Supplementary information for:

Adaptive Multifunctional Supramolecular Assemblies of Glycopeptides Rapidly Enable Morphogenesis

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Materials and Instruments

4-Chloro-7-nitrobenzofurazan (NBD-Cl) was purchased from TCI and amino acids, O-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluorophosphate (HBTU) from GL Biochem (Shanghai) Ltd. Anti-N cadherin (ab76057), anti-integrin β1 (ab52971), anti-fibronectin (ab23750), anti-integrin β_3 (ab197662), anti-integrin $\alpha_v\beta_3$ (ab78289), goat anti-rabbit IgG H&L (Alexa Fluor® 647) (ab150079), goat anti-rabbit IgG H&L (Alexa Fluor® 488) (ab150077) antibodies, Live and Dead Cell Assay (ab115347) were purchased from Abcam. Anti-E cadherin (24E10) mAb was purchased from Cell Signaling Technology, anti-E-selectin (Pa5-29946) from ThermoFisher Scientific, ethylenediaminetetraacetic acid (EDTA, 071M0262V) from Sigma-Aldrich, A205804 (2524) and cilengitide (5870) from Tocris Bioscience, and Actin Cytoskeleton and Focal Adhesion Staining Kit (FAK 100) from EMD Millipore. All the solvents and chemical reagents were used directly as received from the commercial sources without further purification. All the products were purified with Water Delta600 HPLC system, equipped with an XTerra C18 RP column and

an in-line diode array UV detector. Confocal microscopy images were obtained on Leica TCS SP2 or Zeiss 880 spectral confocal microscope. Electron microscopy imaging was performed on a FEI Morgagni 268 TEM with a 1k CCD camera (GATAN, Inc., Pleasanton, CA, USA) or a 300 keV Tecnai F30 intermediate voltage TEM (FEI, Inc., Hillsboro, OR, USA) with a 4k CCD camera (GATAN).

Synthesis (1 as example)

The protected peptide fragments, NBD-Phe-Arg(Pbf)-Gly-Asp(tBu), was first synthesized by solid phase peptide synthesis (SPPS).[54] After being activated by N-hydroxysuccinimide (NHS) and *N,N′*-Diisopropylcarbodiimide (DIC), the C-terminal activated peptide reacts with D-glucosamine to give the intermediate compound, which turns into **1** after the removal of the protecting groups by the mixture of trifluoroacetic acid (TFA), H_2O, triisopropylsilane (TIS) (19:0.5:0.5) (Supplementary Figure S1). The compounds were purified by preparative high-performance liquid chromatography (HPLC) using CH_3CN (0.1% of trifluoroacetic acid) and water (0.1% of trifluoroacetic acid) as the eluents. Other molecules were synthesized following the same/similar procedure.

Light Scattering

The static light scattering (SLS) experiments were performed using an ALV (Langen, Germany) goniometer and correlator system with a 22 mW HeNe (λ = 633 nm) laser and an avalanche photodiode detector. Before testing, all samples were filtered with 0.45 μm PTFE filters after sonicating and heating. The SLS tests were carried out at room temperature, and the angles of light

scattering detection were 30, 60, 90, and 120°. The resulting intensity ratios are proportional to the amount of aggregates in the samples. The test tubes (VWR60825-538) used as sample container was thoroughly cleaned with detergent prior to use.

Transmission Electron Microscopy Imaging (TEM)

Negative staining technique was used in TEM imaging. The 400 mesh copper grids (#1200211, Spi Supplies) coated with continuous thick carbon film (~ 35 nm) were glowed prior to use in order to increase the hydrophilicity. After being loaded on the grid, samples (4 μL) were rinsed by dd-water twice or three times. Immediately after rinsing, the grids containing sample were stained with 2.0 % w/v uranyl acetate three times. The grids were allowed to dry in air prior to imaging.

Cell Culture

All cell lines were purchased from the American Type Culture Collection (ATCC) were propagated in a fully humidified incubator containing 5% CO_2 at 37°C. HS-5 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, #11965092, Gibco™ Life Technology), supplemented with FBS (Gibco 10438026) to a final concentration of 10% and 1% antibiotics (Gibco15140122). The OVSCAR-3 cells were propagated in RPMI-1640 Medium (ATCC® 30-2001™), supplemented with 20% FBS, 0.01 mg/ml bovine insulin (#I0516, Sigma-Aldrich).

LIVE/DEAD Assay

Cells in exponential growth phase were seeded in glass bottomed culture chamber/dish in 2×10^5 cells/well. The cells were allowed for attachment for 12-24 h at 37 ºC, 5% CO_2. The culture medium was removed, and new culture medium containing compounds at certain concentration was added. After incubation for certain time, cells were stained with 2× Live and Dead Dye for 10 min at 37 ºC in dark. After that, cells were ready for imaging.

MTT Cell Viability/Proliferation Assay

Cells in exponential growth phase were seeded in a 96 well plate (#087722C, Fisher HealthCare) at a concentration of 1×10^4 cell/well. The cells were allowed to attach to the wells for 24 h at 37 ºC, 5% CO_2. The culture medium was removed and 100 μL culture medium containing compounds (immediately diluted from fresh prepared stock solution of 10 mM) at gradient concentrations (0 μM as the control) was placed into each well. After culturing at 37 ºC, 5% CO_2 for 24 h, each well was added with 10 μL of 5 mg/mL MTT ((3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, #M5655, Sigma-Aldrich), and the plated cells were incubated at dark for 4 h, 37 ºC, 5% CO_2. 100 μL 10% SDS (# BP166-500, Fisher HealthCare) with 0.01 M HCl was added to each well to stop the reduction reaction and to dissolve the purple precipitates. After incubation of the cells at 37 ºC for overnight, the OD value at 595 nm of the solution was measured in a microplate reader (Beckman Coulter DTX 880 Multimode Detector). Data represent the mean ± standard deviation of three independent experiments. For each concentration, triplicate was conducted to ensure productivity and for calculating standard deviation.

To interpret Figure 6, this assay has wells for plate controls in addition to wells that contain compounds at different concentrations. The 100% viability corresponds to no drug control, and high concentration of a drug known to produce the maximum possible effect (low viability). In Figure 6, the estimated IC_{50} is labeled as the concentration at which the cell viability is 50%.

Cell Clustered Colonies Counting

Cells in exponential growth phase were seeded in 96 well plates with a density of 3×10^4 cells/well. The cells were allowed for attachment for 12-24 h at 37 ºC, 5% CO_2. The culture medium was removed, and fresh culture medium containing compounds at certain concentration was added. After 24 h, clustered colonies were ready for counting and imaging under optical microscope. For each condition, technical triplicate was conducted to ensure reproducibility and to calculate the error bars.

Cell Migration Assay

Cell migration assays were performed by using confluent cell monolayers of HS-5 cells in 3.5-cm petri dishes, wounded by a 1 mL pipette tip. Wounded and unwounded monolayers were washed and refed with fresh complete cell culture medium (with or without the certain compounds), incubated at 37°C for 24 hours, then imaged with optical microscope equipped with Amscop camera.

Confocal Microscope Imaging

The distribution of molecule **1** was reflected by the fluorophore NBD, imaged by 488 nm channel. The dye for actin is Alexa Fluor 647 Phalloidin, imaged by 647 nm channel. Hoechst 33342 for nuclei is imaged by UV. Secondary antibody with Alexa Fluor® 488 is imaged by 488 nm channel.

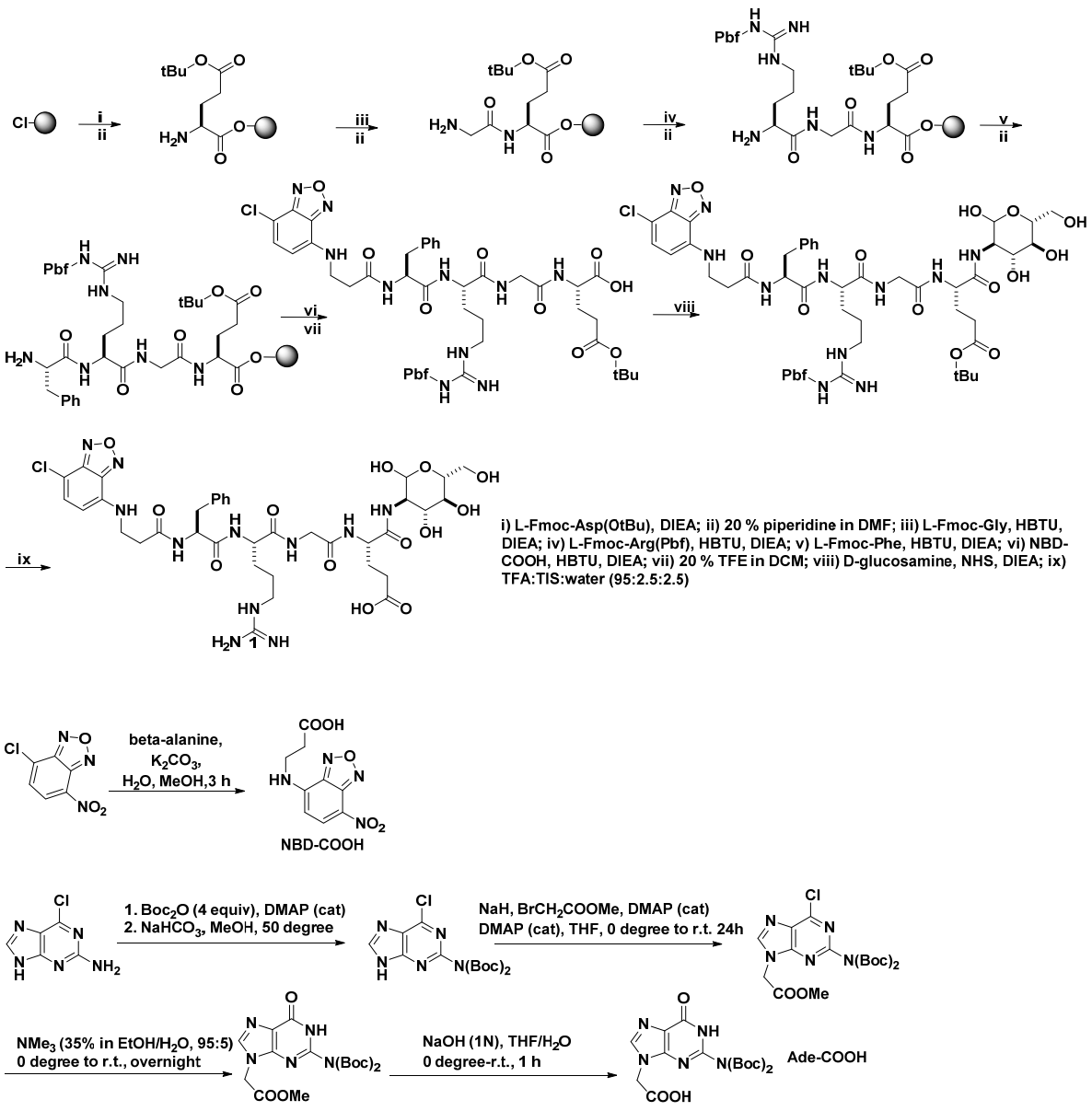
Actin Staining

HS-5 cells in exponential growth phase were seeded in glass bottomed culture chambers/dishes (D35-20-1.5-N, Cellvis) at 5×10^5 cell/well. Cells were allowed to fully attach to the dish bottom for 12-24 hours at 37 ºC, 5% CO_2. The culture medium was then removed, and fresh culture medium containing compounds at different concentration was added. After 24 hours, cells were washed with PBS buffer three times and fixed with 4% formaldehyde for 15 min. Cells were washed with PBS buffer three times, permeabilized with 0.1% Triton X-100 (#9002-93-1, Sigma-Aldrich) in PBS, and incubated in 1.0 % BSA (block non-specific protein-protein interactions, Fisher Scientific BP1600-100) for 15 min. After being washed with PBS buffer three times, 200 µL PBS buffer (with 1% BSA) containing 5 µL Phalloidin stock was added. After 30 min, cell nuclei were stained with Hoechst 33342 (Sigma B2261) for 5min. Cells were washed with PBS buffer three times and mounted for imaging.

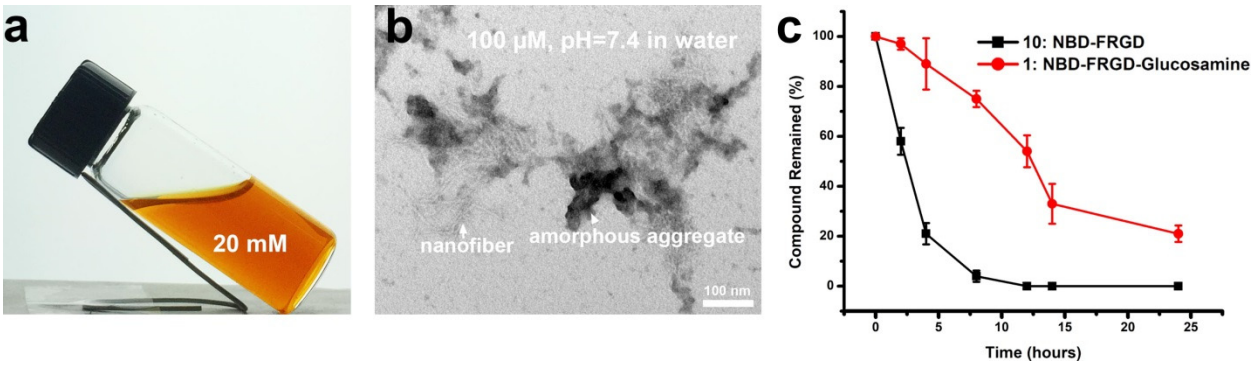
Immunofluorescence Staining

HS-5 cells in exponential growth phase were seeded in glass bottomed culture chambers/dishes at 5×10^5 cell/well density. Cells were allowed to fully attach to the dish bottom for 12-24 hours at 37 ºC, 5% CO_2. The culture medium was then removed, and fresh culture medium containing compounds at different concentrations was added. After 24 hours, cells were washed with PBS buffer three times and fixed with 4% formaldehyde for 15 min. After being washed with PBS

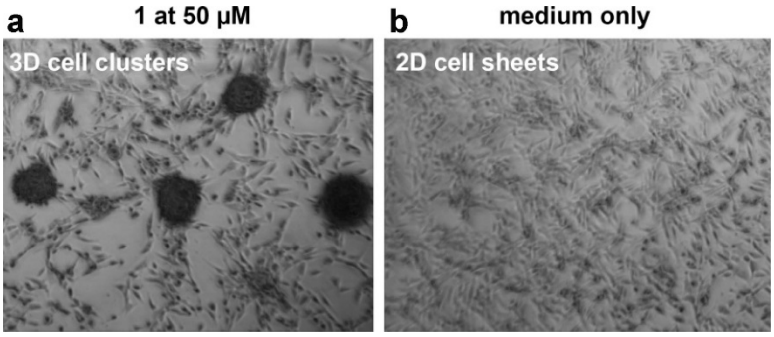
buffer three times and incubated in 1.0 % BSA / 10% normal goat serum / 0.3 M glycine in 0.1% PBS-Tween for 1h (permeabilize the cells membrane and block non-specific protein-protein interactions). After being washed with PBS buffer three times, primary antibodies (1/100) were added and cells were incubated with primary antibodies overnight at +4 °C. Cells were washed with PBS buffer three times and the secondary antibody was (Alexa Fluor® 488/647 goat anti-rabbit IgG (H+L)) added at 2 µg/ml (1/1000) for 1 h. Cell nuclei were stained with Hoechst 33342 for 5min. Cells were washed with PBS buffer three times and mounted for imaging.



