Electronic Supplementary Information

Dual-action gallium-flavonoid compounds for combating *Pseudomonas aeruginosa* infection

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[†]These authors contributed equally to this study.

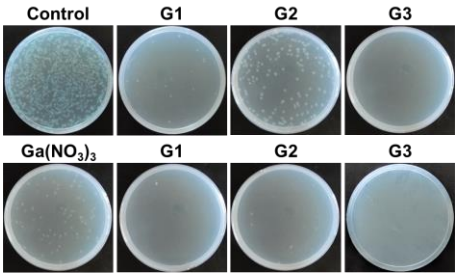


Fig. S1 Bactericidal activity assay of gallium-flavonoid compounds and $Ga(NO_3)_3$ (with MIC concentration) using agar plate counting method.

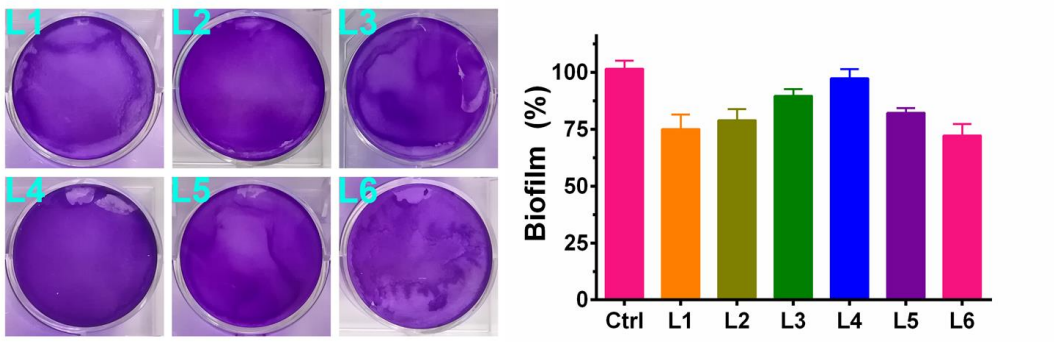


Fig. S2 Inhibition of *P. aeruginosa* biofilm formation by flavonoid ligands.

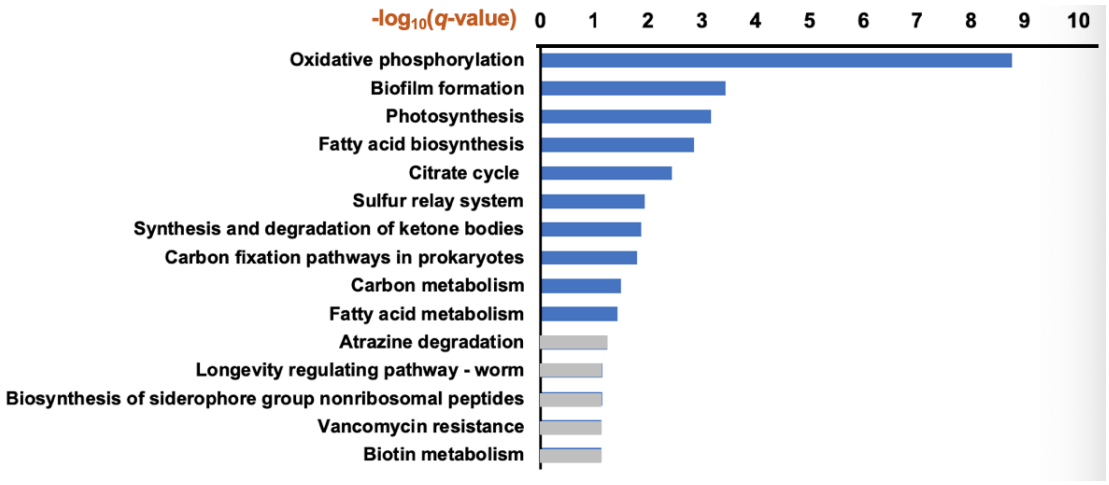


Fig. S3 KEGG enrichment pathways analysis of *P. aeruginosa* exposed to G6 compound according to the significantly altered genes.

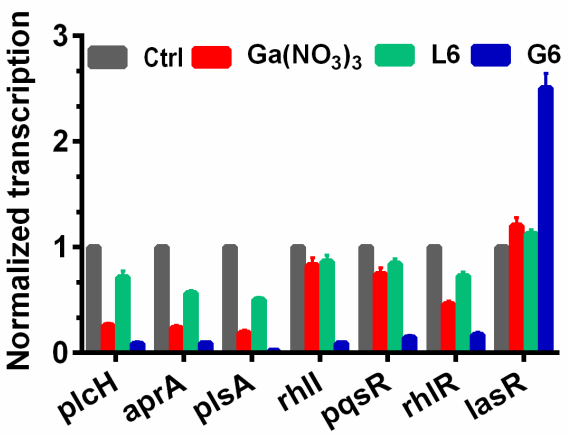


Fig. S4 Quantitative transcript analysis of *plcH*, *aprA*, *plsA*, *rhlR*, *rhlI* and *pqsR* genes of *P. aeruginosa*. The mean values of transcription level in untreated control groups are set as 1. The treated groups are normalized to that of control groups. Results are shown as mean ± sd. The statistical difference is determined by two-tailed Student's t-test.

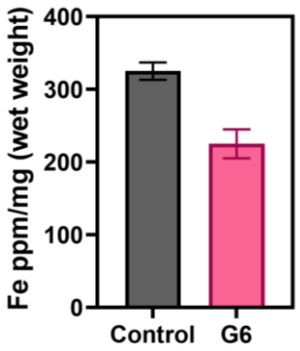


Fig. S5 The intracellular iron level of bacteria is detected by inductively coupled plasma mass spectrometry (ICP-MS) after G6 (0.08 μM) treatment.

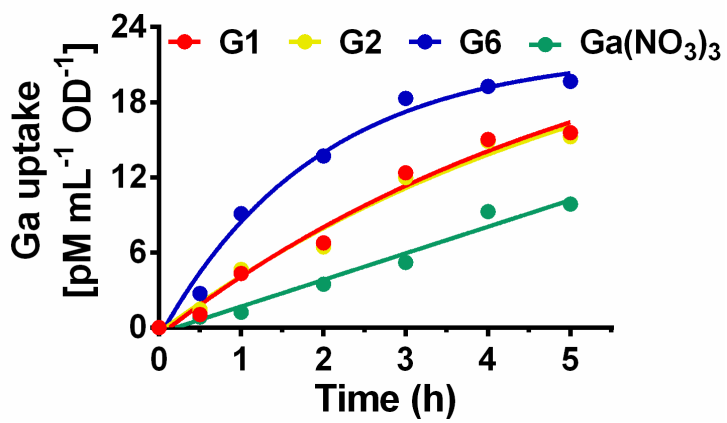


Fig. S6 Gallium uptake by *P. aeruginosa*. *P. aeruginosa* cells were incubated with 0.16 µM gallium compounds (G1, G2, G6) or Ga(NO3)3. The intracellular gallium contents were measured by ICP-MS in the course of time.

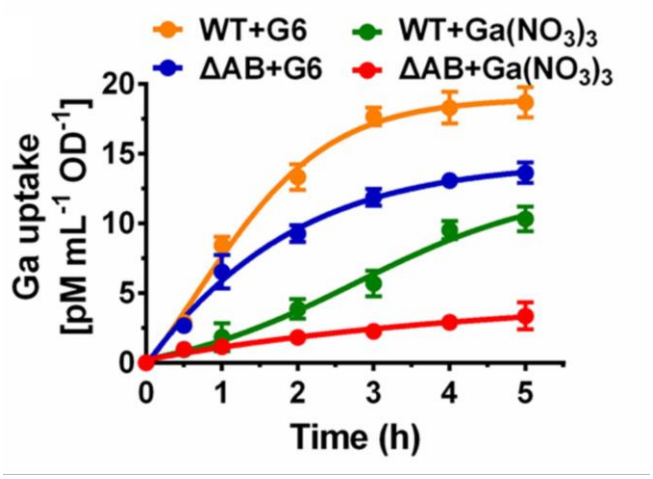


Fig. S7 Gallium uptake by wild-type *P. aeruginosa* and *hitAB* gene knockout mutant. Wild-type *P. aeruginosa* and mutant are incubated with 0.16 μM G6 compound or $Ga(NO_3)_3$. The intracellular gallium contents are measured by ICP-MS in the course of time.

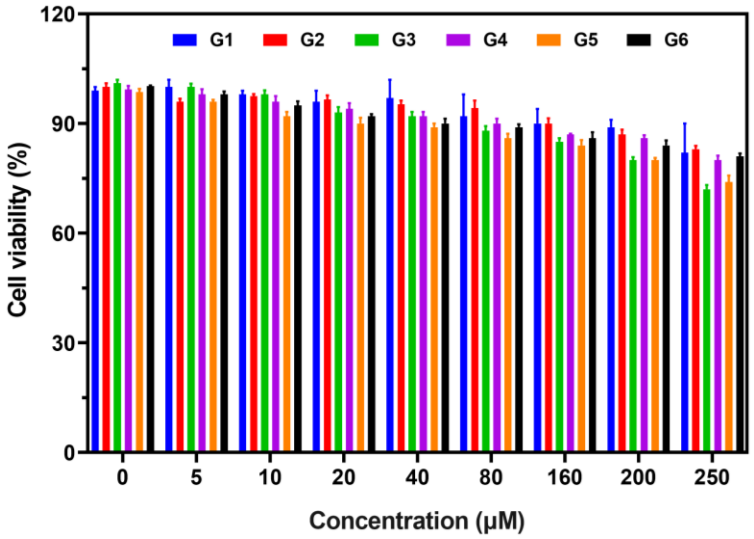


Fig. S8 Relative viabilities of LO2 cell lines after incubation with different concentrations of G1-G6 (0, 5, 10, 20, 40, 80, 160, 200, and 250 μM) for 24 h.

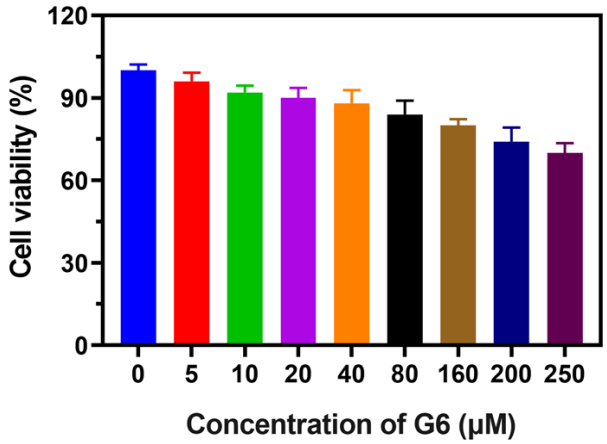


Fig. S9 Relative viabilities of A549 cell lines after incubation with different concentrations of G6 (0, 5, 10, 20, 40, 80, 160, 200, and 250 μM) for 24 h.

Table S1. *In vitro* MIC values of the compounds against bacteria.

