Synthesis of Zwitterionic Asymmetric and Symmetric Carboxy-imidazolium Derivatives and their use in Molecular Interactions with Bovine Serum Albumin

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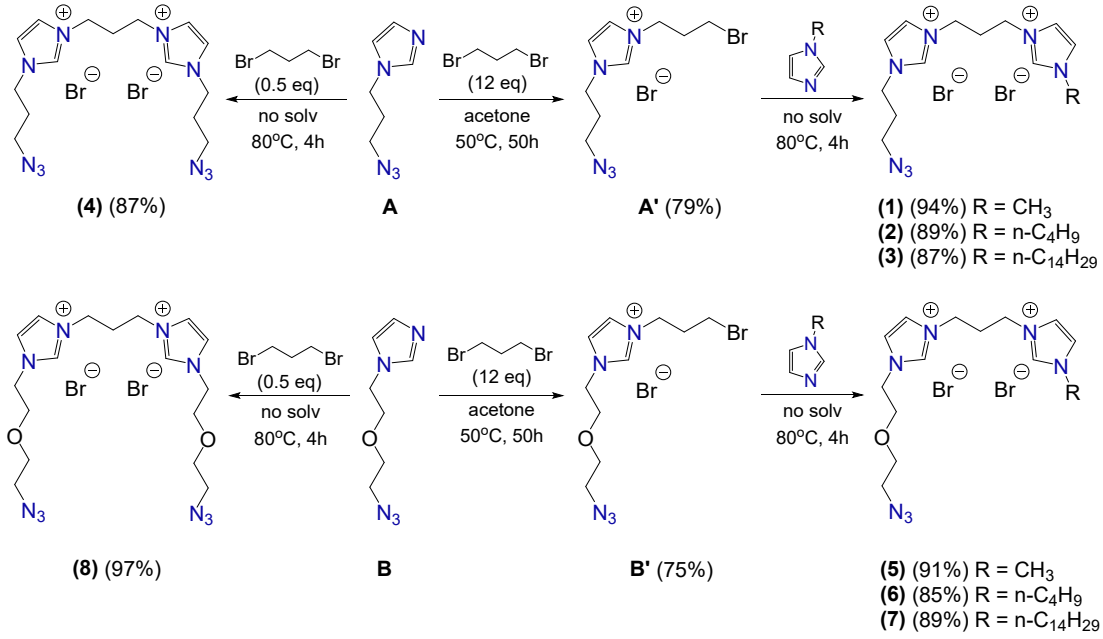
Synthesis of symmetrical and asymmetrical bis-imidazolium salts with azide fragments

Compounds **A**-**A'** and **B**-**B'** were obtained using a literature method [1,2] (Scheme 1S).



Scheme S1. symmetrical and asymmetrical bis-imidazolium salts with azide fragments (compounds **1**–**8**).

General protocol for obtaining bis-imidazolium salts with azide groups.

To obtain the unsymmetrical salt, an equimolar amount of compound **A'** or **B'** was added to 0.5 mmol of N-alkylimidazole (CH_3, *n*-C_4H_9 or *n*-$C_{14}H_{29}$). The mixture was stirred at 80°C for 4 h, after which the mixture was dissolved in 10 ml of water and washed with diethyl ether (3 x 15 ml). The aqueous phase was then removed by rotary evaporation to yield yellow resinous compounds. To obtain the symmetrical salt, 0.25 mmol of 1,3-dibromopropane was added to 0.5 mmol of compound **A** or **B**. The synthesis procedure is similar to the previously described procedure for the preparation of unsymmetrical salts.

3-(3-azidopropyl)-1-(3-(1-methyl-1H-imidazol-3-yl)propyl)-1H-imidazolium dibromide (1). Weight = 0.41 g, yield = 94%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.46 (s, 1H, ImH), 9.36 (s, 1H, ImH), 7.89 (s, 2H, ImH), 7.78 (s, 1H, ImH), 7.76 (s, 1H, ImH), 4.29 – 4.19 (m, 6H, $ImCH_2$), 3.88 (s, 3H, $ImCH_3$), 3.45 (t, *J* = 6.5, 2H, CH_2N_3), 2.46 – 2.37 (m, 2H, CH_2), 2.14 – 2.05 (m, 2H, CH_2). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.):136.9, 136.6, 123.6, 122.5, 122.4, 122.1, 47.6, 46.5, 45.7, 45.6, 36.0, 29.5, 28.6. IR (KBr) ν_{max}cm^{-1}: ν 3087 m (C_{Ar}-H), ν_{as} 2954 m (CH_2), ν_{as} 2101 s (-N_3), ν 1630 m (C=N), ν 1564 s (C=C), ν 1456 s (C-N). HRMS-ESI: found *m/z*: 137.5929 $[M-2Br]^{2+}$; calcd. for. $C_{13}H_{21}N_7^{2+}$ 137.5924.

3-(3-azidopropyl)-1-(3-(1-butyl-1H-imidazol-3-yl)propyl)-1H-imidazolium dibromide (2). Weight = 0.42 g, yield = 89%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.43 – 9.38 (m, 2H, ImH), 7.90 – 7.85 (m, 4H, ImH), 4.31 – 4.23 (m, 6H, CH_2Im), 4.19 (t, *J* = 7.2, 2H, CH_2Im), 3.46 (t, *J* = 6.6, 2H, CH_2N_3), 2.48 – 2.39 (m, 2H, CH_2), 2.14 – 2.04 (m, 2H, CH_2), 1.83 – 1.75 (m, 2H, CH_2), 1.32 – 1.22 (m, 2H, CH_2), 0.90 (t, *J* = 7.4, 3H, CH_3). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.): 136.9, 136.3, 122.5, 122.4, 122.3, 48.6, 47.6, 46.5, 45.8, 45.8, 31.2, 29.5, 28.6, 18.8, 13.3. IR (KBr) ν_{max}cm^{-1}: ν 3070 m (C_{Ar}-H), ν_{as} 2936 m (CH_2), ν_{as} 2101 s (-N_3), ν 1644 m (C=N), ν 1564 s (C=C), ν 1458 m (C-N). HRMS-ESI: found *m/z*: 158.6158 [M-2Br]$^{2+}$; calcd. for. $C_{16}H_{27}N_7^{2+}$ 158.6158.

3-(3-azidopropyl)-1-(3-(1-tetradecyl-1H-imidazol-3-yl)propyl)-1H-imidazolium dibromide (3). Weight = 0.54 g, yield = 87%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.41 (br s, 2H, ImH), 7.91 – 7.85 (m, 4H, ImH), 4.31 – 4.22 (m, 6H, CH_2Im), 4.17 (t, *J* = 7.3, 2H, CH_2Im), 3.46 (t, *J* = 6.6, 2H, CH_2N_3), 2.48 – 2.36 (m, 2H, CH_2), 2.13 – 2.04 (m, 2H, CH_2), 1.84 – 1.74 (m, 2H, CH_2), 1.34 – 1.18 (m, 22H, CH_2), 0.84 (t, *J* = 6.7, 3H, CH_3). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.): 136.8, 136.3, 122.5, 122.5, 122.5, 122.4, 64.9, 48.9, 47.6, 46.5, 45.8, 45.8, 31.3, 29.4, 29.3, 29.0, 29.00, 29.00, 28.9, 28.7, 28.6, 28.4, 25.6, 22.0, 15.1, 13.9. IR (KBr) ν_{max}cm^{-1}: ν 3052 m (C_{Ar}-H), ν_{as} 2957 m (CH_2), ν_{as} 2101 s (-N_3), ν 1628 m (C=N), ν 1561 m (C=C), ν 1451 m (C-N). HRMS-ESI: found *m/z*: 228.6942 [M-2Br]$^{2+}$; calcd. for. $C_{26}H_{47}N_7^{2+}$ 228.6941.

3,3'-(propane-1,3-diyl)bis(1-(3-azidopropyl)-1H-imidazol-3-ium) dibromide (4). Weight = 0.24 g, yield = 97%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.29 (s, 2H, ImH), 7.85 (br s, 2H, ImH), 7.84 (br s, 2H, ImH), 4.30 – 4.20 (m, 8H, CH_2Im), 3.45 (t, *J* = 6.6, 4H, CH_2N_3), 2.46 – 2.37 (m, 2H, CH_2), 2.13 – 2.03 (m, 4H, CH_2). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.): 136.5, 122.6, 122.5, 47.6, 46.6, 45.9, 29.4, 28.6. IR (KBr) ν_{max}cm^{-1}: ν 3079 m (C_{Ar}-H), ν_{as} 2942 m (CH_2), ν_{as} 2101 s (-N_3), ν 1621 m (C=N), ν 1545 s (C=C), ν 1453 m (C-N). HRMS-ESI: found *m/z*: 172.1089 [M-2Br]$^{2+}$; calcd. for. $C_{15}H_{24}N_{10}^{2+}$ 172.1087.

1-(2-(2-azidoethoxy)ethyl)-3-(3-(1-methyl-1H-imidazol-3-yl)propyl)-1H-imidazolium dibromide (5). Weight = 0.42 g, yield = 91%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.25 (s, 1H, ImH), 9.19 (s, 1H, ImH), 7.84 (s, 1H, ImH), 7.81 (s, 1H, ImH), 7.79 (s, 1H, ImH), 7.72 (s, 1H, ImH), 4.39 (t, *J* = 4.8, 2H, ImCH_2), 4.24 (t, *J* = 7.0, 4H, ImCH_2), 3.87 (s, 3H, ImCH_3), 3.83 (t, *J* = 4.9, 2H, CH_2O), 3.64 (t, *J* = 4.6, 2H, CH_2O), 3.40 (t, *J* = 4.9, 2H, CH_2N_3), 2.44 – 2.35 (m, 2H, CH_2). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.): 137.8, 137.6, 124.4, 123.7, 123.1, 123.1, 70.3, 68.9, 51.1, 50.2, 47.0, 46.9, 37.0, 30.9. IR (KBr) ν_{max}cm^{-1}: ν 3056 cp. (C_{ap}-H), ν_{as} 2955 cp. (CH_2), ν_{as} 2102 c. (-N_3), ν 1631 cp. (C=N), ν 1556 c. (C=C), ν 1441 cp. (C-N), ν_{as} 1073 c. (C-O-C). HRMS-ESI: found *m/z*: 152.5976 [M-2Br]$^{2+}$; calcd. for. $C_{14}H_{23}N_7O_2^{2+}$ 152.5977.

1-(2-(2-azidoethoxy)ethyl)-3-(3-(1-butyl-1H-imidazol-3-yl)propyl)-1H-imidazolium dibromide (6). Weight = 0.43 g, yield = 85%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.27 (s, 1H, ImH), 9.24 (s, 1H, ImH), 7.84 (br s, 2H, ImH), 7.81 (br s, 2H, ImH), 4.39 (t, *J* = 4.5, 2H, CH_2Im), 4.27 – 4.22 (m, 4H, CH_2Im), 4.18 (t, *J* = 7.2, 2H, CH_2Im), 3.83 (t, *J* = 4.8, 2H, CH_2O), 3.64 (t, *J* = 4.6, 2H, CH_2O), 3.40 (t, *J* = 4.8, 2H, CH_2N_3), 2.46 – 2.36 (m, 2H, CH_2), 1.85 – 1.73 (m, 2H, CH_2), 1.34 – 1.22 (m, 2H, CH_2), 0.91 (t, *J* = 7.4, 3H, CH_3). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.): 136.7, 136.3, 122.8, 122.5, 122.3, 122.2, 69.2, 67.8, 49.8, 48.8, 48.6, 45.8, 31.2, 29.6, 18.8, 13.3. IR (KBr) ν_{max}cm^{-1}: ν 3061 m (C_{Ar}-H), ν_{as} 2952 cp. (CH_2), ν_{as} 2110 s (-N_3), ν 1629 m (C=N), ν 1564 s (C=C), ν 1448 m (C-N), ν_{as} 1072 m (C-O-C). HRMS-ESI: found *m/z*: 173.6212 [M-2Br]$^{2+}$; calcd. for. $C_{17}H_{29}N_7O^{2+}$ 173.6211.

