A Solanesol-derived Scaffold for Multimerization of Bioactive Peptides

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SUPPORTING INFORMATION

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General Experimental

Solanesol (**3**) and solanesyl bromide (**4**) were purchased from NetChem, New Brunswick, NJ. Serine amide hydrochloride was purchased from Senn Chemicals USA. Diethyl ether (ether), tetrahydrofuran (THF), and dichloromethane (DCM) were dried by passage through columns of activated alumina. For moisture sensitive reactions, glassware was flame-dried under argon. Analytical thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 F-254 plates with visualization by UV exposure, by exposure to I_2 vapor, or by staining with 10% phosphomolybdic acid solution in EtOH. Flash column chromatography was accomplished using silica gel 60 (4.0–63 μm). Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz or 500 MHz for ^{1}H NMR and at 75 MHz or 125 MHz for ^{13}C NMR. Chemical shifts (δ) are expressed in ppm and are internally referenced (7.24 ppm for $CDCl_3$ and 3.31 ppm for CD_3OD for ^{1}H NMR and 77.0 ppm for $CDCl_3$ and 49.15 ppm for CD_3OD for ^{13}C NMR). Fast-atom bombardment (FAB) mass spectra were measured with *m*-nitrobenzyl alcohol (NBA) or 1% trifluoroacetic acid in a matrix of 50% of glycerol, 25% of thioglycerol, and 25% of NBA (MIX). Electrospray ionization (ESI) was also used to ionize some of the samples. The samples were dissolved in 1:1 methanol:water at a concentration of ca 50 uM. Standard ESI conditions were applied to detect positively charged ions. Analytical HPLC was performed on a 4.6 × 75 mm Waters Symmetry® C_{18} column and preparative HPLC was performed on a 19 × 256 mm Waters X-Bridge Preparative C_{18} column. The mobile phase was 10-90% acetonitrile and water containing 1% TFA within 50 min, the flow rates were 1 mL/min and 15 mL/min for analytical and preparative runs, respectively. The dual UV detector system operated at 230 and 280 nm. ArgoGel™ Rink NH-Fmoc resin (0.68 mmol/g) was purchased from Novabiochem.

Non-standard Abbreviations:

APT, attached proton test

BSA, bovine serum albumin

Cl-HOBt, 6-chloro-1-hydroxybenzotriazole

CuAAC, copper(I)-catalyzed azide-alkyne cycloaddition

DIC, diisopropyl carbodiimide

DIEA, diisopropylethylamine

DMEM, Dulbecco's Modified Eagle Medium

DTPA, diethylenetriaminepentaacetic acid

FBS, fetal bovine serum

HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

hMC4R, human melanocortin 4 receptor

HOBt, 1-hydroxybenzotriazole

MSH(4), His-DPhe-Arg-Trp

NDP-α-MSH, Ser-Tyr-Ser-Nle-Glu-His-DPhe-Arg-Trp-Gly-Lys-Pro-Val

Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-ylsulfonyl

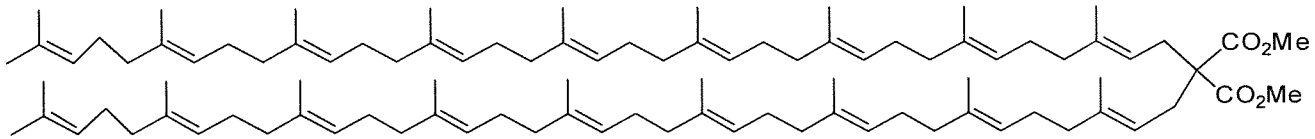
PEGO, 19-amino-5-oxo-3,10,13,16-tetraoxa-6-azanonadecan-1-oic acid

PVA, poly(vinyl alcohol)

TBTA, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine

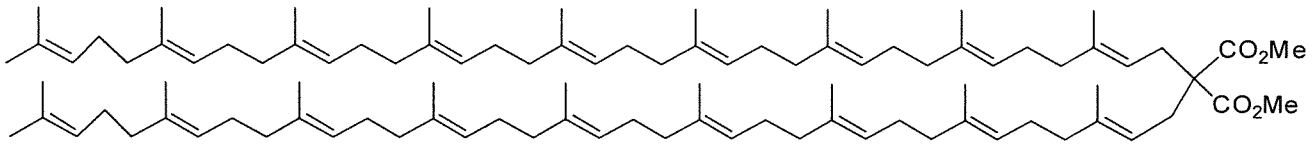
Tris-HCl, tris(hydroxymethyl)amino methane hydrochloride