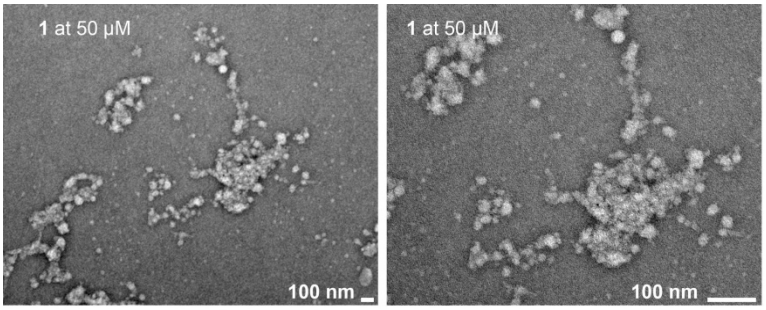
Supplementary Figure S1. Synthetic route of molecule 1, NBD-COOH and Ade-COOH



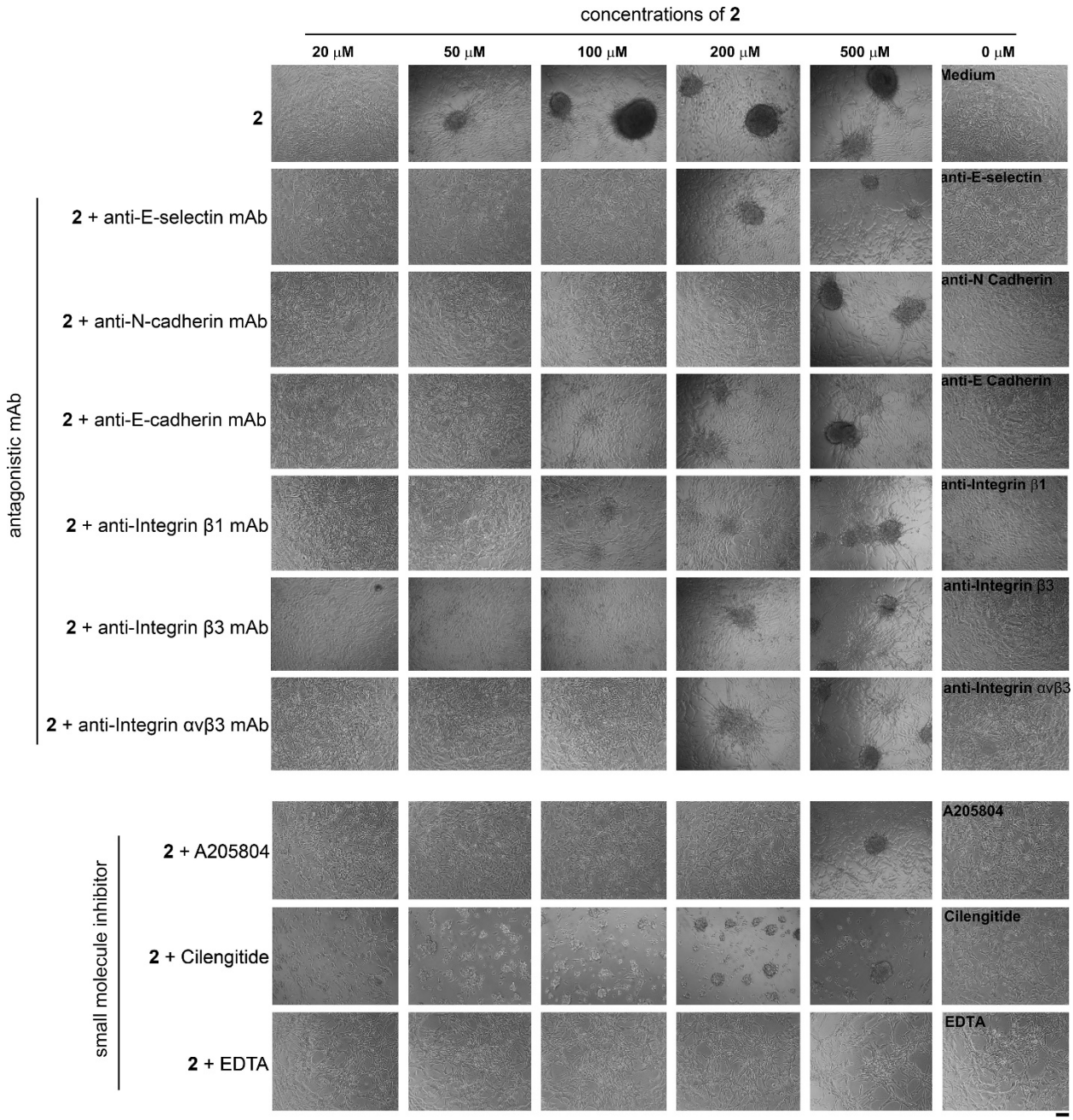
Supplementary Figure S2. (a) **1** dissolves in water to give a clear orange solution even at 20 mM (pH = 7.4 in water). (b) TEM image shows amorphous aggregates (arrow head) and nanofibers (arrow, d = 5 ± 2 nm) in the solution of **1** (100 μM, pH 7.4, water). (c) Digestions of compounds **1** (NBD-FRGD-Glucosamine) and **10** (NBD-FRGD) over the course of the incubation with proteinase K for 24 h. All the compounds are at the concentration of 1.0 mM.



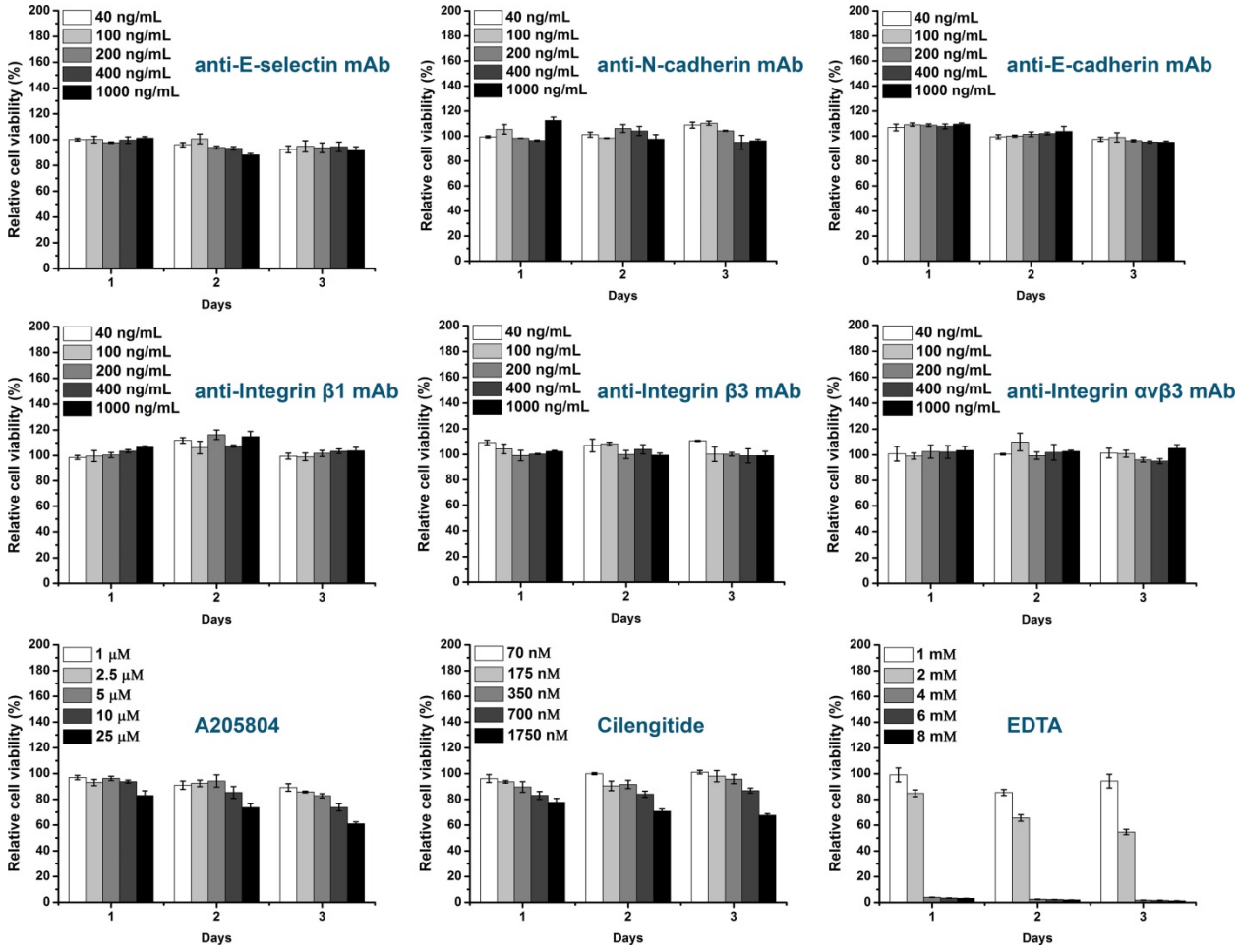
Supplementary Figure S3. (a) Incubation **1** with HS-5 cells induces monolayer-spheroid cell reorganization within 24 hours even at the concentration as low as 50 μM, while cells, maintained in (b) medium only, remain as a monolayer.



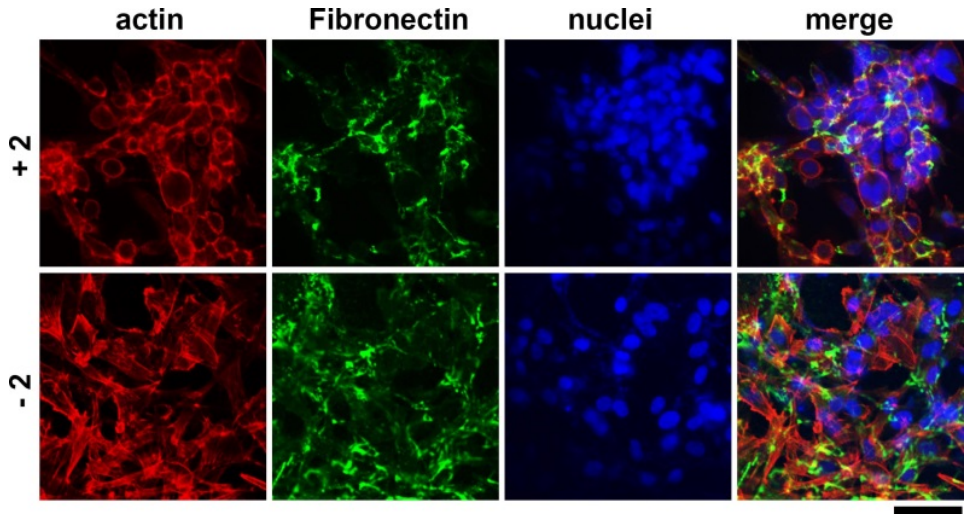
Supplementary Figure S4. TEM images show aggregates or nanoparticles in the solution of **2** (50 μM, pH 7.4, in water). The scale bar is 100 nm.



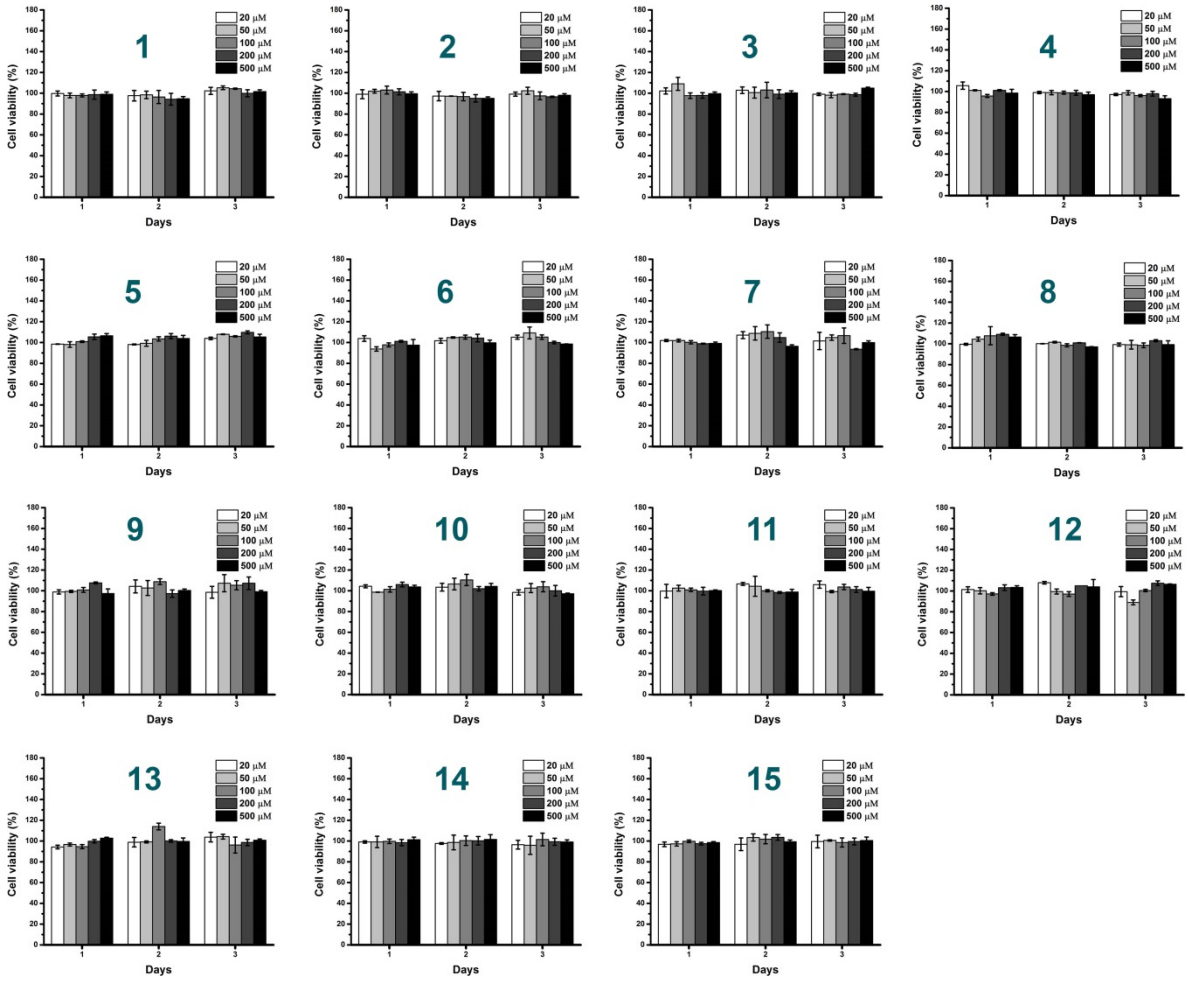
Supplementary Figure S5. Optical images of HS-5 cells treated with **2** at different concentrations with/without different antagonistic mAbs (i.e., anti-E-selectin, anti-N/E-cadherin, anti-Integrin β1/β3/α_vβ3, 400 ng/mL) or different small molecule inhibitors (i.e., A205804 (10 μM), Cilengitide (175 nM) and EDTA (1 mM)). The incubation time is 24 h. The initial cell number is 3.0×10^4/well in a 96-well plate. The scale bar is 100 μm.



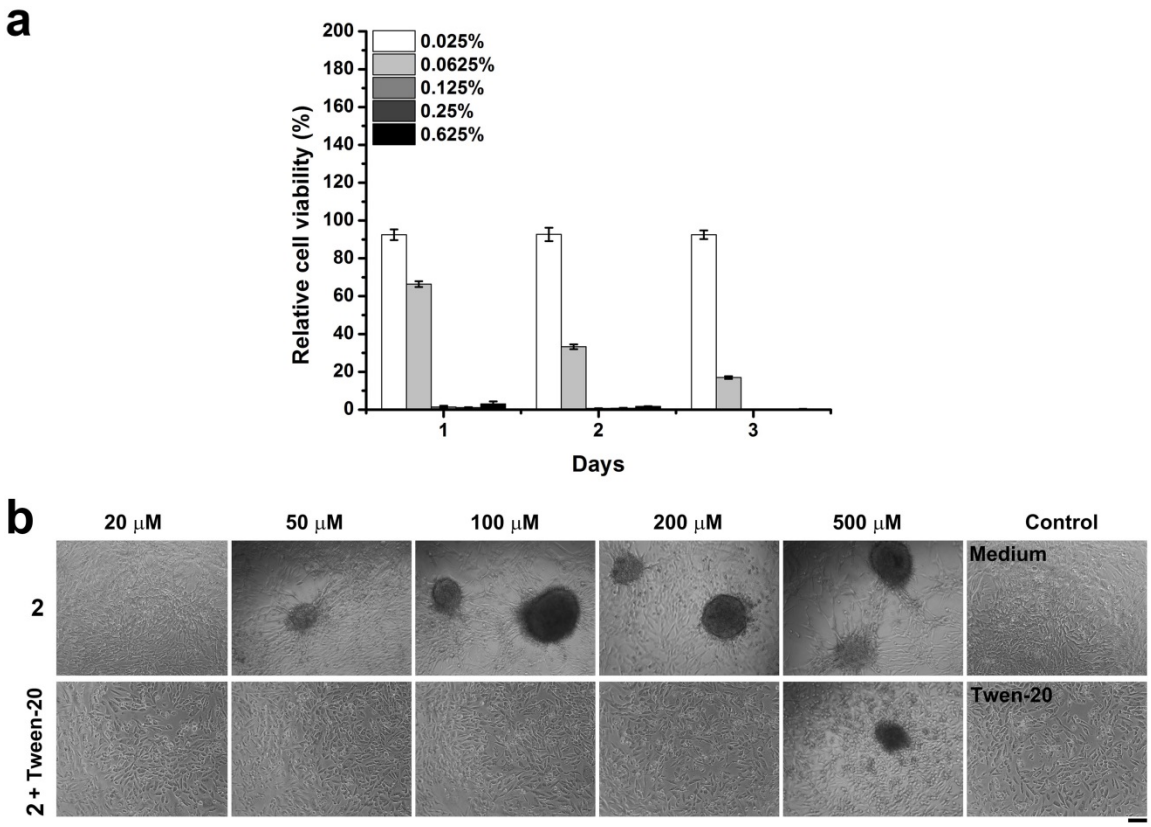
Supplementary Figure S6. Relative cell viability (determined by MTT assay; 100% represents the control, i.e., 0 μM of the compound) of HS-5 cells incubated with mAbs (anti-E-selectin, anti-N/E-cadherin, anti-Integrin β1/β3/α_vβ3 Abs) or small molecule inhibitors (A205804, Cilengitide, EDTA) at different concentrations for 24, 48 and 72 hours. The initial cell number is 1.0×10^4/well.