1-(2-(2-azidoethoxy)ethyl)-3-(3-(1-tetradecyl-1H-imidazol-3-yl)propyl)-1H-imidazolium dibromide (7). Weight = 0.58 g, yield = 89%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.41 (s, 1H, ImH), 9.37 (s, 1H, ImH), 7.91 – 7.85 (m, 3H, ImH), 7.82 (s, 1H, ImH), 4.41 (t, *J* = 4.6, 2H, CH_2Im), 4.32 – 4.25 (m, 4H, CH_2Im), 4.17 (t, *J* = 7.2, 2H, CH_2Im), 3.85 (t, *J* = 4.7, 2H, CH_2O), 3.65 (t, *J* = 4.6, 2H, CH_2O), 3.40 (t, *J* = 4.8, 2H, CH_2N_3), 2.48 – 2.37 (m, 2H, CH_2), 1.86 – 1.74 (m, 2H, CH_2), 1.35 – 1.10 (m, 22H, CH_2), 0.84 (t, *J* = 6.4, 3H, CH_3). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.): 137.8, 137.4, 123.8, 123.4, 123.2, 123.2, 70.5, 69.0, 51.3, 50.5, 50.5, 47.2, 47.1,

32.6, 30.8, 30.5, 30.3, 30.3, 30.3, 30.2, 30.1, 30.0, 29.6, 26.7, 23.3, 14.3. IR (KBr) $\nu_{max}cm^{-1}$: ν 3069 m (C_{Ar}-H), ν_{as} 2946 m (CH_2), ν_{as} 2104 s (-N_3), ν 1628 m (C=N), ν 1559 s (C=C), ν 1442 s (C-N), ν_{as} 1079 m (C-O-C). HRMS-ESI: found *m/z*: 243.6996 $[M-2Br]^{2+}$; calcd. for. $C_{27}H_{49}N_7O^{2+}$ 243.6994.

3,3'-(propane-1,3-diyl)bis(1-(2-(2-azidoethoxy)ethyl)-1H-imidazol-3-ium) dibromide (8). Weight = 0.27 g, yield = 97%. NMR ^{1}H (400 MHz, DMSO-d_6, 25°C, δ, ppm, *J*/Hz): 9.41 (br d, 2H, ImH), 7.95 – 7.88 (m, 2H, ImH), 7.84 (br s, 2H, ImH), 4.41 (t, *J* = 4.7, 4H, ImCH_2), 4.29 (t, *J* = 6.6, 4H, CH_2Im), 3.85 (t, *J* = 4.9, 4H, CH_2O), 3.64 (t, *J* = 4.6, 4H, CH_2O), 3.40 (t, *J* = 4.9, 4H, CH_2N_3), 2.46 – 2.38 (m, 2H, CH_2). NMR ^{13}C-{^{1}H} (100.9 MHz, DMSO-d_6, 25°C, δ, ppm.): 137.1, 123.3, 122.7, 69.7, 68.3, 50.2, 49.3, 46.2, 30.1. IR (KBr) $\nu_{max}cm^{-1}$: ν 3086 m (C_{Ar}-H), ν_{as} 2949 m. (CH_2), ν_{as} 2102 s (-N_3), ν 1627 s (C=N), ν 1551 m (C=C), ν 1458 s (C-N), ν_{as} 1086 m (C-O-C). HRMS-ESI: found *m/z*: 202.1194 $[M-2Br]^{2+}$; calcd. for. $C_{17}H_{28}N_{10}O_2^{2+}$ 202.1193.

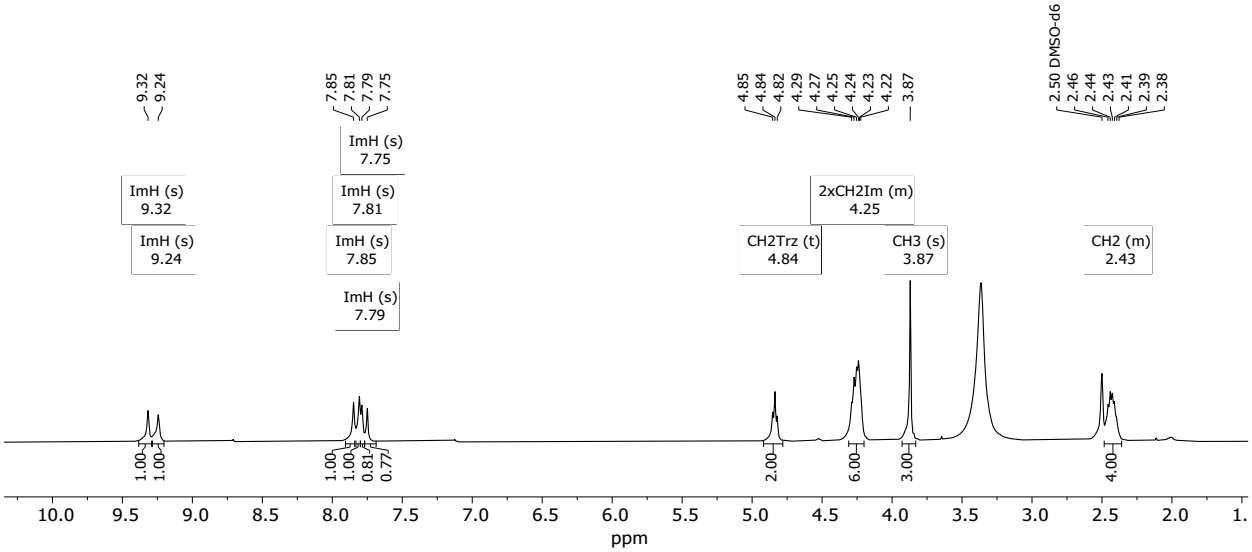


Fig. S1. ^{1}H NMR spectra (400 MHz) of compound **COOH-Imd-C1** in DMSO-d_6

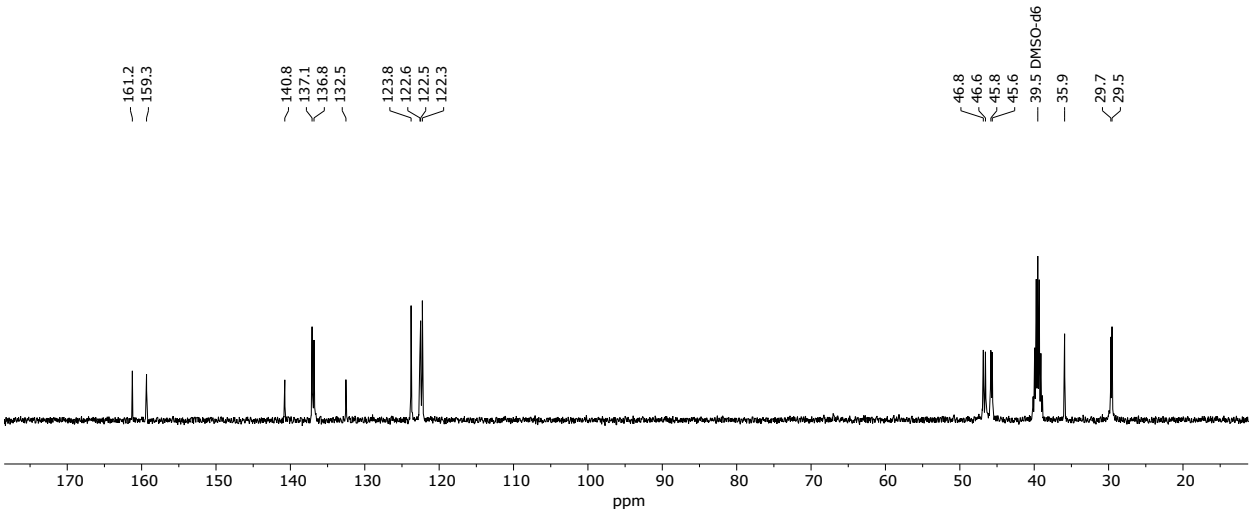


Fig. S2. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-Imd-C1** in DMSO-d_6.

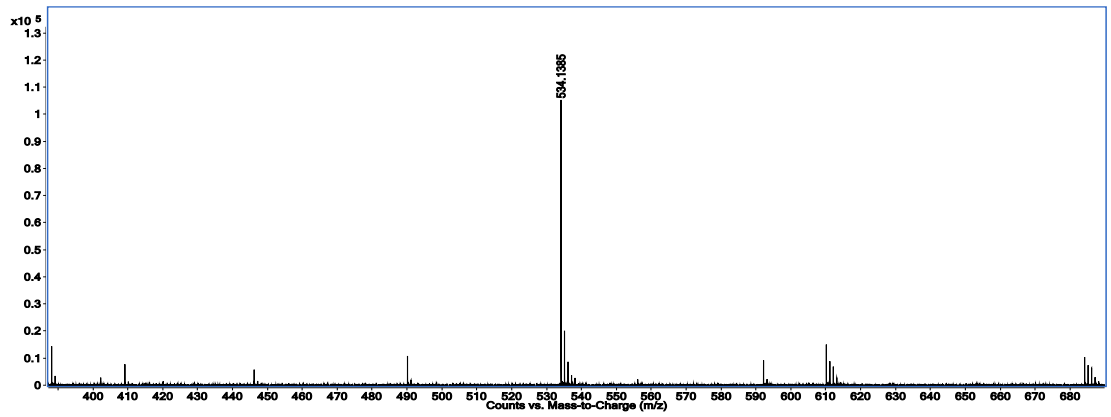


Fig. S3. ESI HR mass spectra of compound **COOH-Imd-C1**.

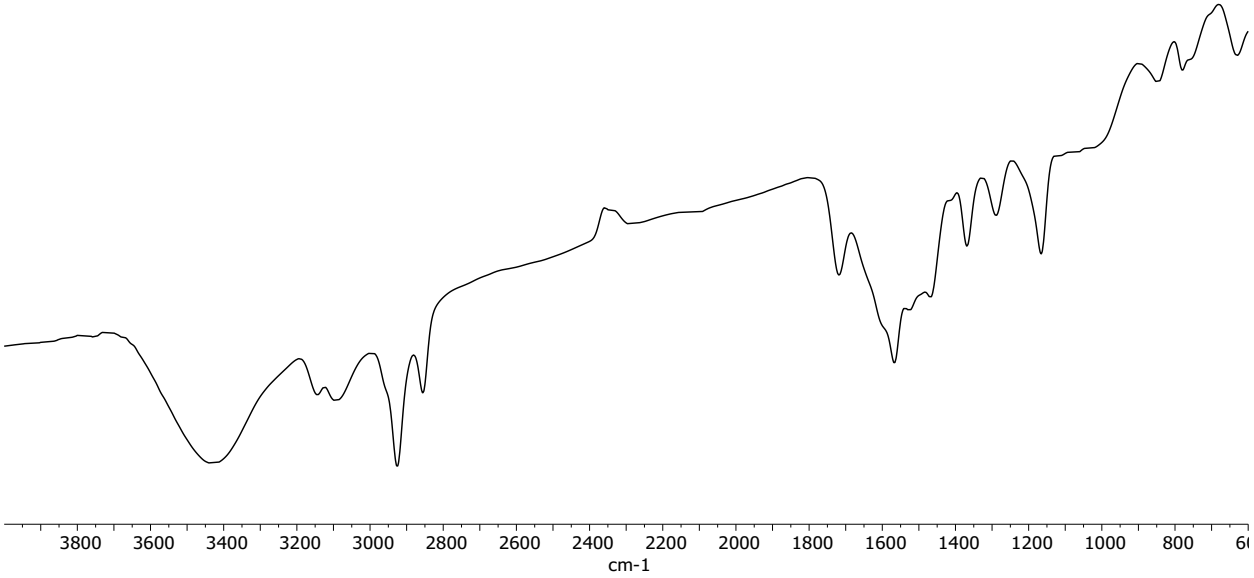


Fig. S4. FTIR spectra of compound **COOH-Imd-C1**.

