9 Hz), 7.90 (1H, d, *J*=8.3 Hz), 7.67 (2H, dd, *J*=8.3 and 8.9 Hz), 7.58 (3H, br-s), 7.41 (2H, d, *J*=8.6 Hz), 7.37 (6H, d, *J*=8.3 Hz), 7.07-7.24 (10H, m), 6.66 (1H, br-s), 4.98 (6H, s), 4.80 (2H, s), 4.53-4.57 (1H, m), 4.31-4.46 (3H, m), 4.08 (1H, t, *J*=7.8 and 7.8 Hz), 3.95 (2H, s), 3.56-3.60 (3H, t, *J*=6.0 and 5.7 Hz), 3.01-3.05 (1H, dd, *J*=5.45 and 5.45 Hz), 2.87-3.00 (2H, m), 2.70-2.75 (3H, m), 1.91 (2H, s), 1.82-1.86 (1H, m), 1.75 (4H, s), 1.64-1.67 (1H, m), 1.45-1.57 (5H, m), 1.36-1.41 (1H, m), 1.23-1.28 (5H, m), 0.83 (3H, d, *J*=6.6 Hz), 0.79 (3H, d, *J*=6.6 Hz), 0.69 (6H, dd, *J*=2.9 and 3.2 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6); δ 172.6, 171.5, 171.5, 171.0, 170.9, 170.6, 170.3, 169.0, 167.9, 157.8, 157.7, 137.5, 137.2, 136.3, 135.4, 134.2, 134.0, 129.0, 128.2, 127.9, 126.5, 126.4, 126.1, 119.6, 113.3, 113.3, 113.1, 70.3, 69.8, 69.4, 69.0, 67.2, 64.8, 57.5, 53.3, 53.3, 51.0, 40.7, 40.4, 39.6, 39.5, 39.3, 39.0, 38.7, 38.4, 38.3, 37.6, 36.7, 32.0, 30.7, 26.7, 24.1, 23.0, 22.5, 22.0, 21.6, 19.1, 17.9.

Thioflavin T (Th T) binding assay.

Inhibition of amyloid aggregation was measured using a Thioflavin T (ThT) binding assay.[3] a) Without photoirradiation: Th T binding assay was performed with $A\beta_{42}$ (40.0 μM) and **1** or **2** in 10% DMF/20 mM Tris-HCl buffer (pH 8.0, 25.0 μL) at 37 °C in an atmosphere of 5% CO_2 for 24 h. **1** or **2** was tested over a broad concentration range (10.0 nM to 300 μM) using 8-10 concentration points. After the incubation period, a solution of 6.67 μM Th T in water (75.0 μL) was added to each sample. The sample was

measured on Safire (TECAN) micro plate reader with excitation and emission wavelengths at 430 nm and 491 nm, respectively. Data were analyzed using GraphPad Prism®. b) With photoirradiation: Th T assay was performed with $A\beta_{42}$ (40.0 μM) and **1** or **2** in 10% DMF/20 mM Tris-HCl buffer (pH 8.0, 25.0 μL) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the sample, and an additional incubation of the mixture at 37 °C in an atmosphere of 5% CO_2 for 22 h. **1** or **2** was tested over a broad concentration range (10.0 nM to 300 μM) using 8-10 concentration points. After the incubation period, a solution of 6.67 μM Th T in water (75.0 μL) was added to the sample. Fluorescence was measured and data were analyzed by the same ways mentioned above.

Photodegradation of $A\beta_{42}$ monomer and oligomers with photoirradiation.

To a mixture of $A\beta_{42}$ monomer and oligomers (5.0 μM/$A\beta_{42}$ monomer), which was prepared by incubation of $A\beta_{42}$ monomer in 20 mM Tris-HCl buffer (pH 8.0, 8 μL) at 25 °C for 14 h,[4] was added a solution of each porphyrin derivative (50-0.5 μM) in 50% DMF/Tris-HCl buffer (pH 8.0, 20 mM, 2 μL). The mixture was then incubated at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed 10 cm from the sample.

Polyacrylamide gel electrophoreses and immunoblotting.

