

Supporting Information

Design, Synthesis and Biological Evaluations of Asymmetric Bow-Tie PAMAM Dendrimer-Based Conjugates for Tumor-Targeted Drug Delivery

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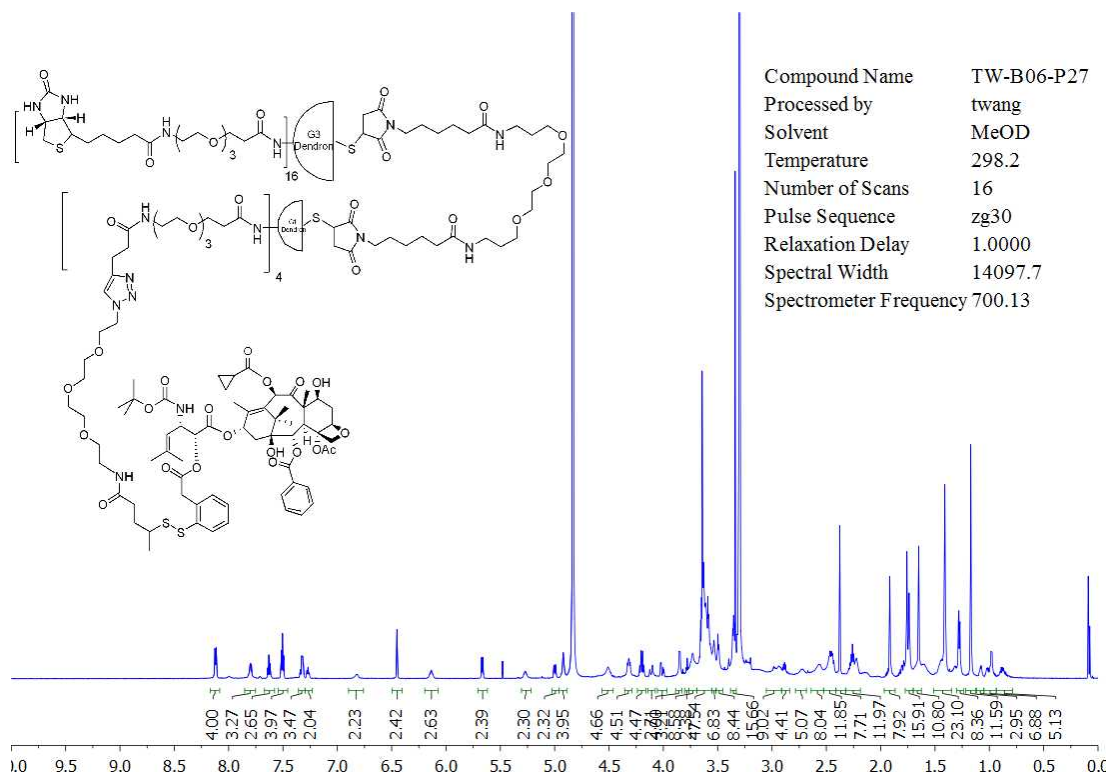