TRF, time-resolved fluorescence



5

1H-NMR of Compound **5**



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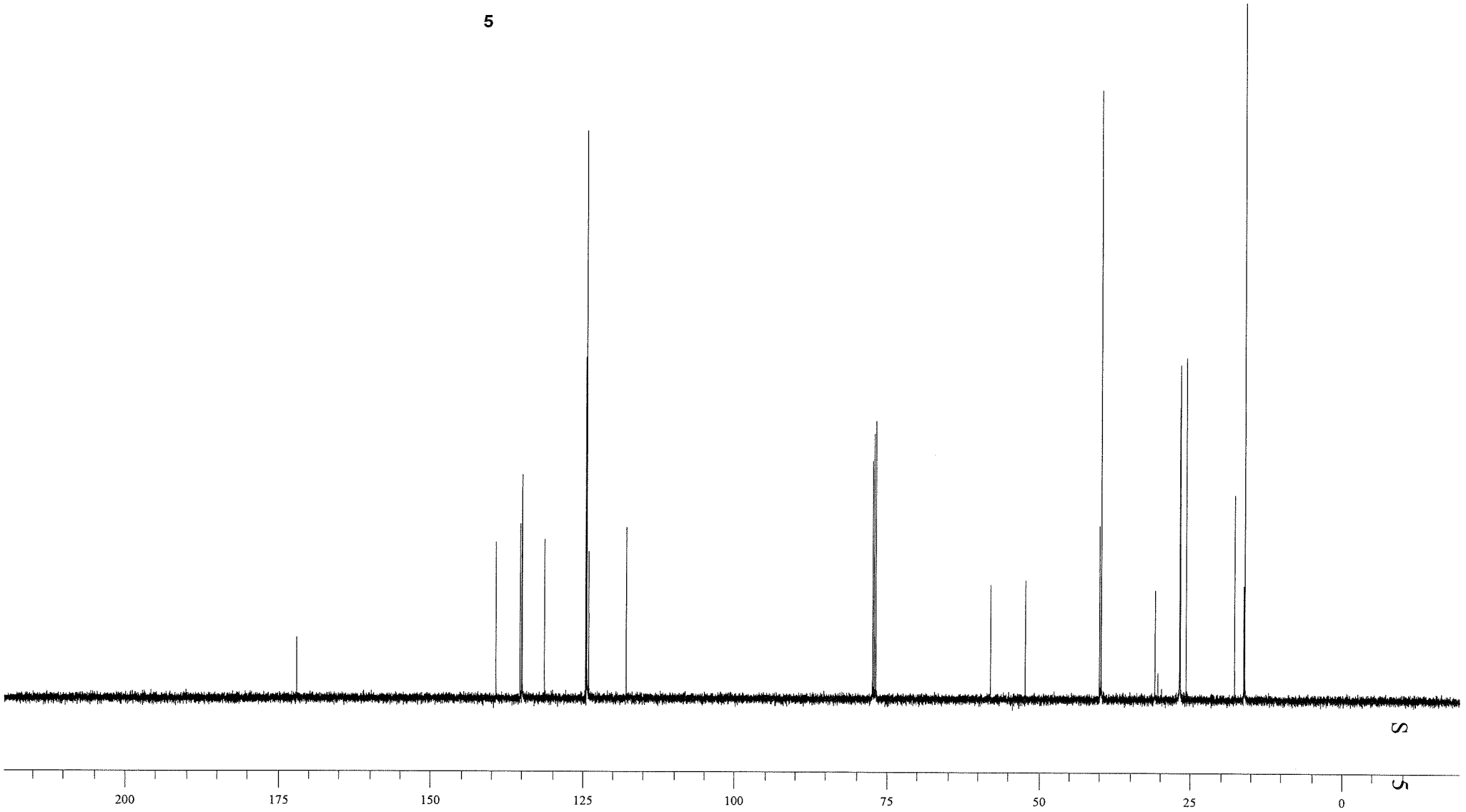
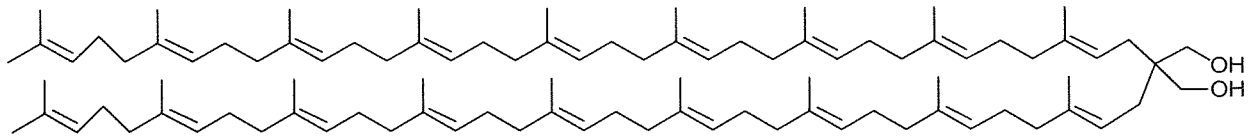
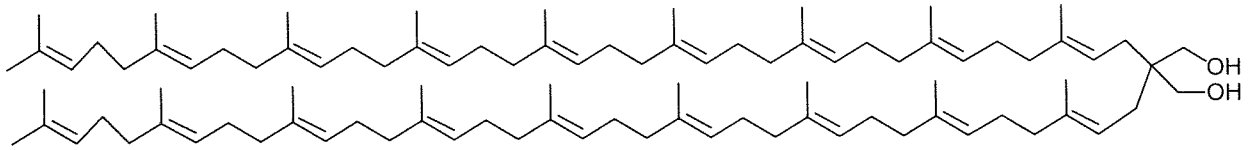


13C-NMR of Compound **5**



6

1H-NMR of Compound **6**



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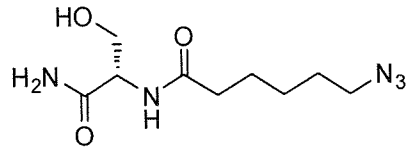
13C-NMR of Compound **6**



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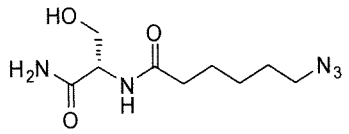
1H-NMR of Compound **10**



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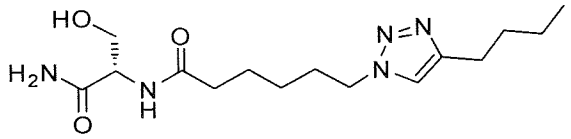
13C-NMR of Compound **10**