Compound	MIC [μg mL^{-1}](μM)		
	S. aureus (G+)	*E. coli* (G-)	*P. aeruginosa* (G-)
(G1) Ga(III)$(L1)_2$	128	256	1.0 (1.7)
(G2) Ga(III)$(L2)_2$	64	128	2.0 (3.3)
(G3) Ga(III)$(L3)_2$	>256	>256	8.0 (12.5)
(G4) Ga(III)$(L4)_2$	256	128	16.0 (23.8)
(G5) Ga(III)$(L5)_2$	256	128	4.0 (6.6)
(G6) Ga(III)$(L6)_2$	64	256	0.5 (0.8)
(Ga) Ga$(NO_3)_3$	128	128	2.0 (7.8)
Streptomycin	--	--	64.0
Sodium cefoperazone	--	--	4.0
Gentamicin	--	--	4.0
Polymyxin B sulfate	--	--	2.0
Ofloxacin	--	--	1.0
(L1) Chrysin	--	--	128.0
(L2) Apigenin	--	--	128.0
(L3) Luteolin	--	--	256.0
(L4) Quercetin	--	--	256.0
(L5) Baicalein	--	--	256.0
(L6) Naringenin	--	--	128.0

Table S2. LogP values for different gallium compounds

Compound	Absorption	Linear relation	R^2	LogP
G1	318 nm	$Y=8.58\times10^{-3}X+0.29$	0.9946	1.78
G2	339 nm	$Y=13.04\times10^{-3}X+0.21$	0.9965	1.56
G3	352 nm	$Y=13.01\times10^{-3}X+0.20$	0.9946	0.95
G4	377 nm	$Y=6.73\times10^{-3}X+0.08$	0.9946	0.46
G5	377 nm	$Y=7.02\times10^{-3}X+0.24$	0.9946	1.38
G6	290 nm	$Y=15.45\times10^{-3}X+0.98$	0.9946	1.86

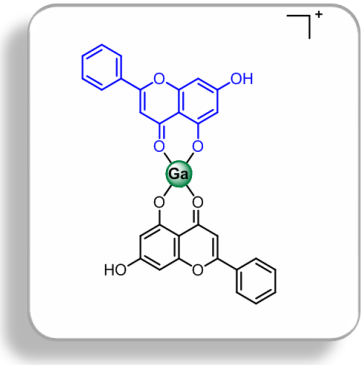
Table S3. Cytotoxicity of gallium compounds toward LO2 cell

Gallium compound	**IC_{50}** LO2
G1	>200 μM
G2	>200 μM
G3	>200 μM
G4	>200 μM
G5	>200 μM
G6	>200 μM

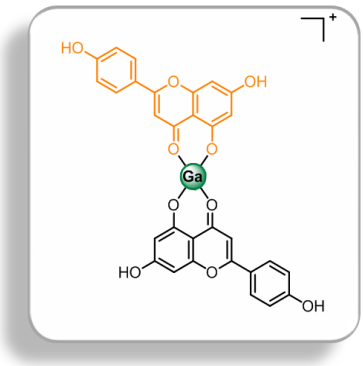
Table S4. qPCR primer sequence used in this study

Primer	Sequence(5'-3')
qPCR-pqsR-For	AACATGTTCCTCCAGGTCAT
qPCR-pqsR-Rev	GTTGAGATTGAAGGCGATGT
qPCR-lasR-For	CTGTGGATGCTCAAGGACTAC
qPCR-lasR-Rev	AACTGGTCTTGCCGATGG
qPCR-aprA-For	GCTTCAGCCAGAACCAGAAGAT
qPCR-aprA-Rev	TCGACACATTGCCCTTCAAC
qPCR-plcH-For	CATATGCCTACCCTCGCGGTGC
qPCR-plcH-Rev	TTACAGGTTGCCGTGGAACTCG
qPCR-rhlR-For	GCCAGCGTCTTGTTCGG
qPCR-rhlR-Rev	CGGTCTGCCTGAGCCATC
qPCR-rhlI-For	GCTCTCTGAATCGCTGGA
qPCR-rhlI-Rev	TCACACCGCCATCGACAG
qPCR-plsA-For	AAGATCAAGAAACGCGTGGAAT
qPCR-plsA-Rev	TGTAGAGGTCGAACCACACCG

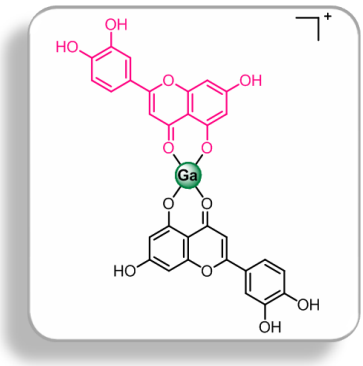
Characterization of Gallium compounds：



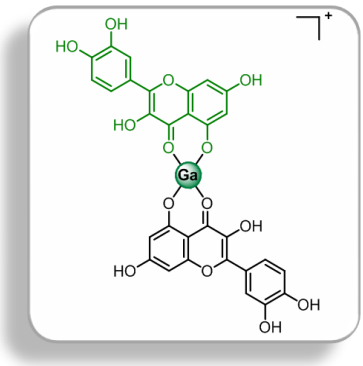
Compound G1. Yield: 61.0%. **^{1}H NMR** (400 Hz, DMSO-*d*6, δ, ppm): 6.23 (d, 2H,), 6.53 (d, 2H,), 6.96 (s, 2H), 7.59 (m, 6H), 8.06 (d, *J* = 4.0 Hz, 4H), 10.92 (s, 2H). **^{13}C NMR** (500 Hz, DMSO-*d*6, δ, ppm): 182.34, 164.91, 163.64, 161.93, 157.93, 132.48, 131.18, 129.60, 126.87, 105.64, 104.44, 99.50, 94.60. **IR:** 3600-2600 (υOH), 1642, 1604, 1578, 1535, 1498, 1450, 1365, 1311, 1245, 1167, 1101, 1030, 995, 909, 841, 806, 778, 732, 676, 641, 611, 576, 507. **Anal.** Calcd for $C_{30}H_{18}GaO_8$: C, 62.54; H, 3.15; Ga, 12.10. Found: C, 61.50; H, 3.60; Ga, 11.98.**ESI-MS (m/z):** calcd for $[M]^+$: 575.03, found: 575.35.



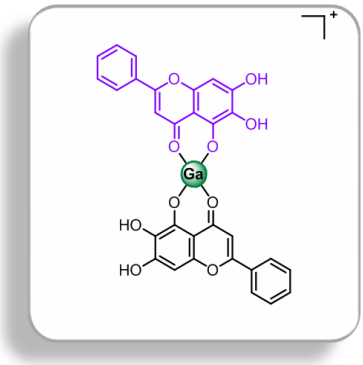
Compound G2. Yield: 56.7%. **^{1}H NMR** (400 Hz, DMSO-*d*6, δ, ppm): m 6.19 (d, J=1.96 Hz, 2 H), 6.48 (d, J=1.96 Hz, 2 H), 6.78 (s, 2 H), 6.92 (d, J=8.80 Hz, 4 H), 7.92 (d, J=8.80 Hz, 4 H), 10.83 (s, 2 H), 12.96 (s, 2 H). **^{13}C NMR** (100 Hz, DMSO-*d*6, δ, ppm):182.22, 164.61, 161.93, 161.65, 159.07, 157.78, 129.28, 128.93, 116.42, 105.18, 104.16, 96.89, 94.82. **IR:** 3600-2600 (υOH), 1654, 1606, 1586, 1556, 1498, 1356, 1298, 1270, 1243, 1179, 1104, 1033, 983, 907, 831, 806, 740, 692, 672, 646, 614, 576, 505. **Anal.** Calcd for $C_{30}H_{18}GaO_{10}$: C, 59.25; H, 2.98; Ga 11.46. Found: C, 58.92; H, 2.63; Ga, 11.14. **ESI-MS (m/z):** calcd for $[M]^+$: 607.02, found: 607.41.



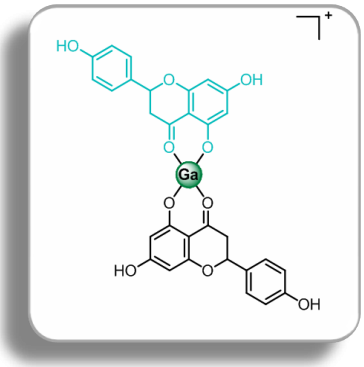
Compound G3. Yield: 61.8%. **^{1}H NMR** (400 Hz, DMSO-*d*6, δ, ppm):6.17 - 6.23 (m, 4 H), 6.24 (d, J=1.70 Hz, 2H), 6.48 (d, J=1.70 Hz, 2 H), 7.08 (s, 2 H), 7.59 (d, J=1.47 Hz, 2 H), 10.69 (br.s., 2 H), 10.90 (br. s., 2 H), 12.71 (br. s., 2H). **^{13}C NMR** (100 Hz, DMSO-*d*6, δ, ppm):182.19, 165.02, 164.09, 162.04, 158.16, 151.42, 148.16, 139.30, 119.95, 117.98, 112.91, 108.89, 104.29, 99.60, 94.25. **IR:** 3600-2600 (υOH), 1654, 1611, 1531, 1498, 1445, 1365, 1265, 1217, 1165, 1118, 1099, 1032, 999, 952, 862, 838, 814, 791, 686, 639, 601, 563, 520. **Anal.** Calcd for $C_{30}H_{18}GaO_{12}$: C, 56.28; H, 2.83; Ga, 10.89. Found: C, 56.26; H, 2.86; Ga, 11.05. **ESI-MS (m/z):** calcd for $[M]^+$: 639.01, found: 639.48.



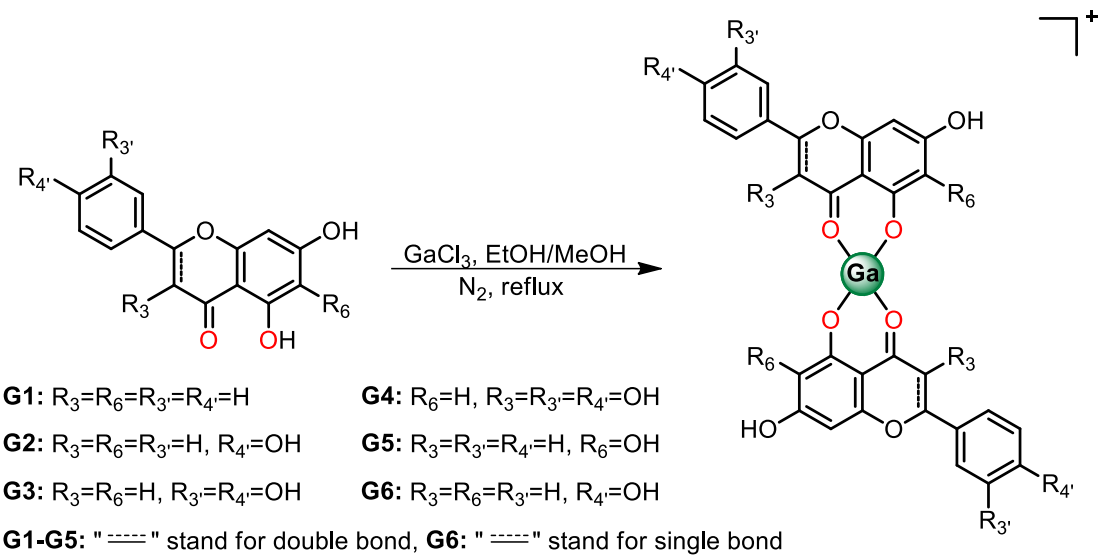
Compound G4. Yield: 55.7%.**^{1}H NMR** (400 Hz, DMSO-*d*6, δ, ppm): 6.18 (d, *J* = 1.90 Hz, 2 H), 6.40 (d, *J* = 1.90 Hz, 2 H), 6.88 (d, *J* = 8.44 Hz, 2 H), 7.54 (dd, *J* = 8.44, 2.08 Hz, 2 H), 7.67 (d, *J* = 2.08 Hz, 2 H), 9.35 (s, 2 H), 9.59 (s, 2 H), 10.78 (s, 2 H), 12.48 (s, 2 H).**^{13}C NMR** (100 Hz, DMSO-*d*6, δ, ppm): 176.31, 164.36, 161.20,156.61, 148.17, 147.26, 145.52, 136.21, 122.43, 120.44, 116.05, 115.52, 103.48, 98.64,93.82. **IR:** 3600-2600 (υOH), 1650, 1602, 1555, 1512, 1460, 1413, 1356, 1318, 1242, 1199, 1166, 1114, 1090, 1009, 1000, 934, 881, 820, 791, 692, 654, 635, 602, 521. **Anal.** Calcd for $C_{30}H_{18}GaO_{14}$: C, 53.60; H, 2.70; Ga, 10.37. Found: C, 53.68; H, 2.72; Ga, 10.67. **ESI-MS (m/z):** calcd for $[M]^+$: 671.00, found: 671.38.



Compound G5. Yield: 59.3%. **^{1}H NMR** (400 Hz, DMSO-*d*6, δ, ppm): 6.65 (s, 2 H), 6.95 (s, 2 H), 7.59 (m, 6 H), 8.08 (d, J=6.60 Hz, 4 H), 10.61 (br. s., 2 H), 12.68 (br. s., 2 H). **^{13}C NMR** (100 Hz, DMSO-*d*6, δ, ppm):182.62, 163.39, 154.12, 150.32, 147.45, 132.34, 131.44, 129.81, 129.61, 126.79, 104.97, 104.77, 94.52. **IR:** 3600-2600 (υOH), 1644, 1615, 1573, 1539, 1497, 1473, 1449, 1386, 1359 1340, 1302, 1278, 1245, 1208, 1184, 1160, 1084, 1037, 999, 913, 895, 852, 823, 771, 733, 681, 657, 638, 615, 572, 515. **Anal.** Calcd for $C_{30}H_{18}GaO_{10}$: C, 59.25; H, 2.98; Ga, 11.46. Found: C, 48.12; H, 2.65; Ga, 11.09.**ESI-MS (m/z):** calcd for $[M]^{+}$: 607.02, found: 607.29.