Figure S1. ¹H NMR spectrum of 1

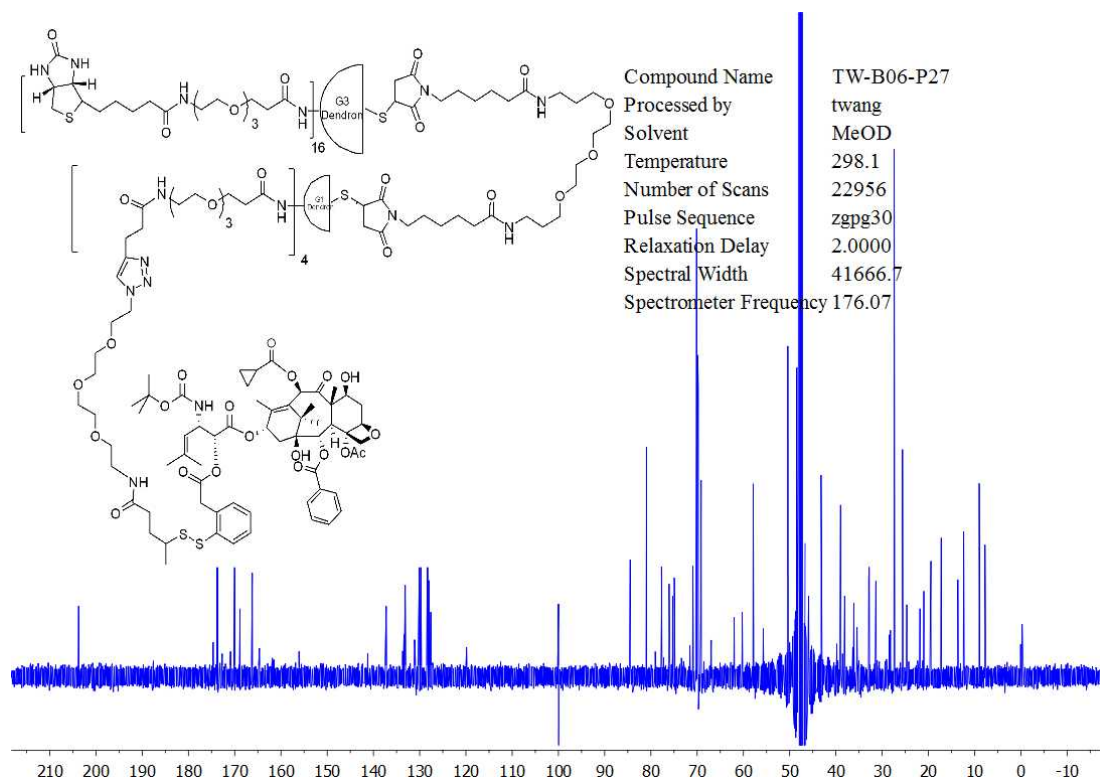


Figure S2. ¹³C NMR spectrum of 1

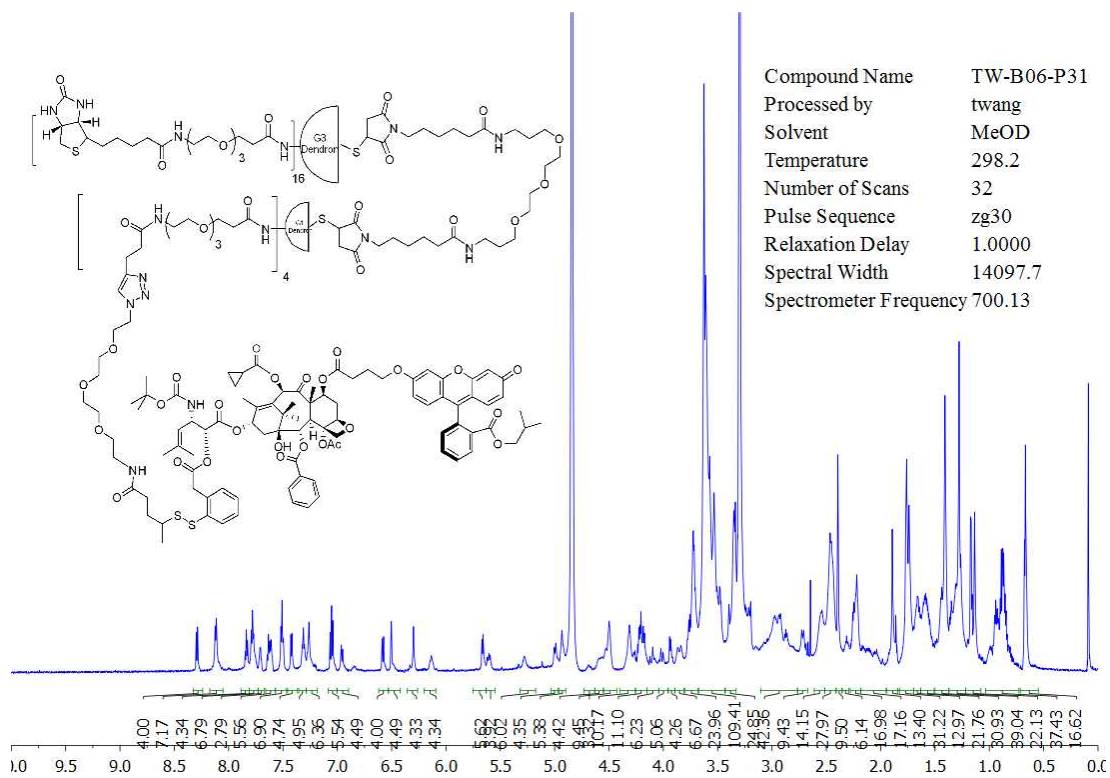


Figure S3. ¹H NMR spectrum of 2

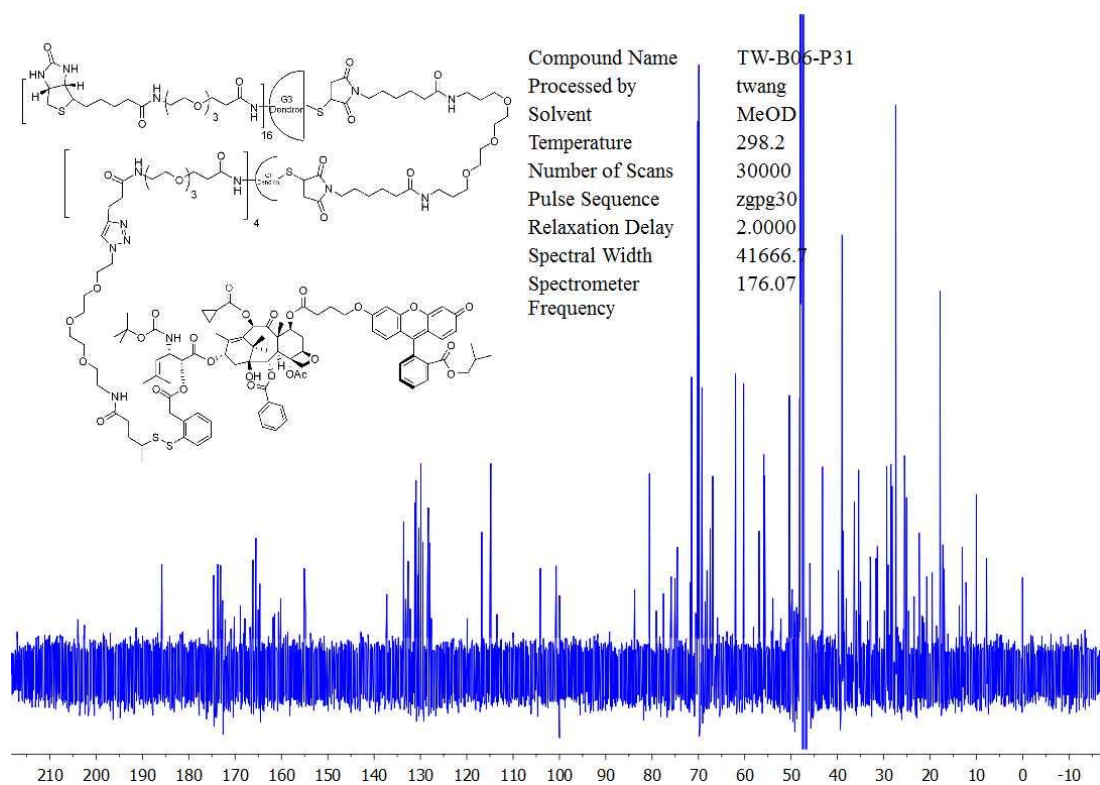


Figure S4. ¹³C NMR spectrum of 2

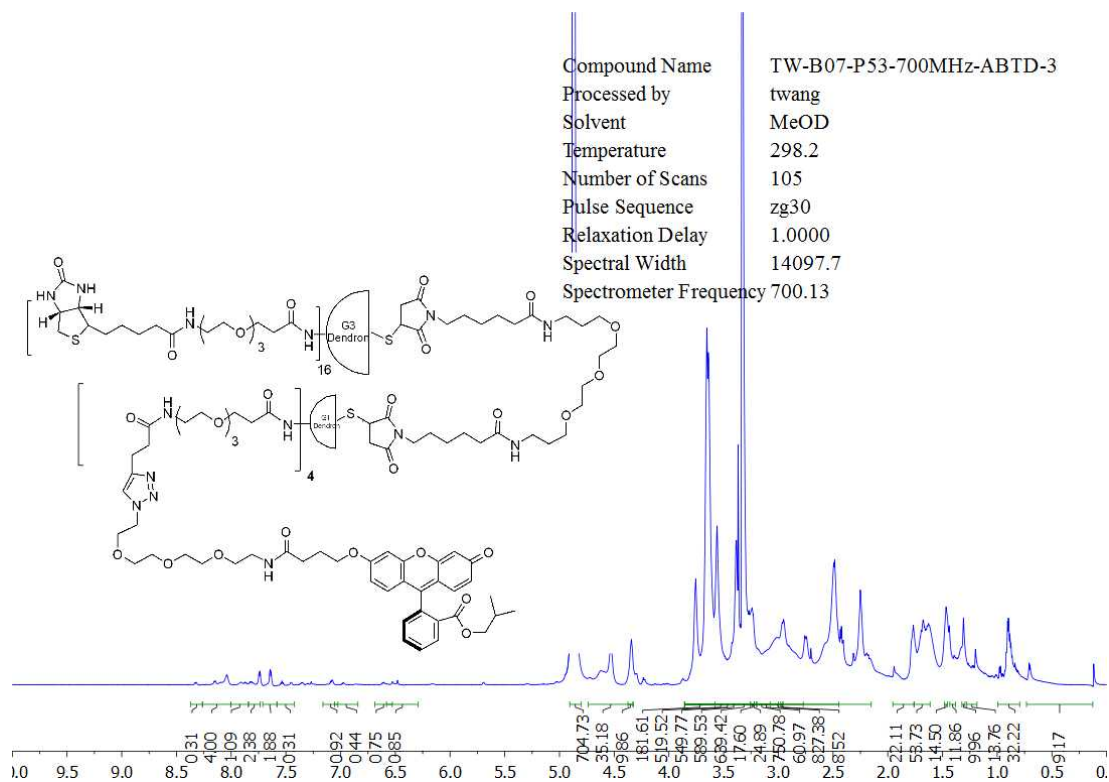