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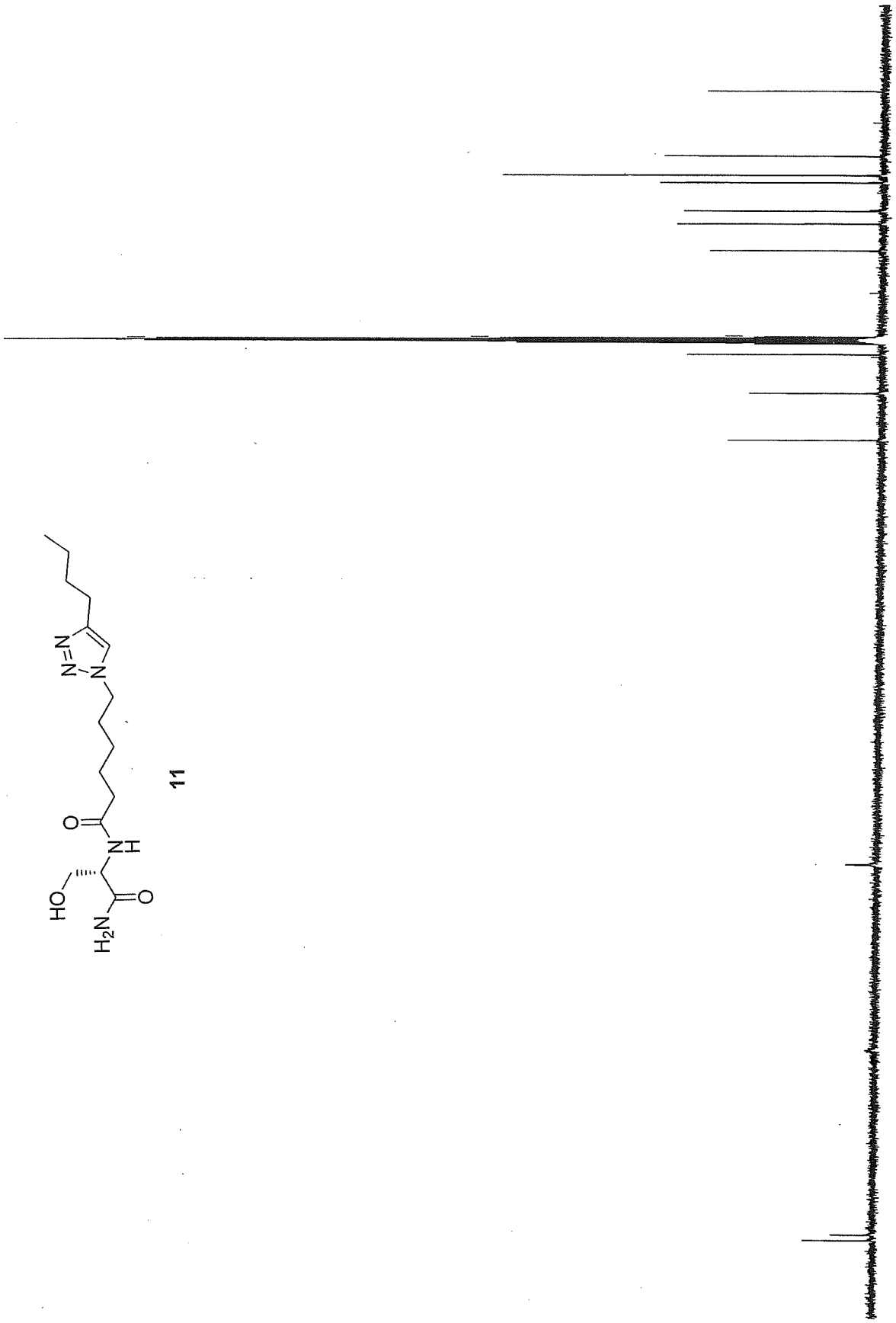
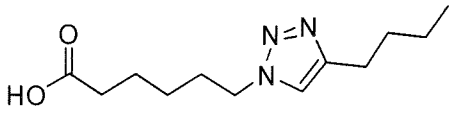
1H-NMR of Compound **11**



13C-NMR of Compound **11**



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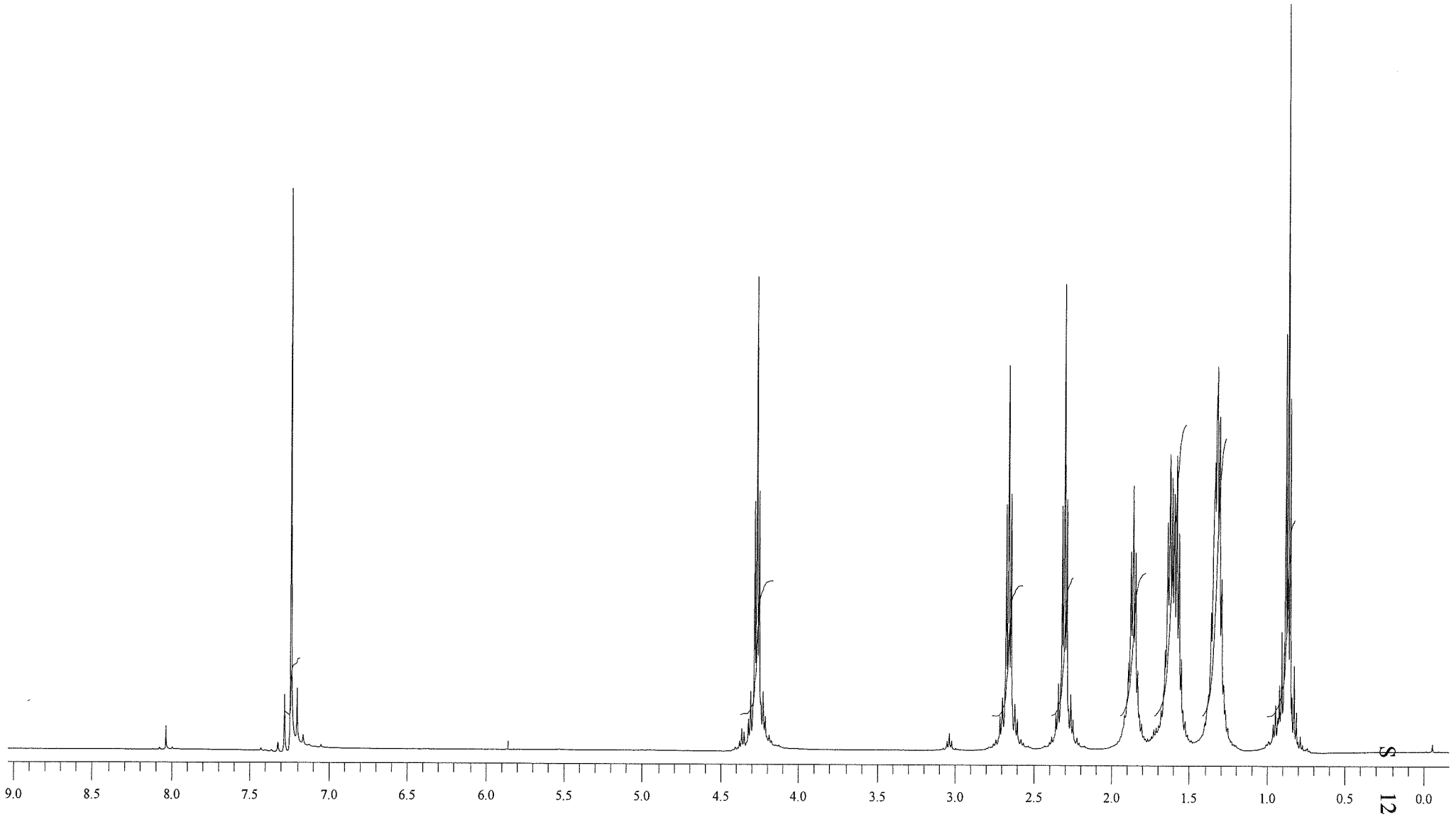
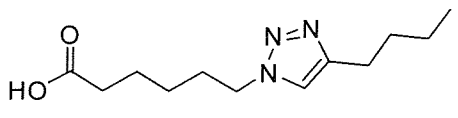


1H-NMR of Compound **12**



12

13C-NMR of Compound **12**

Figure S11. APT of **7**.