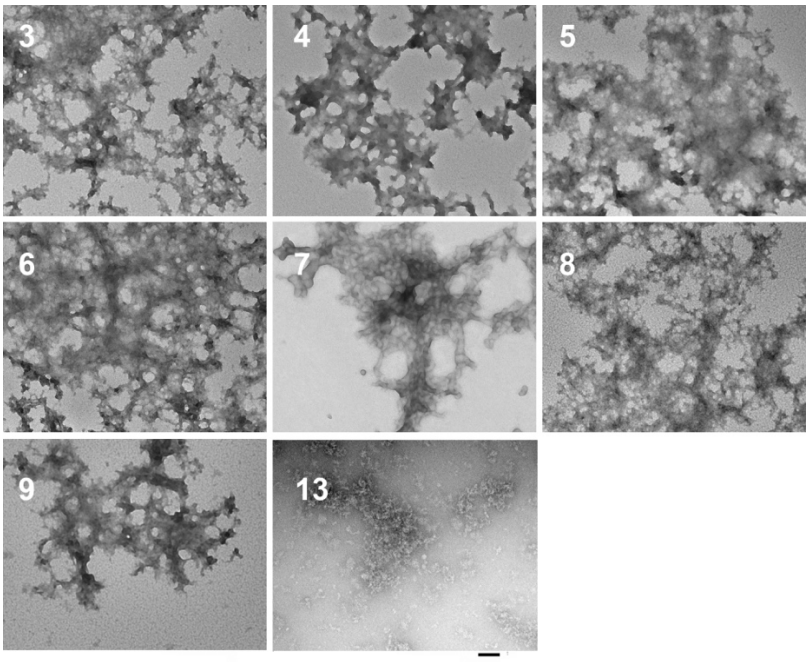
Supplementary Figure S7. Immunofluorescence antibody staining reveals the distribution of fibronectin in HS-5 cell culture treated with and without **2** (500 μM) in culture medium for 24 hours. The scale bar is 50 μm.



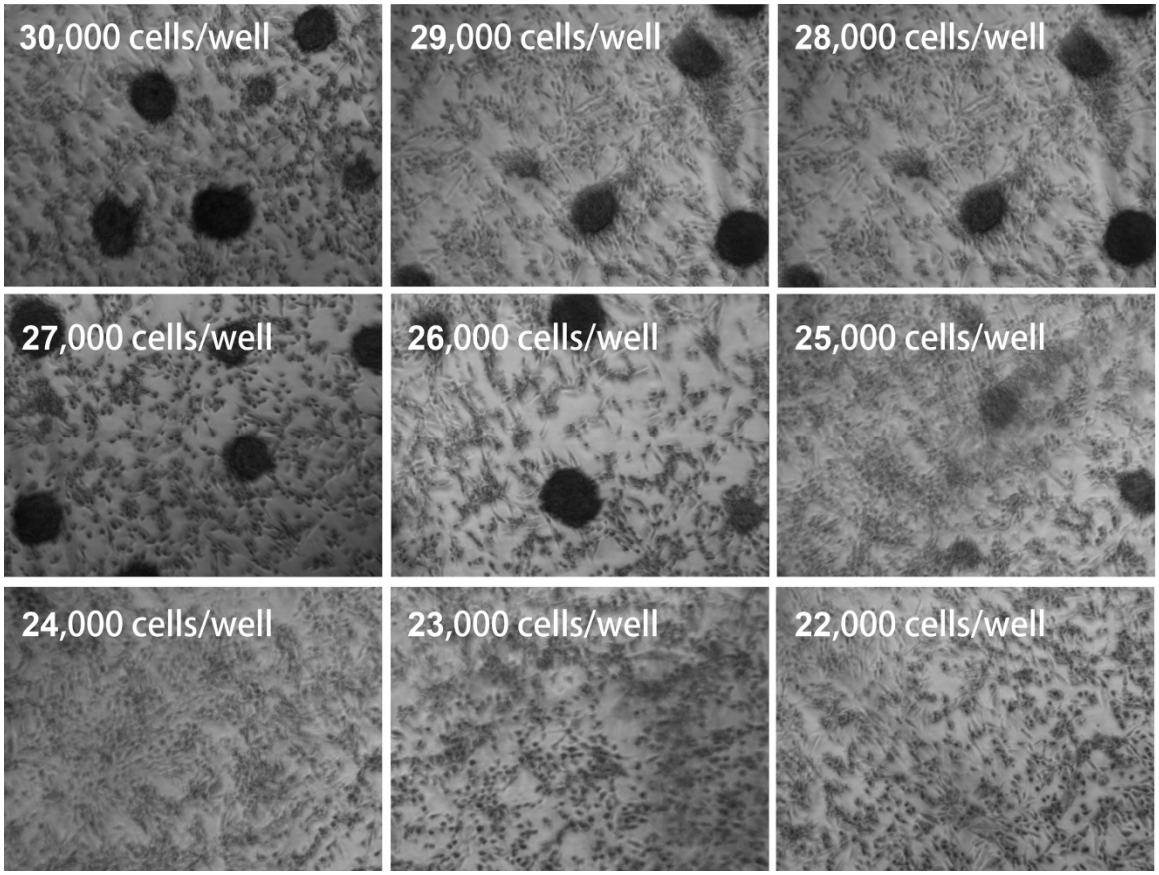
Supplementary Figure S8. Relative cell viability (determined by MTT assay; 100% represents the control, i.e., 0 μM of the compound) of HS-5 cells incubated with **1-15** at different concentrations for 24, 48 and 72 hours. The initial number of cells is 1.0×10^4/well. All the 15 compounds are cell compatible.



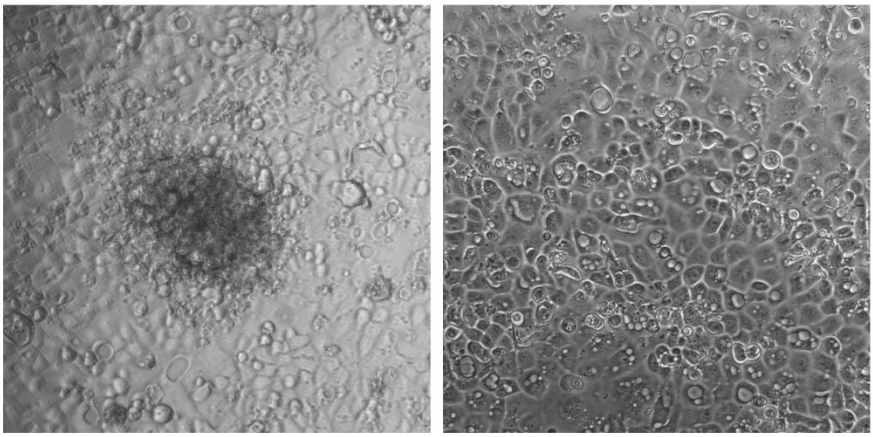
Supplementary Figure S9. (a) Relative cell viability (determined by MTT assay; 100% represents the control, i.e., 0 μM of the compound) of HS-5 cells incubated with Tween-20 at different concentrations. The initial cell number is 1.0×10^4/well. (b) Optical images of HS-5 cells treated with **2** at different concentrations with/without different tween-20 (0.025%). The incubation time is 24 h. The initial cell number is 3.0×10^4/well in a 96-well plate. The scale bar is 100 μm.



Supplementary Figure S10. TEM images of the nanoscale structures formed by molecule **3-9** and **13** at the concentration of 500 μM in water. pH = 7.4. The scale bar is 100 nm.



Supplementary Figure S11.Optical images of HS-5 cells at different cell density in a 96-well plate treated by **2** (500 μM) for 24 hours.



Supplementary Figure S12. Incubation **1** with HS-5 cells induces monolayer to spheroid cell reorganization within 24 hours even at the concentration as low as 50 μM, while cells, maintained in medium only, remain as a monolayer.

Movie S1. Z-scan of a 3D cell spheroid formed by HS-5 cells treated with **1** at 500 μM in culture medium for 24 hours. Actin (red) is stained for visualizing cell morphologies. Nuclei (blue) are stained by Hoechst 33342. The assemblies of **1** are yellow fluorescent.

Movie S2. Smaller cell clusters merge to form a larger one during the treatment of glycopeptide assemblies of **1**.

Movie S3. 3D projection of a cell spheroid formed by HS-5 cells treated with **1** at 500 μM in culture medium for 24 hours. Actin (red) is stained for visualizing cell morphologies. Nuclei (blue) are stained by Hoechst 33342. The assemblies of **1** are yellow fluorescent.

Movie S4. Z-scan of a 3D cell spheroid formed by HS-5 cells treated with **1** at 500 μM in culture medium for 24 hours. The assemblies of **1** are of green fluorescence. The fibronectin is of red fluorescence. Nuclei (blue) are stained by Hoechst 33342.

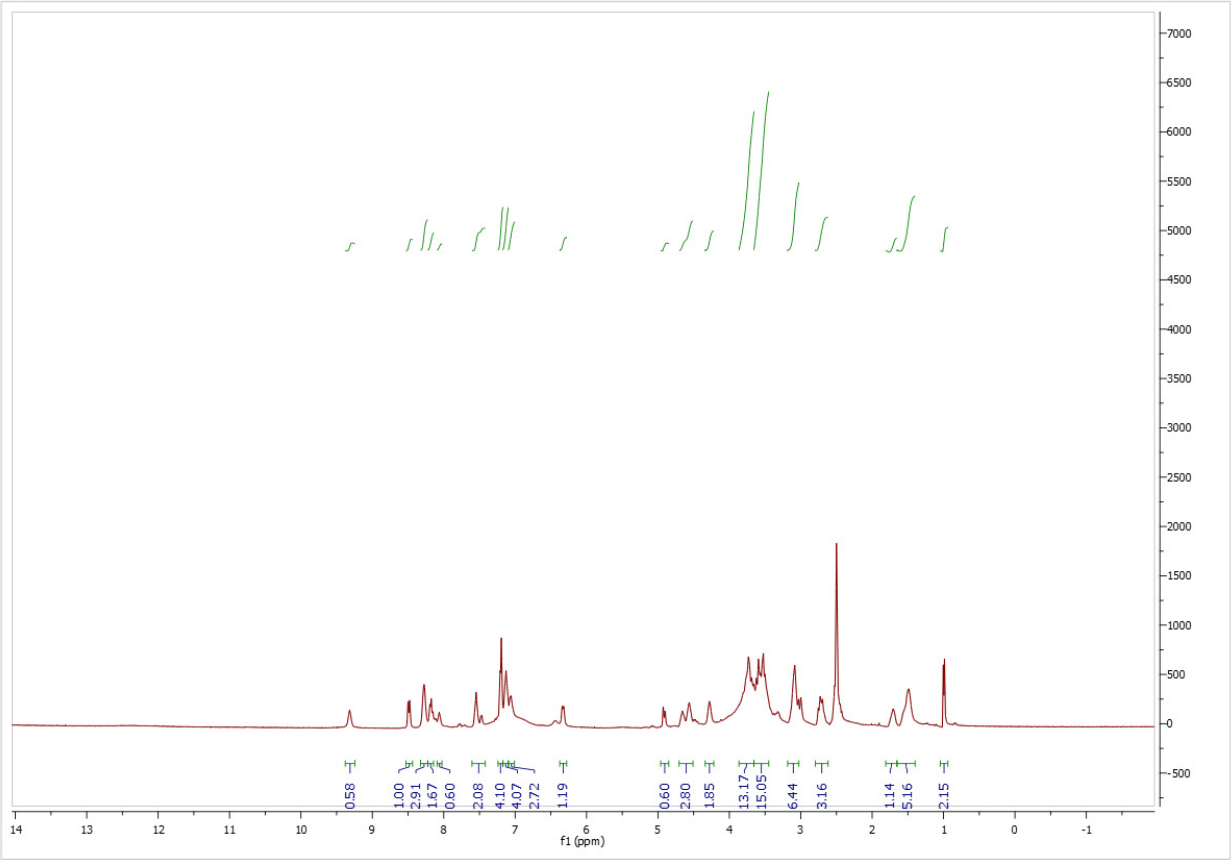
Movie S5. 3D projection of a cell spheroid formed by HS-5 cells treated with **1** at 500 μM in culture medium for 24 hours. The assemblies of **1** are of green fluorescence. The fibronectin is of red fluorescence. Nuclei (blue) are stained by Hoechst 33342.

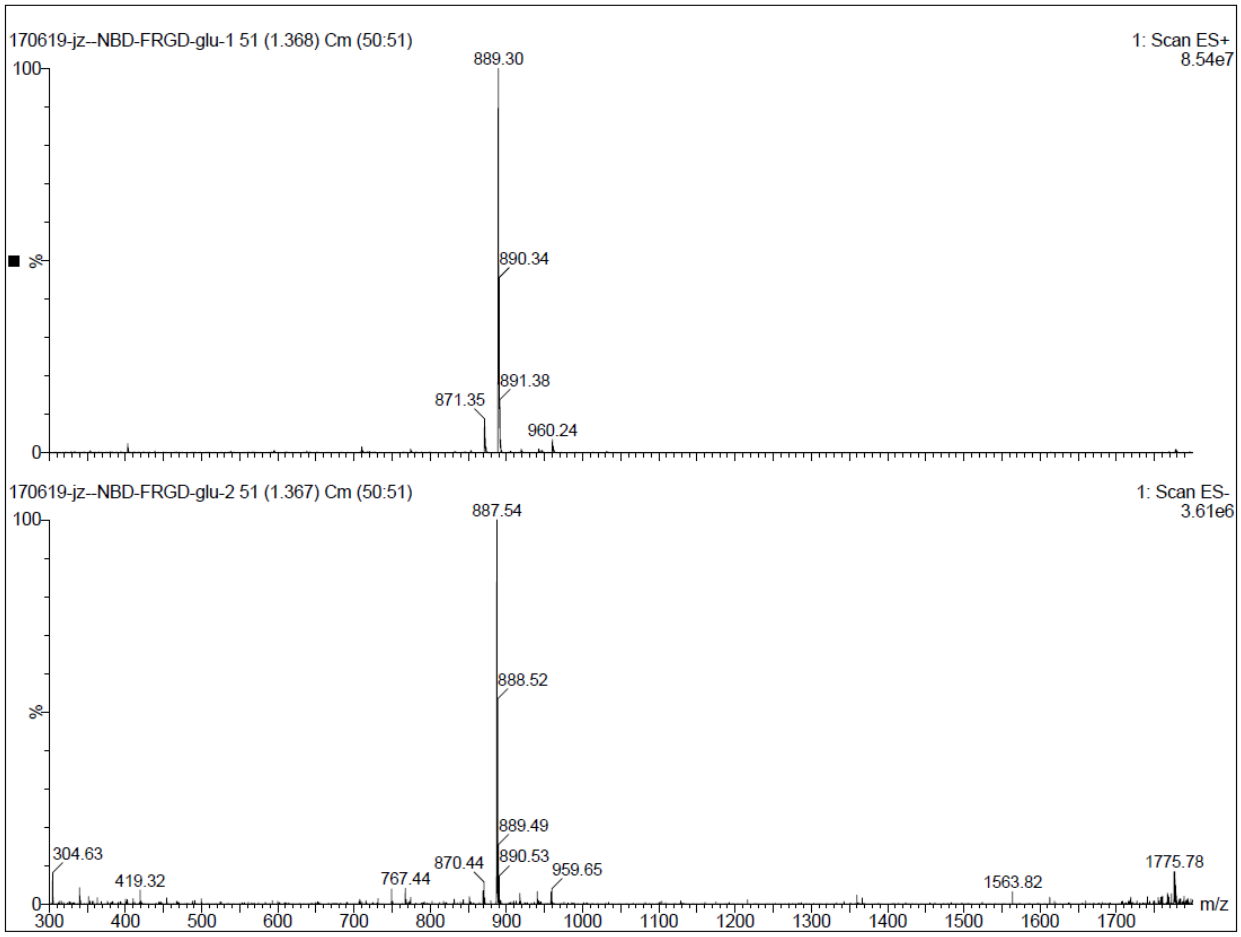
Supplementary Table S1. LC-MS characterization of molecule **1-15.**

#	compound	calculated M.W.	LCMS detected M.W.	
			ES-	ES+
1	NBD-FRGD-Glucosamine	888.34	887.54	889.30
2	Ac-FRGD-Glucosamine	696.31	695.47	697.29
3	Ade-FRGDS-Glucosamine	916.38	915.58	917.40
4	Ade-FRGD-Glucosamine	829.35	828.58	830.60
5	Ade-FRGD-Galactosamine	829.35	828.57	830.32
6	Ade-FRGD-Condrosine	1005.38	1004.58	1007.60
7	Ade-FRGDF-Glucosamine	976.42	975.52	977.40
8	Ade-FFRGD-Glucosamine	976.42	975.58	977.31
9	Thy-FRGD-Glucosamine	820.34	819.53	821.35
10	NBD-FRGD	727.27	726.42	728.17
11	Ade-FRGD	668.28	667.44	669.33
12	Thy-FRGD	659.27	658.40	660.29
13	Ade-Glucosamine	354.13	353.22	355.04
14	FRGD-Glucosamine	654.30	653.46	877.33
15	Ade-FRGE-Glucosamine	843.36	842.61	844.37

NMR and LCMS spectra of the glycopeptides. Other compounds (e.g., **3, 6, 13**) were previously reported.