Figure S5. ^1H NMR spectrum of **3**

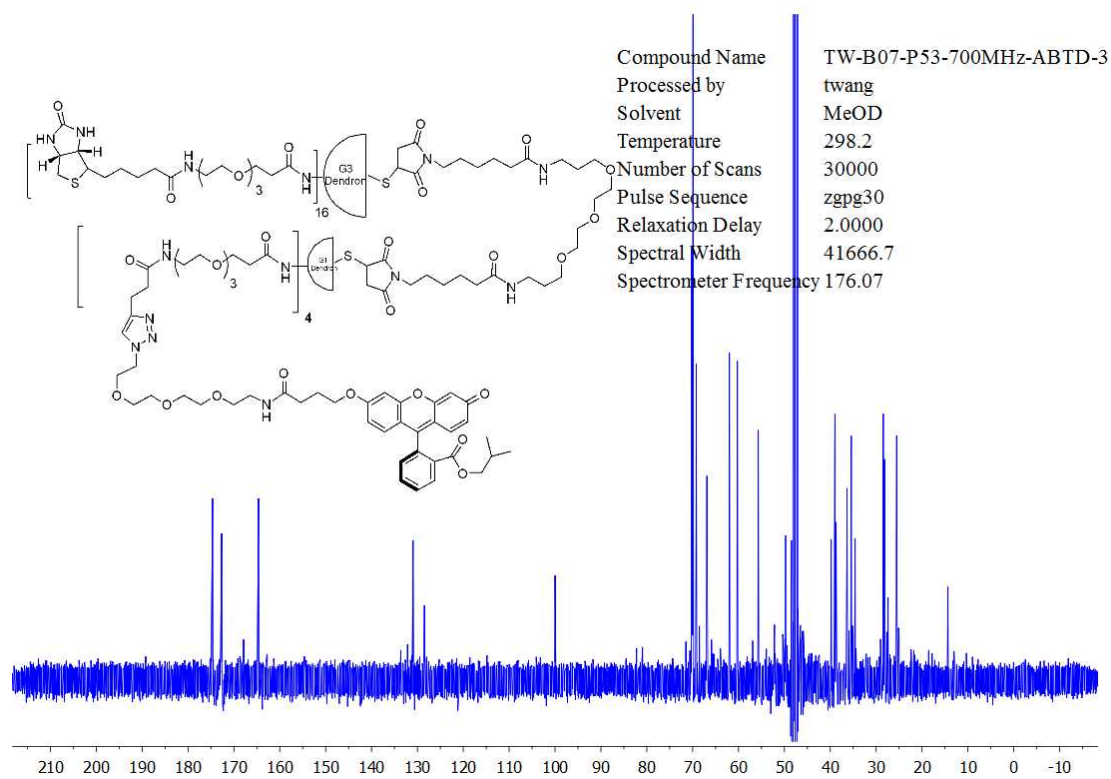


Figure S6. ^{13}C NMR spectrum of **3**

Compound Name TW-B05-P42
 Processed by twang
 Solvent CDCl3
 Temperature 298.2
 Number of Scans 16
 Pulse Sequence zg30
 Relaxation Delay 1.0000
 Spectral Width 10000.0
 Spectrometer Frequency 499.89