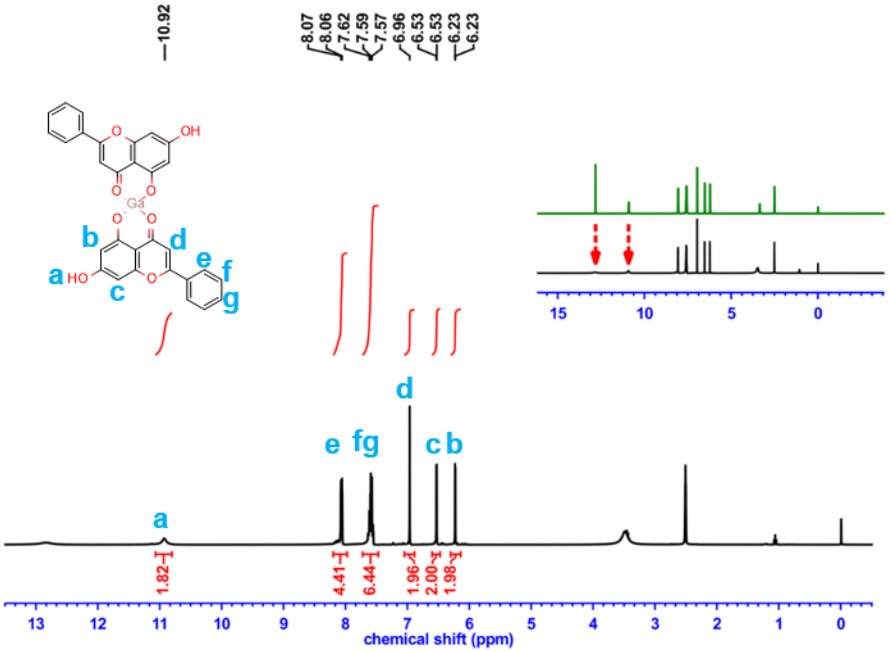
Yield: 62.4%.**^{1}H NMR** (400 Hz, DMSO-*d*6, δ, ppm): 2.68 (dd, *J*=17.15, 2.90 Hz, 2 H), 3.26 (dd, *J*=17.15, 12.90 Hz, 2 H), 5.44 (dd, *J* = 12.71, 2.90 Hz, 2 H), 5.88 (s, 4 H), 6.79 (d, J=8.56 Hz, 4 H), 7.31 (d, J=8.44 Hz, 4 H), 10.79 (s, 2 H), 12.15 (s, 2 H). **^{13}C NMR** (100 Hz, DMSO-*d*6, δ, ppm): 196.89, 167.12, 163.97, 163.42, 158.21, 129.33, 128.83, 115.64, 102.25, 96.27, 95.45, 78.89, 42.45.**IR:** 3600-2600 (υOH), 1658, 1630, 1601, 1521, 1492, 1459, 1430, 1364, 1312, 1250, 1179, 1155, 1084, 1065, 1013, 970, 890, 833, 757, 729, 710, 667, 629, 615, 558, 529. **Anal.** Calcd for $C_{30}H_{22}GaO_{10}$: C, 8.86; H, 3.62; Ga, 11.39. Found: C, 58.23; H, 2.45; Ga, 11.21. **ESI-MS (m/z):** calcd for $[M]^{+}$: 611.05, found: 611.48.



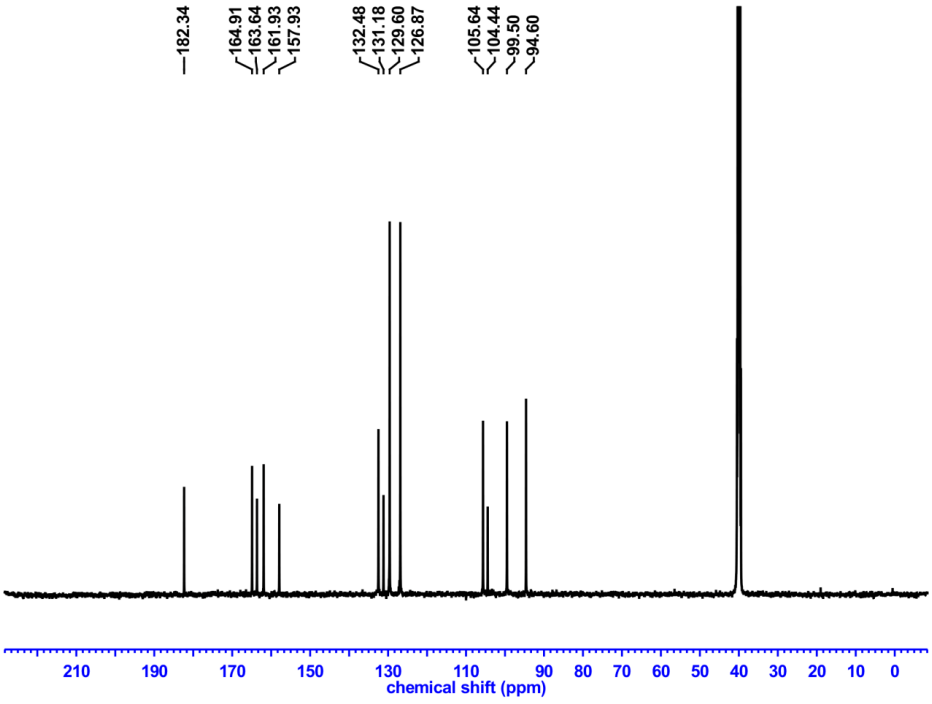
Scheme S1. The reaction scheme for the synthesis of the gallium compounds.