1：NBD-FRGD-Glucosamine

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%
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890.34
891.38
871.35
960.24
170619-jz--NBD-FRGD-glu-2 51 (1.367) Cm (50:51)
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100
%
0
887.54
888.52
889.49
890.53
304.63
419.32
870.44
767.44
959.65
1563.82
1775.78
300
400
500
600
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1000
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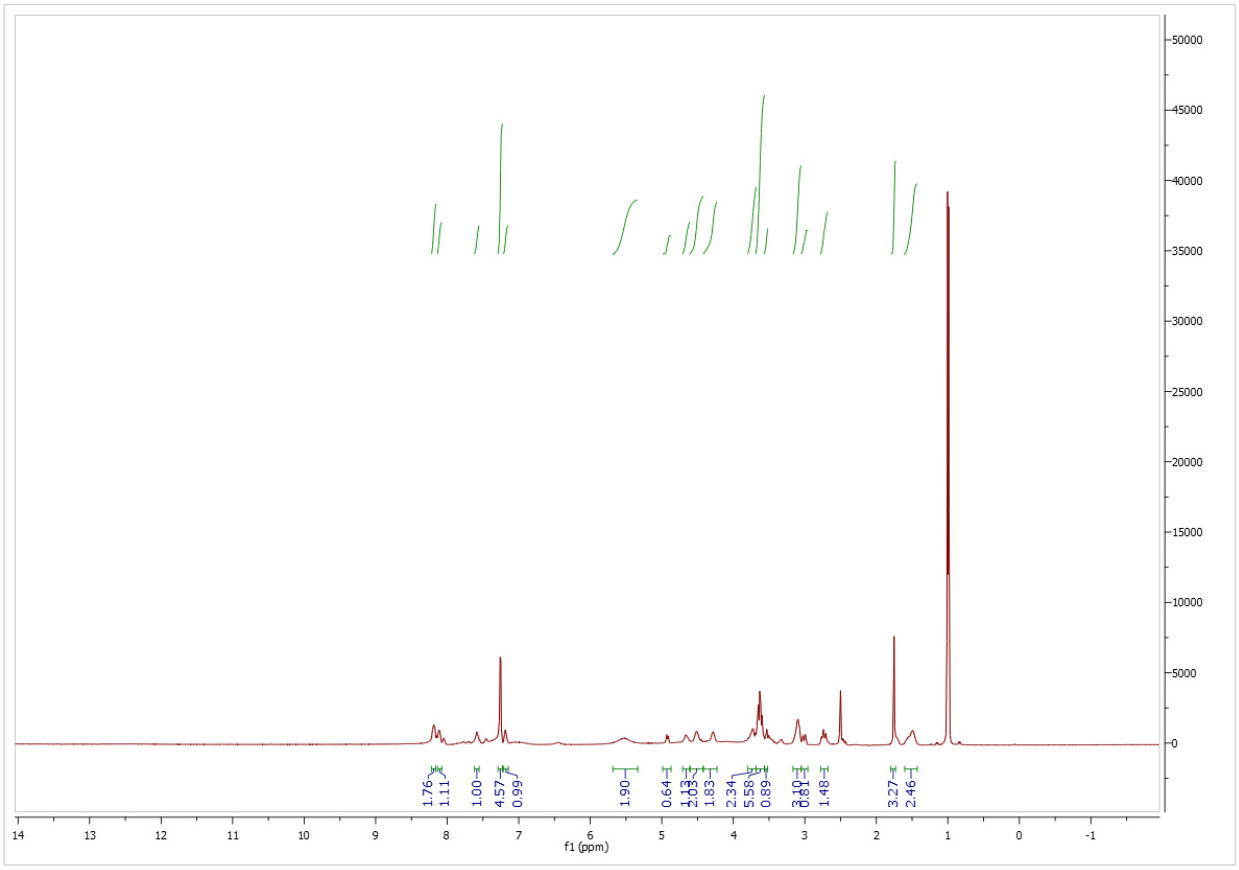
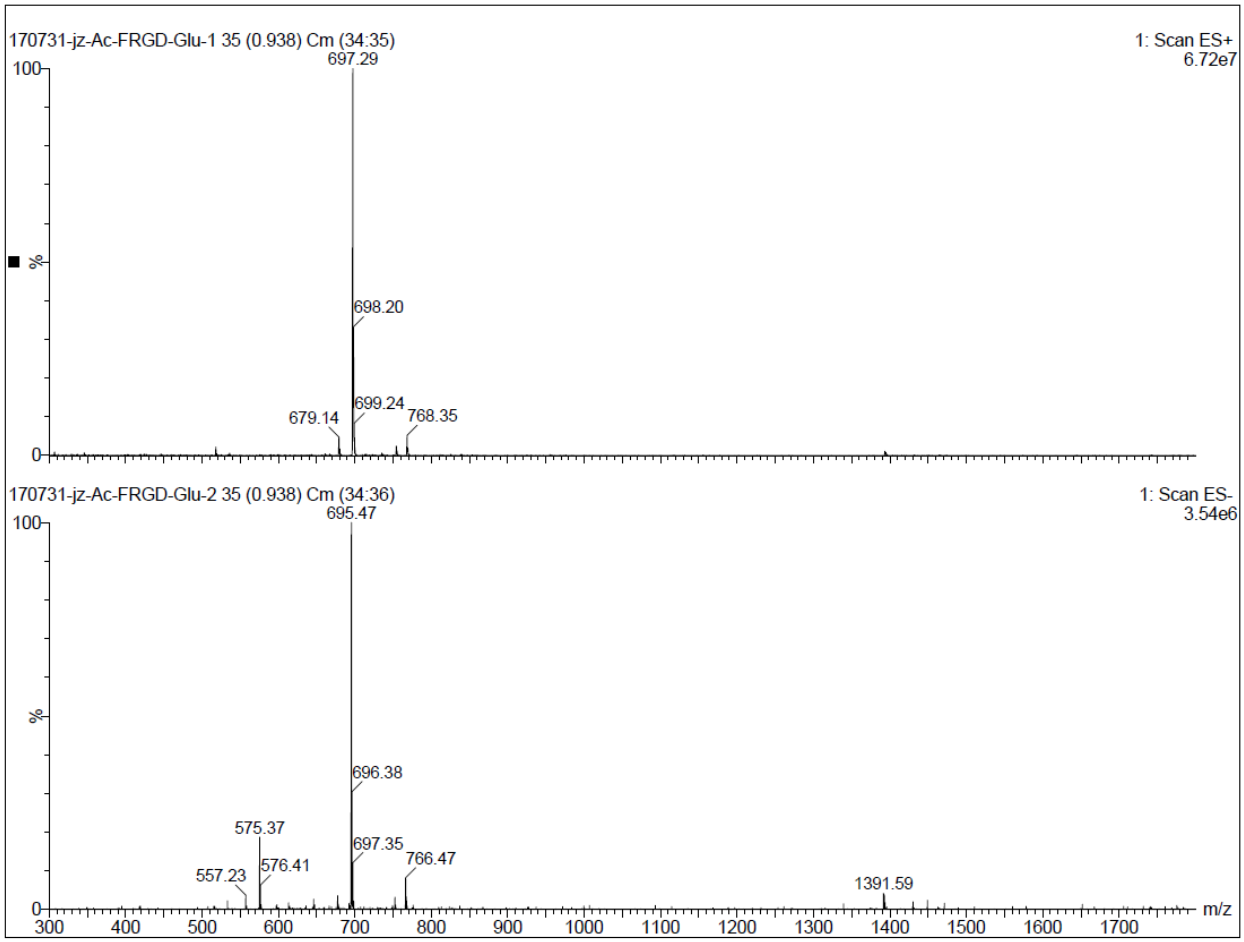
1600
1700
m/z

2： Ac-FRGD-Glucosamine

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698.20
679.14
699.24
768.35
170731-jz-Ac-FRGD-Glu-2 35 (0.938) Cm (34:36)
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%
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696.38
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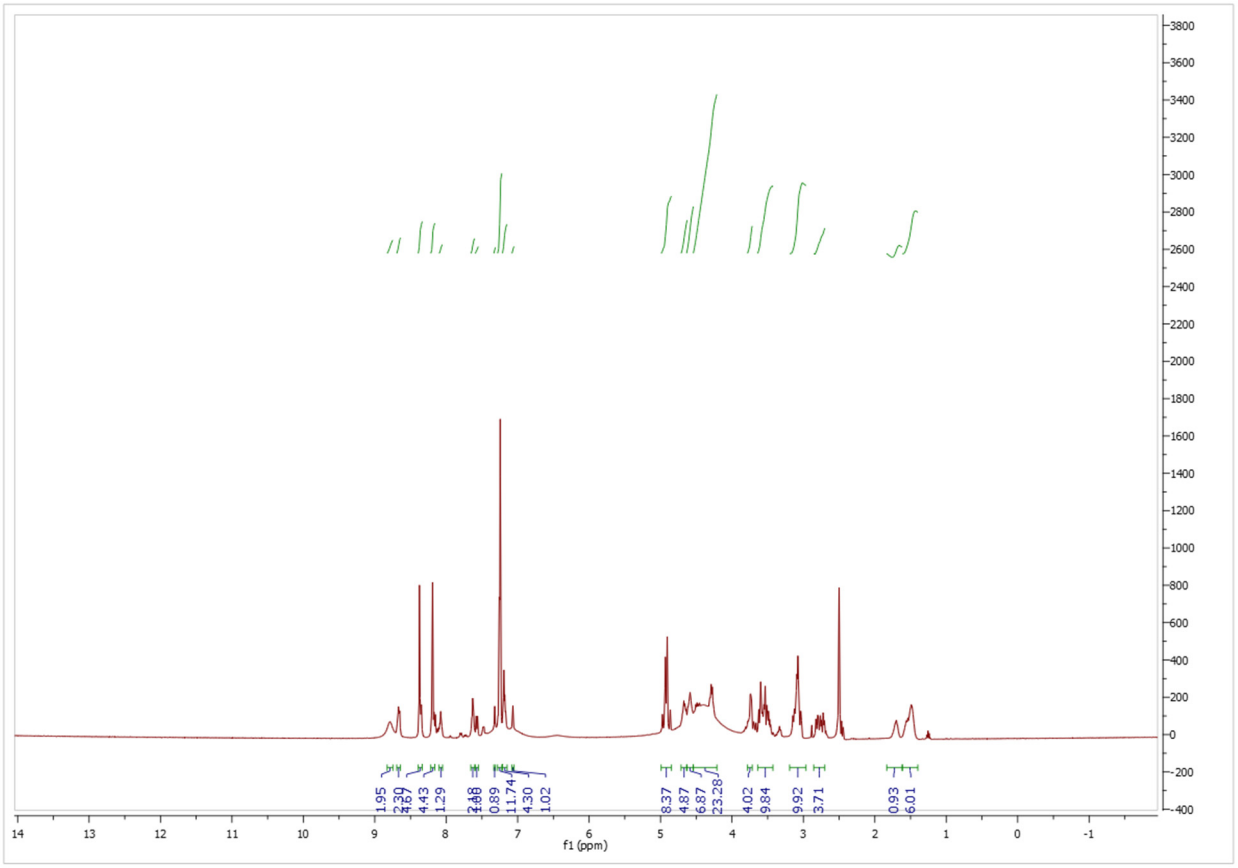
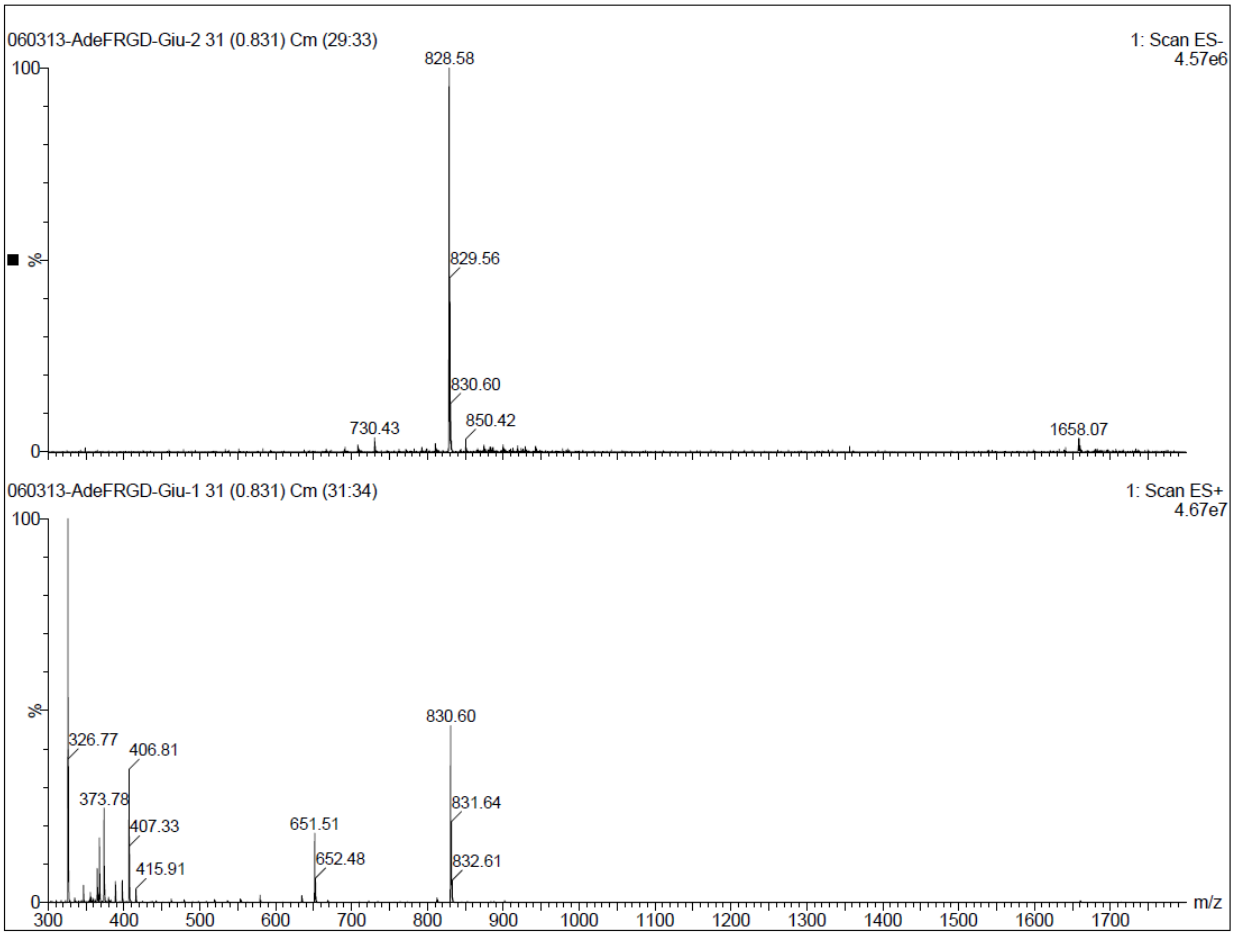
m/z