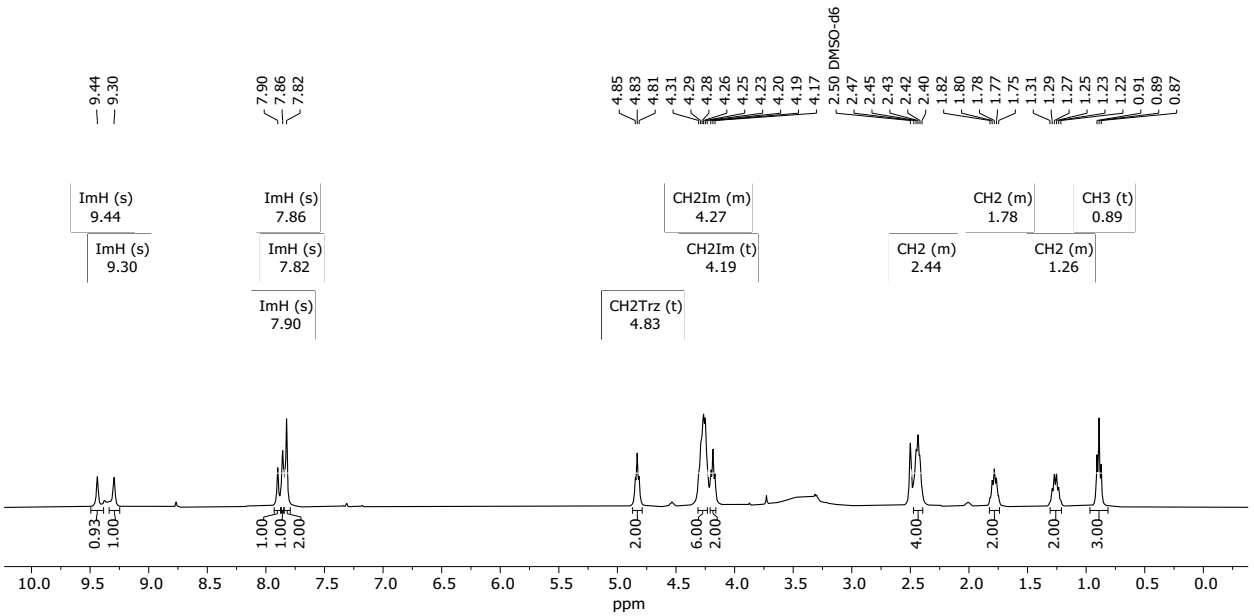


Fig. S5. ^{1}H NMR spectra (400 MHz) of compound **COOH-Imd-C4** in DMSO-d_6

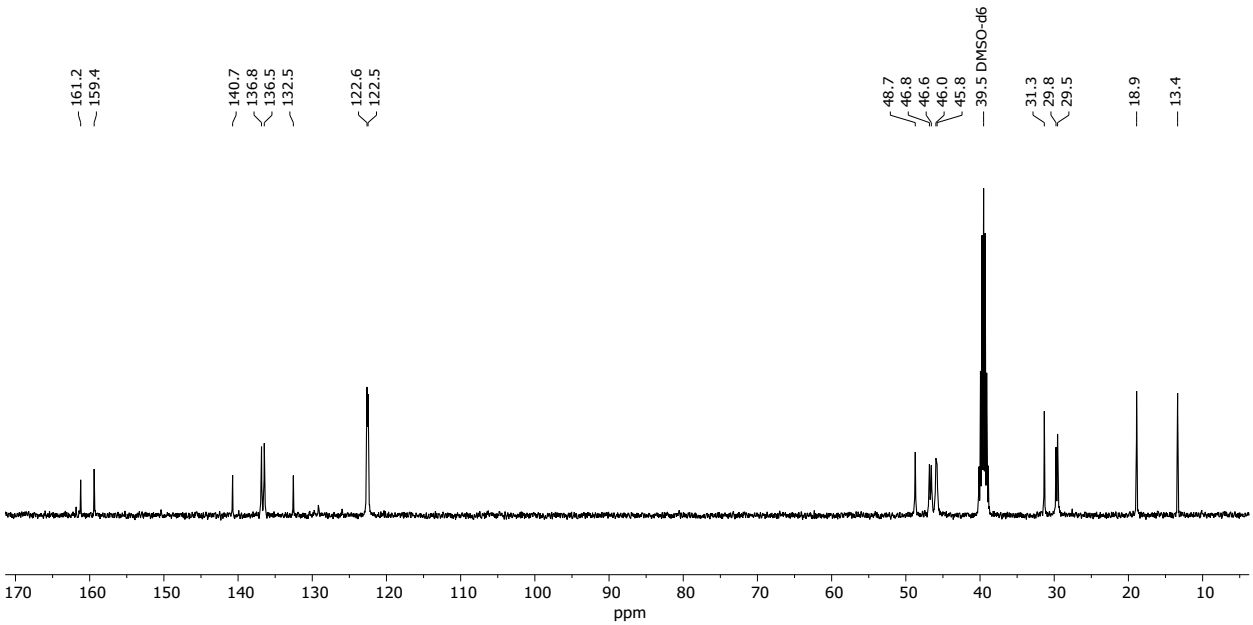


Fig. S6. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-Imd-C4** in DMSO-d_6.

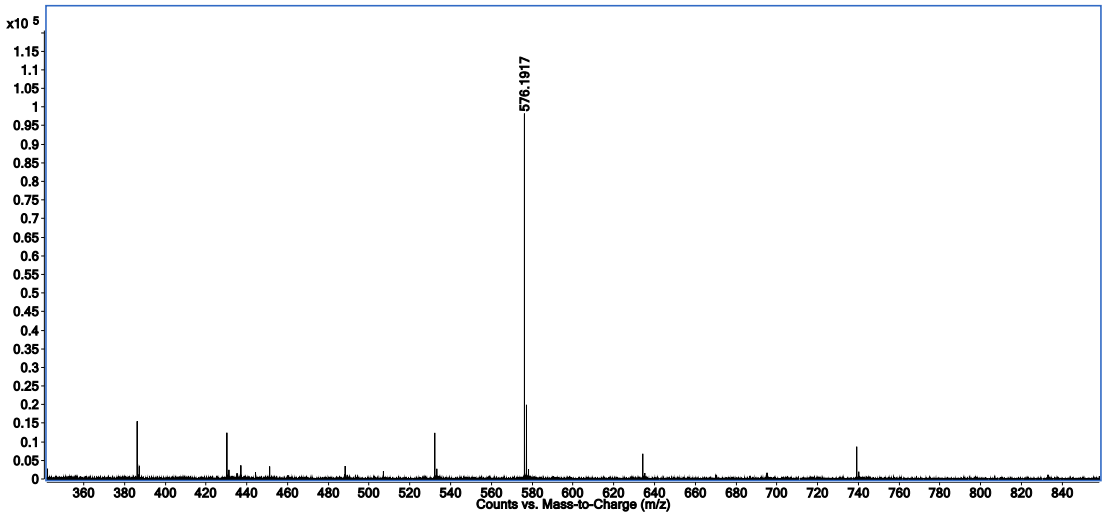


Fig. S7. ESI HR mass spectra of compound **COOH-Imd-C4**.

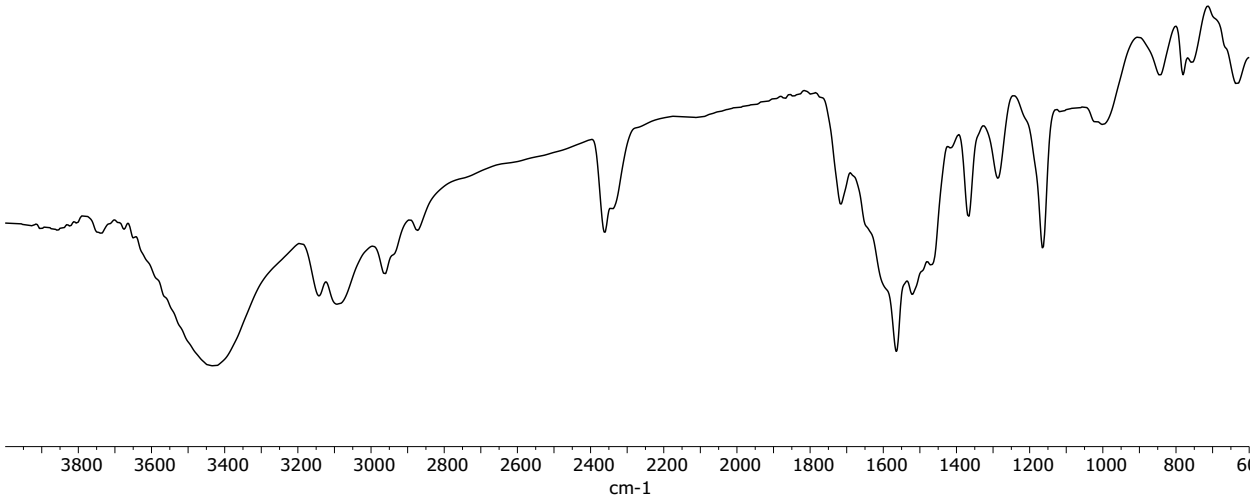


Fig. S8. FTIR spectra of compound **COOH-Imd-C4**.

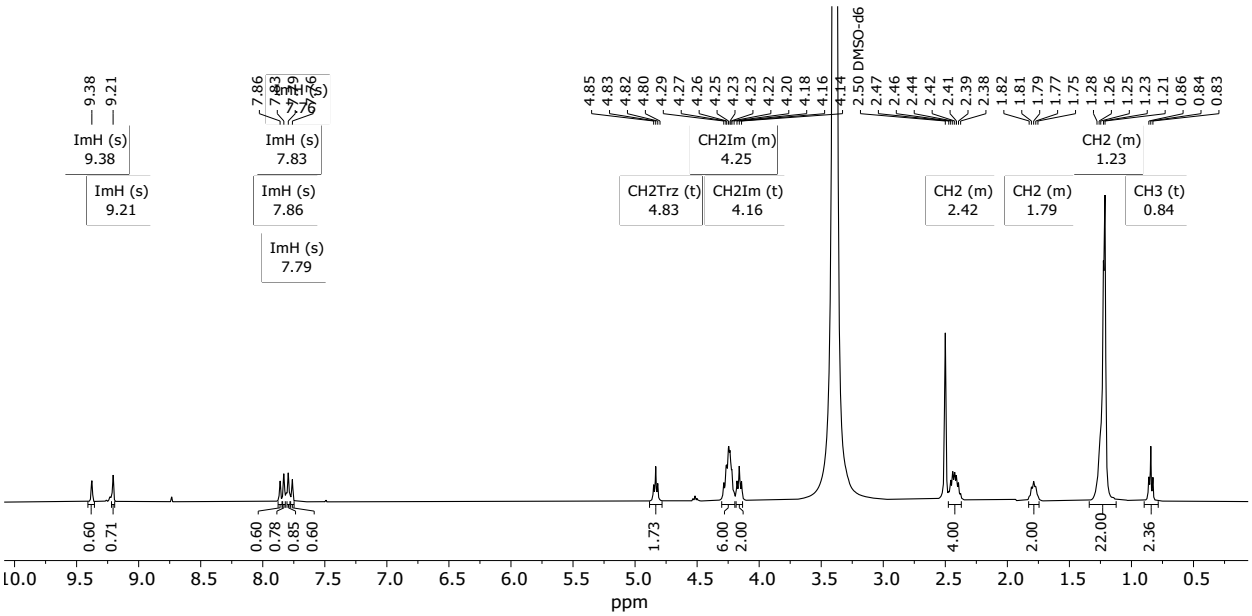


Fig. S9. ^{1}H NMR spectra (400 MHz) of compound **COOH-Imd-C14** in DMSO-d_6

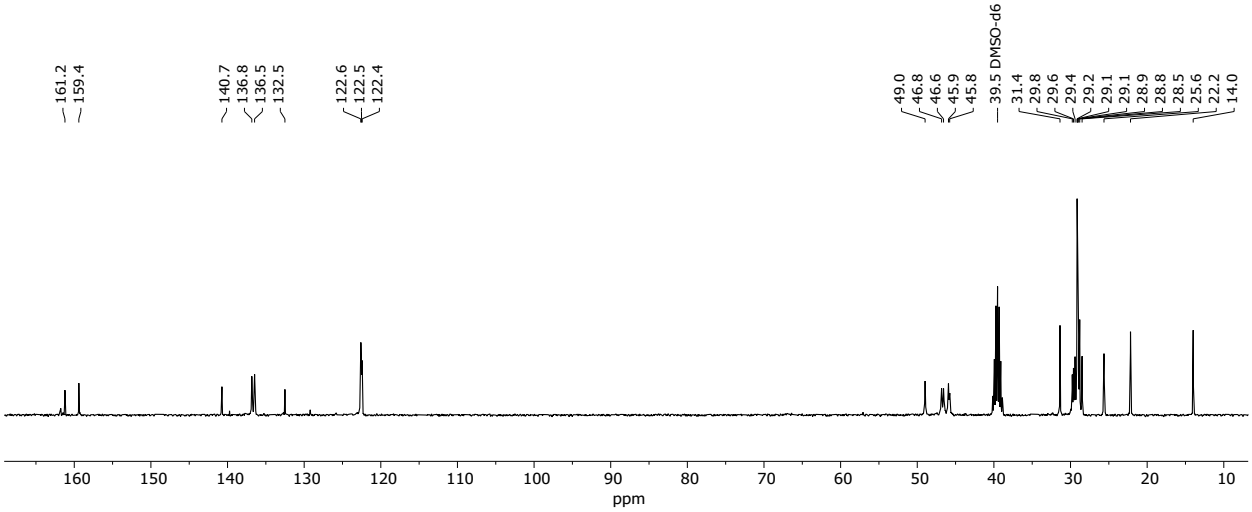


Fig. S10. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-Imd-C14** in DMSO-d_6