^{1}H NMR and ^{13}C NMR Data：

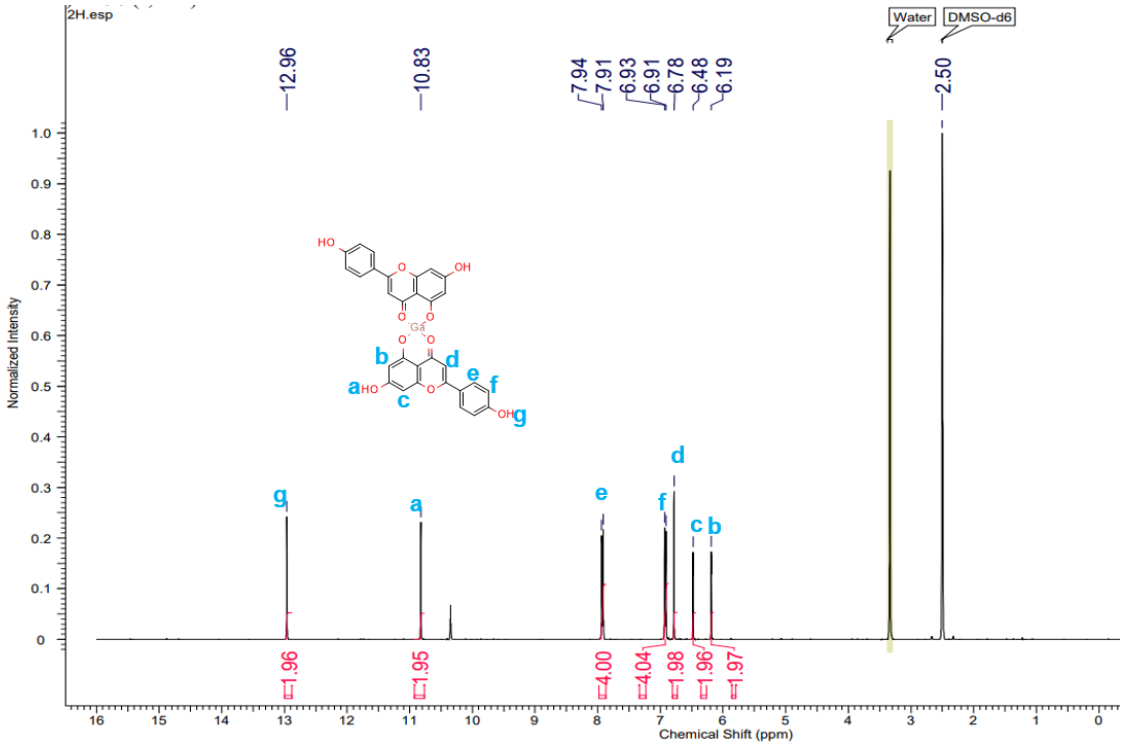
^{1}H NMR：G1



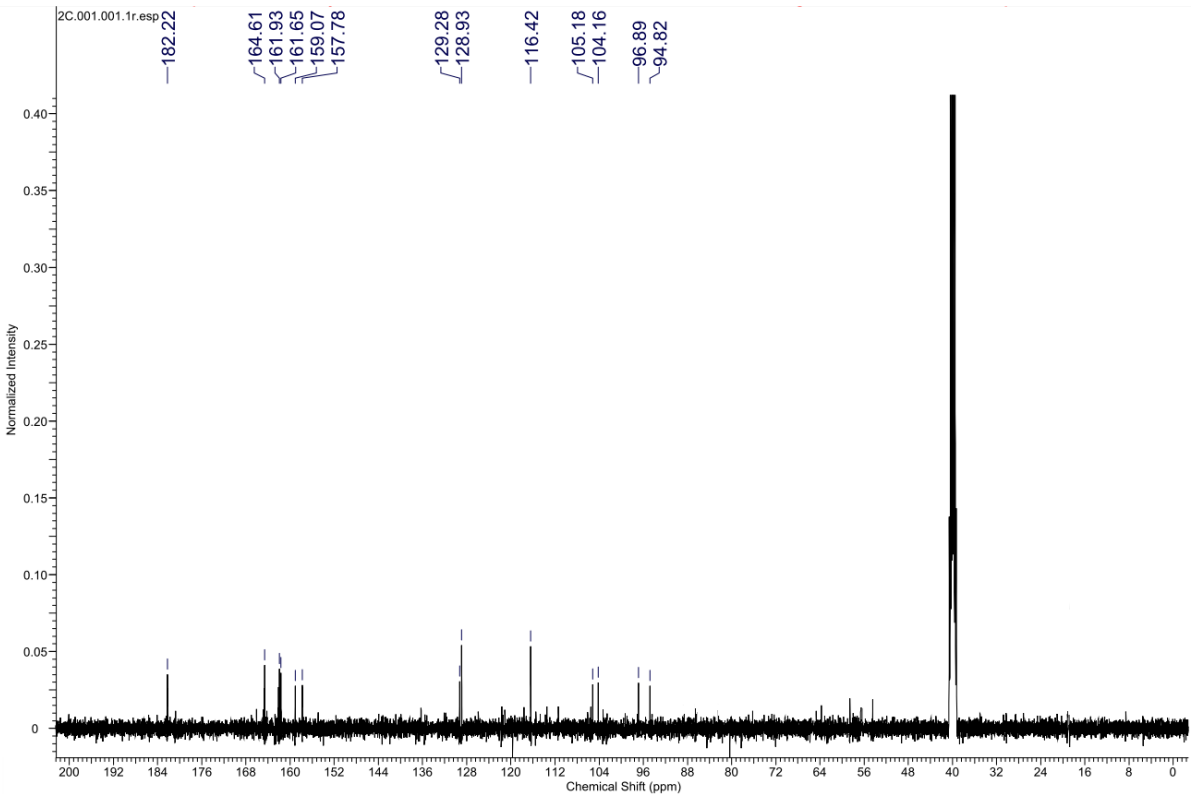
^{13}C NMR：G1



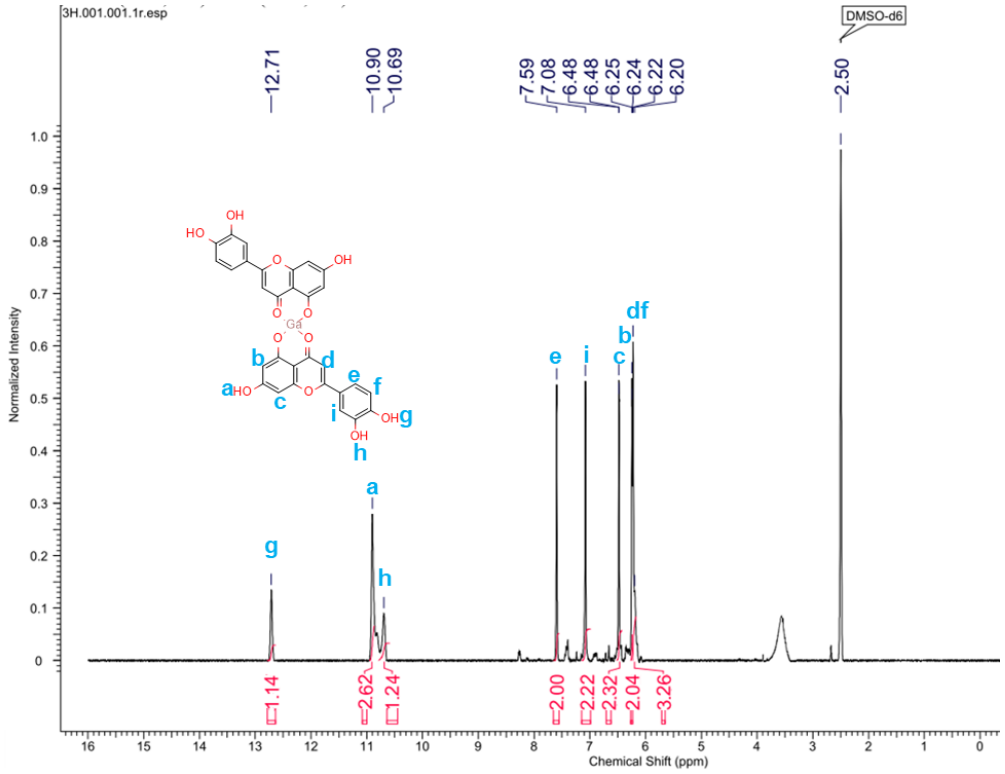
^{1}H NMR：G2



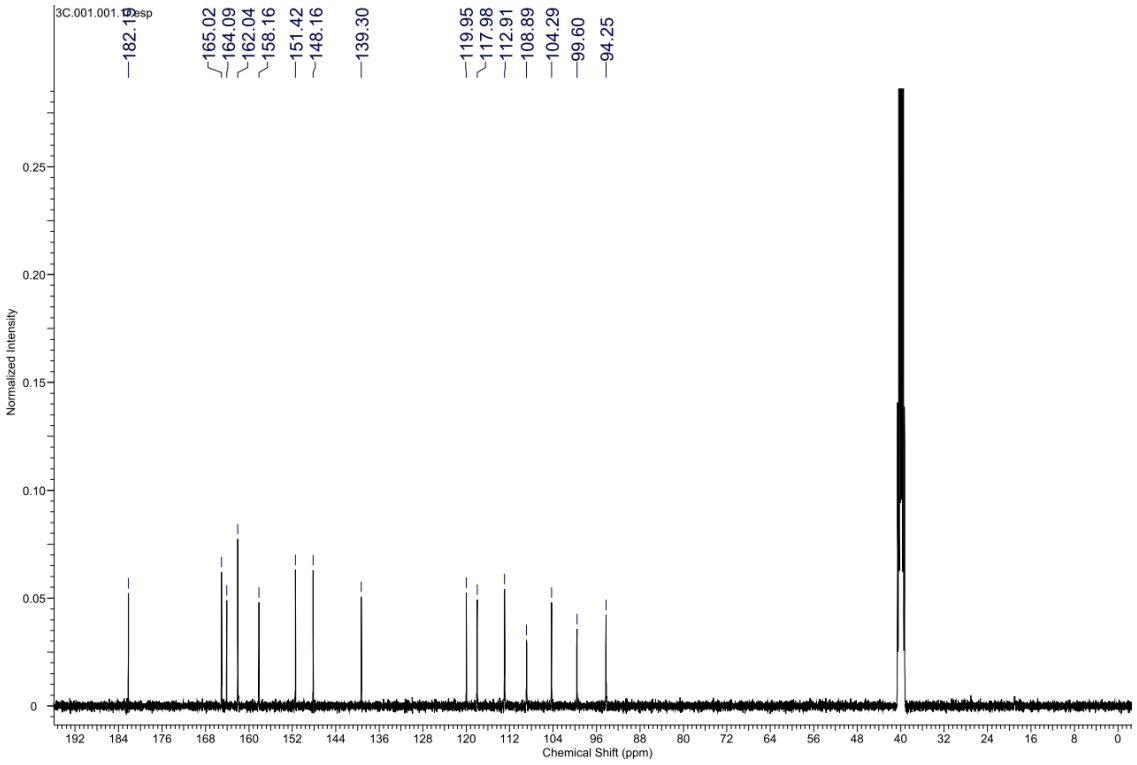
^{13}C NMR：G2



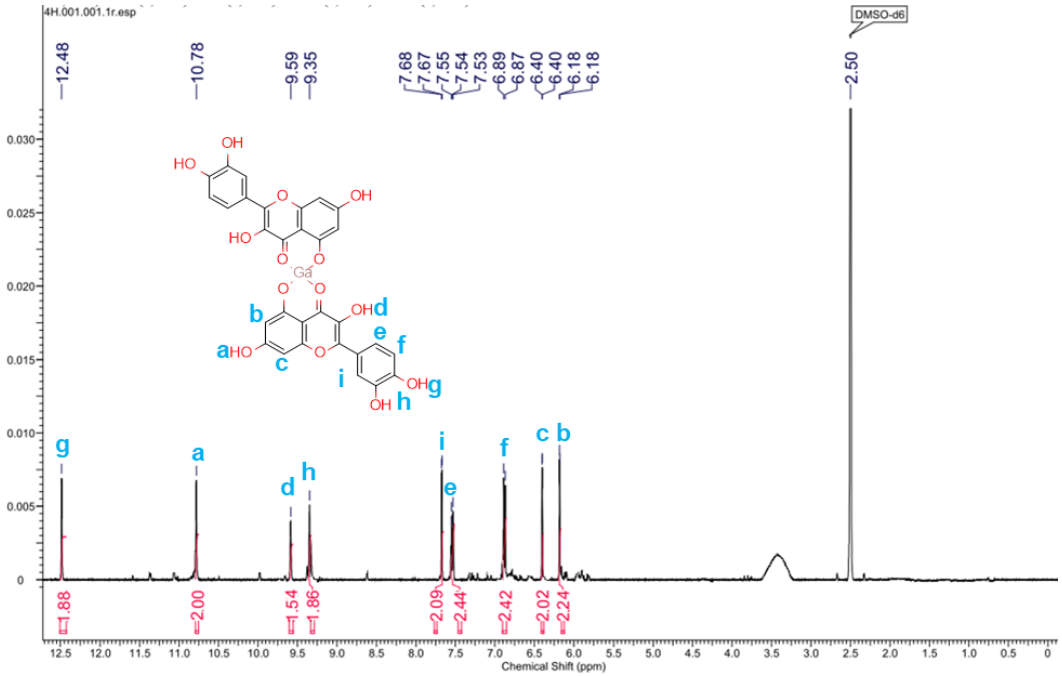
^{1}H NMR：G3



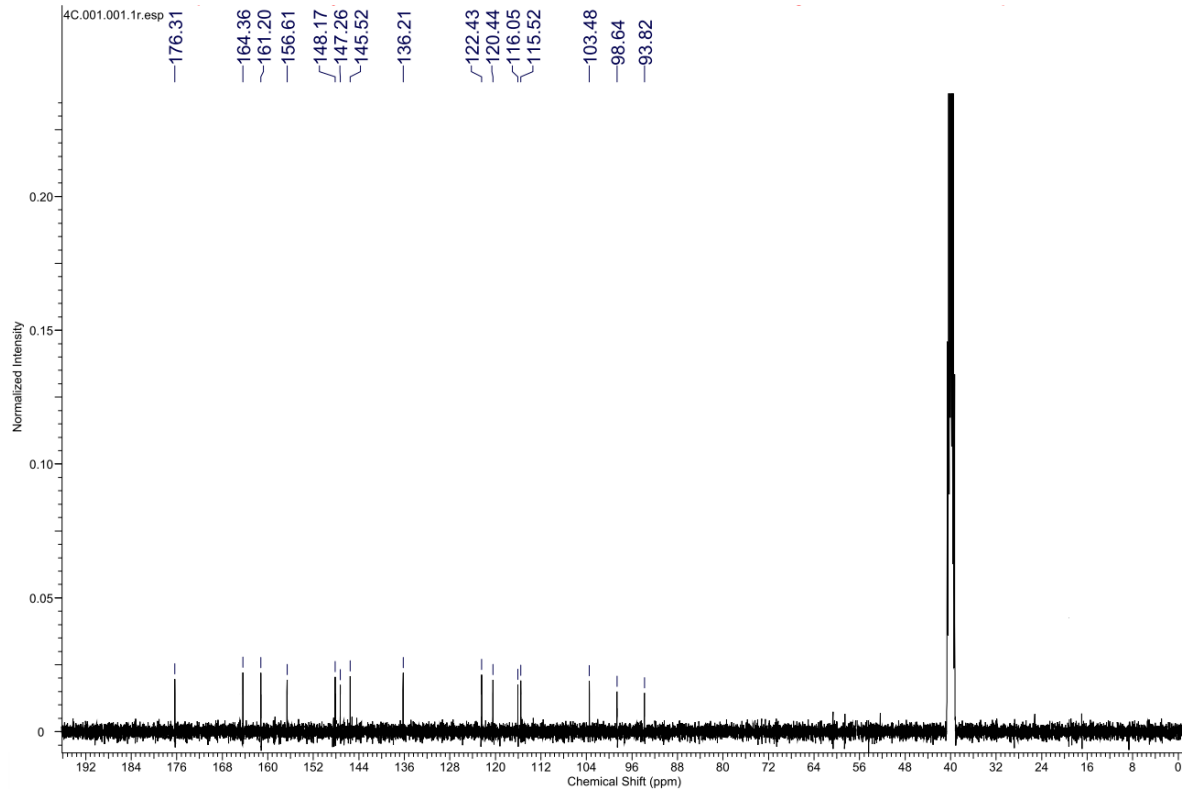
^{13}C NMR：G3



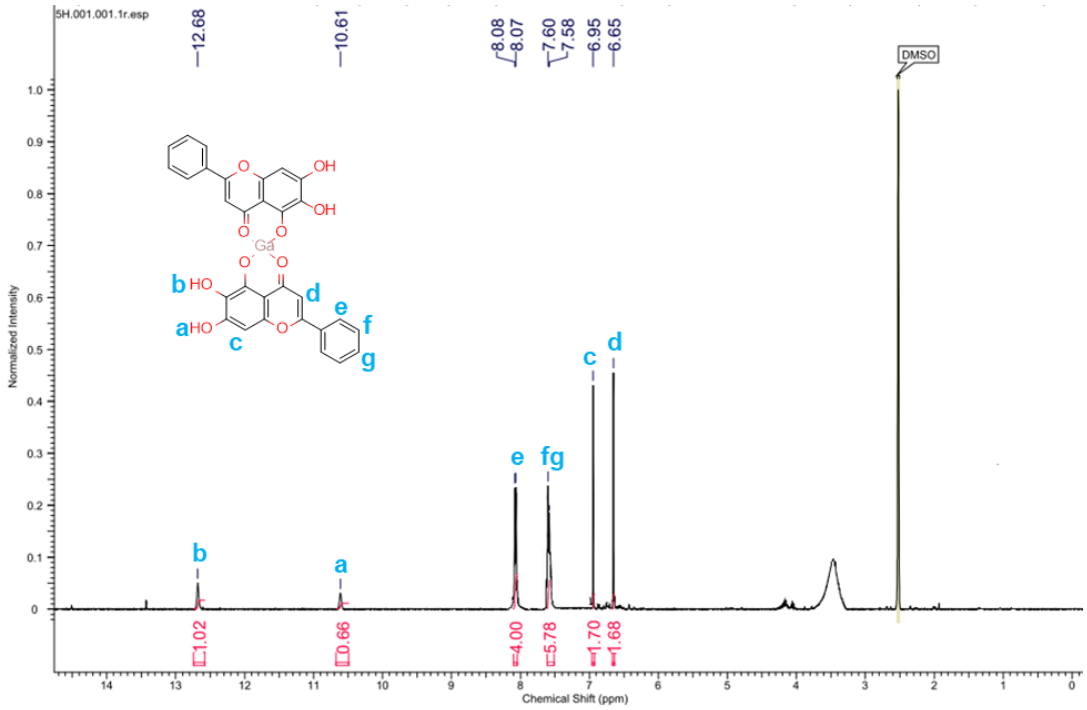