4： Ade-FRGD-Glucosamine

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%
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829.56
830.60
850.42
730.43
1658.07
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%
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407.33
415.91
651.51
652.48
830.60
831.64
832.61
m/z
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400
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600
700
800
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1000
1100
1200
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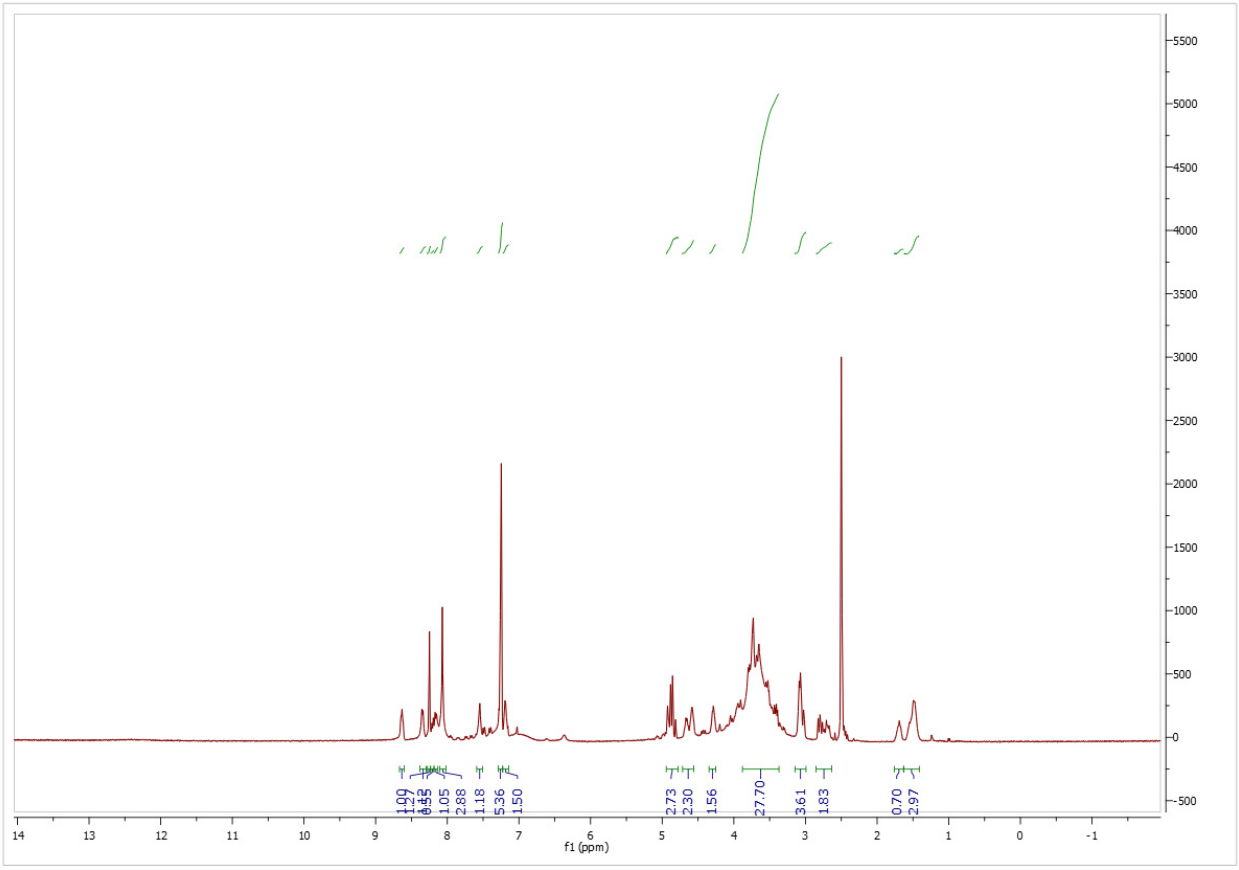
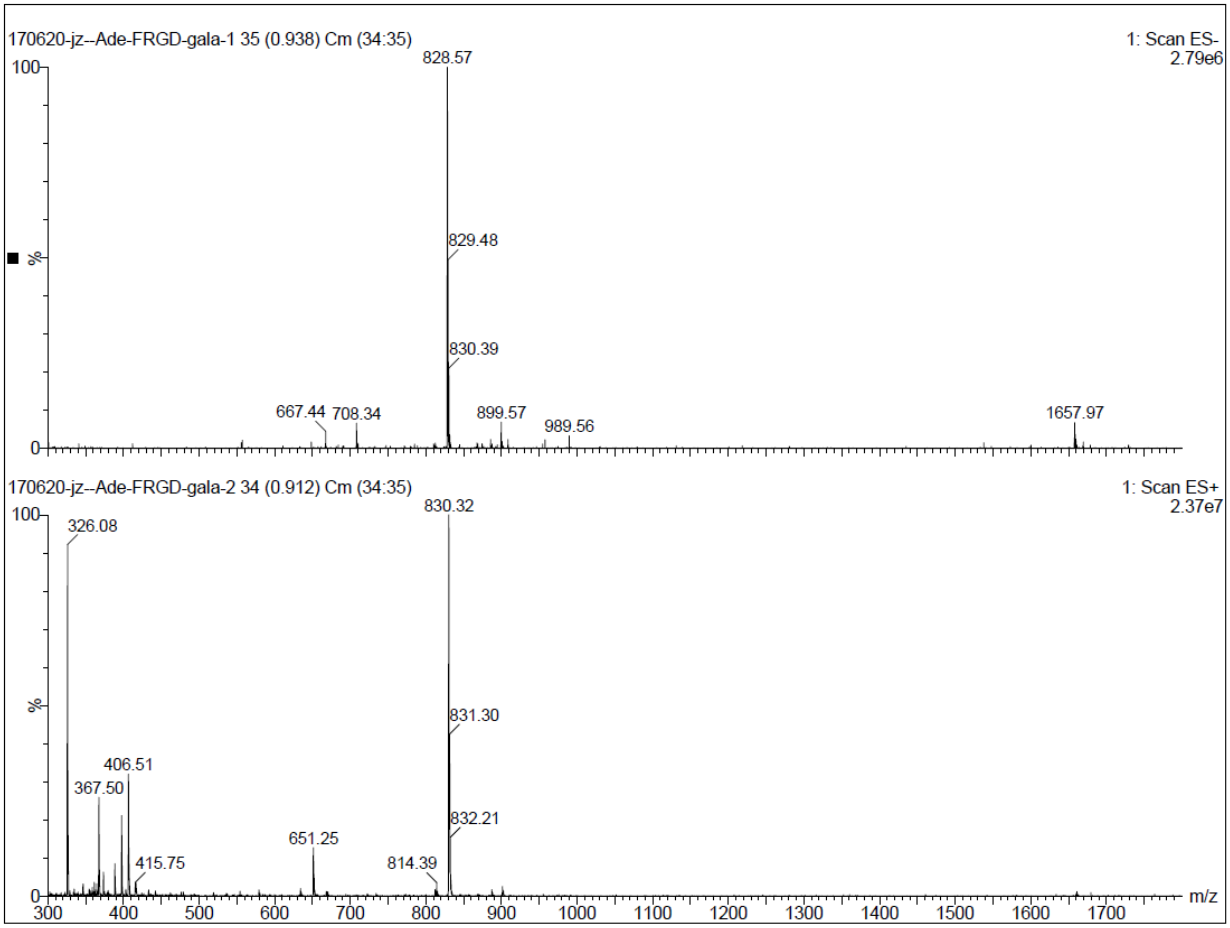
1600
1700

5： Ade-FRGD-Galactosamine

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100
%
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829.48
830.39
667.44
708.34
899.57
989.56
1657.97
170620-jz--Ade-FRGD-gala-2 34 (0.912) Cm (34:35)
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%
0
326.08
830.32
831.30
406.51
367.50
832.21
651.25
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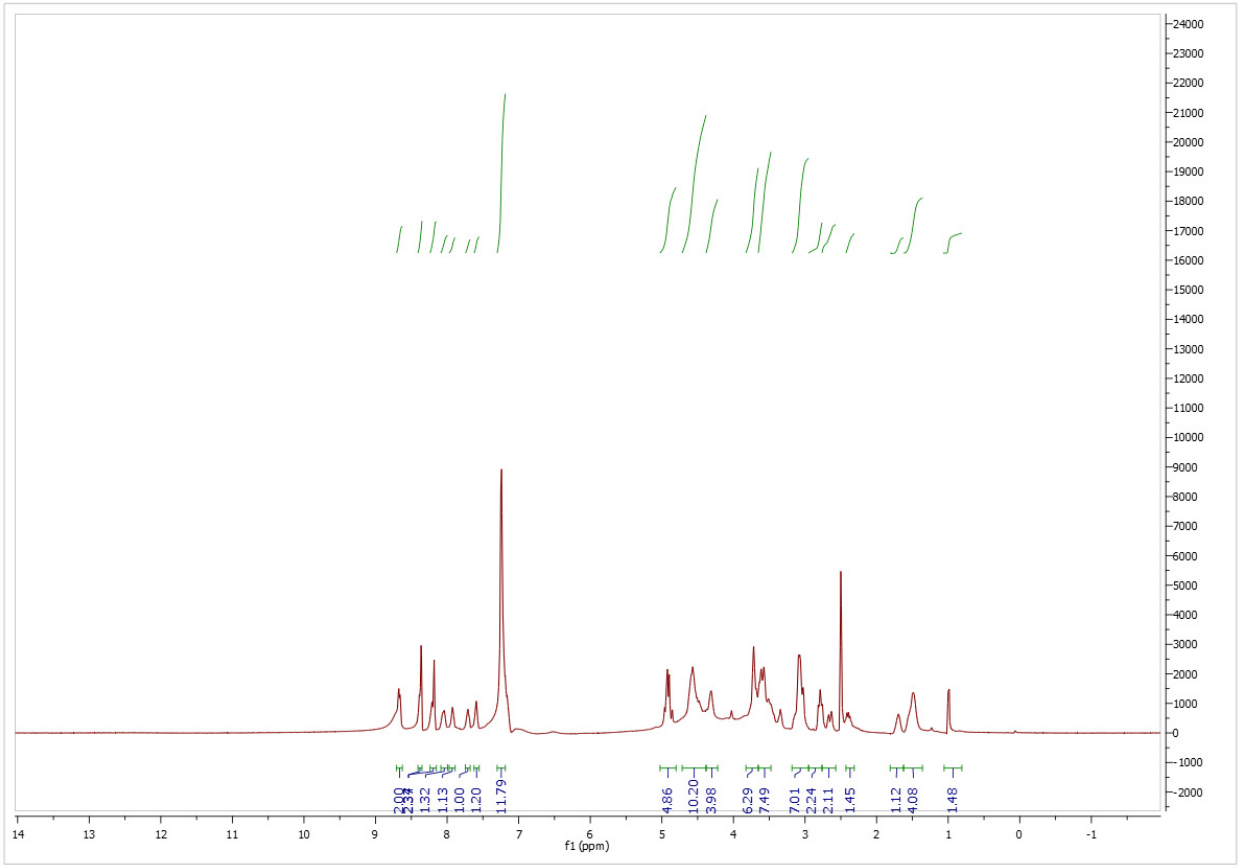
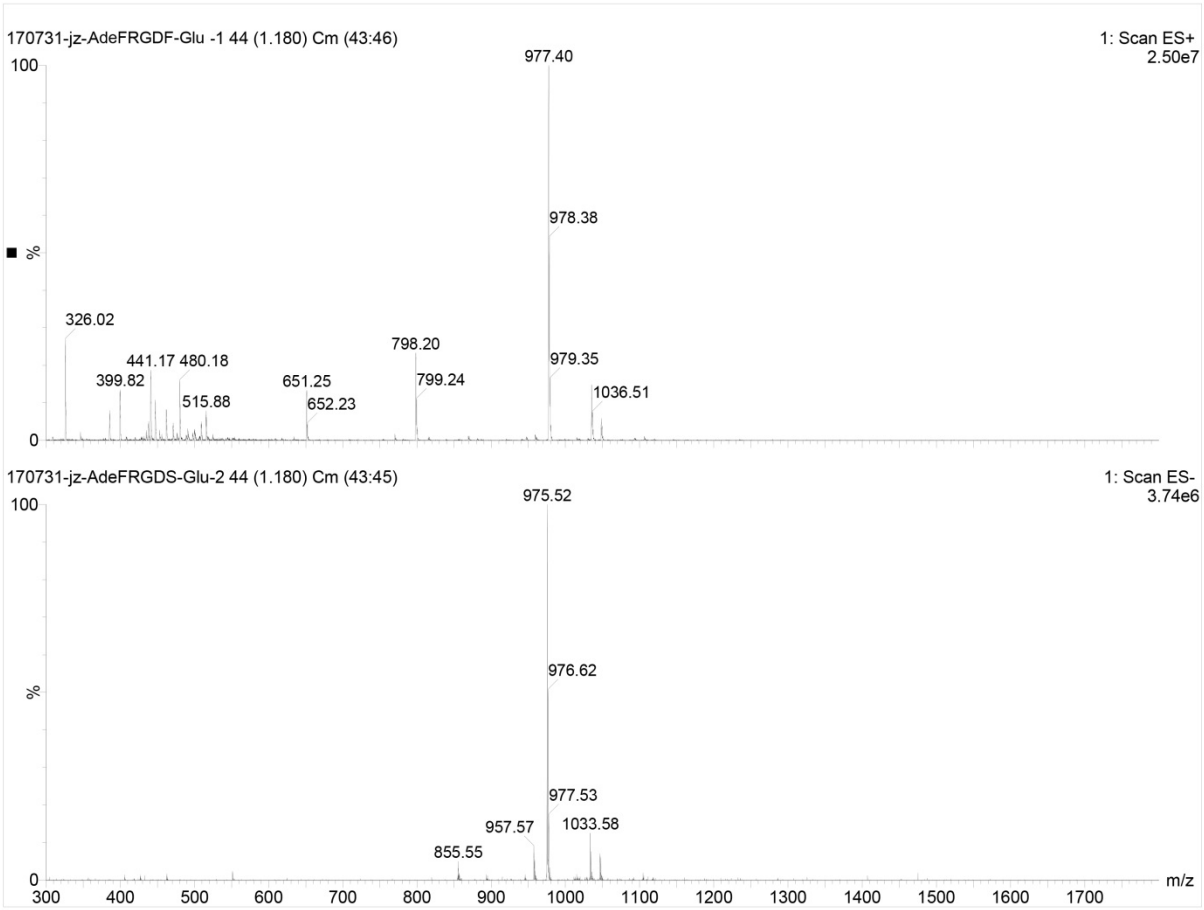
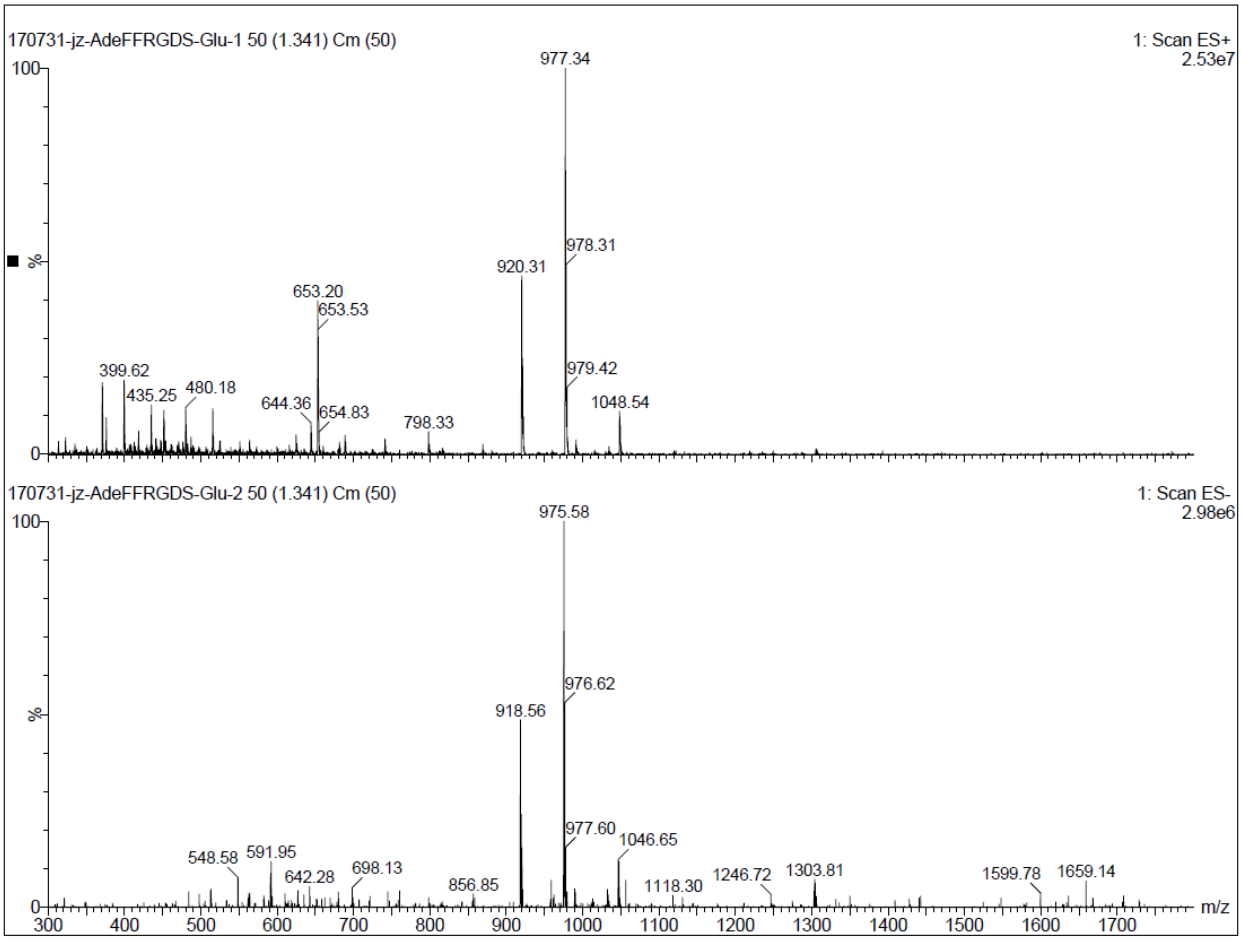
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z