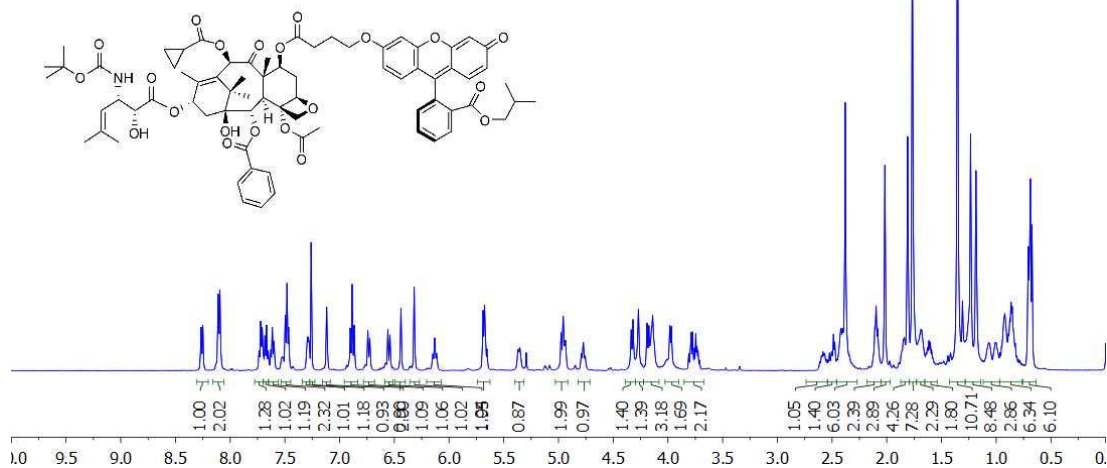


Figure S7. ¹H NMR spectrum of 5

Compound Name TW-B05-P42
 Processed by twang
 Solvent CDCl3
 Temperature 298.3
 Number of Scans 904
 Pulse Sequence zgpg30
 Relaxation Delay 2.0000
 Spectral Width 29761.9
 Spectrometer Frequency 125.71