^{1}H NMR：G4



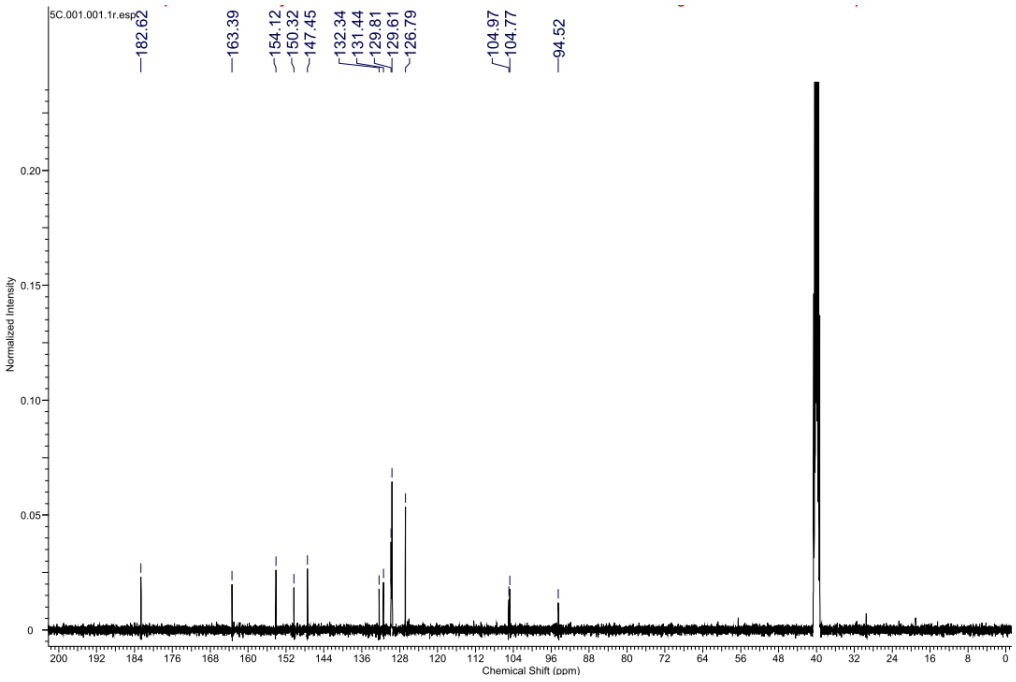
^{13}C NMR：G4



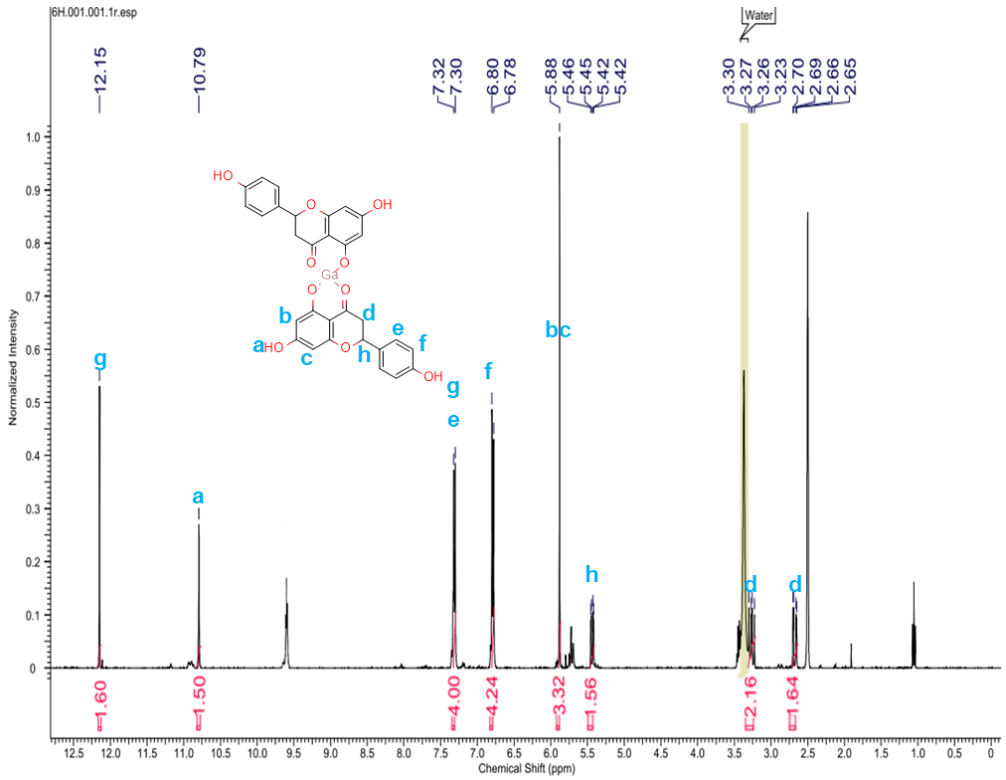
^{1}H NMR：G5



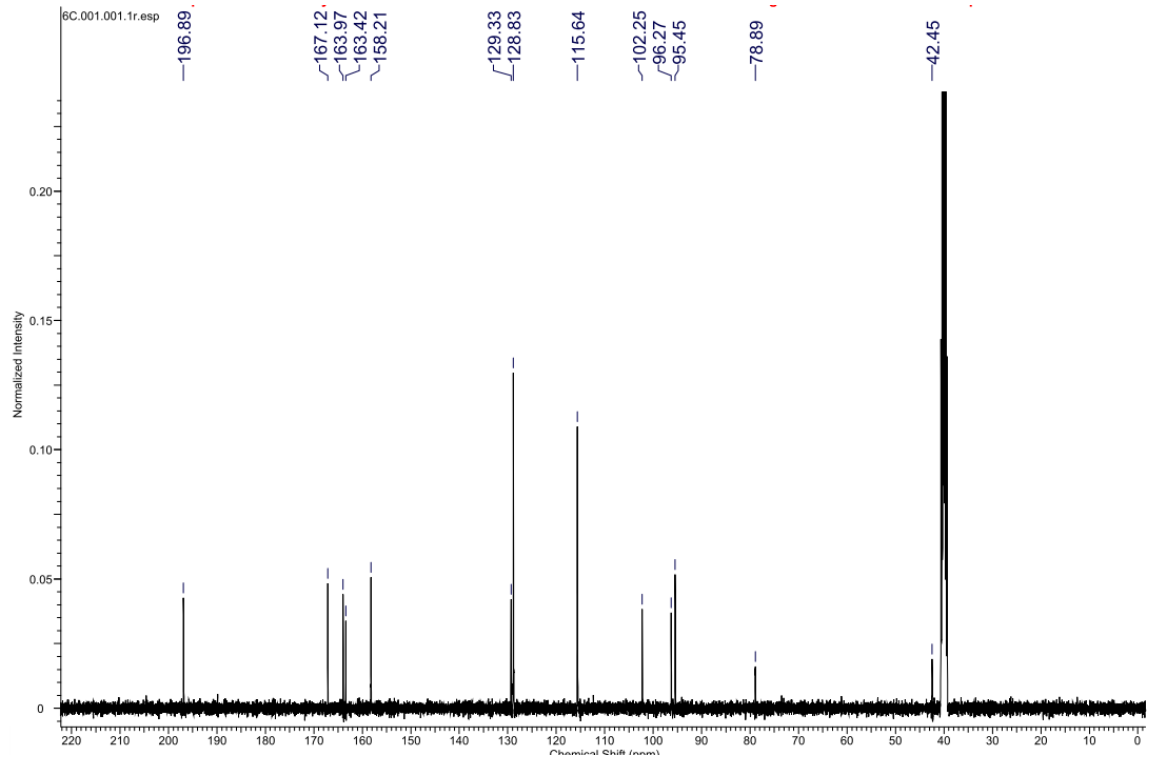
^{13}C NMR：G5



^{1}H NMR：G6

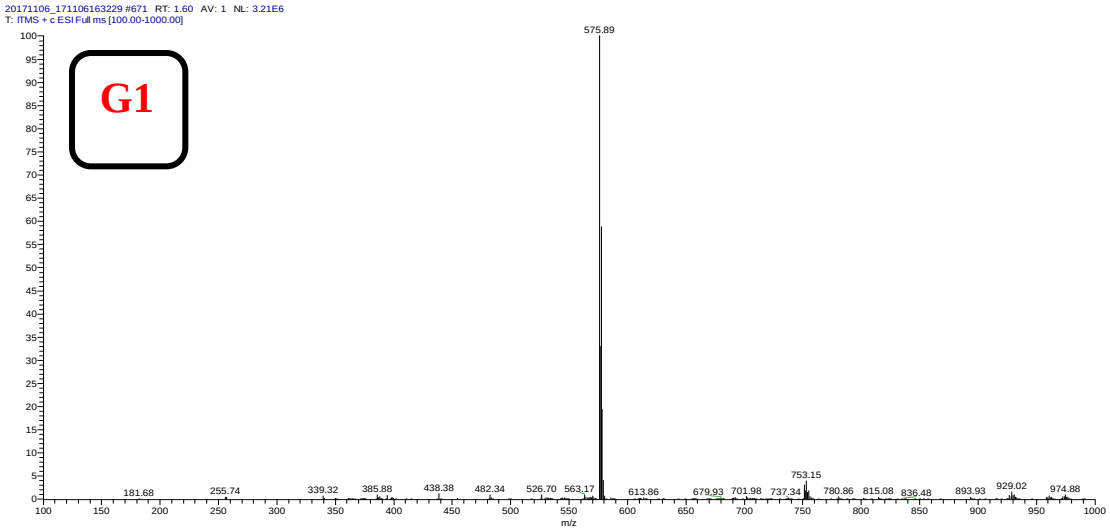


^{13}C NMR：G6

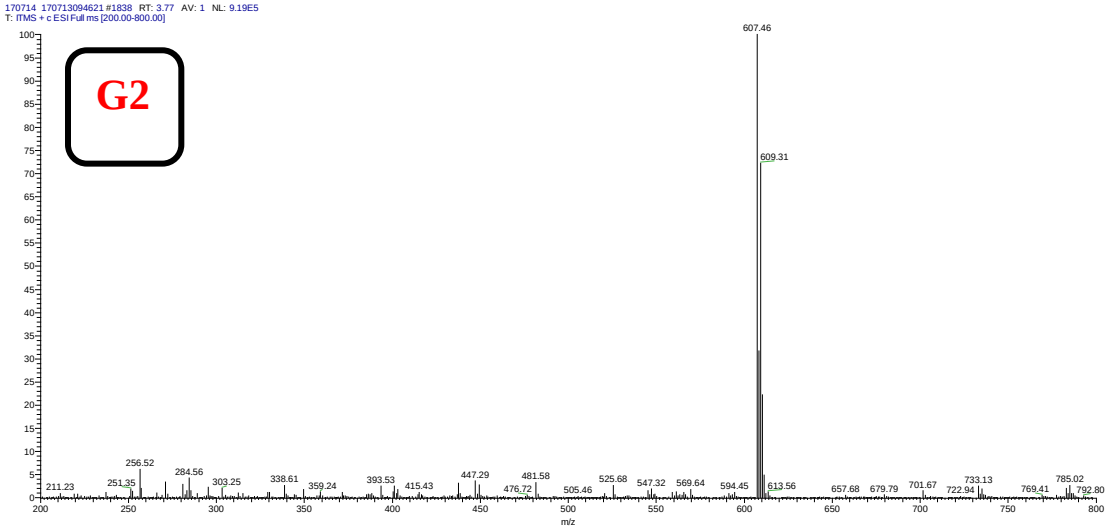


Mass spectrometric Data：

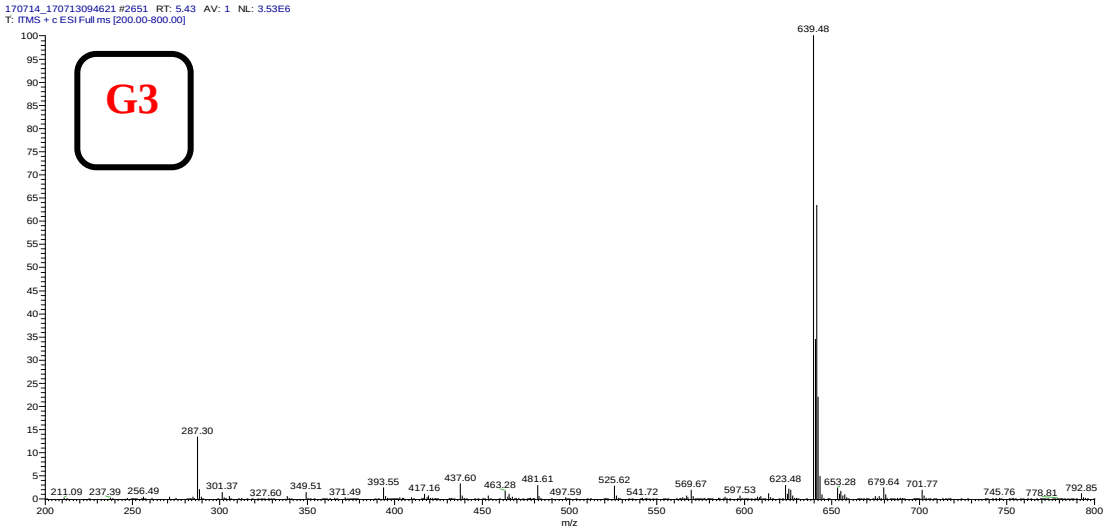
MS (ESI, theoretical mass: 575.03):575.89 $[M]^+$