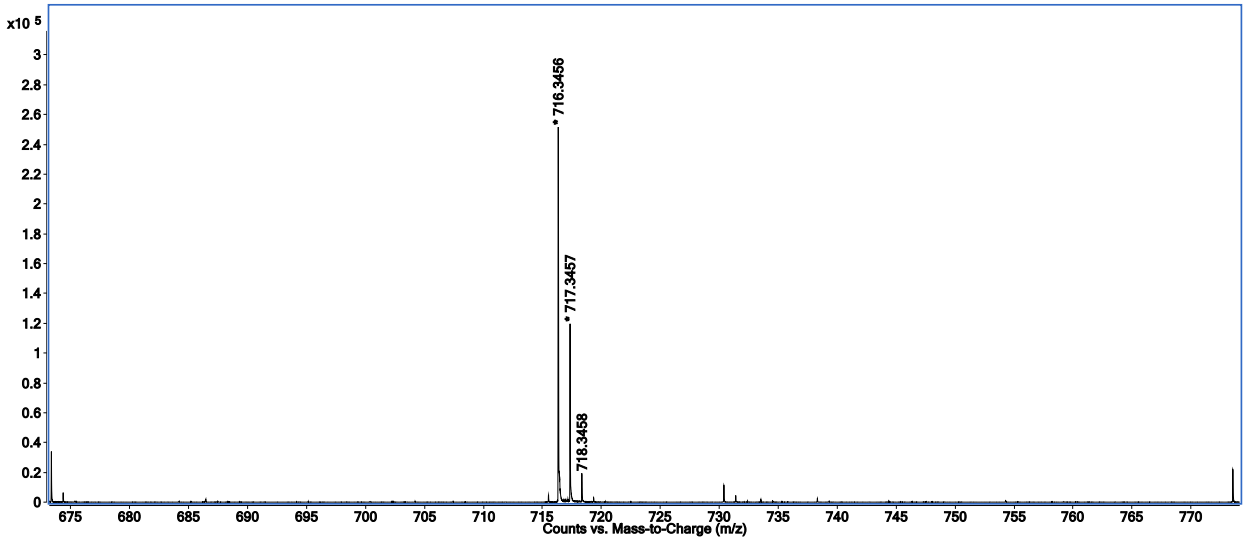


Fig. S11. ESI HR mass spectra of compound **COOH-Imd-C14**.

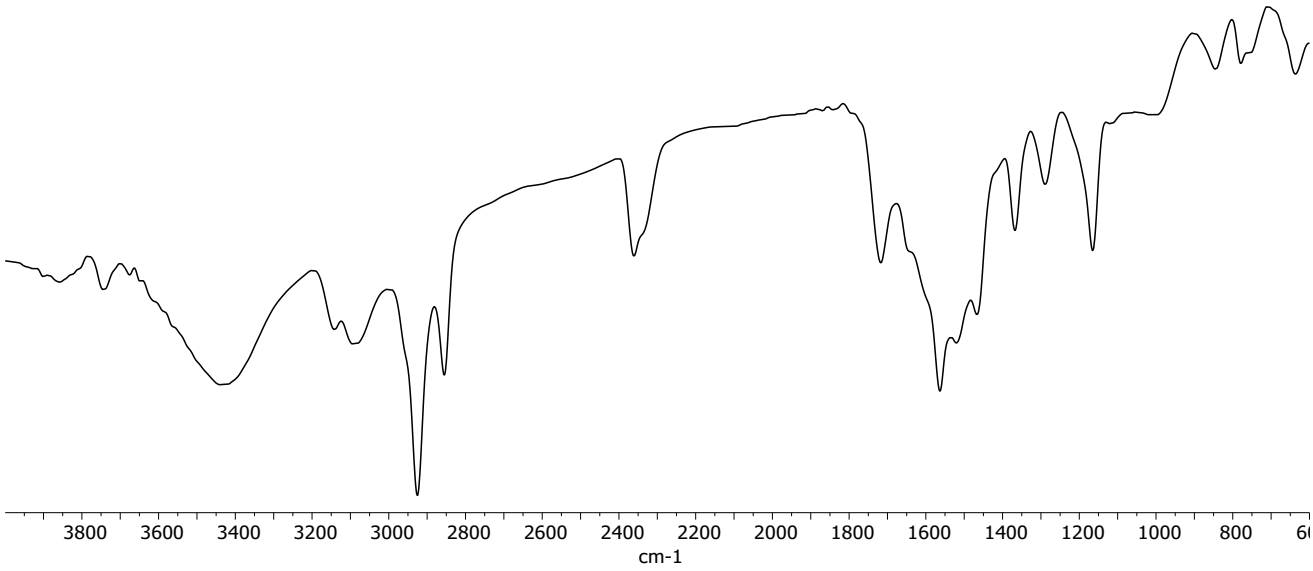


Fig. S12. FTIR spectra of compound **COOH-Imd-C14**.

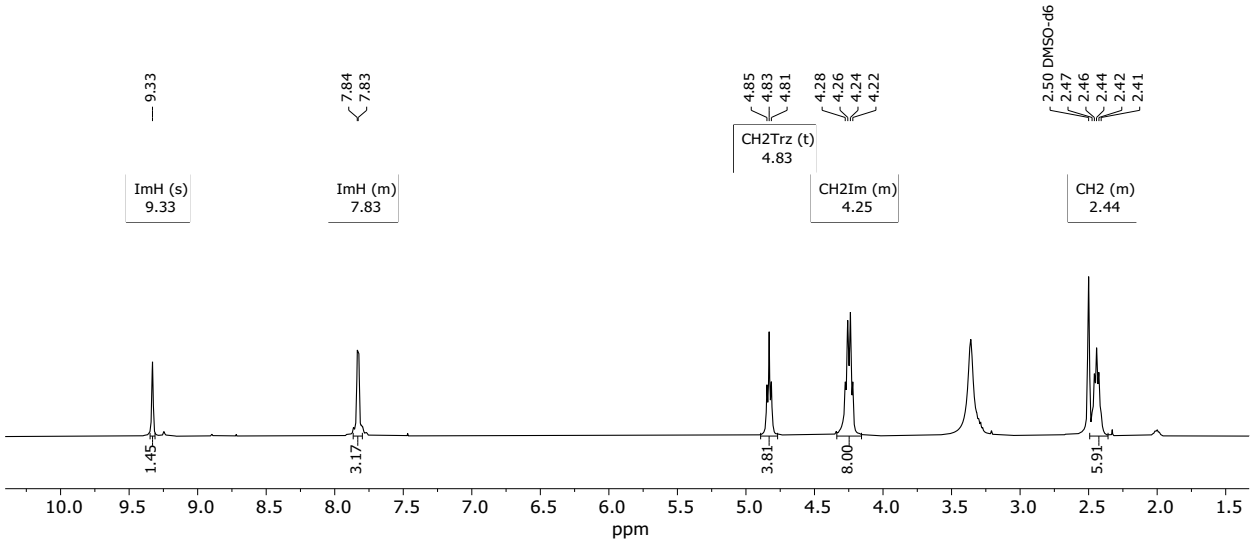


Fig. S13. ^{1}H NMR spectra (400 MHz) of compound **COOH-Imd- COOH** in DMSO-d_6

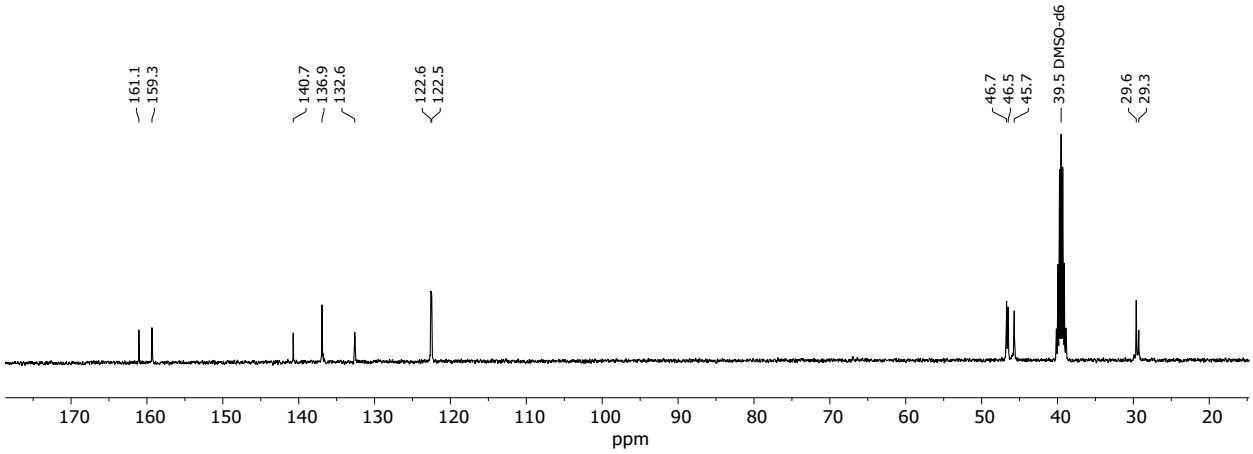


Fig. S14. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-Imd- COOH** in DMSO-d_6.

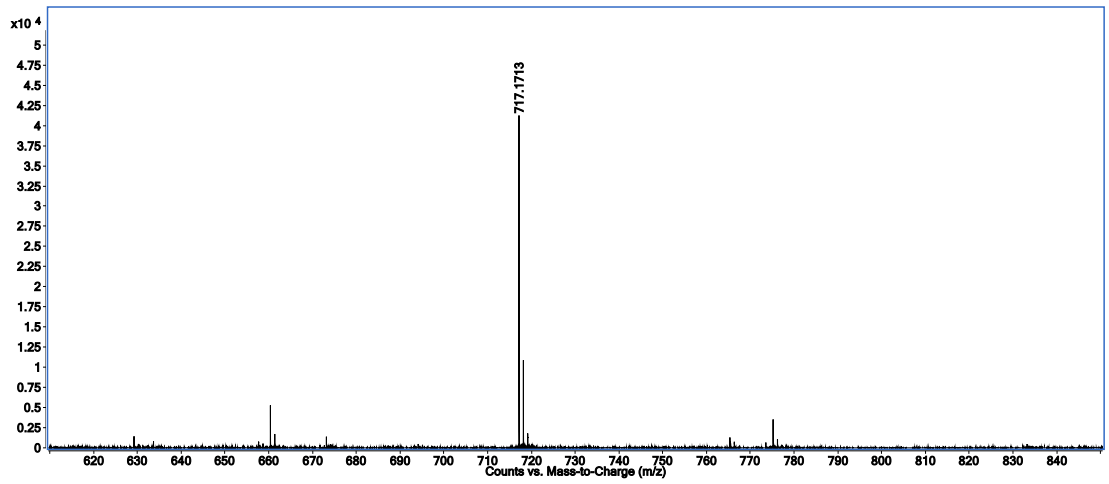


Fig. S15. ESI HR mass spectra of compound **COOH-Imd- COOH**.

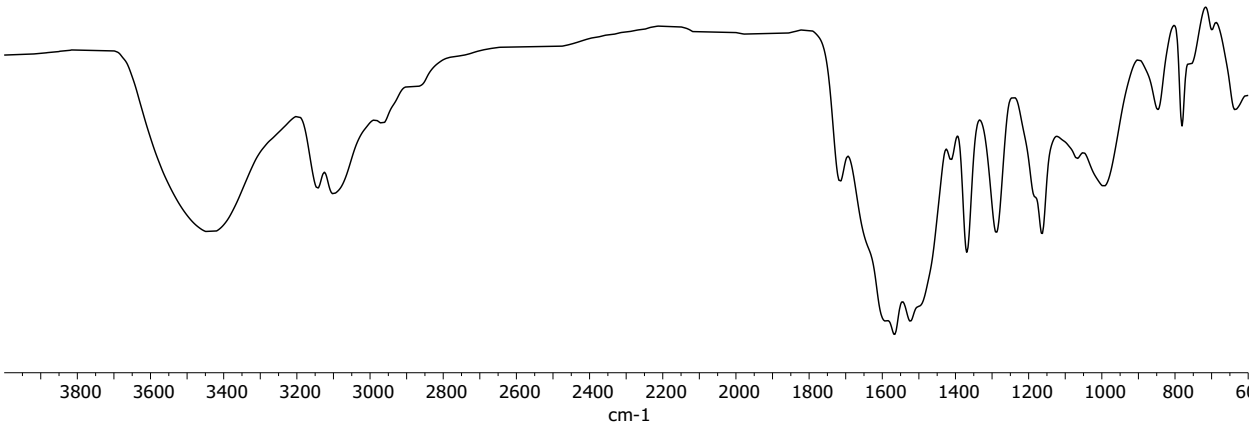


Fig. S16. FTIR spectra of compound **COOH-Imd-COOH**.