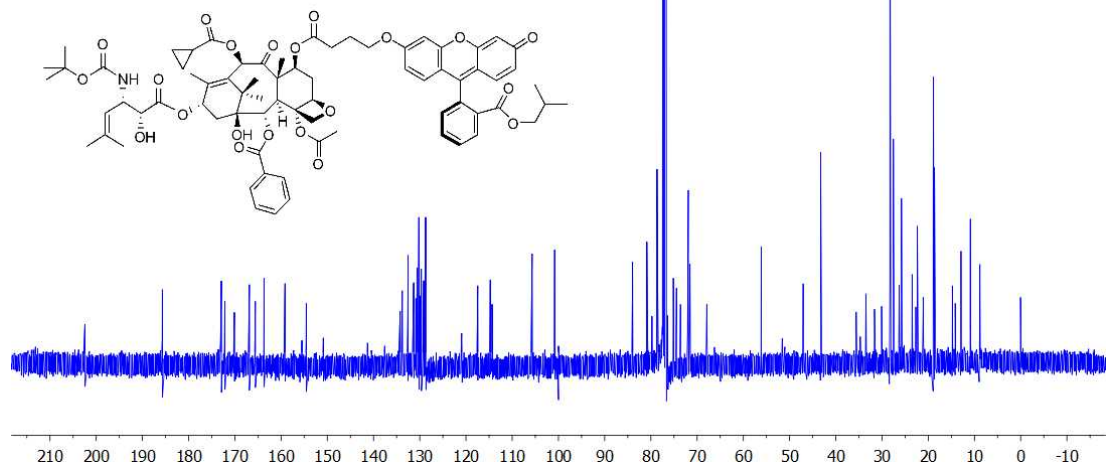


Figure S8. ¹³C NMR spectrum of 5

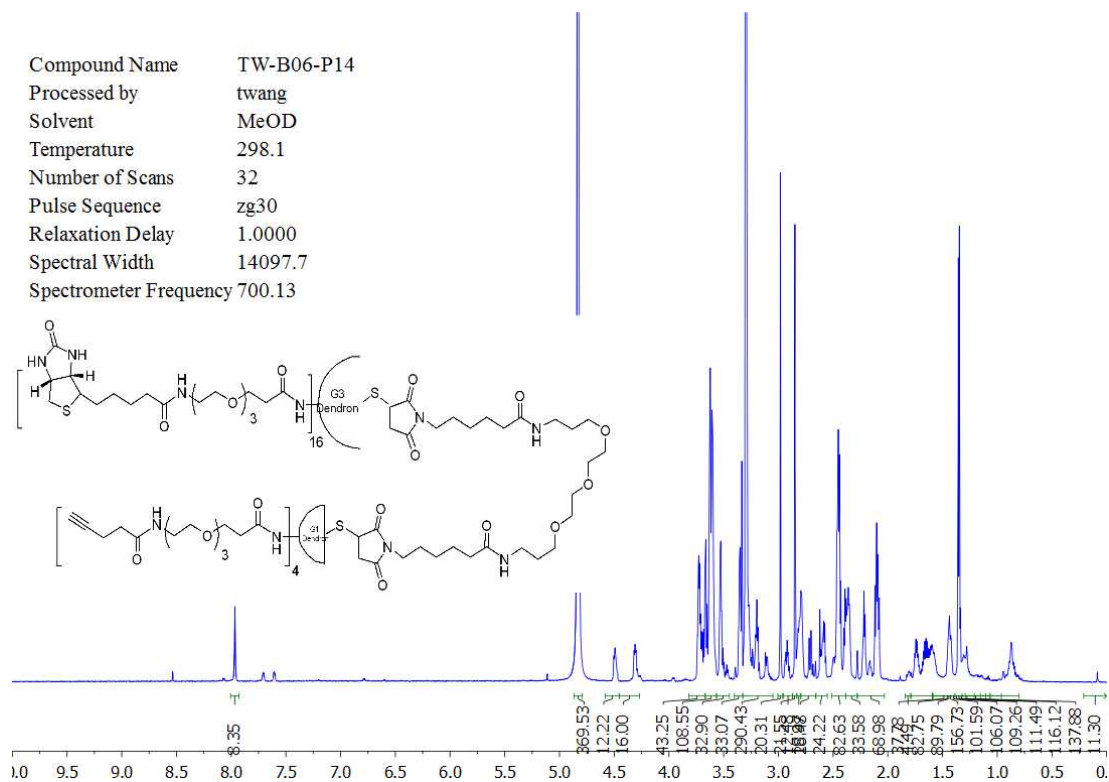


Figure S9. ^1H NMR spectrum of **6**

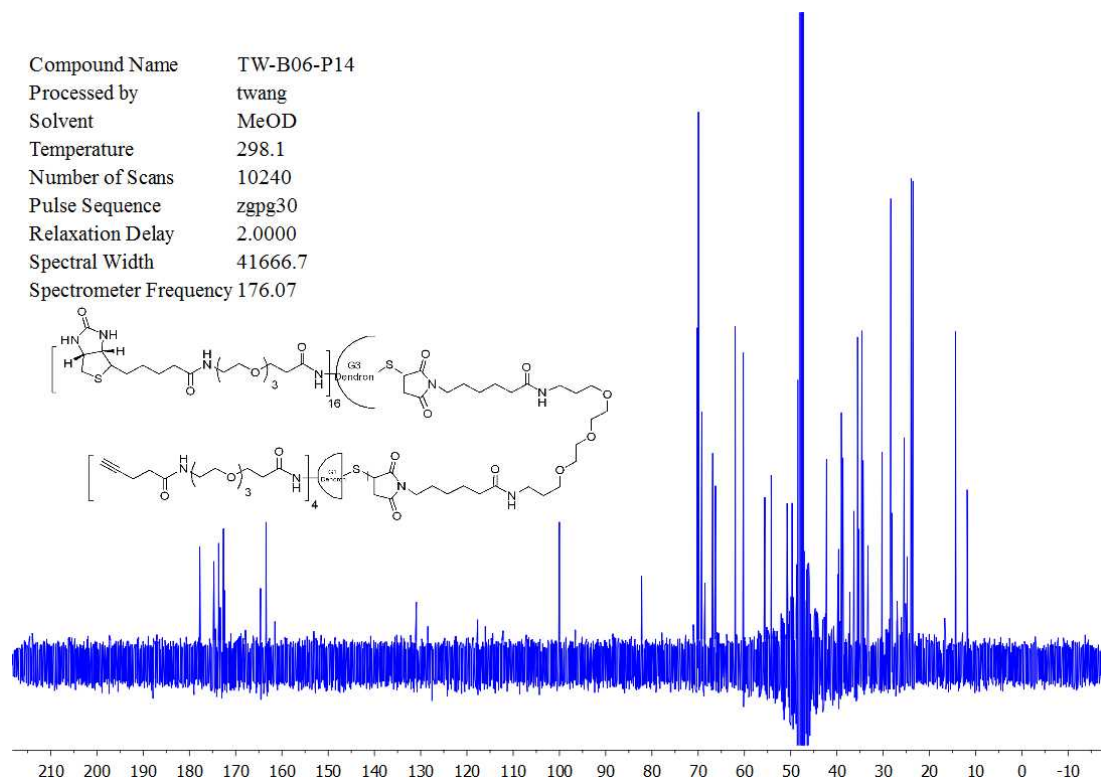


Figure S10. ^{13}C NMR spectrum of **6**

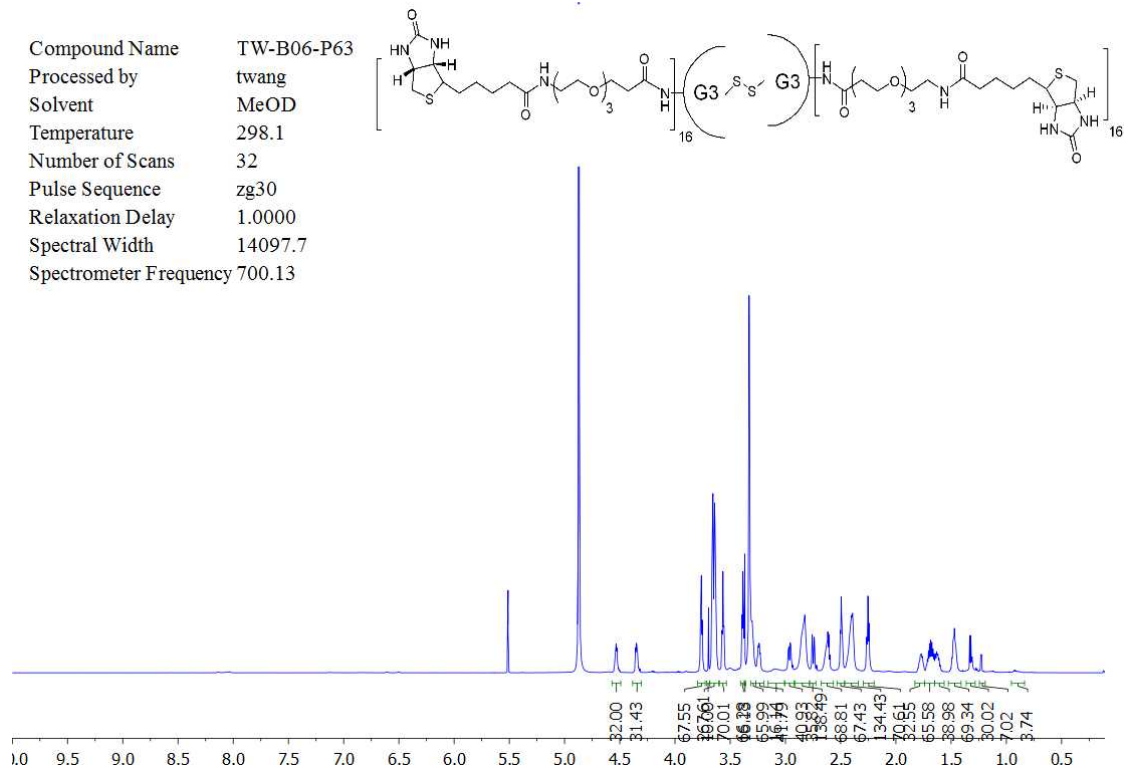


Figure S11. ^1H NMR spectrum of **7**