7：Ade-FRGDF-Glucosamine

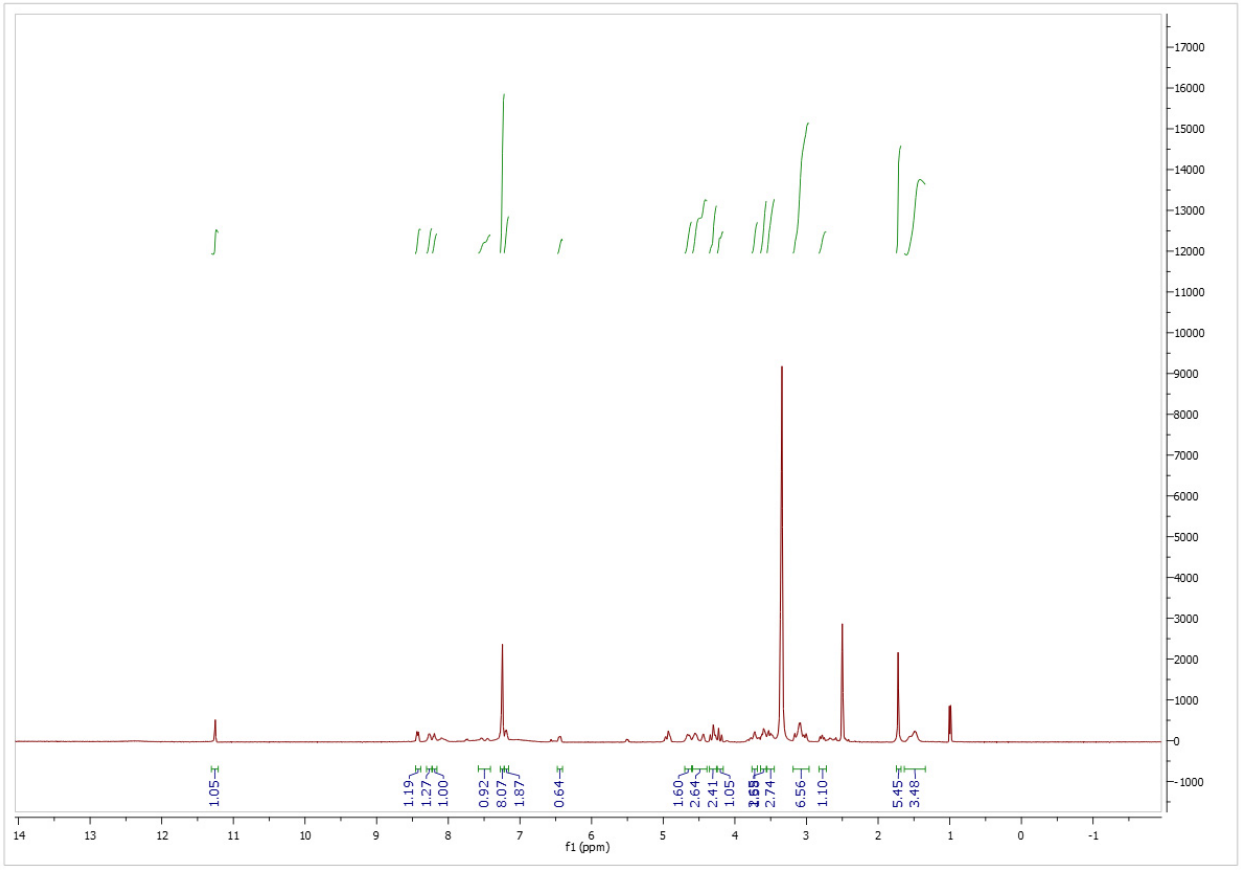


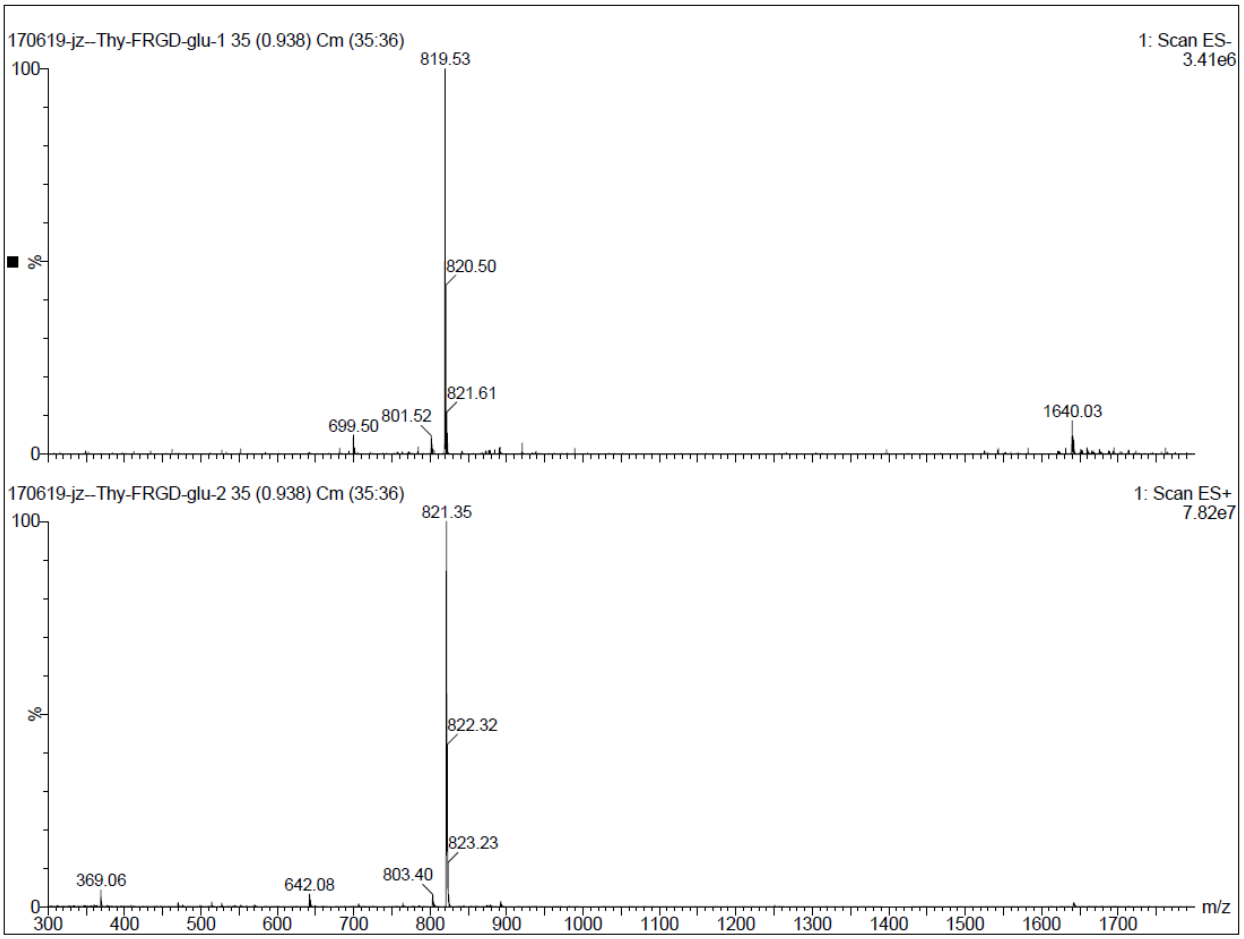

170731-jz-AdeFRGDF-Glu -1 44 (1.180) Cm (43:46)
1: Scan ES+
2.50e7
100
%
0
977.40
978.38
326.02
798.20
441.17
480.18
979.35
399.82
651.25
799.24
1036.51
515.88
652.23
170731-jz-AdeFRGDS-Glu-2 44 (1.180) Cm (43:45)
1: Scan ES-
3.74e6
100
%
0
975.52
976.62
977.53
957.57
1033.58
855.55
m/z
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