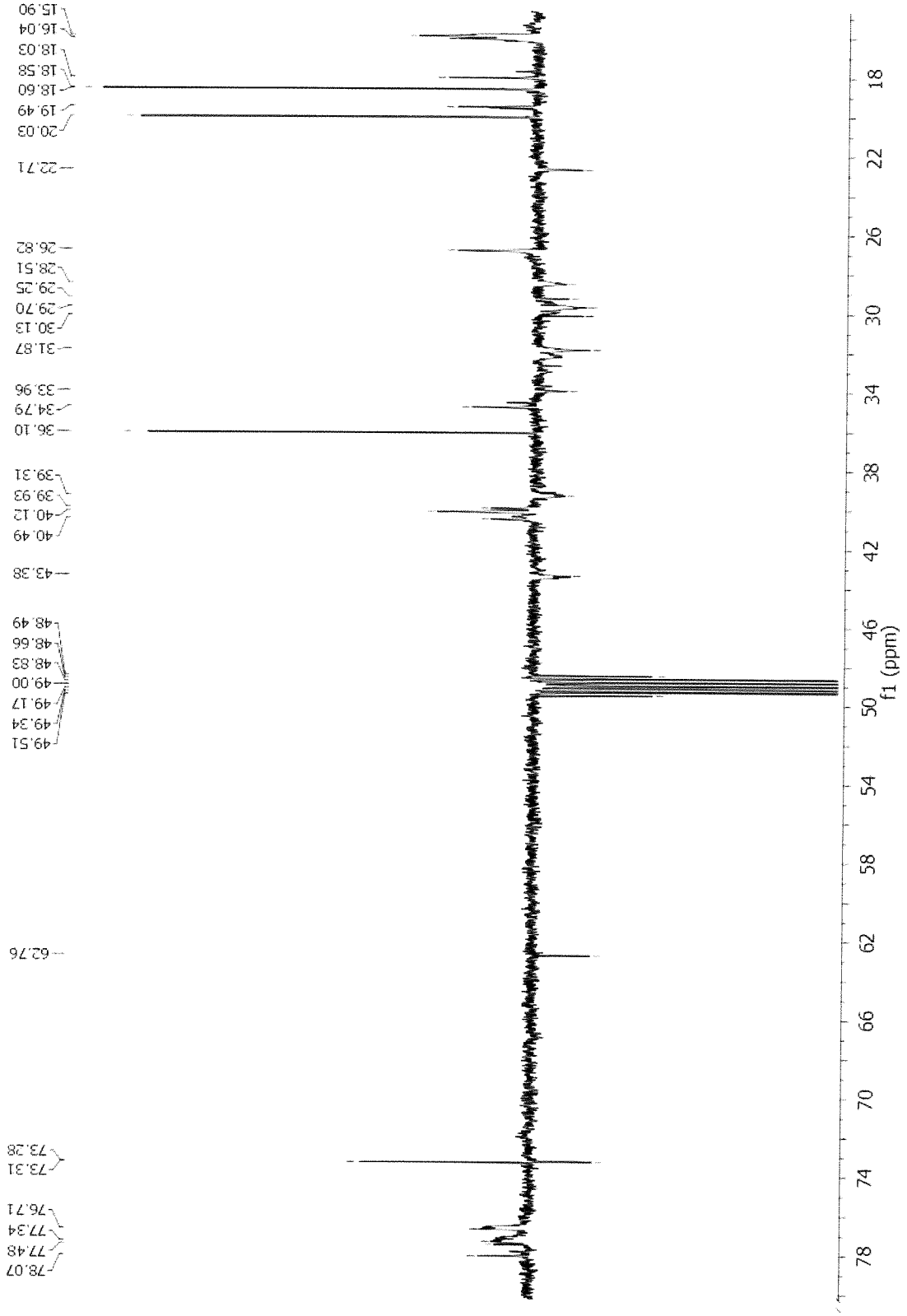
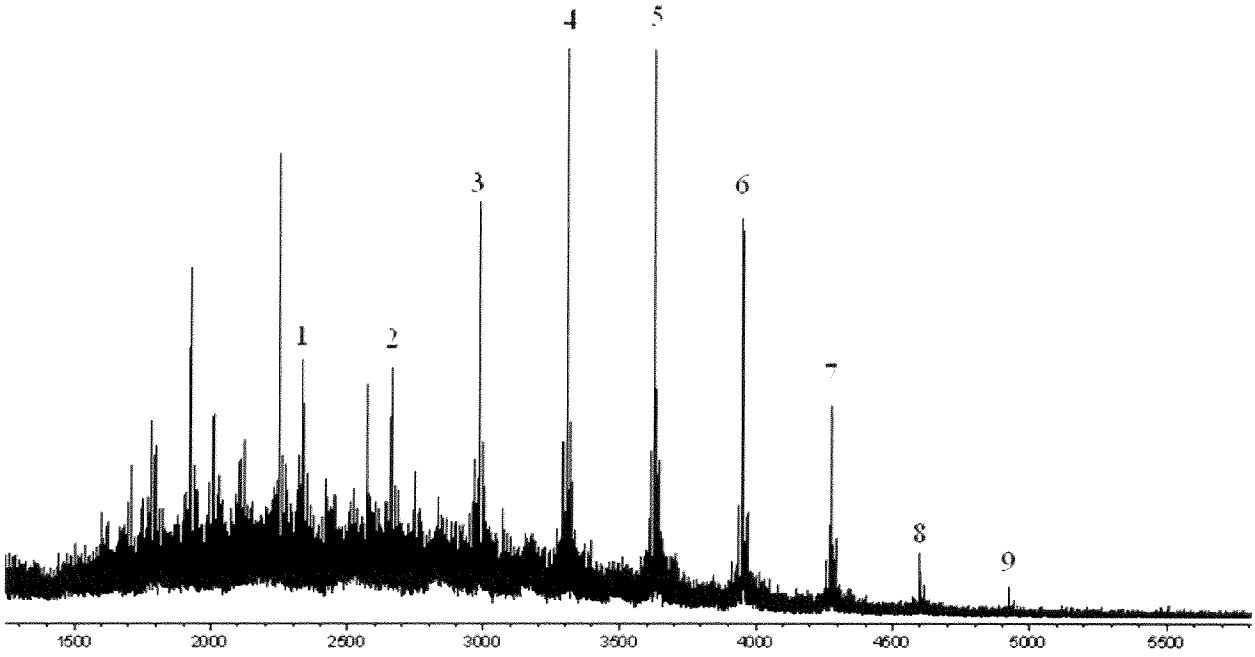
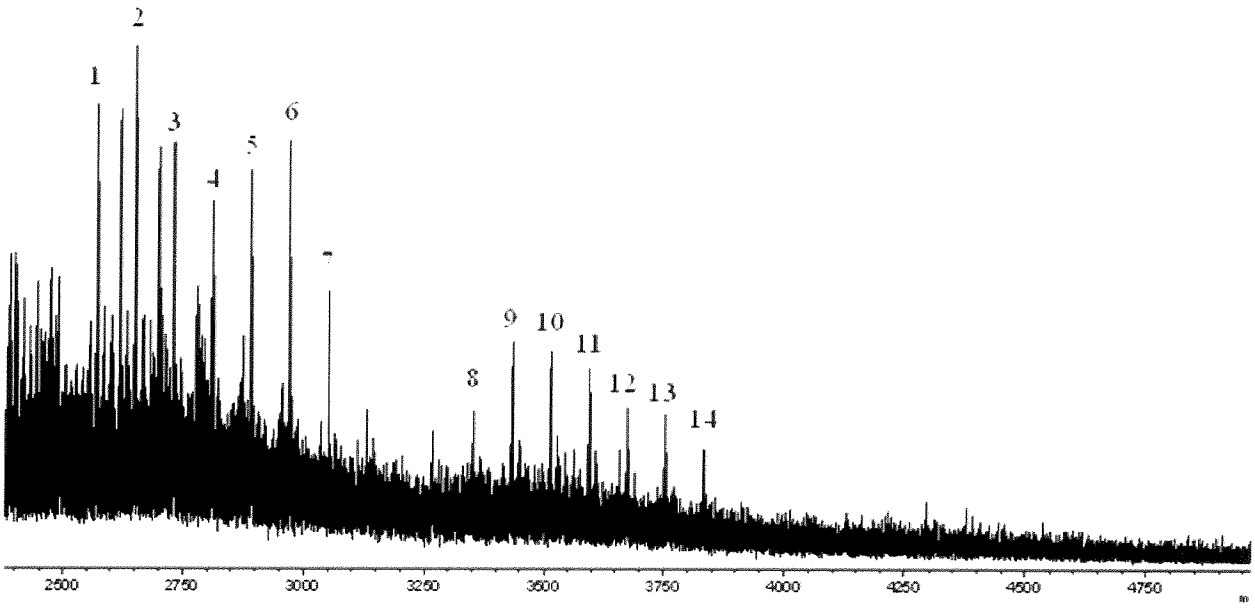