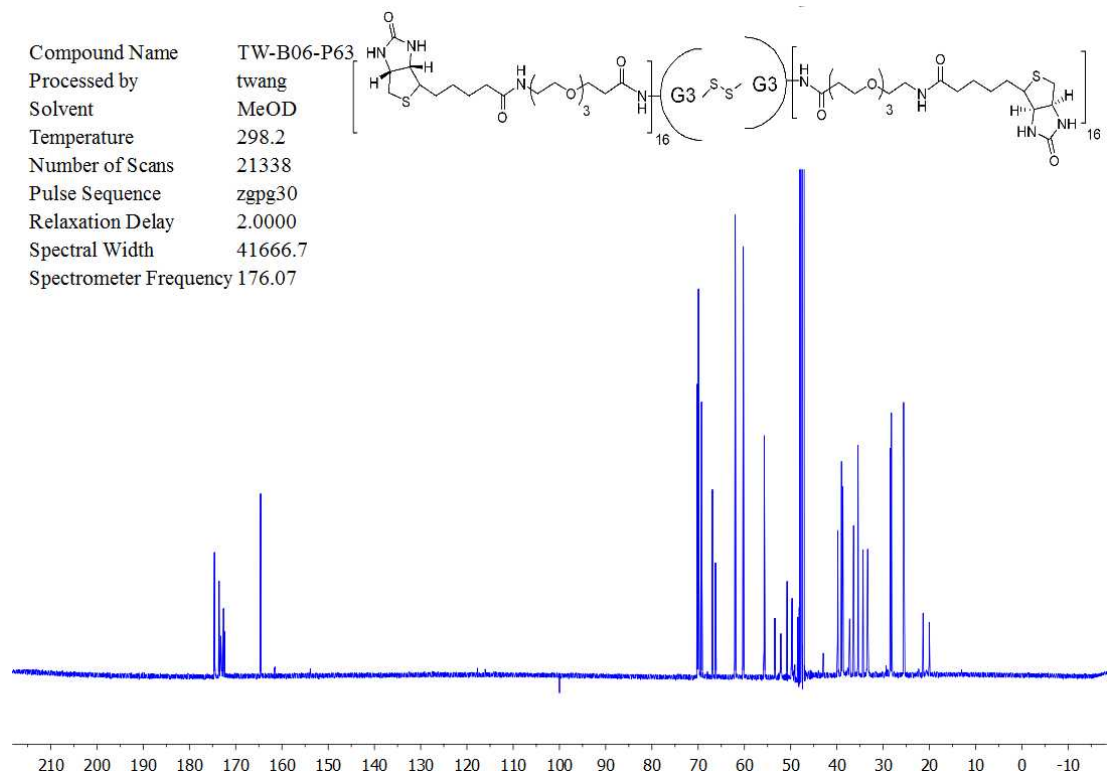


Figure S12. ^{13}C NMR spectrum of **7**

Compound Name TW-B07-P98-700MHz-MeOD
 Processed by twang
 Solvent MeOD
 Temperature 298.2
 Number of Scans 32
 Pulse Sequence zg30
 Relaxation Delay 1.0000
 Spectral Width 14097.7
 Spectrometer Frequency 700.13