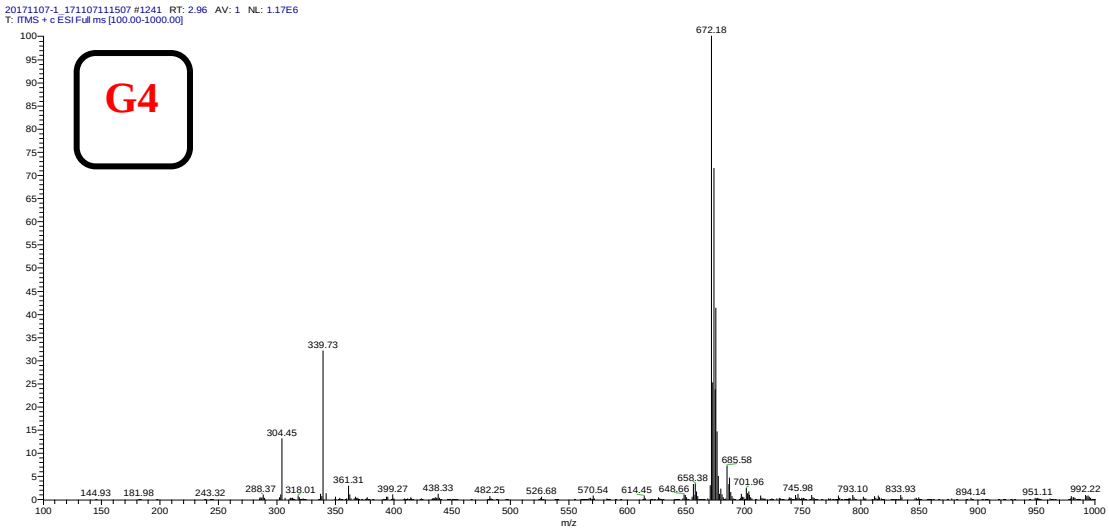
MS (ESI, theoretical mass: 607.02):607.48 $[M]^+$



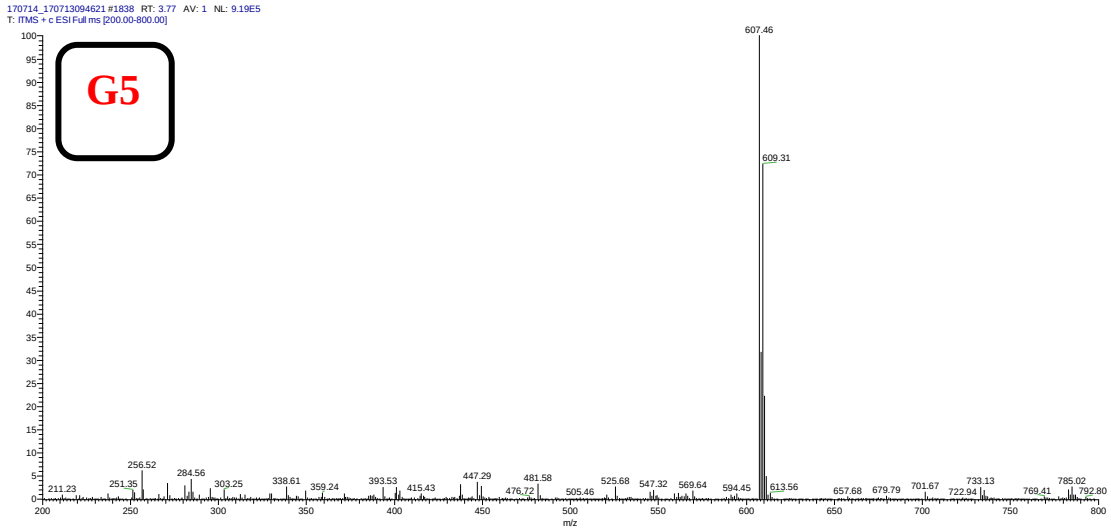
MS (ESI, theoretical mass: 639.01):639.48 [M]$^+$



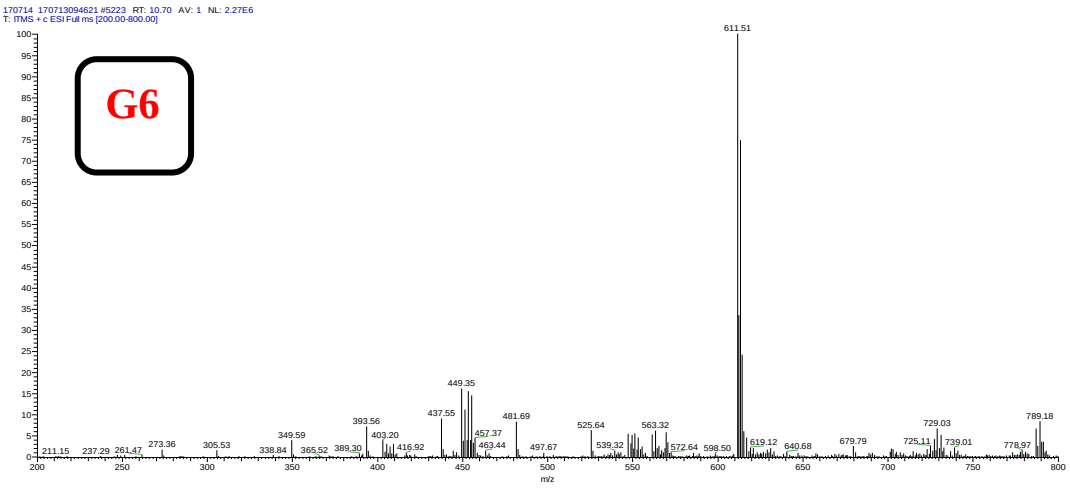
MS (ESI, theoretical mass: 671.00):672.48 [M+H]$^+$



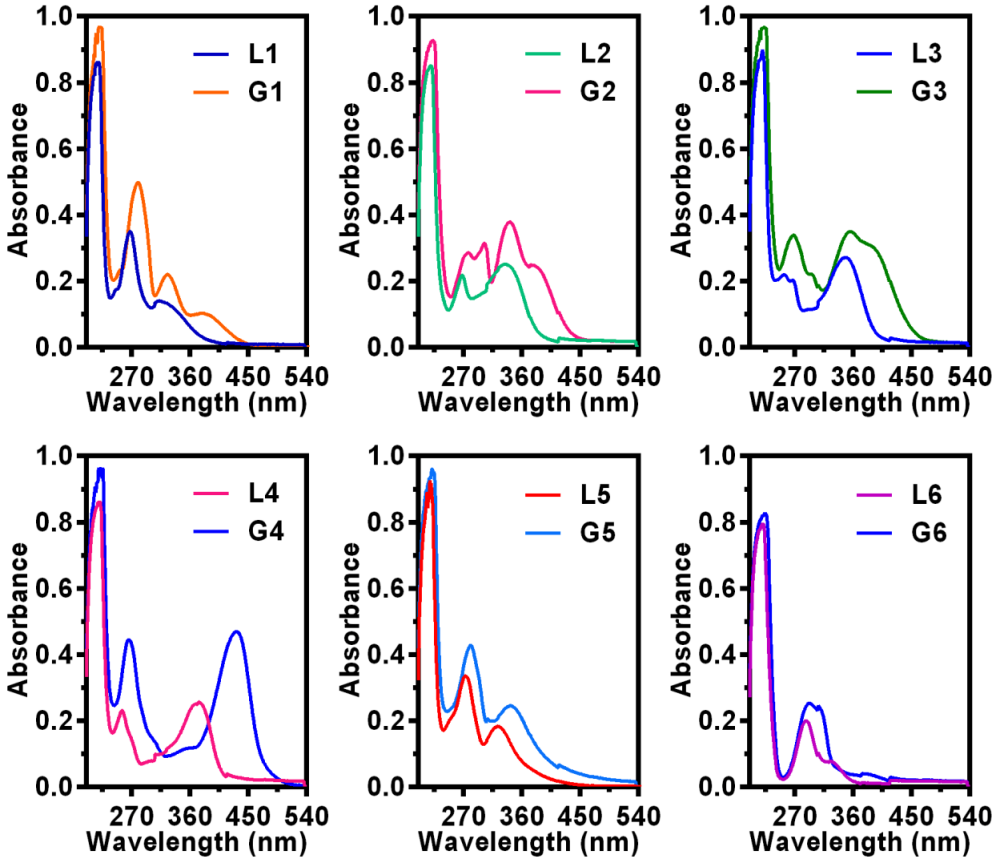
MS (ESI, theoretical mass: 607.02):607.46 $[M]^+$



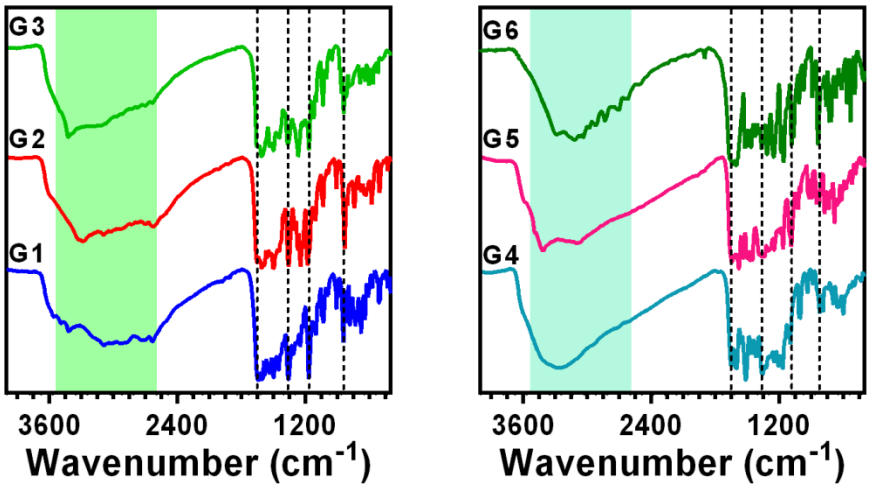
MS (ESI, theoretical mass: 611.05):611.51[M]$^+$



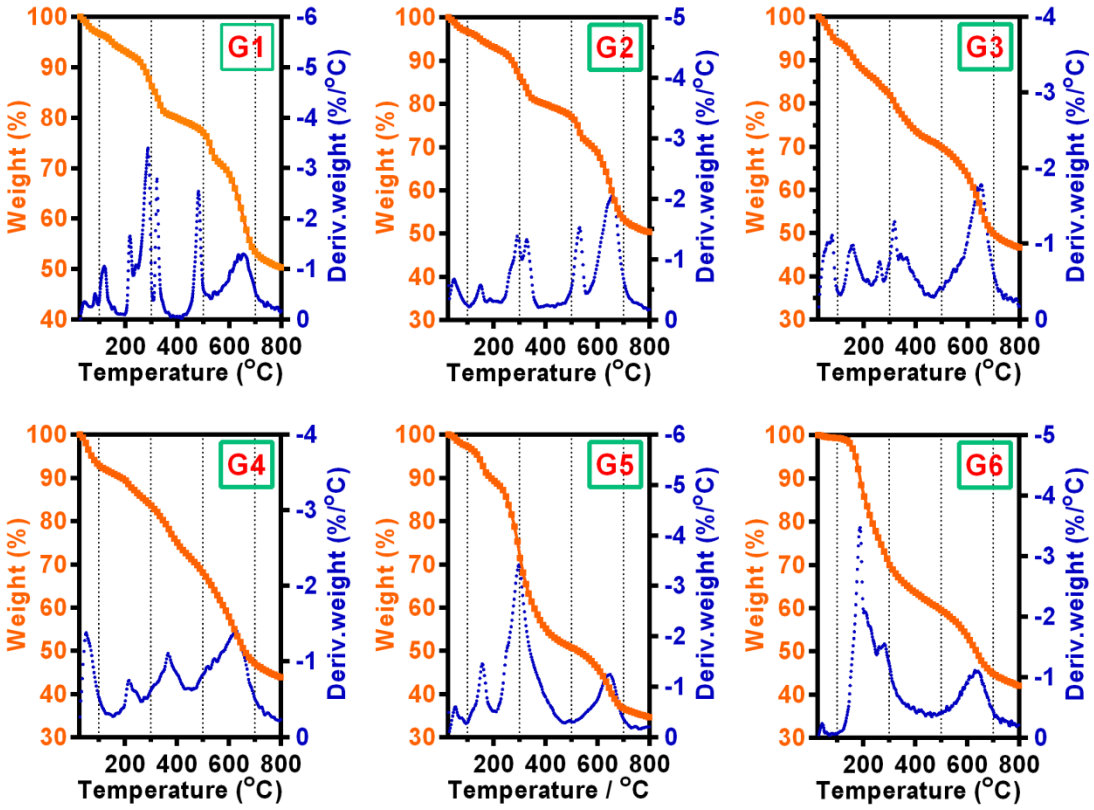
UV-vis spectra of ligands and gallium compounds in 10 μM PBS buffer.