Figure S12. MALDI-TOF of **15**.



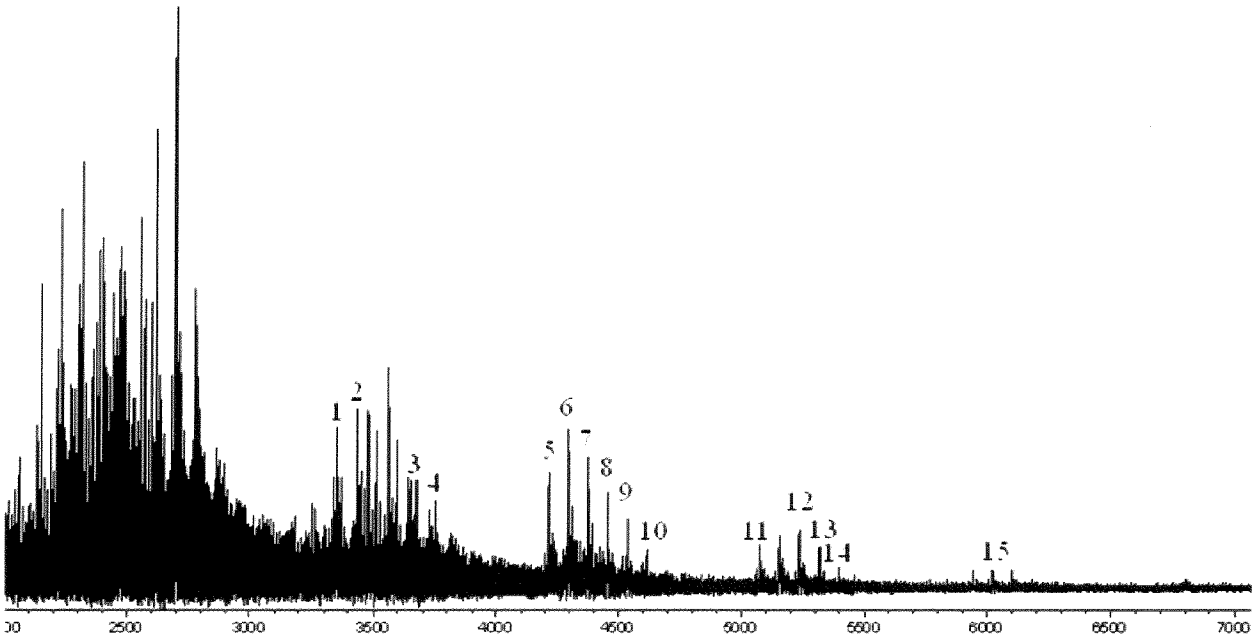
Peak number	Number of side chains	Number of triazole rings	Number of MSH(4) ligands	Number of serinamide residues
1	2	2	0	2
2	3	3	0	3
3	4	4	0	4
4	5	5	0	5
5	6	6	0	6
6	7	7	0	7
7	8	8	0	8
8	9	9	0	9
9	10	10	0	10

Figure S13. MALDI-TOF of **16a**.