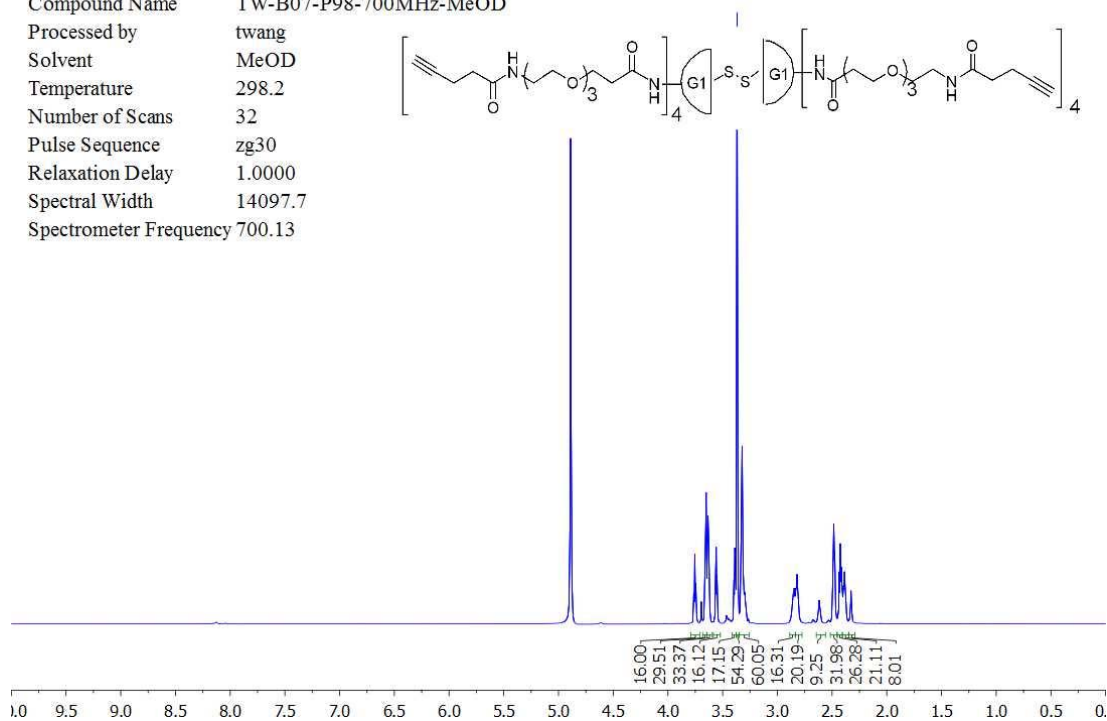


Figure 13. ^1H NMR spectrum of **8**

Compound Name TW-B07-P98-700MHz-MeOD
 Processed by twang
 Solvent MeOD
 Temperature 298.2
 Number of Scans 2186
 Pulse Sequence zgpg30
 Relaxation Delay 2.0000
 Spectral Width 41666.7
 Spectrometer Frequency 176.07