8：Ade-FFRGD-Glucosamine

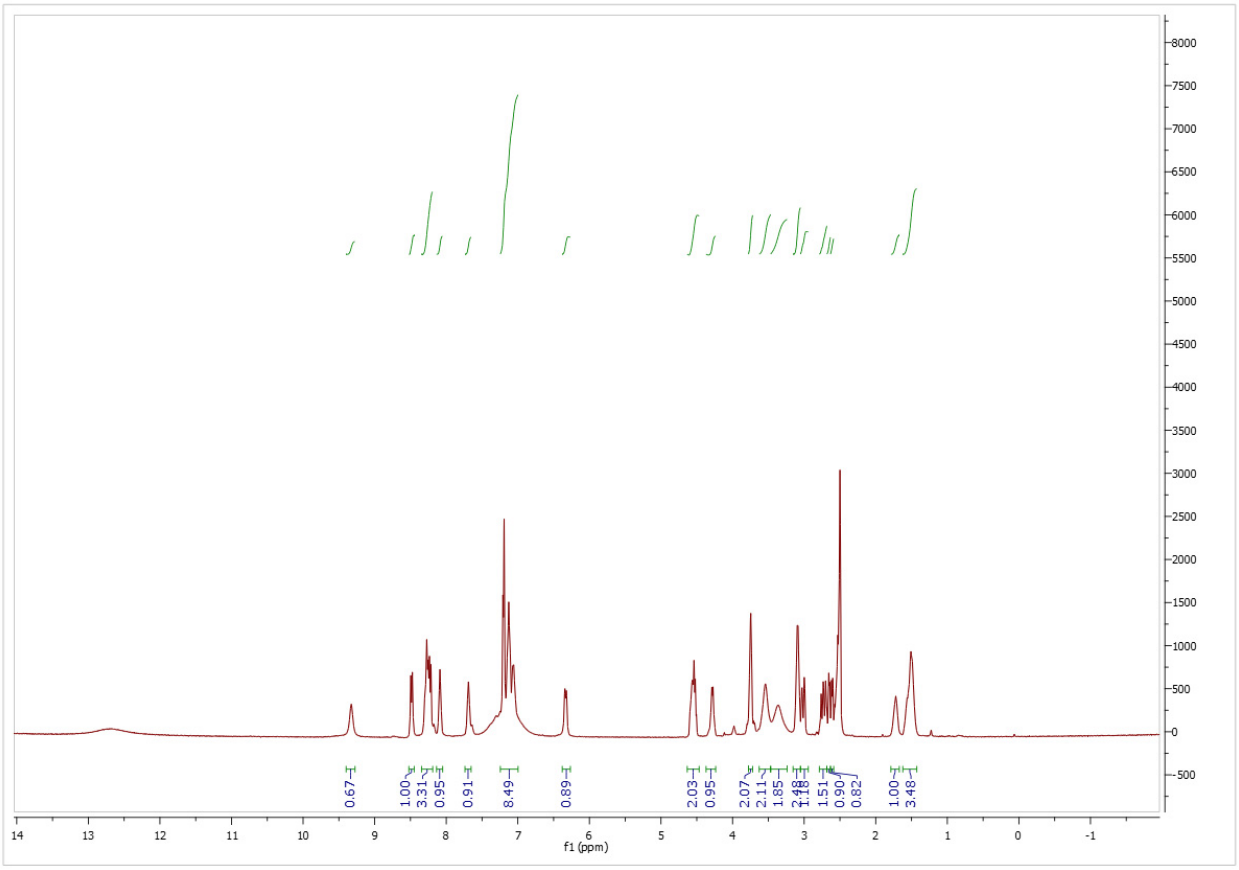


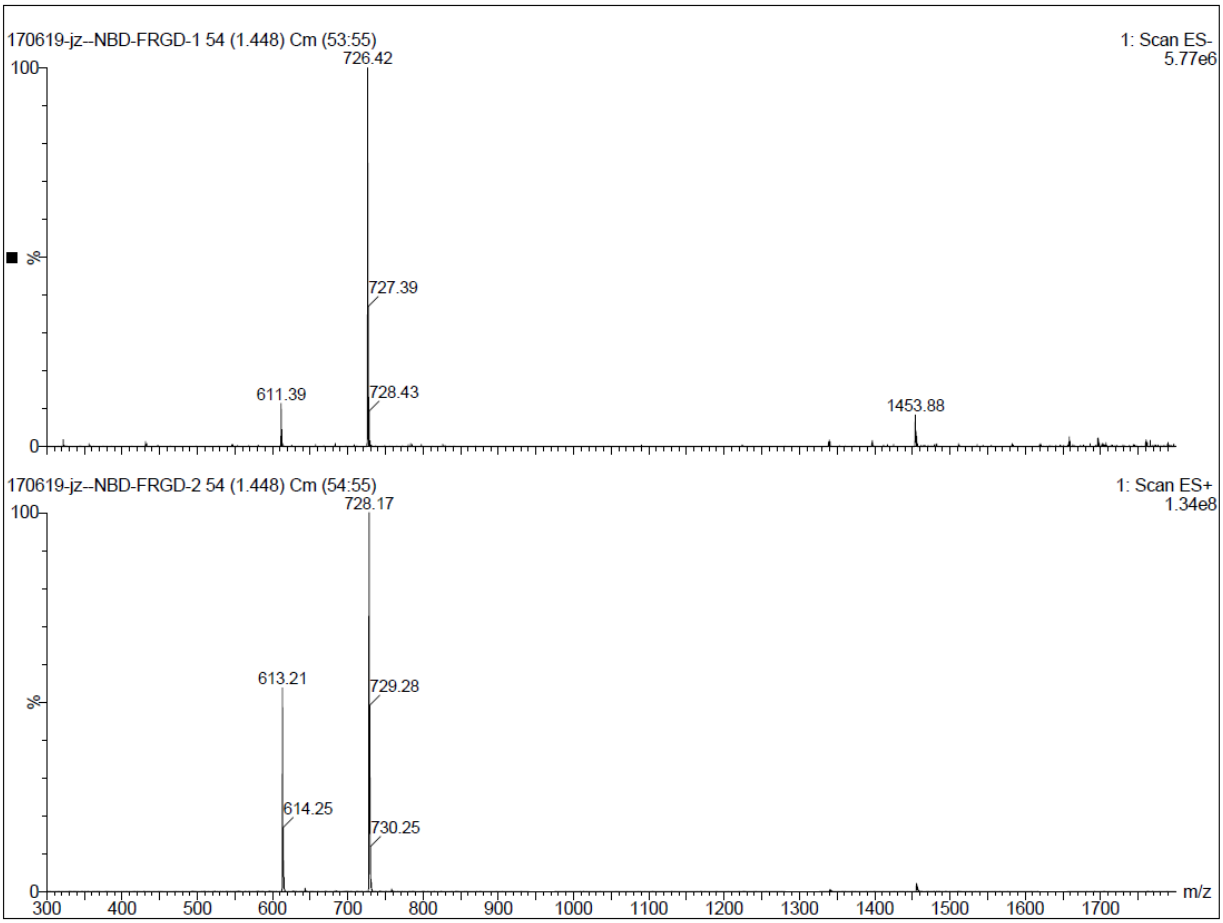
9： Thy-FRGD-Glucosamine



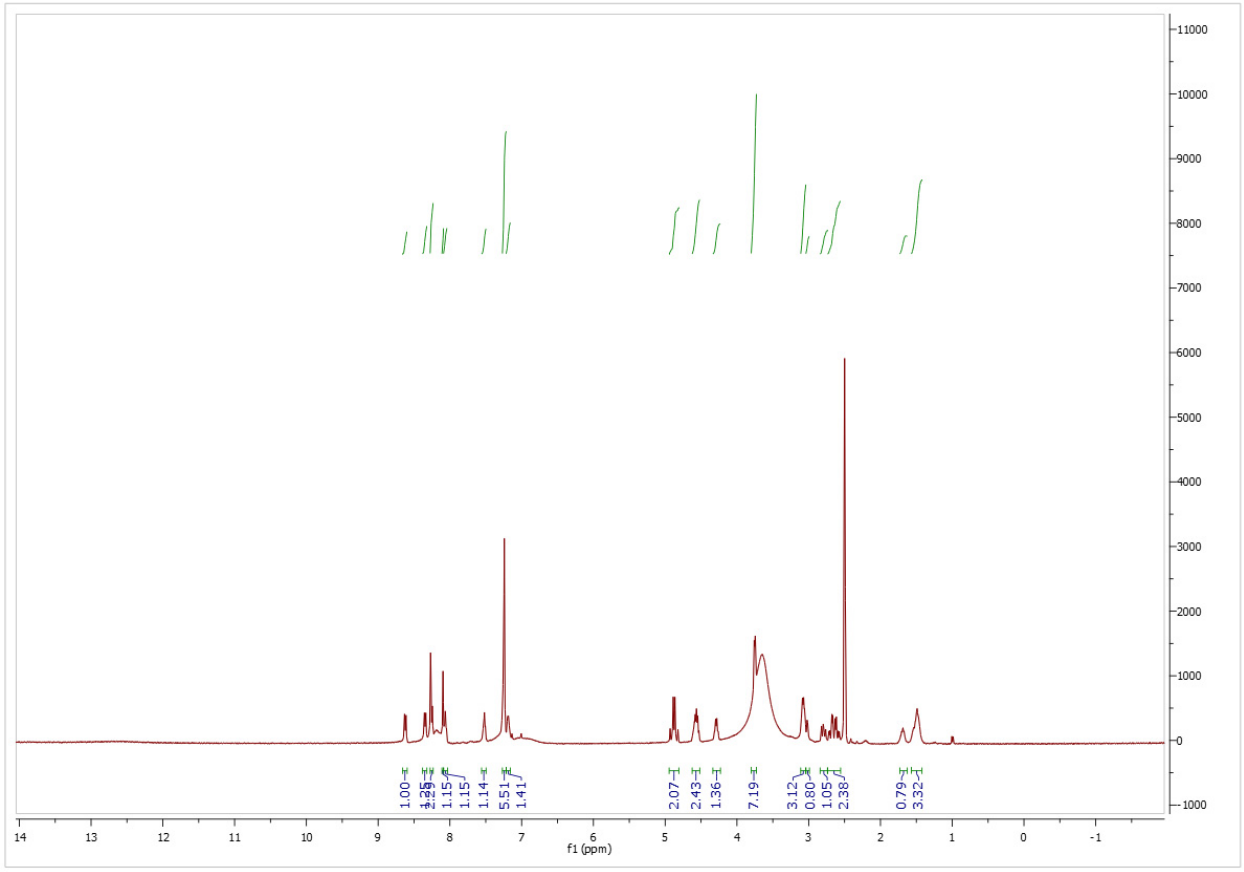

170619-jz--Thy-FRGD-glu-1 35 (0.938) Cm (35:36)
1: Scan ES-
3.41e6
100
%
0
819.53
820.50
821.61
699.50
801.52
1640.03
170619-jz--Thy-FRGD-glu-2 35 (0.938) Cm (35:36)
1: Scan ES+
7.82e7
100
%
0
821.35
822.32
823.23
369.06
642.08
803.40
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z

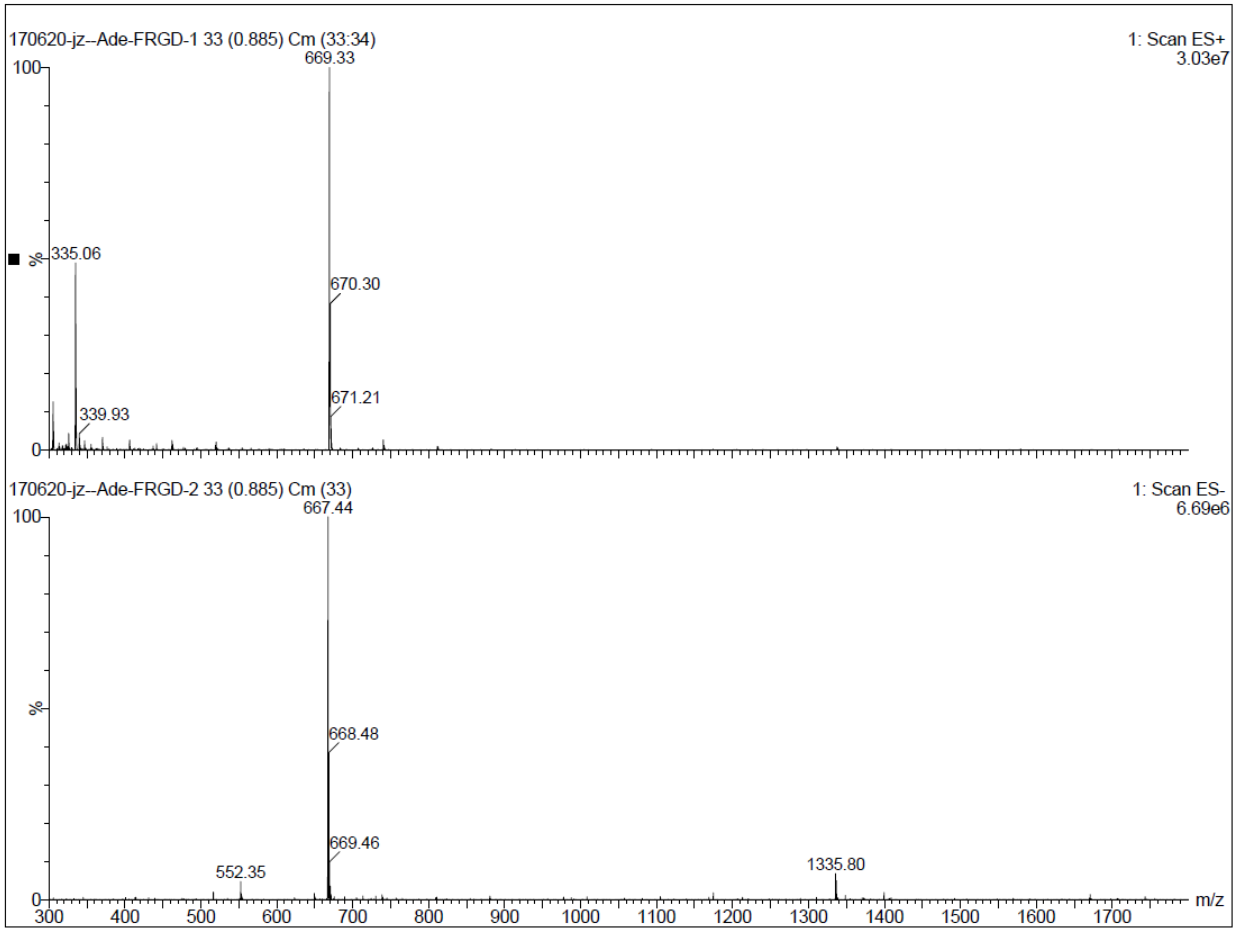
10： NBD-FRGD


0.67
1.00
3.31
0.95
0.91
8.49
0.89
2.03
0.95
2.07
2.11
1.85
2.48
1.18
1.51
0.90
0.82
1.00
3.48
f1 (ppm)

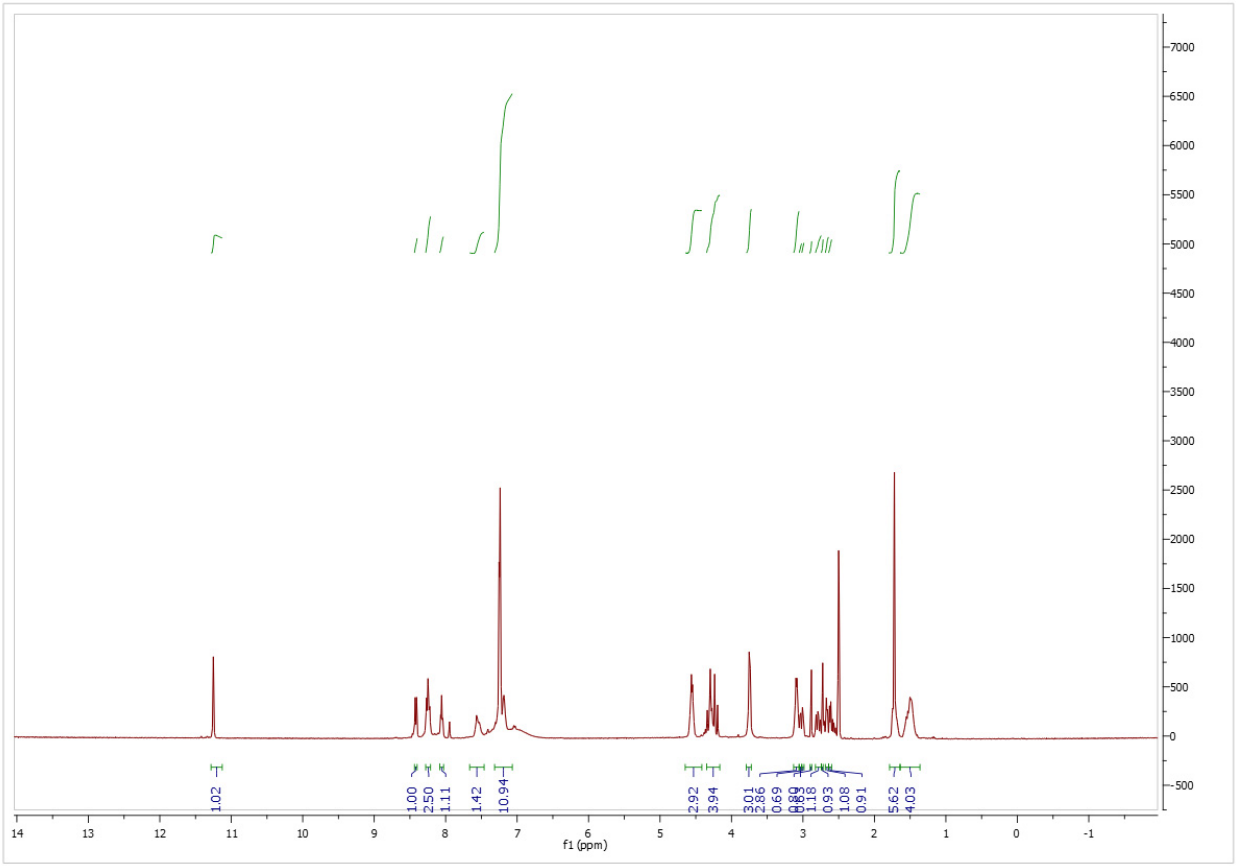

170619-jz--NBD-FRGD-1 54 (1.448) Cm (53:55)
1: Scan ES-
5.77e6
100
%
0
726.42
727.39
611.39
728.43
1453.88
170619-jz--NBD-FRGD-2 54 (1.448) Cm (54:55)
1: Scan ES+
1.34e8
100
%
0
728.17
613.21
729.28
614.25
730.25
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z

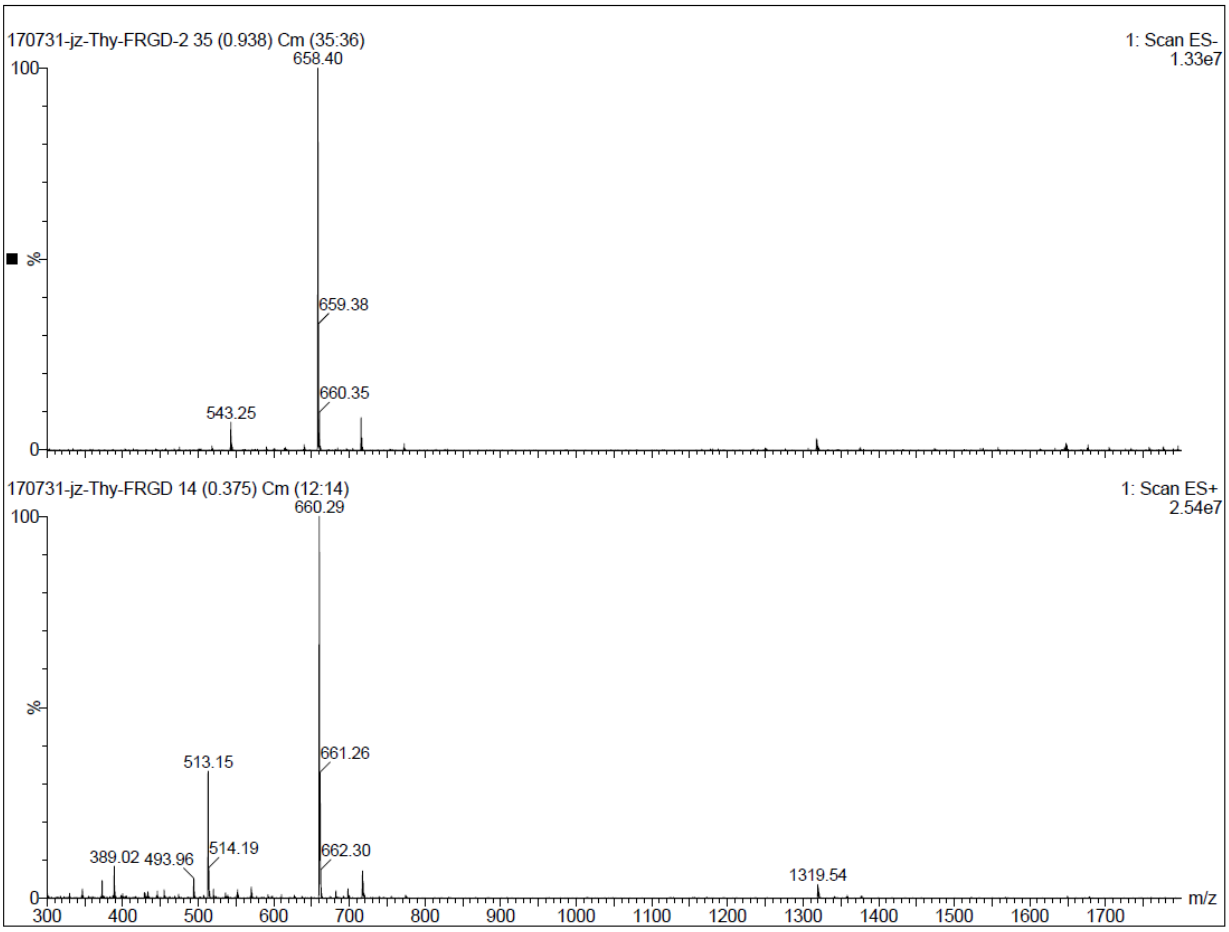
11： Ade-FRGD


11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
1.00
1.25
1.29
1.15
1.15
1.14
5.51
1.41
2.07
2.43
1.36
7.19
3.12
0.80
1.05
2.38
0.79
3.32
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