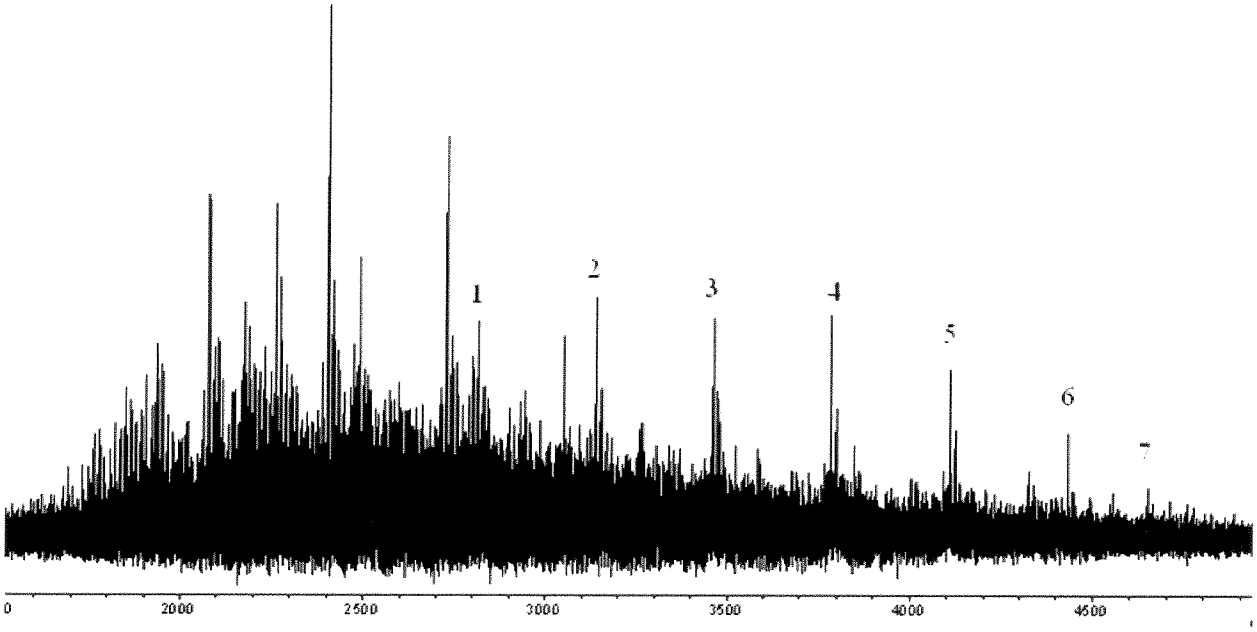
Peak number	Number of side chains	Number of triazole rings	Number of MSH(4) ligands	Number of serinamide residues
1	2	1	1	0
2	3	1	1	0
3	4	1	1	0
4	5	1	1	0
5	6	1	1	0
6	7	1	1	0
7	8	1	1	0
8	2	2	2	0
9	3	2	2	0
10	4	2	2	0
11	5	2	2	0
12	6	2	2	0
13	7	2	2	0
14	8	2	2	0

Figure S14. MALDI-TOF of **16b**.



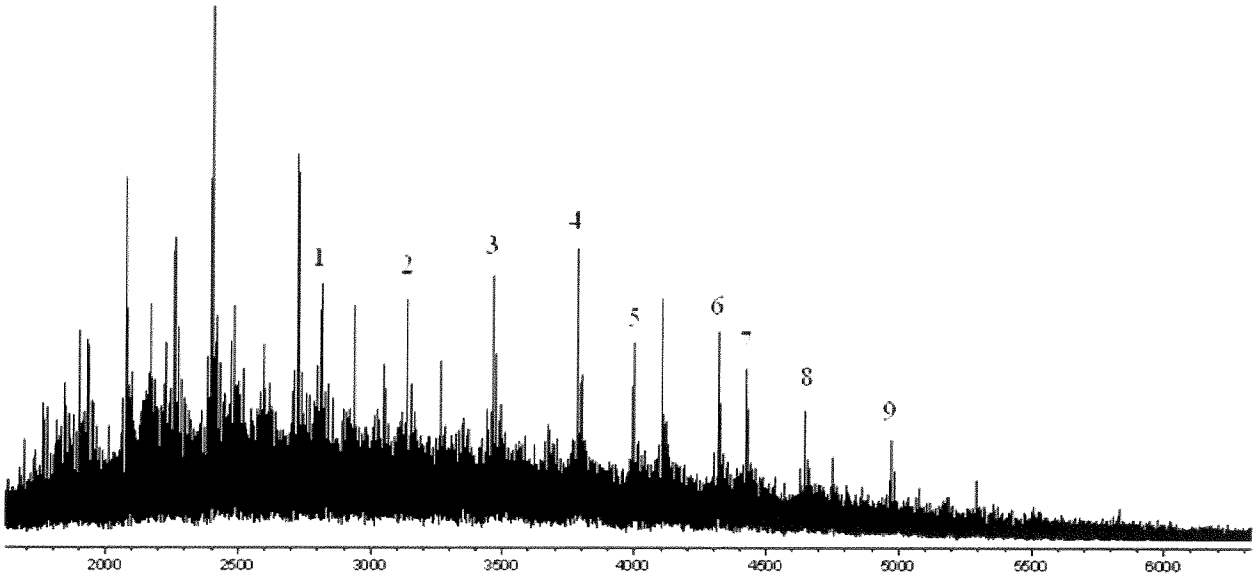
Peak number	Number of side chains	Number of triazole rings	Number of MSH(4) ligands	Number of serinamide residues
1	2	2	2	0
2	3	2	2	0
3	6	2	2	0
4	7	2	2	0
5	3	3	3	0
6	4	3	3	0
7	5	3	3	0
8	6	3	3	0
9	7	3	3	0
10	8	3	3	0
11	4	4	4	0
12	6	4	4	0
13	7	4	4	0
14	8	4	4	0
15	6	5	5	0

Figure S15. MALDI-TOF of **16c**.