Electrophoresis buffer consisted of SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue (0.007%, wt/vol); 4.8 μL of buffer was added to samples. Gels (10%) were run by applying 60 V for 1.5 h. $A\beta_{42}$ monomer and oligomers were transferred at 200 mA for 2 h onto Amersham Hybond ECL Nitrocellulose Membrane (GE Healthcare). Nonspecific binding sites were blocked for 0.5 h by immersing the membrane in a blocking solution, Tris-buffered saline with Tween 20 (TBST): 10 mM Tris-HCl, (pH 8.0) containing 150 mM NaCl, 0.1% Tween 20 (vol/vol), and 5% (wt/vol) nonfat dry milk. After a short wash in TBST, the membrane was incubated in a 1:2500 dilution of a primary antibody 6E10 (Covance Research Products) in TBST for 14 h at 4 °C followed by 30 min of washing with TBST. The bound antibody was then detected with horseradish peroxidase-conjugated secondary antibody Anti-mouse lgG (GE healthcare) diluted at 1:3000 in TBST by incubation with it for 2 h at 25 °C. After having been washed for 30 min in TBST, the immunocomplexes were detected by using Immobilon Western (Millipore, Billerica, MA). Exposure to RX-U films (Fujifilm Co.) was carried out for 30 s to 2 min.

MALDI TOF MS analysis.

2.00 μL of sample was mixed with a matrix solution (18.0 μL) of 3,5-dimethoxy 4-hydroxycinnamic acid (in 50:50 MeCN/H_2O containing 0.1% TFA). Analyses by MALDI TOF MS were performed in the positive ion mode on a Ultra flex (Bruker).

Cell culture.

The PC12 cell line was routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% (v/v) Fetal bovine serum, 10% (v/v) horse serum, 0.5% (v/v) penicillin and streptomycin. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell cytotoxicity assay.

PC12 cells (4.0×10^4 cells/mL) were cultured in 96-well microplates (100 μL/well) and incubated for 24 h at 37 °C. The PC12 cells were washed once with serum-free DMEM containing 0.5% (v/v) NGF to exclude the effect of the serum and incubated for 48 h at 37 °C. To each well was added 10 μL of $A\beta_{42}$ monomer (5 μM) in 10% DMF/serum-free DMEM with each porphyrin derivative (0.003-1.0 μM). The PC12 cells were incubated at 25 °C for 15 min. with or without irradiation using a UV lamp (365 nm, 30 W) placed 15 cm form the sample, and the mixture was further incubated at 37 °C for 24 h. Cell viability was evaluated using the MTT assay.[5] 10 μL of 5 mg/mL MTT dissolved in PBS was added to each well. After incubation for 3 h at 37 °C, 50 μL of DMSO was added to each well, and color intensity was measured using Safire (TECAN) micro plate reader at 570 nm.

References

1. C. Visintin, A. E. Aliev, D. Riddall, D. Baker, M. Okuyama, P. M. Hoi, R. Hiley and R, D. L. Selwood, *Org. Lett*., 2005, **7**, 1699.
2. M. Sibrian-Vazquez, T. J. Jensen, R. P. Hammer and M. G. H. Vicente, *J. Med. Chem*., 2006, **49**, 1364.
3. H. LeVine, III, *Protein Sci.,* 1993, **2**, 404.
4. V. Rangachari, B. D. Moore, D. K. Reed, L. K. Sonoda, A. W. Bridges, E. Conboy, D. Hartigan and T. Rosenberry, *Biochemistry,* 2007, **46**, 12451.
5. M. B. Hansen, S. E. Nielsen and K. Berg, *J. Immunol. Methods,* 1989, **119**, 203.

^{1}H- and ^{13}C-NMR spectra of 2.