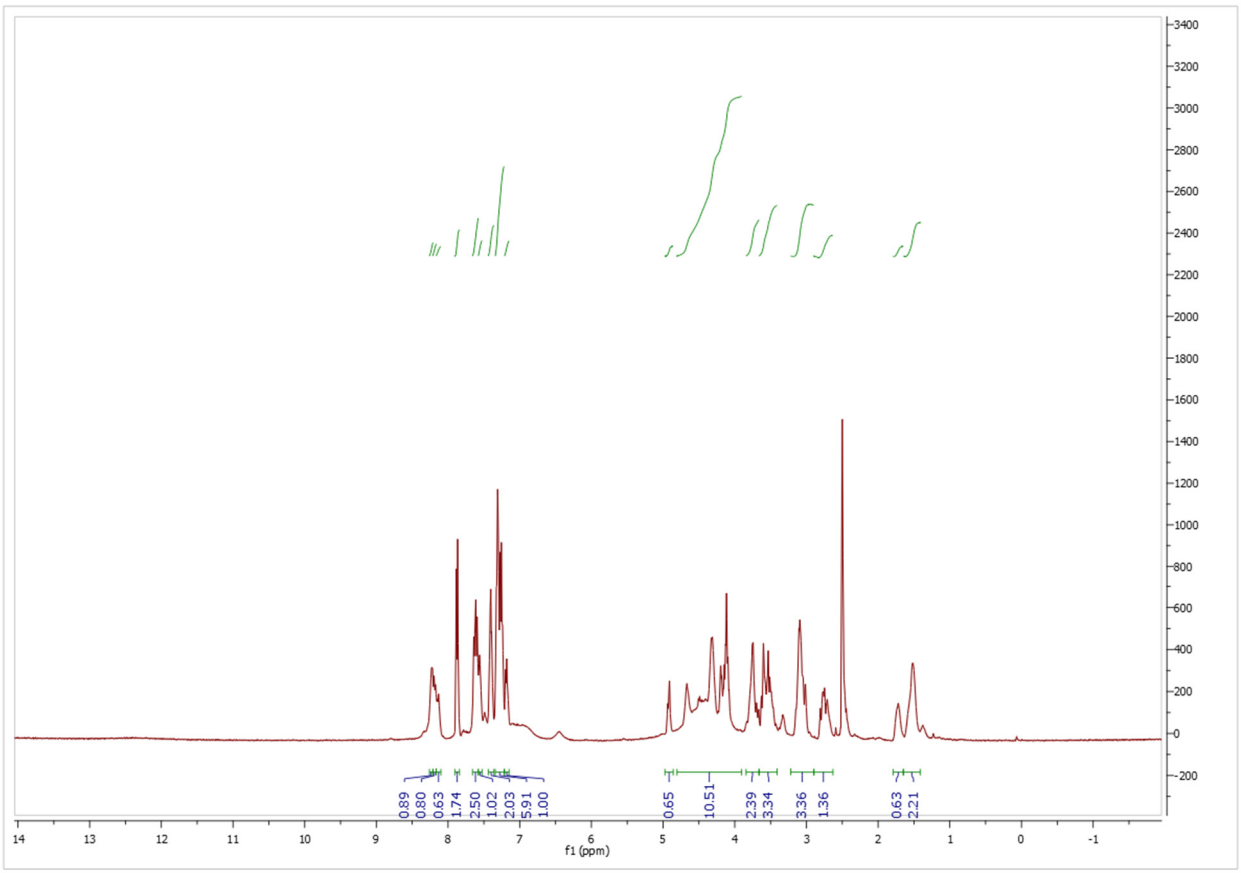

170620-jz--Ade-FRGD-1 33 (0.885) Cm (33:34)
1: Scan ES+
3.03e7
669.33
335.06
670.30
671.21
339.93
170620-jz--Ade-FRGD-2 33 (0.885) Cm (33)
1: Scan ES-
6.69e6
667.44
668.48
669.46
552.35
1335.80
100
%
0
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z

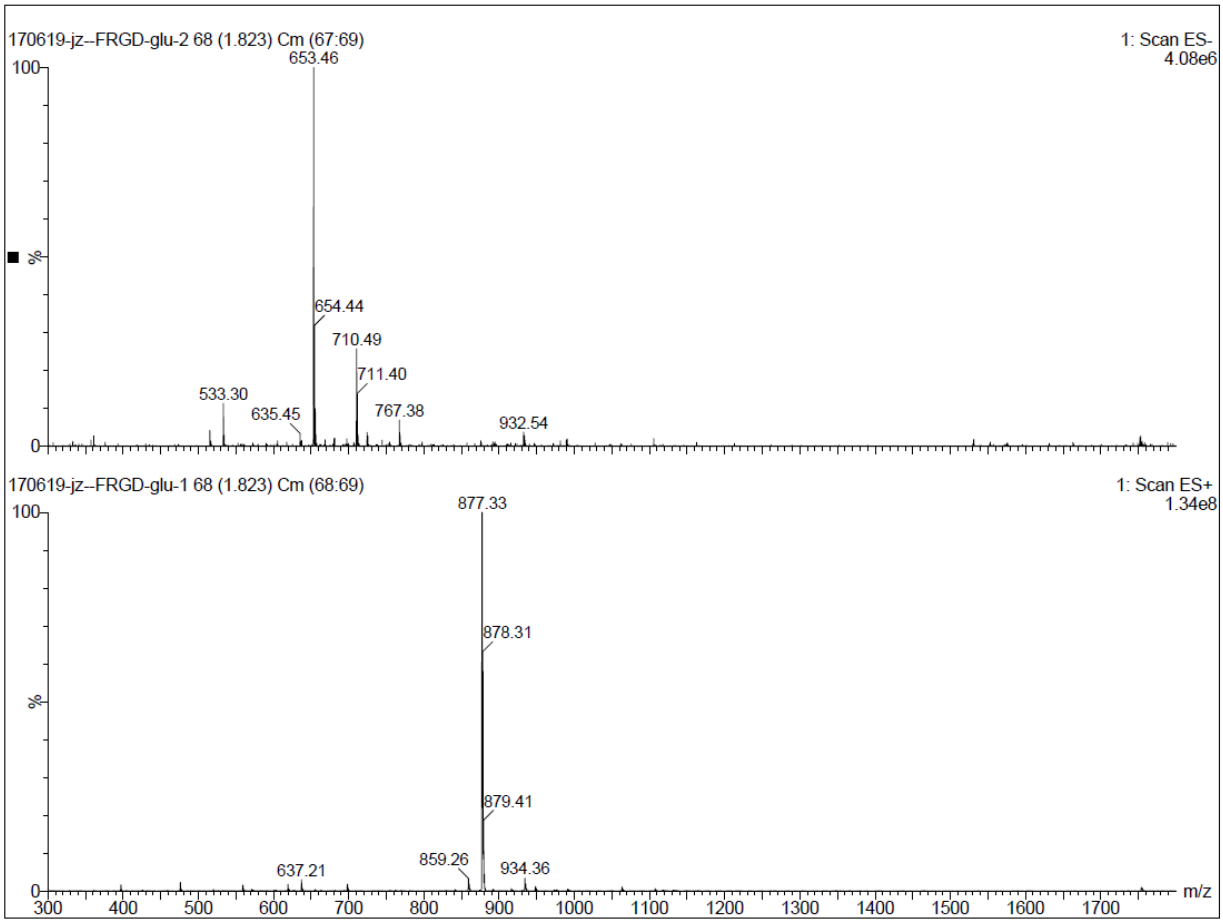
12： Thy-FRGD


1.02
1.00
2.50
1.11
1.42
10.94
2.92
3.94
3.01
2.86
0.69
0.80
0.83
1.18
0.93
1.08
0.91
5.62
4.03
f1 (ppm)

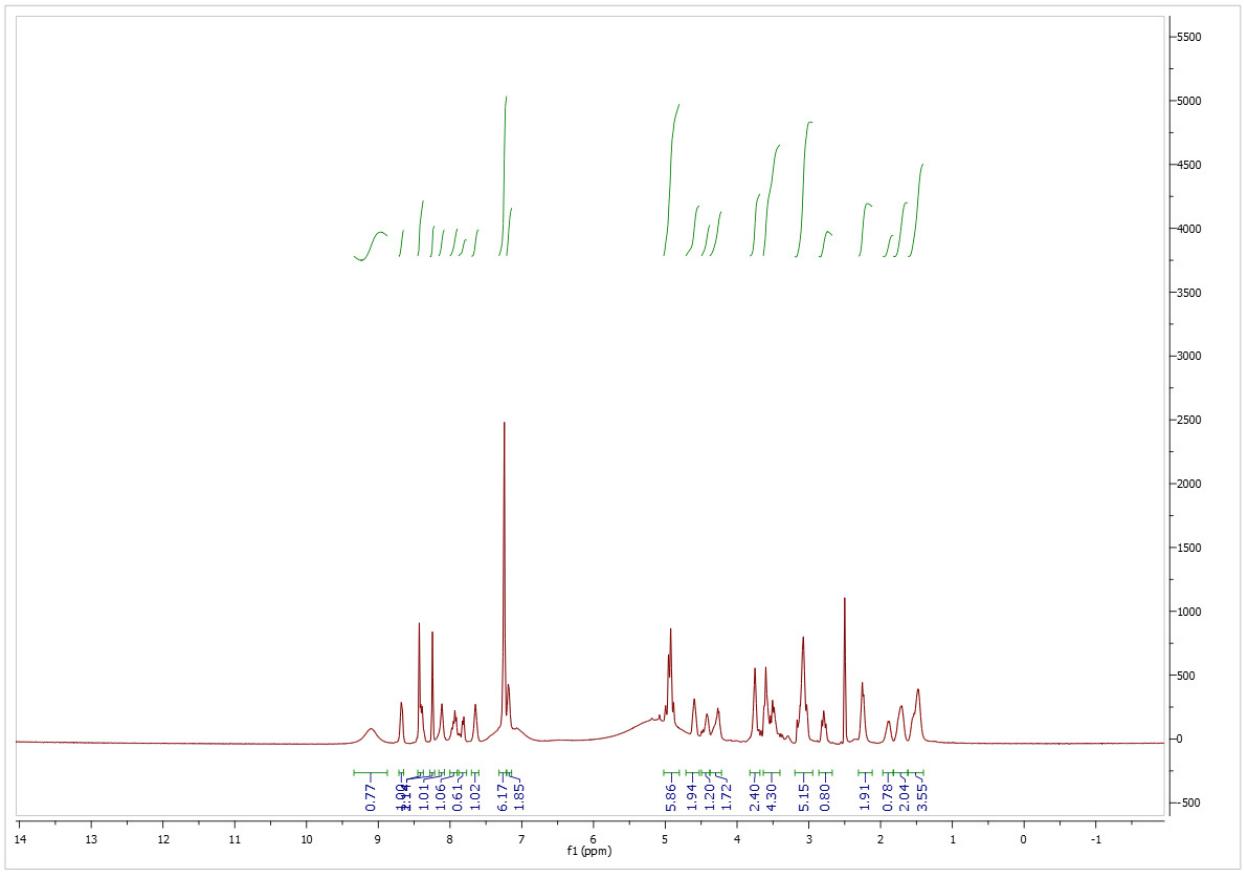

170731-jz-Thy-FRGD-2 35 (0.938) Cm (35:36)
1: Scan ES-
1.33e7
100
%
0
658.40
659.38
660.35
543.25
170731-jz-Thy-FRGD 14 (0.375) Cm (12:14)
1: Scan ES+
2.54e7
100
%
0
660.29
661.26
513.15
389.02
493.96
514.19
662.30
1319.54
m/z
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

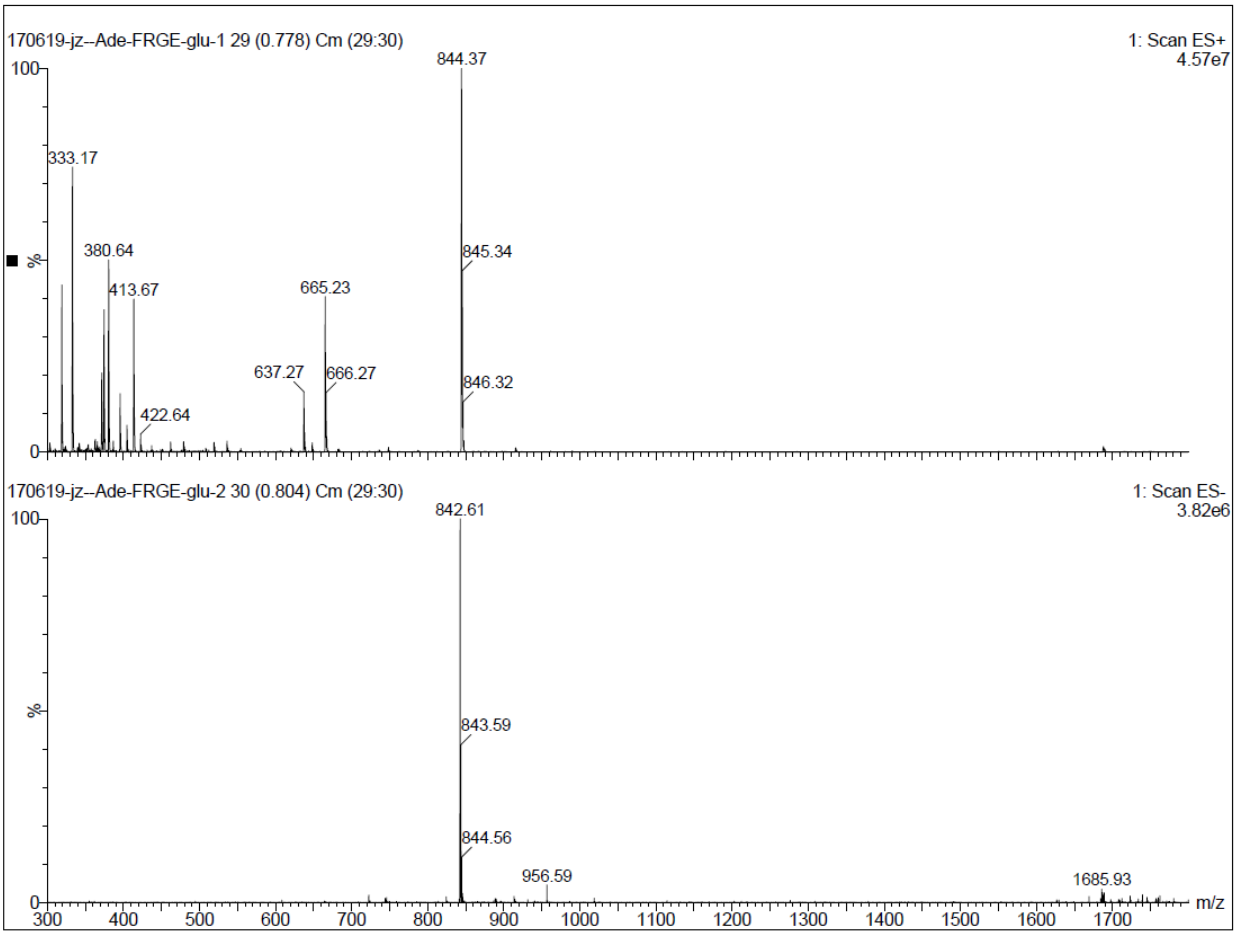
14：FRGD-Glucosamine




170619-jz--FRGD-glu-2 68 (1.823) Cm (67:69)
1: Scan ES-
4.08e6
653.46
654.44
710.49
711.40
533.30
635.45
767.38
932.54
170619-jz--FRGD-glu-1 68 (1.823) Cm (68:69)
1: Scan ES+
1.34e8
877.33
878.31
879.41
637.21
859.26
934.36
%
m/z
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700

15： Ade-FRGE-Glu




170619-jz--Ade-FRGE-glu-1 29 (0.778) Cm (29:30)
1: Scan ES+
4.57e7
100
%
0
844.37
333.17
380.64
845.34
413.67
665.23
637.27
666.27
846.32
422.64
170619-jz--Ade-FRGE-glu-2 30 (0.804) Cm (29:30)
1: Scan ES-
3.82e6
100
%
0
842.61
843.59
844.56
956.59
1685.93
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
m/z