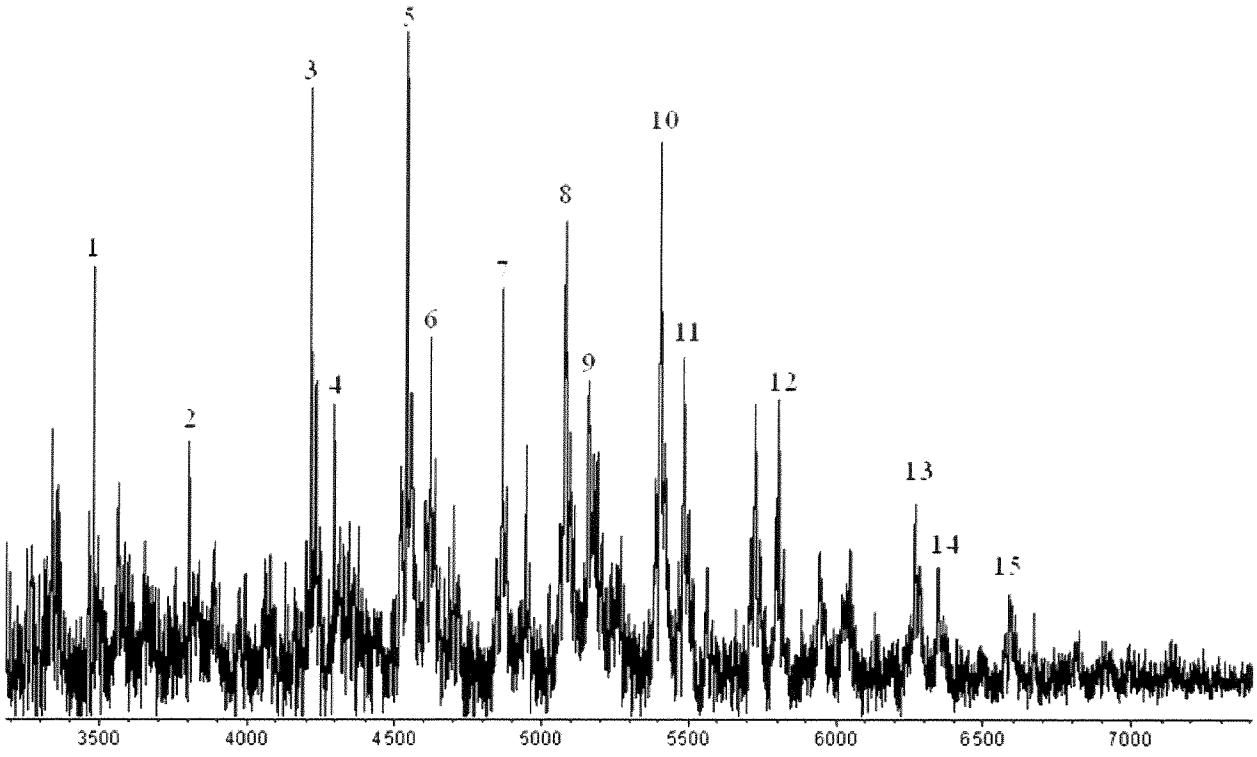
Peak number	Number of side chains	Number of triazole rings	Number of MSH(4) ligands	Number of serinamide residues
1	2	2	1	1
2	3	3	1	2
3	4	4	1	3
4	5	5	1	4
5	6	6	1	5
6	7	7	1	6
7	6	6	2	4

Figure S16. MALDI-TOF of **16d**.



Peak number	Number of side chains	Number of triazole rings	Number of MSH(4) ligands	Number of serinamide residues
1	2	2	1	1
2	3	3	1	2
3	4	4	1	3
4	5	5	1	4
5	4	4	2	2
6	5	5	2	3
7	7	7	1	6
8	6	6	2	4
9	7	7	2	5

Figure S17. MALDI-TOF of **16e**.



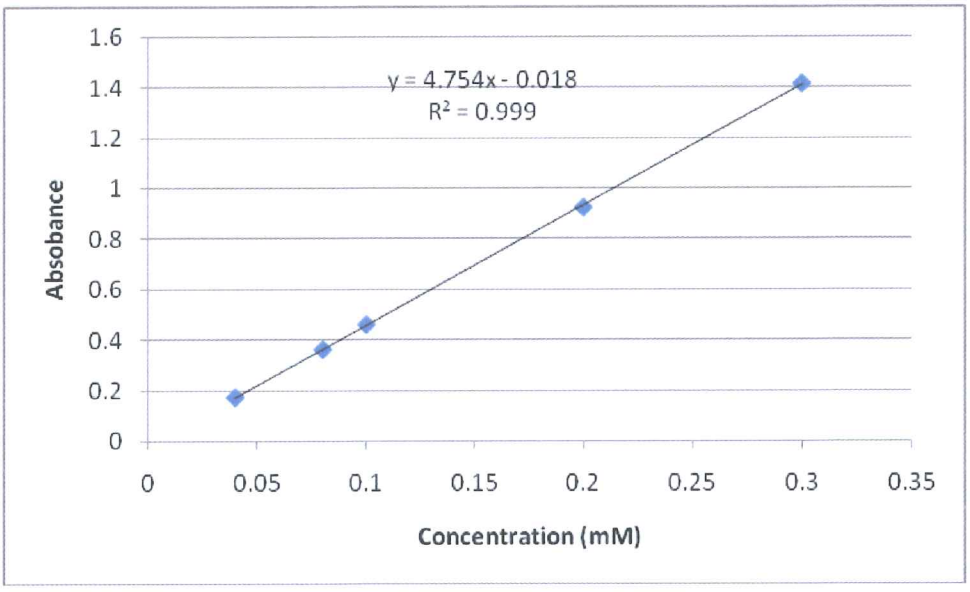
Peak number	Number of side chains	Number of triazole rings	Number of MSH(4) ligands	Number of serinamide residues
1	4	4	1	3
2	5	5	1	4
3	3	3	3	0
4	4	3	3	0
5	4	4	3	1
6	5	4	3	1
7	5	5	3	2
8	4	4	4	0
9	5	4	4	0
10	5	5	4	1
11	6	5	4	1
12	7	6	4	2
13	6	6	5	1
14	7	6	5	1
15	7	7	5	2