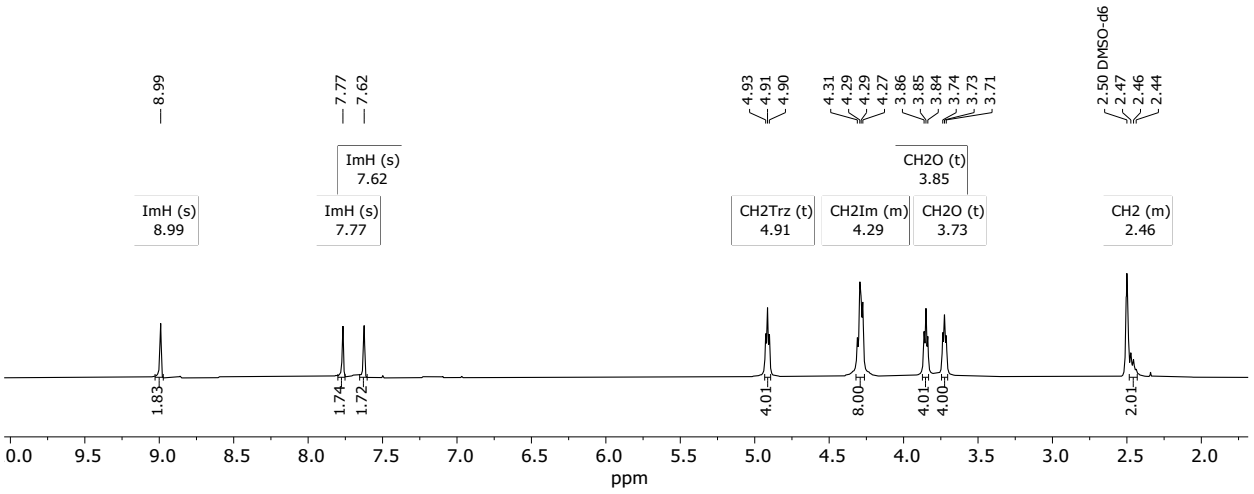


Fig. S17. ^{1}H NMR spectra (400 MHz) of compound **COOH-EG-Imd-EG-COOH** in DMSO-d_6

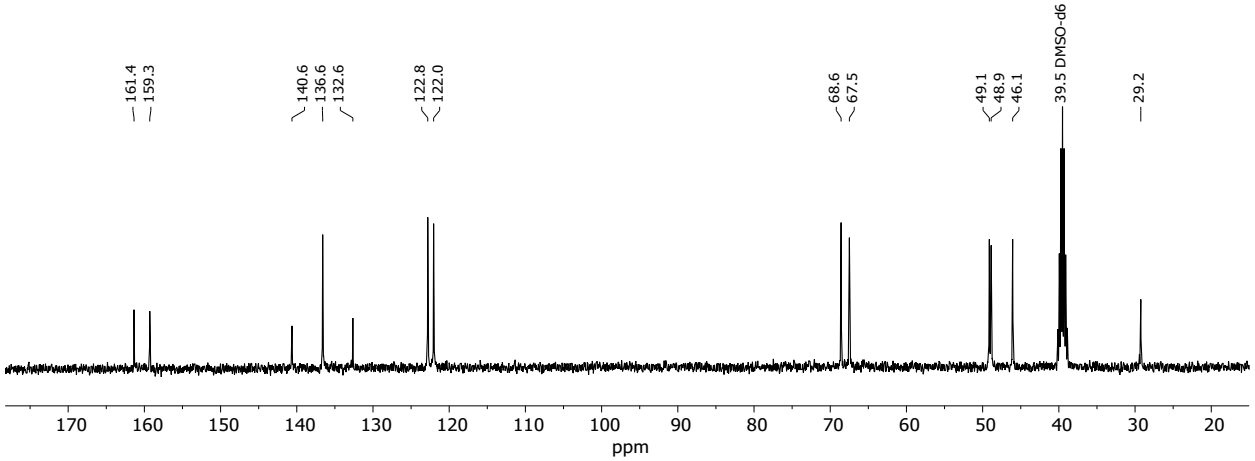


Fig. S18. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-EG-Imd-EG-COOH** in DMSO-d_6.

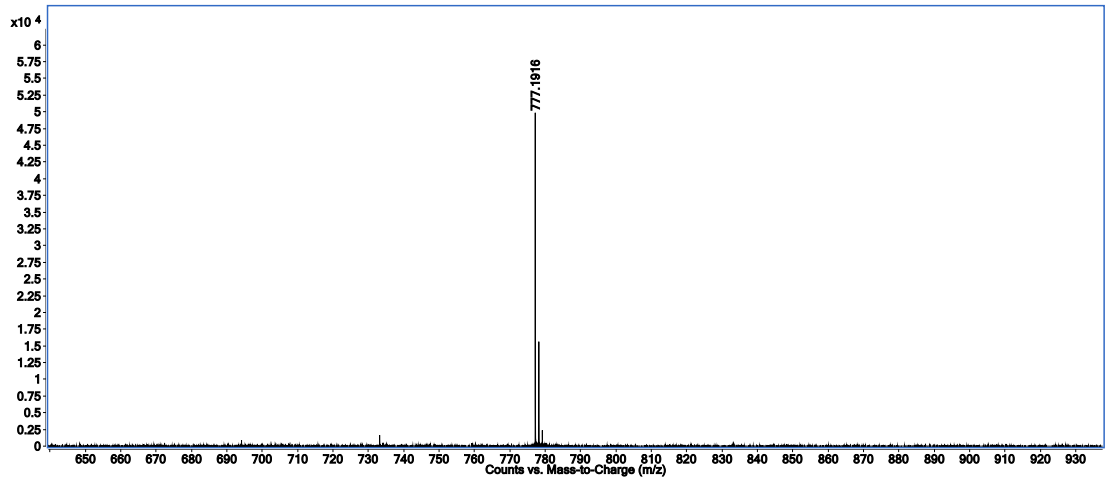


Fig. S19. ESI HR mass spectra of compound **COOH- EG-Imd-EG-COOH**.

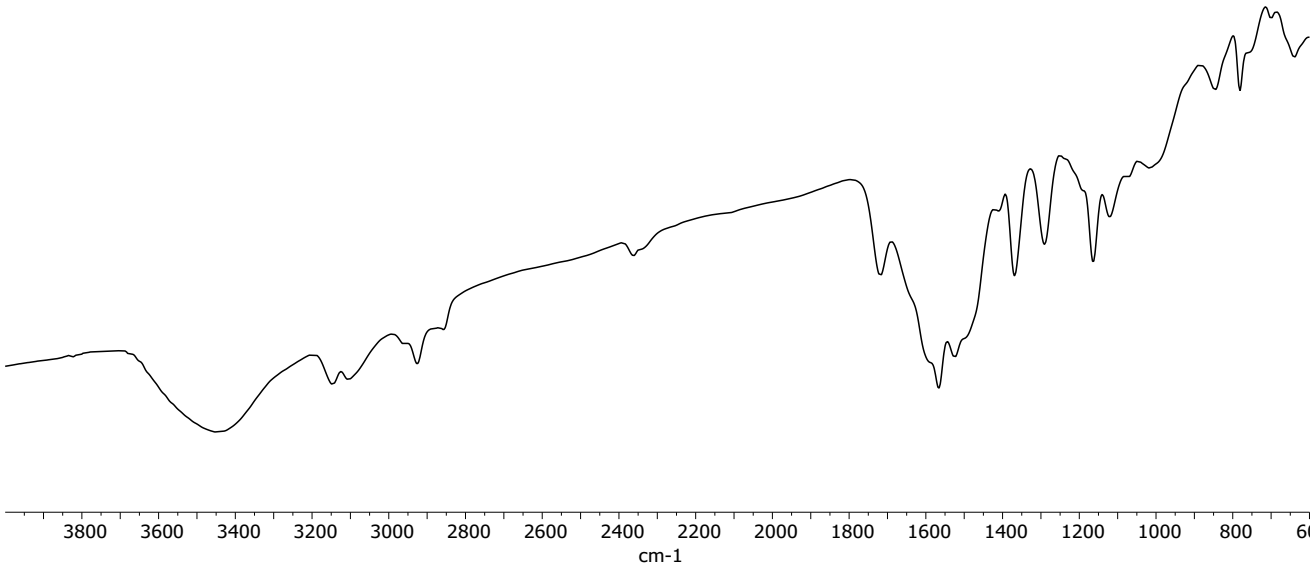


Fig. S20. FTIR spectra of compound **COOH-EG-Imd-EG-COOH**.

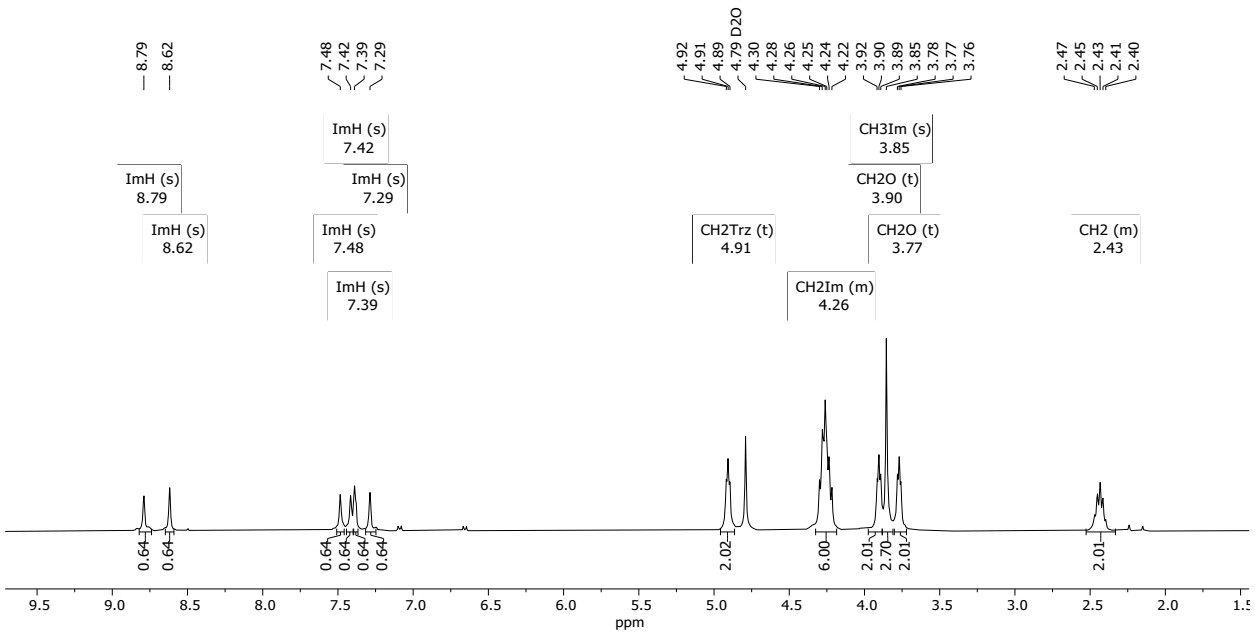


Fig. S21. ^{1}H NMR spectra (400 MHz) of compound **COOH-EG-Imd-C1** in D_2O.