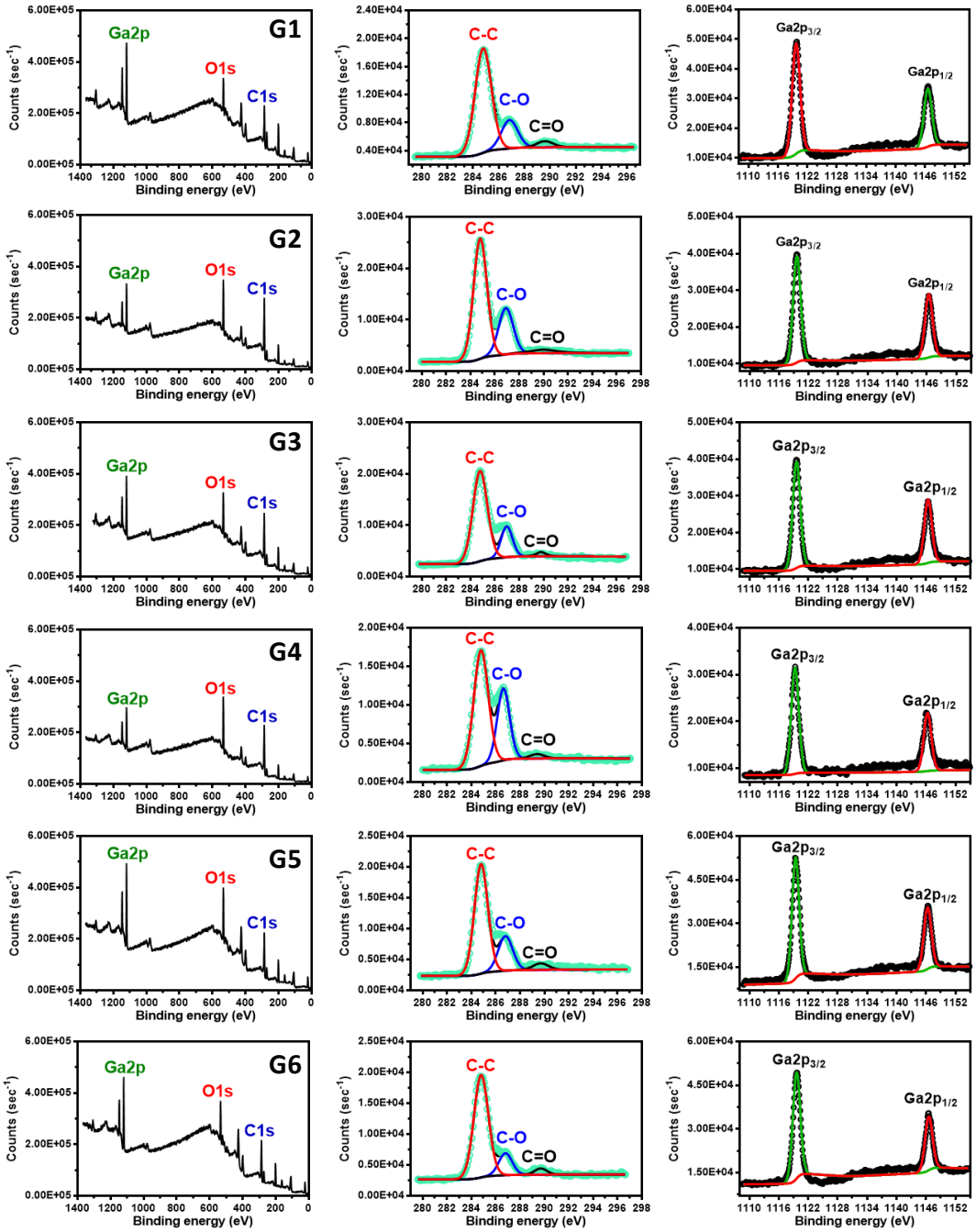
Infrared spectra of gallium compounds



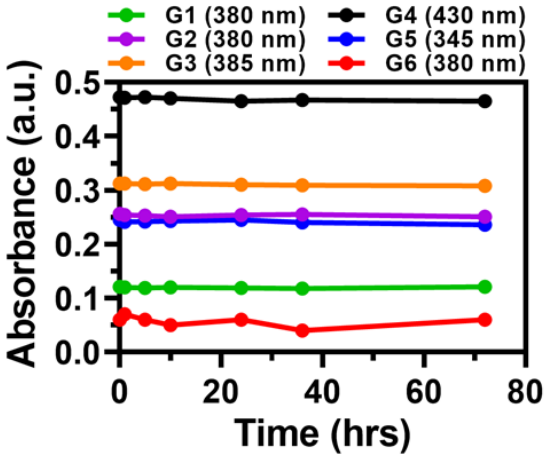
Differential thermal analysis of gallium compounds



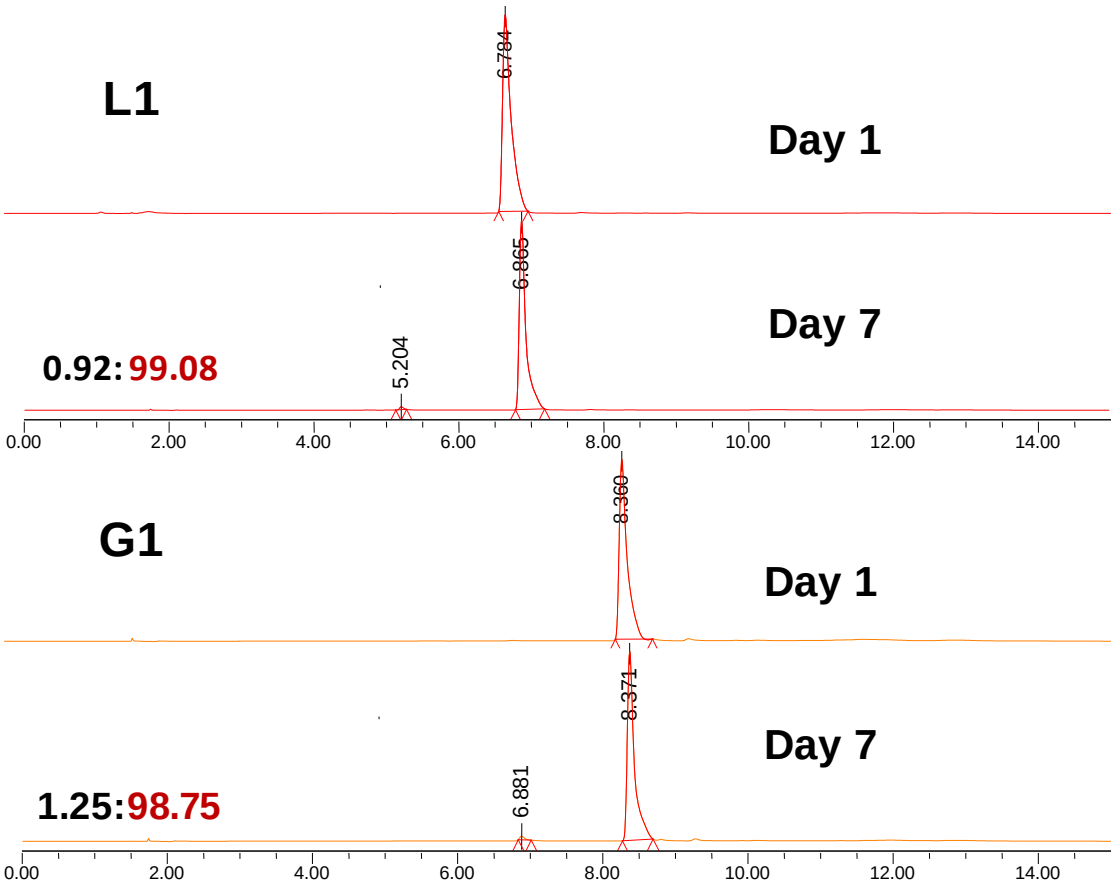
X-ray photoelectron spectra of gallium compounds

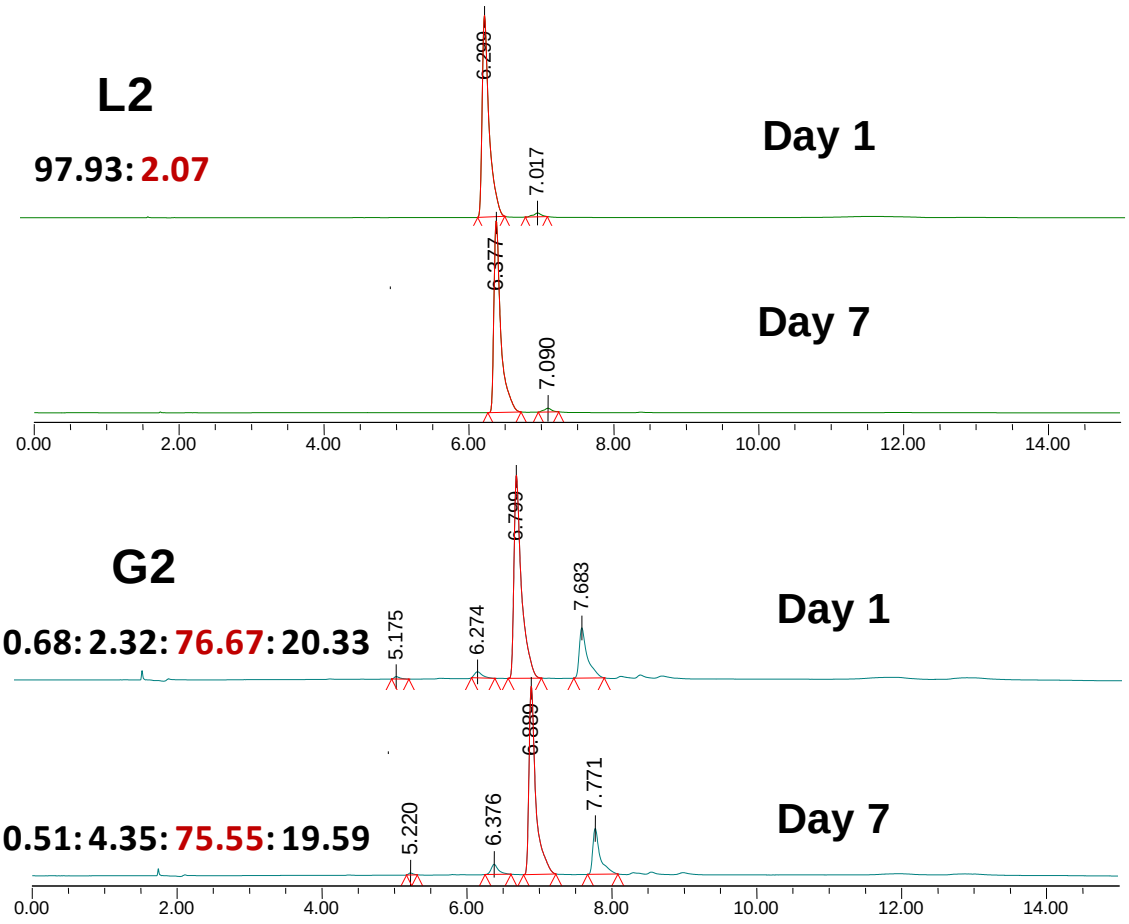


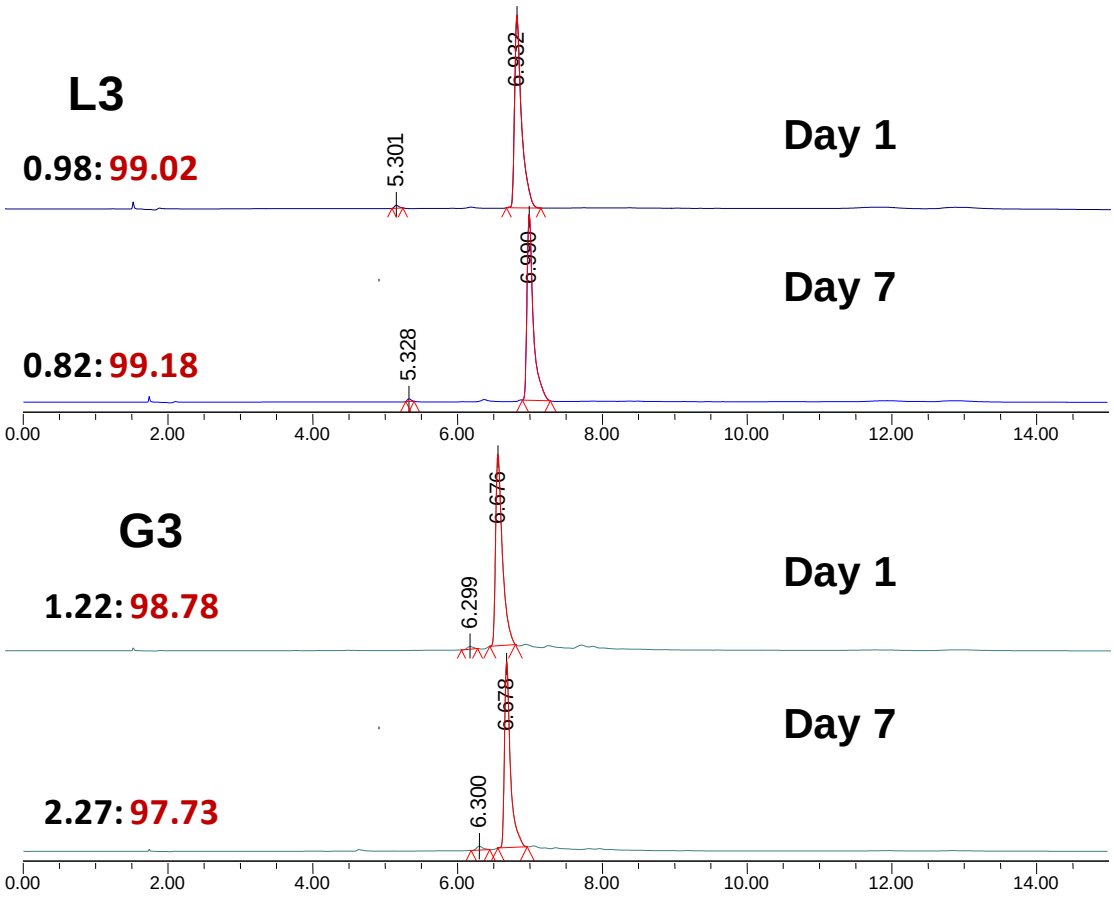
The stability of the gallium-flavonoid complexes (G1 to G6) in aqueous solution