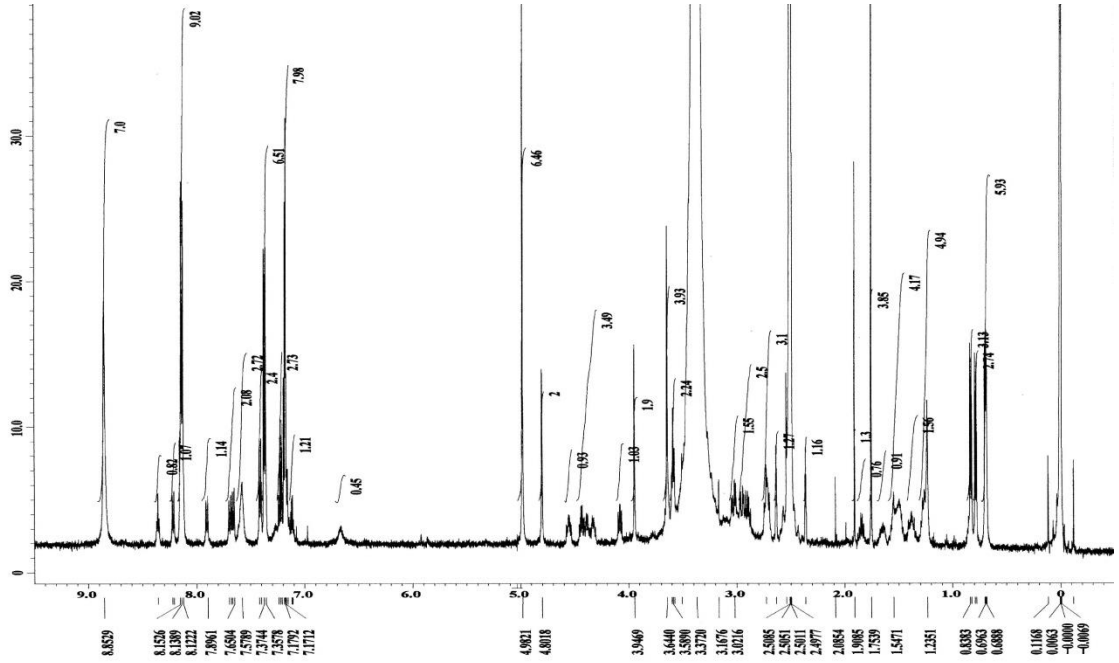


Fig. S1 ^{1}H-NMR spectrum of **2**

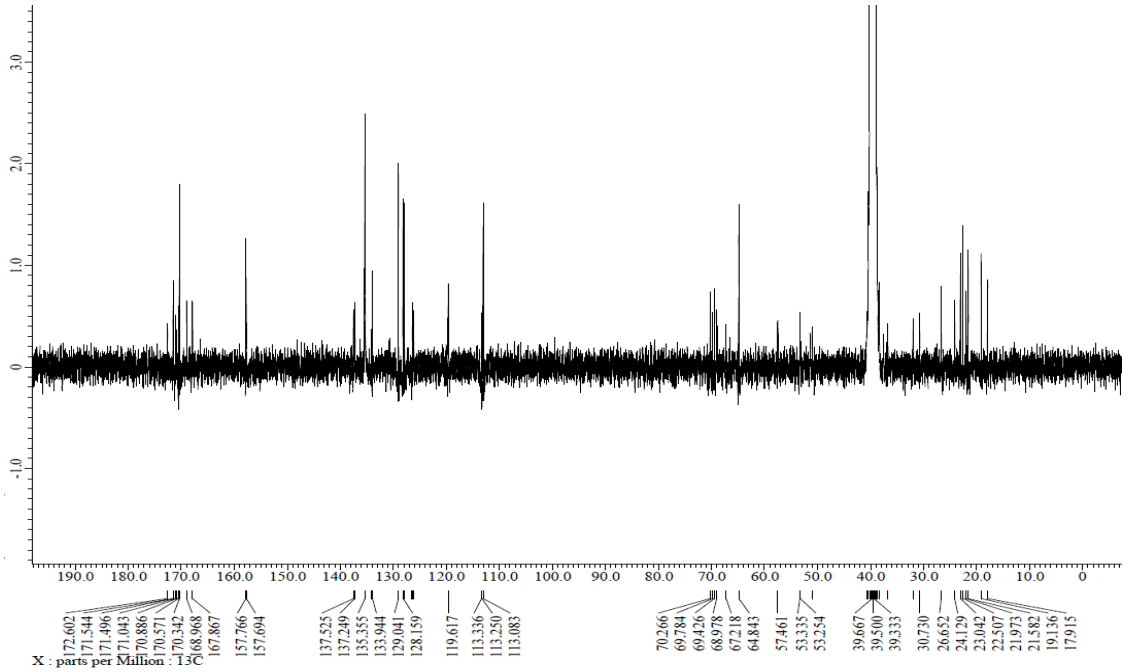


Fig. S2 ^{13}C-NMR spectrum of **2**