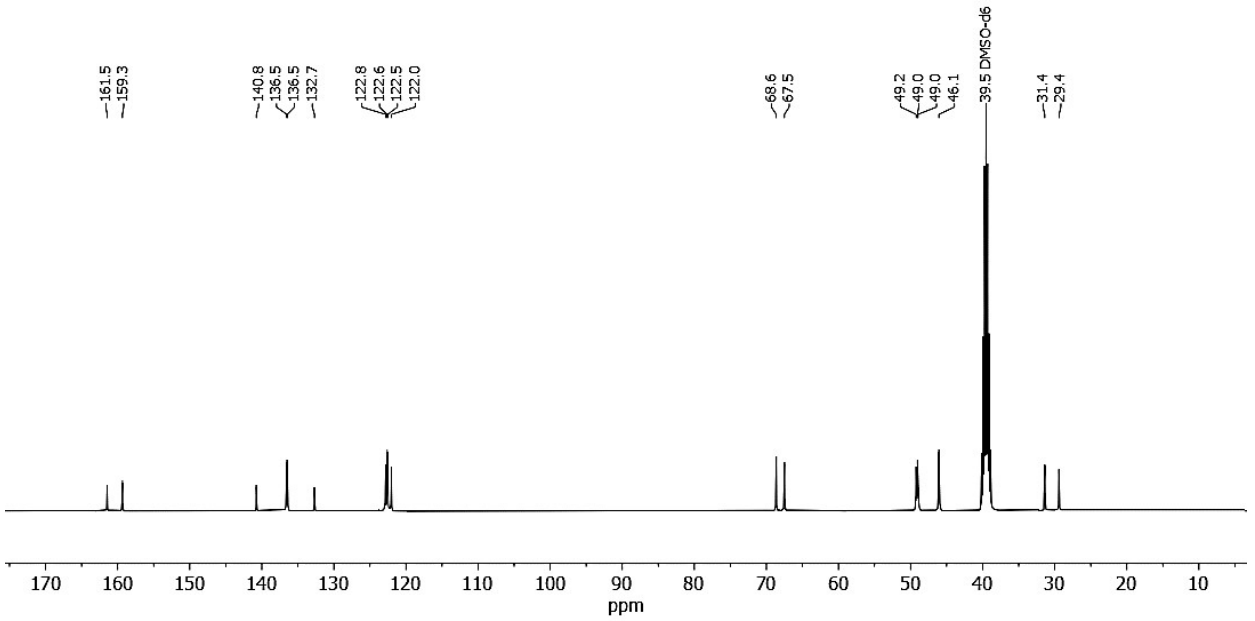


Fig. S22. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-EG-Imd-C1** in DMSO-d_6.

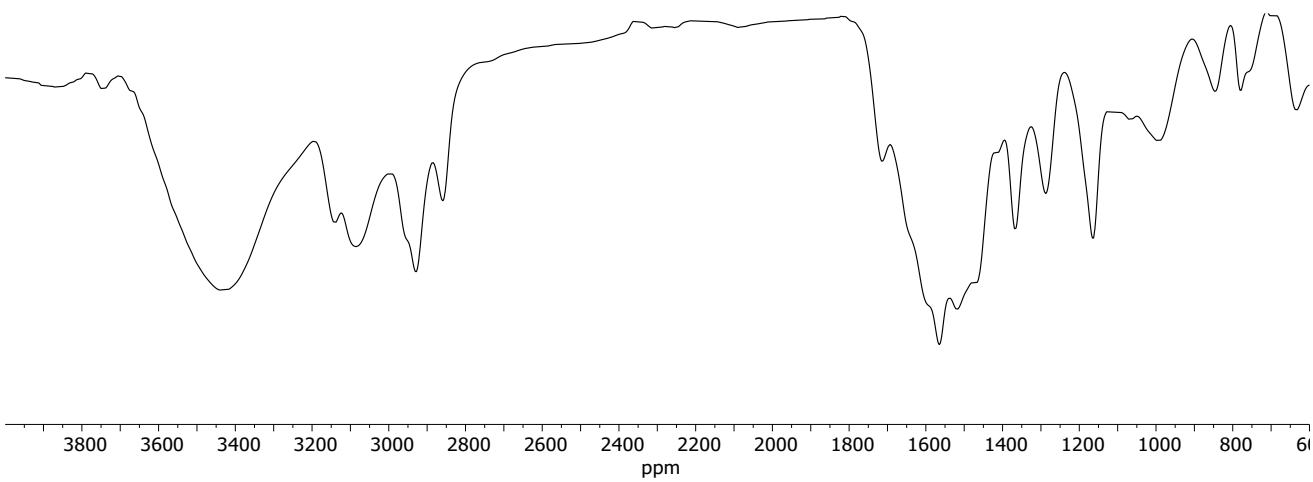


Fig. S23. FTIR spectra of compound **COOH-EG-Imd-C1**.

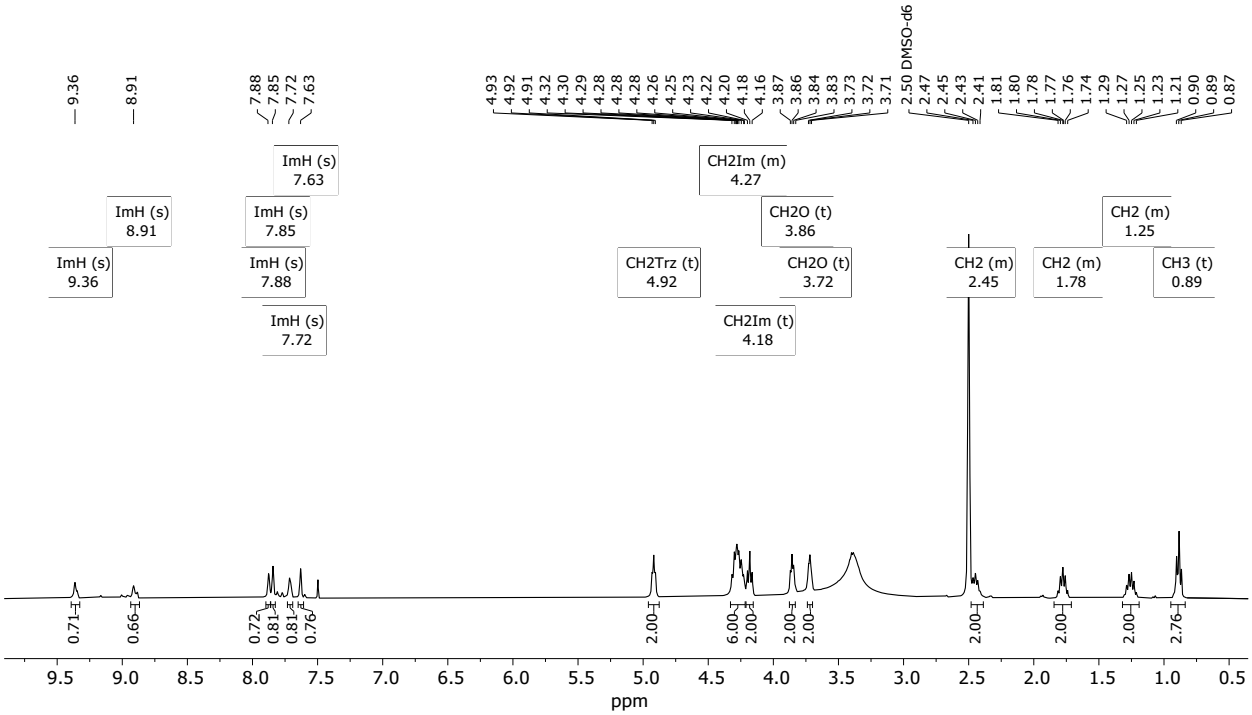


Fig. S24. ^{1}H NMR spectra (400 MHz) of compound **COOH-EG-Imd-C4** in DMSO-d_6.

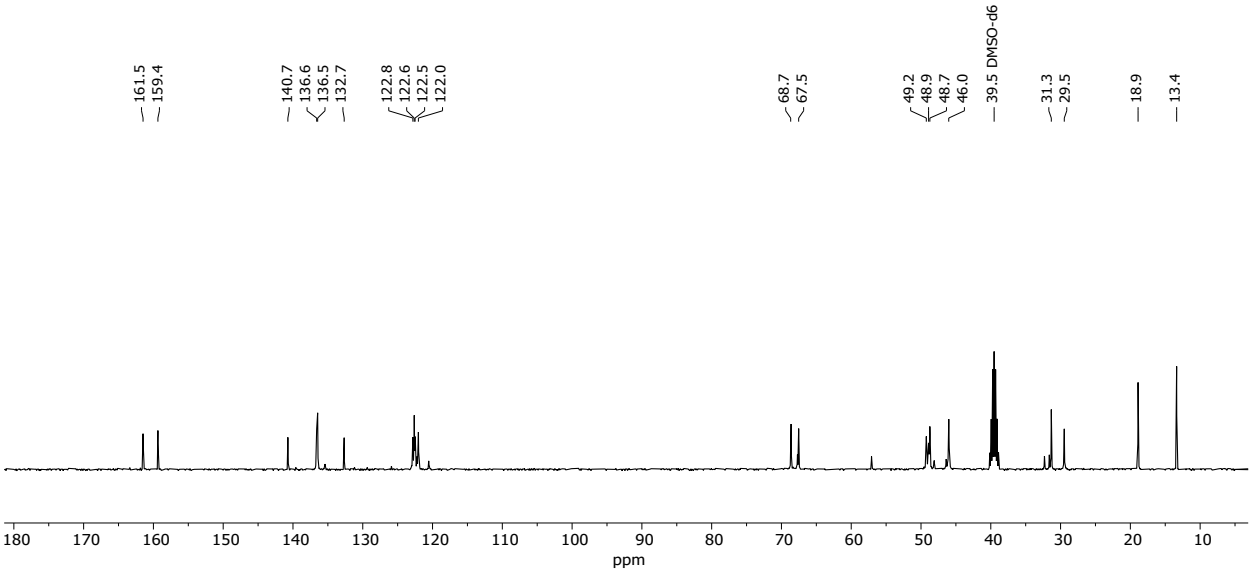


Fig. S25. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-EG-Imd-C4** in DMSO-d_6.

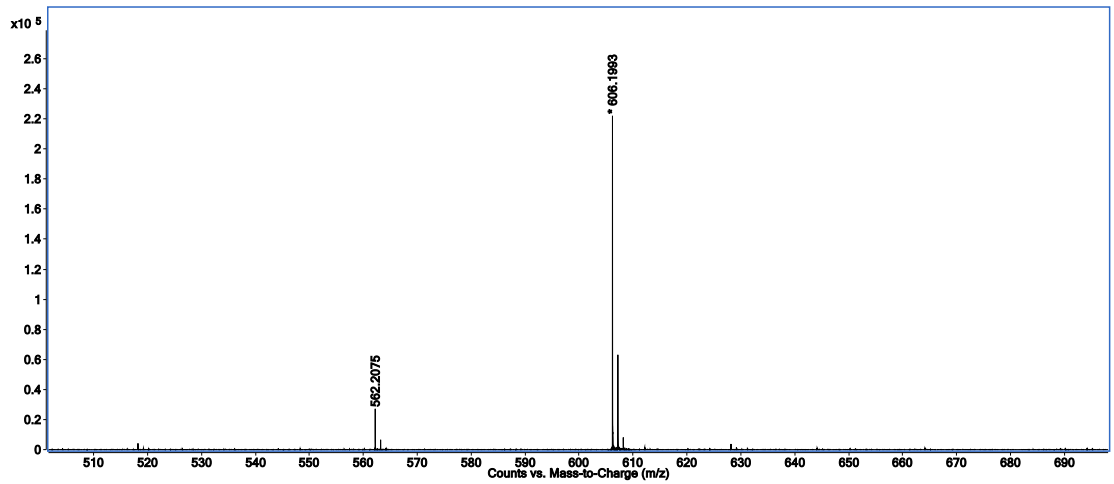


Fig. S26. ESI HR mass spectra of compound **COOH-EG-Imd-C4**

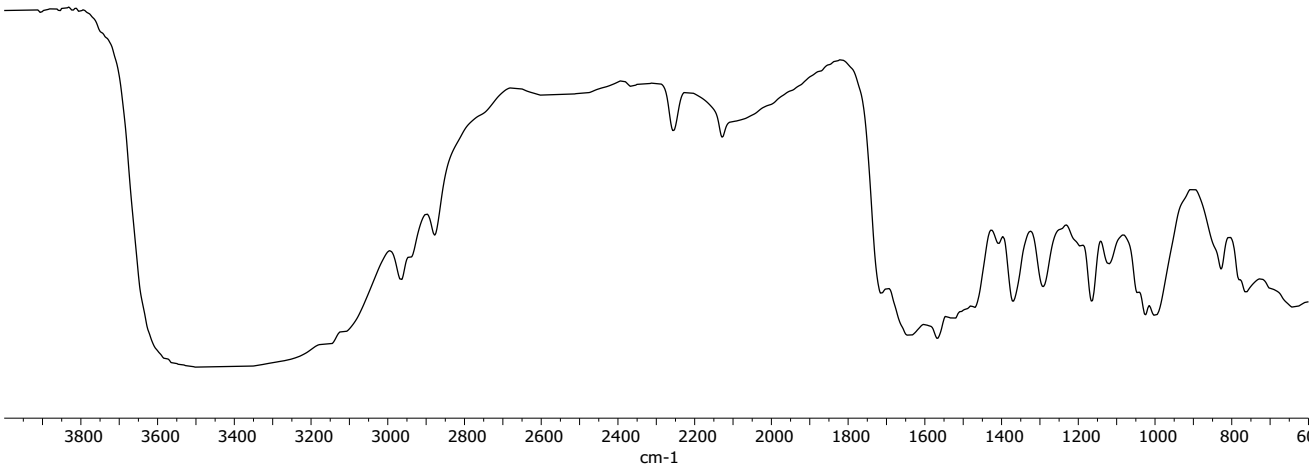


Fig. S27. FTIR spectra of compound **COOH-EG-Imd-C4**.