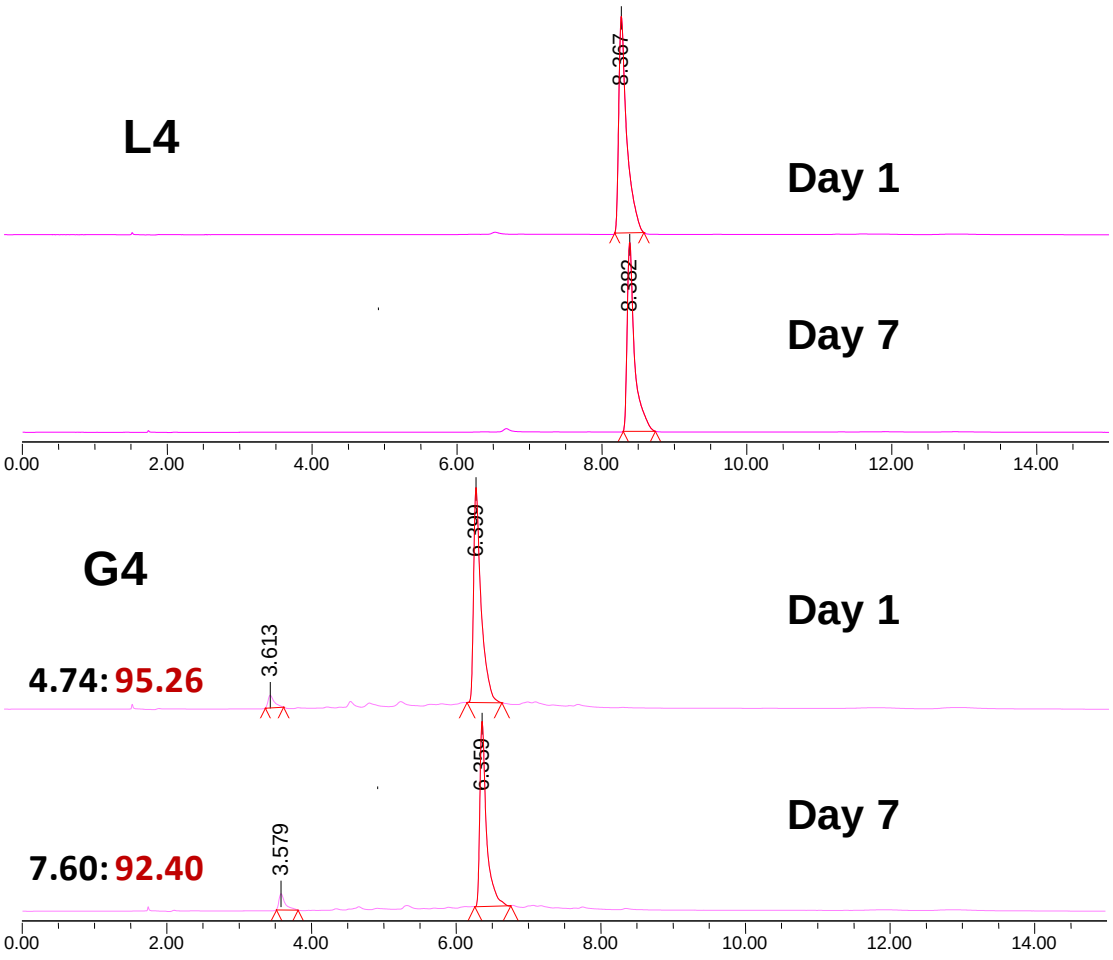


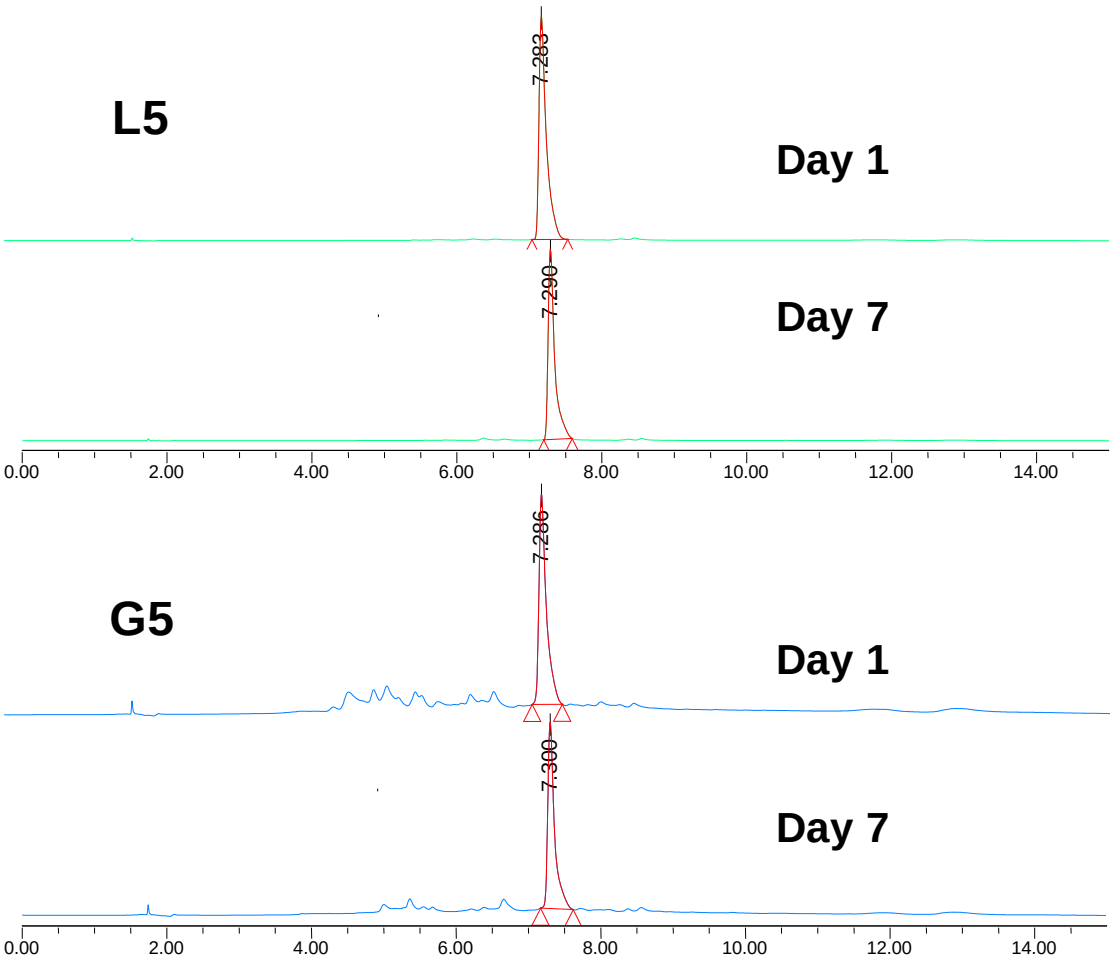
HPLC profiles of ligands and gallium compounds

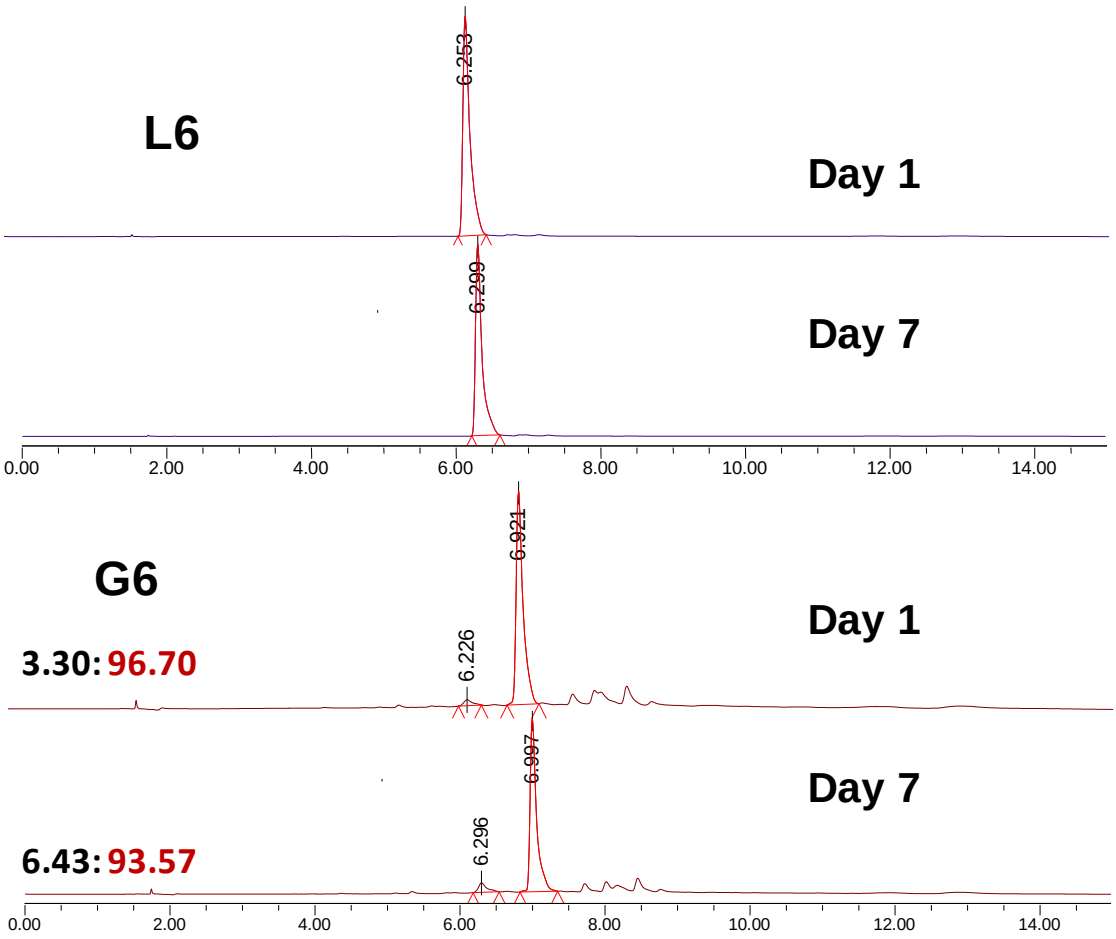



L2
97.93: 2.07
6.299
7.017
Day 1
6.377
7.090
Day 7
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
G2
0.68: 2.32: 76.67: 20.33
5.175
6.274
6.799
7.683
Day 1
0.51: 4.35: 75.55: 19.59
5.220
6.376
6.889
7.771
Day 7
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00


L3
0.98: 99.02
5.301
6.932
Day 1
6.990
Day 7
0.82: 99.18
5.328
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
G3
1.22: 98.78
6.299
6.676
Day 1
6.678
Day 7
2.27: 97.73
6.300
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00


L4
8.367
Day 1
8.382
Day 7
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
G4
6.399
Day 1
3.613
4.74: 95.26
6.359
Day 7
3.579
7.60: 92.40
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00


L5
7.283
Day 1
7.290
Day 7
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
G5
7.286
Day 1
7.300
Day 7
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00


L6
6.253
Day 1
6.299
Day 7
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
G6
6.921
Day 1
3.30: 96.70
6.226
6.997
Day 7
6.43: 93.57
6.296
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00