UV Studies

MSH(4) (Ac-His-DPhe-Arg-Trp-NH_2)

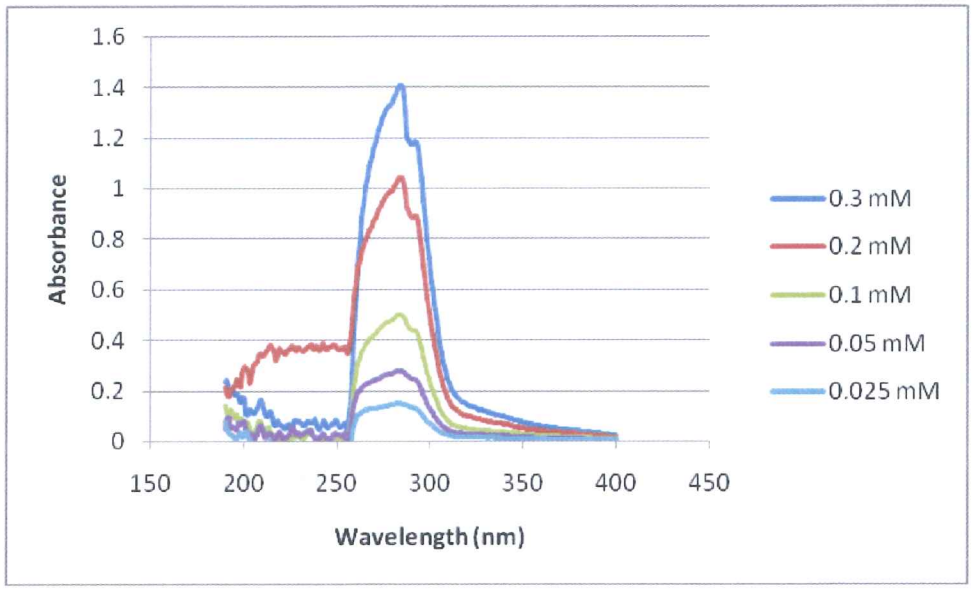
Solutions of Ac-MSH(4) (0.3, 0.2, 0.1, 0.08, 0.04 mM) were prepared in dry DMSO by dilution. A plot of concentration vs absorbance at λ_{max} = 282 appears below.

Concentration (mM)	Absorbance (λ_{max} = 282 nm)
0.04	0.173
0.08	0.362
0.1	0.46
0.2	0.923
0.3	1.413



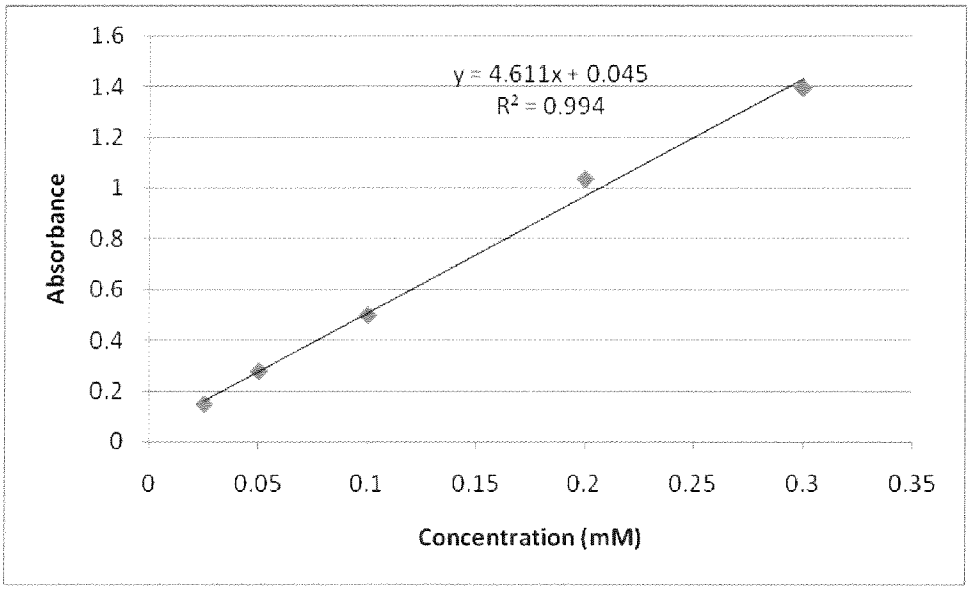
MSH(4) derivative 14.

Solutions of compound **14** (0.3, 0.2, 0.1, 0.05, 0.025 mM) were prepared in dry DMSO by dilution. The UV spectra of these solutions appear below.



A plot of concentration vs absorbance at λ_{max} = 282 appears below.

Concentration (mM)	Absorbance (λ_{max} = 282 nm)
0.025	0.146
0.05	0.276
0.1	0.496
0.2	1.033
0.3	1.391


y = 4.611x + 0.045
R² = 0.994
Absorbance
Concentration (mM)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35