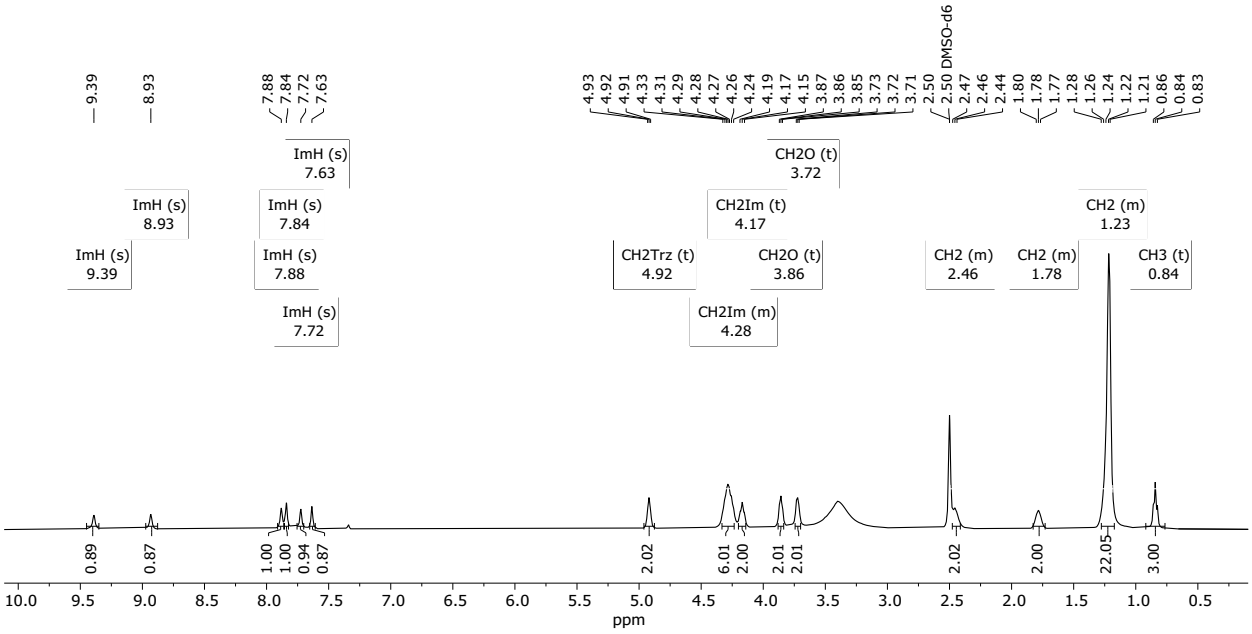


Fig. S28. ^{1}H NMR spectra (400 MHz) of compound **COOH-EG-Imd-C14** in DMSO-d_6.

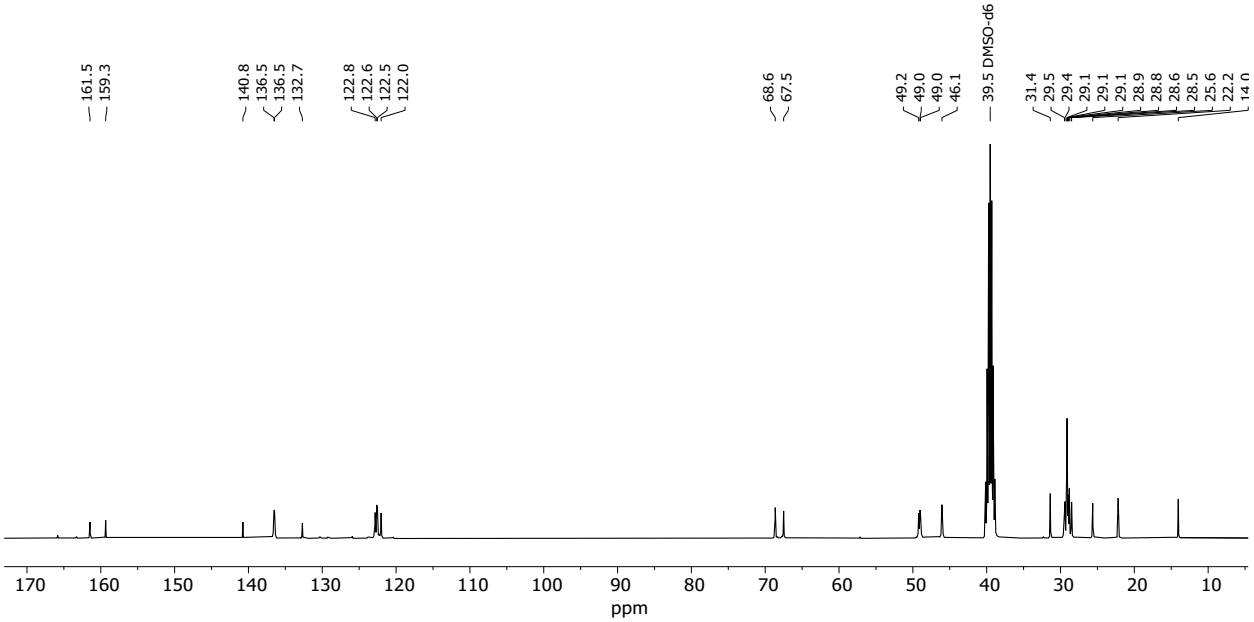


Fig. S29. ^{13}C-{^{1}H} NMR spectra (100.9 MHz) of compound **COOH-EG-Imd-C14** in DMSO-d_6.

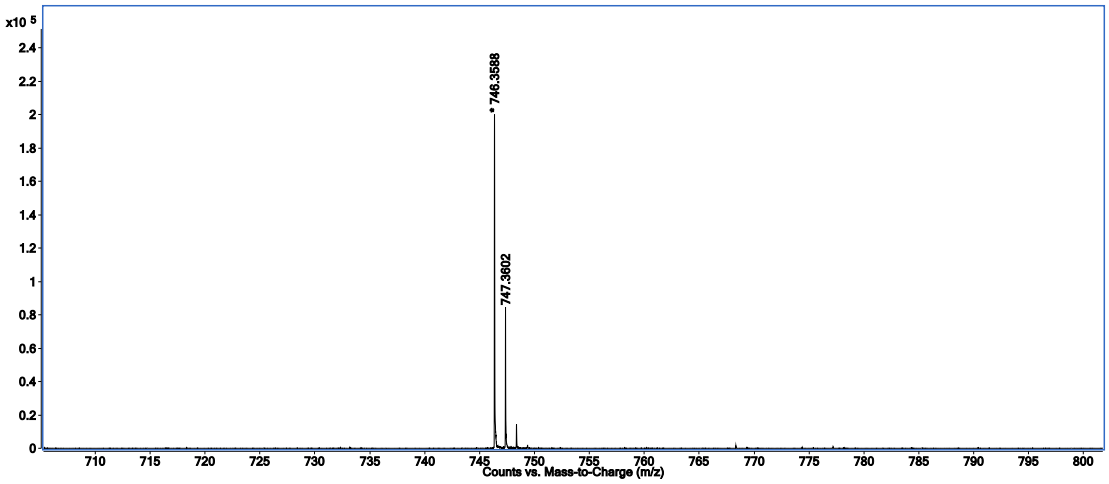


Fig. S30. ESI HR mass spectra of compound **COOH-EG-Imd-C14**

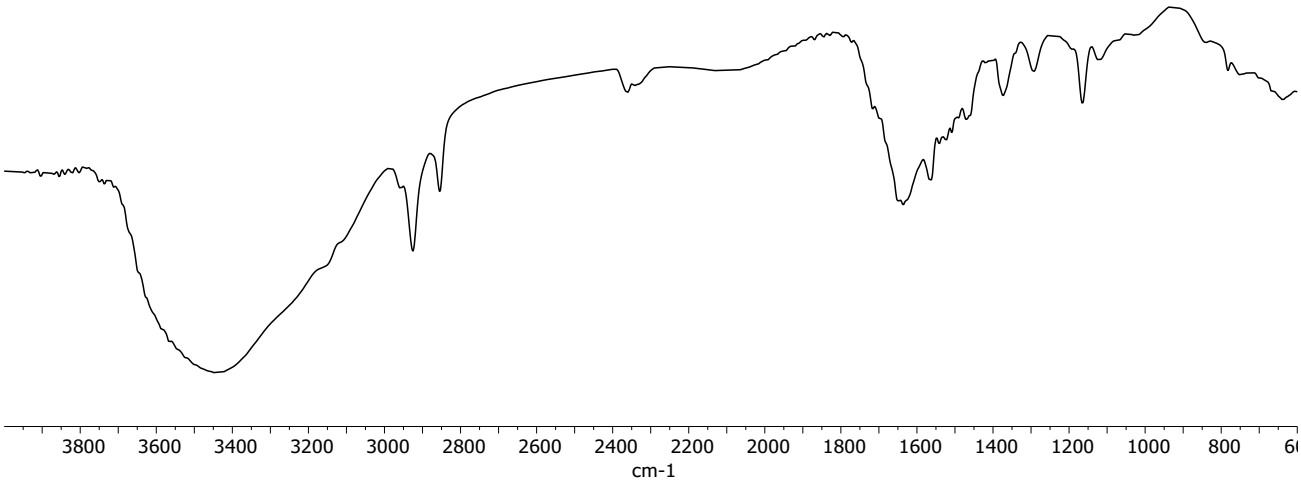


Fig. S31. FTIR spectra of compound **COOH-EG-Imd-C4**.

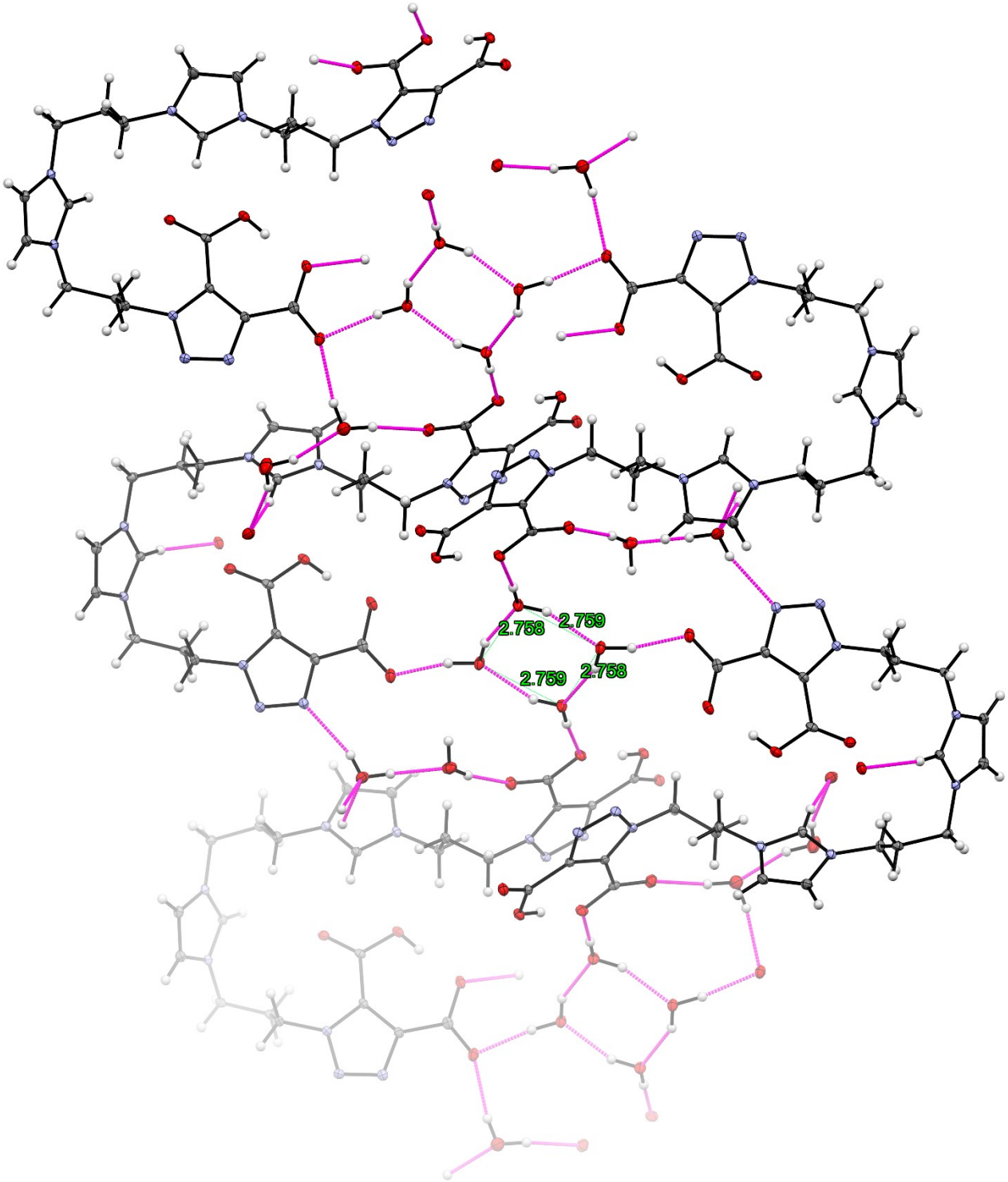


Fig. S32. ORTEP diagram of COOH-Imd-COOH showing 30% probability thermal ellipsoids. C atoms –grey, O atoms – red and N atoms - blue. The pink dotted line indicates close contacts, Green line – distance between oxygen atoms.

Table S1. Parameters from Eq. 1 Boltzmann.

Model	Boltzmann	
Equation	y = A2 + (A1-A2)/(1 + exp((x-x0)/dx))	
Surfactants	parameters	
COOH-Imd-C1	A1	3.29±0.15
	A2	9.93±0.08
	x0	0.62±0.02
	dx	0.23±0.01
	span	6.64±0.19
	R2	0.9977
COOH-Imd-C4	A1	3.68±0.18
	A2	9.84±0.09
	x0	0.67±0.02
	dx	0.23±0.02
	span	6.16±0.23
	R2	0.9964
COOH-Imd-C14	A1	3.20±0.06
	A2	9.36±0.05
	x0	1.68±0.02
	dx	0.53±0.02
	span	6.13±0.11
	R2	0.9984
COOH-Imd-COOH	A1	3.270±0.17
	A2	10.30±0.20
	x0	2.21±0.06
	dx	0.70±0.07
	span	7.02±0.33
	R2	0.9952
COOH-EG-Imd-COOH	A1	3.64±0.22
	A2	10.08±0.14
	x0	1.31±0.04
	dx	0.52±0.05
	span	6.44±0.33
	R2	0.9940
COOH-EG-Imd-C1	A1	3.39±0.10
	A2	9.75±0.06
	x0	0.79±0.01
	dx	0.28±0.01
	span	6.36±0.15
	R2	0.9987
COOH-EG-Imd-C4	A1	3.53±0.14
	A2	9.61±0.13
	x0	0.82±0.02
	dx	0.21±0.02
	span	6.08±0.22
	R2	0.9957
COOH-EG-Imd-C14	A1	3.49±0.07
	A2	10.29±0.04

	x0	1.79±0.01
	dx	0.42±0.02
	span	6.81±0.09
	R2	0.9988

Table S2. Topological polar surface area (TPSA)

	TPSA
COOH-Imd-C1	122.95
COOH-Imd-C4	122.95
COOH-Imd-C14	122.95
COOH-Imd-COOH	228.27
COOH-EG-Imd-COOH	246.74
COOH-EG-Imd-C1	132.19
COOH-EG-Imd-C4	132.19
COOH-EG-Imd-C14	132.19

Table S3. Estimated structural content (%) from CD spectra of BSA in the absence and presence of zwitterion derivatives.

System	Helix1 (regular)	Helix2 (distorted)	Antiparallel	Parallel	Turns	Others	RMSD
BSA	34.8	17.9	0	0	9.8	37.5	0.0742
BSA/HOOC-imd-C1	36.6	18.0	0	0	9.3	36.0	0.0952
BSA/HOOC-imd-C4	36.9	18.9	0	0	9.7	34.6	0.0909
BSA/HOOC-imd-C14	37.8	18.7	0	0	9.4	34.1	0.0911
BSA/HOOC-imd-COOH	36.4	18.1	0	0	10.1	35.4	0.0885
BSA/HOOC-EG-imd-EG-COOH	37.2	18.4	0	0	9.4	35.0	0.1176
BSA/HOOC-EG-imd-C1	37.8	18.5	0	0	9.5	34.2	0.0967
BSA/HOOC-EG-imd-C4	34.8	17.9	0	0	9.8	37.5	0.0742
BSA/HOOC-EG-imd-C14	36.5	19.2	0	0	9.2	35.1	0.0978

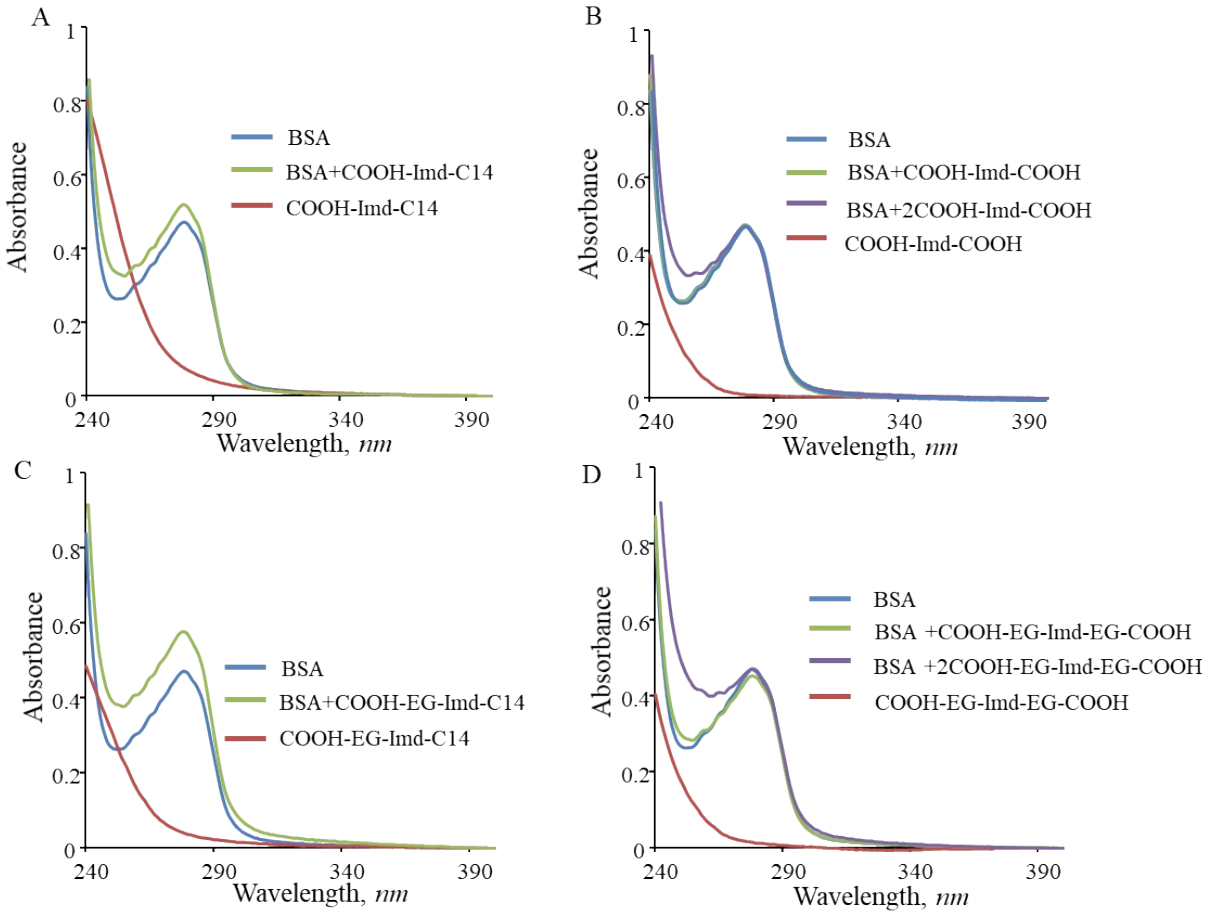


Fig. S33. UV-visible spectra of BSA in the absence and presence of A) COOH-Imd-C14, B) COOH-Imd-COOH, C) COOH-EG-Imd-C14 and D) COOH-EG-Imd-EG-COOH, C(BSA) = 0.165 mg/ml, *C*(carboxyimidazolium compounds) = 0.05 мМ), C(COOH-Imd-COOH) = C(COOH-EG-Imd-EG-COOH) = 0.1 mM, 25 °C.

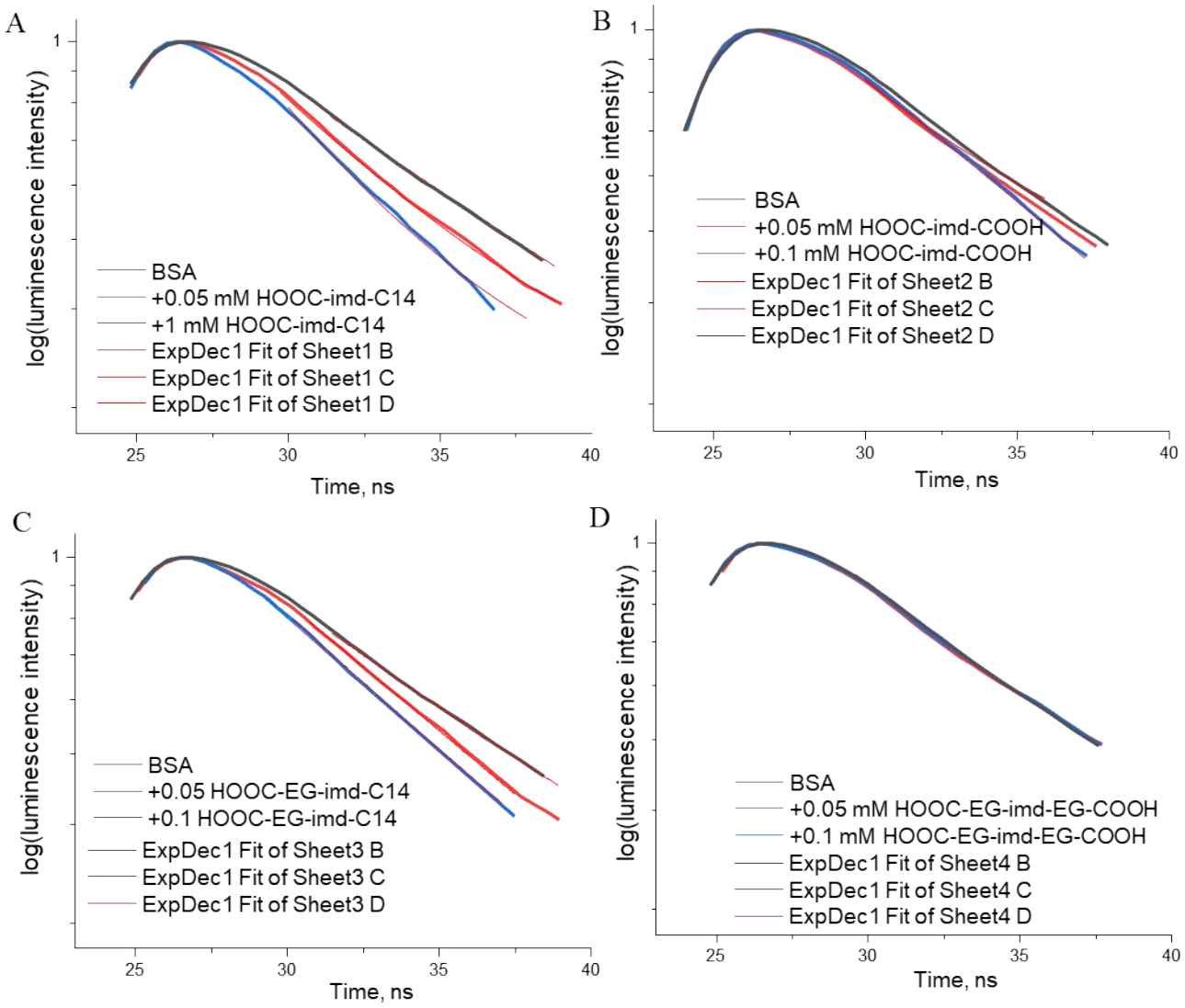


Fig. S34. Luminescence decayof BSA in the absence and presence of A) COOH-Imd-C14, B) COOH-Imd-COOH, C) COOH-EG-Imd-C14 and D) COOH-EG-Imd-EG-COOH, C(BSA) = 0.165 mg/ml, C(carboxyimidazolium compounds) = 0.05 мМ and 0.1 mM), Ex = 266 nm.