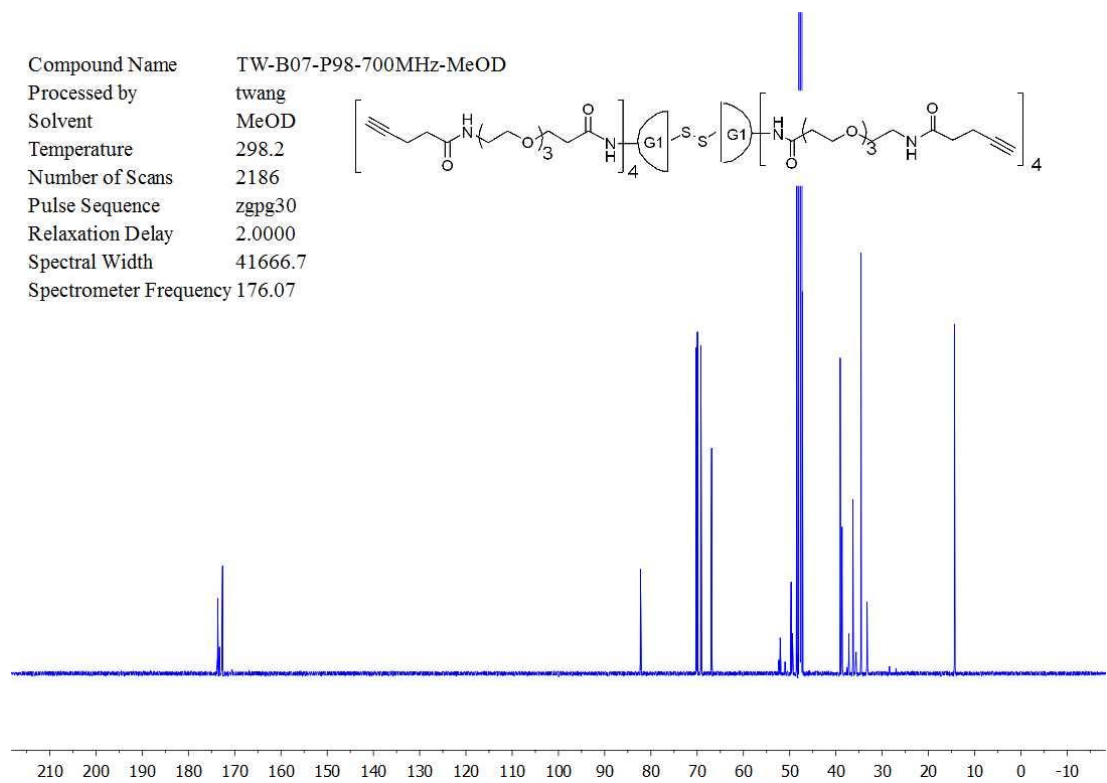


Figure S14. ^{13}C NMR spectrum of **8**

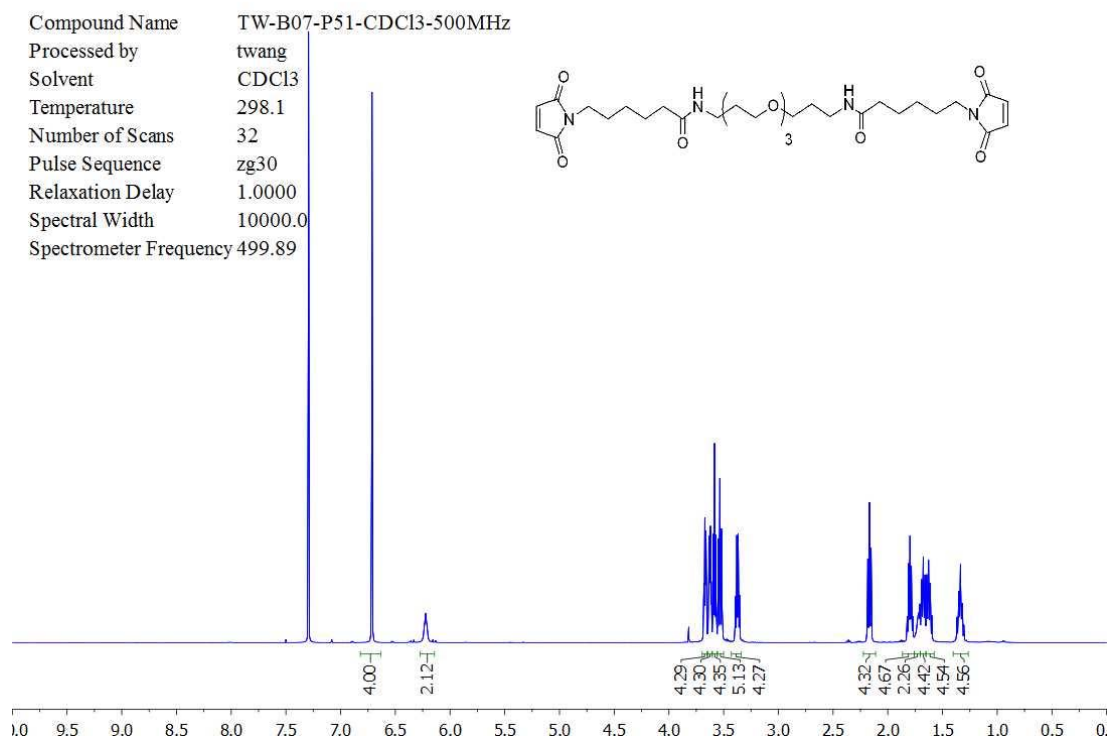


Figure S15. ^1H NMR spectrum of **9**

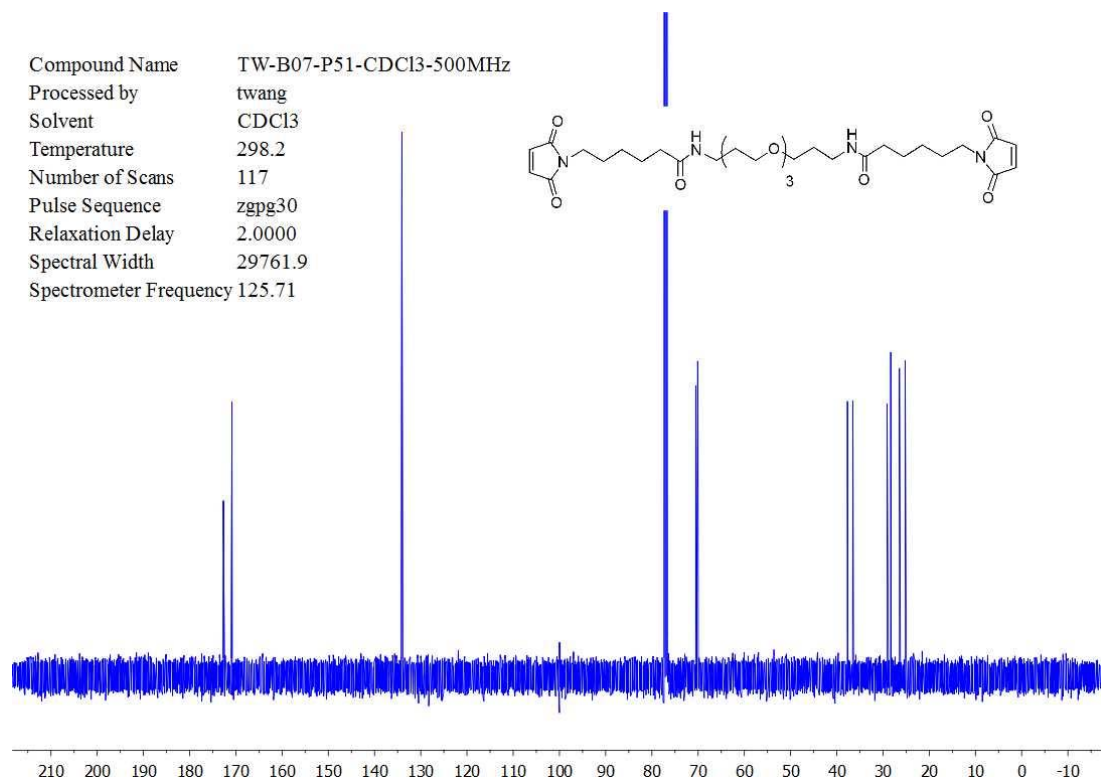


Figure S16. ^{13}C NMR spectrum of **9**

Compound Name TW-B05-P104
 Processed by twang
 Solvent MeOD
 Temperature 298.1
 Number of Scans 32
 Pulse Sequence zg30
 Relaxation Delay 1.0000
 Spectral Width 14097.7
 Spectrometer Frequency 700.13

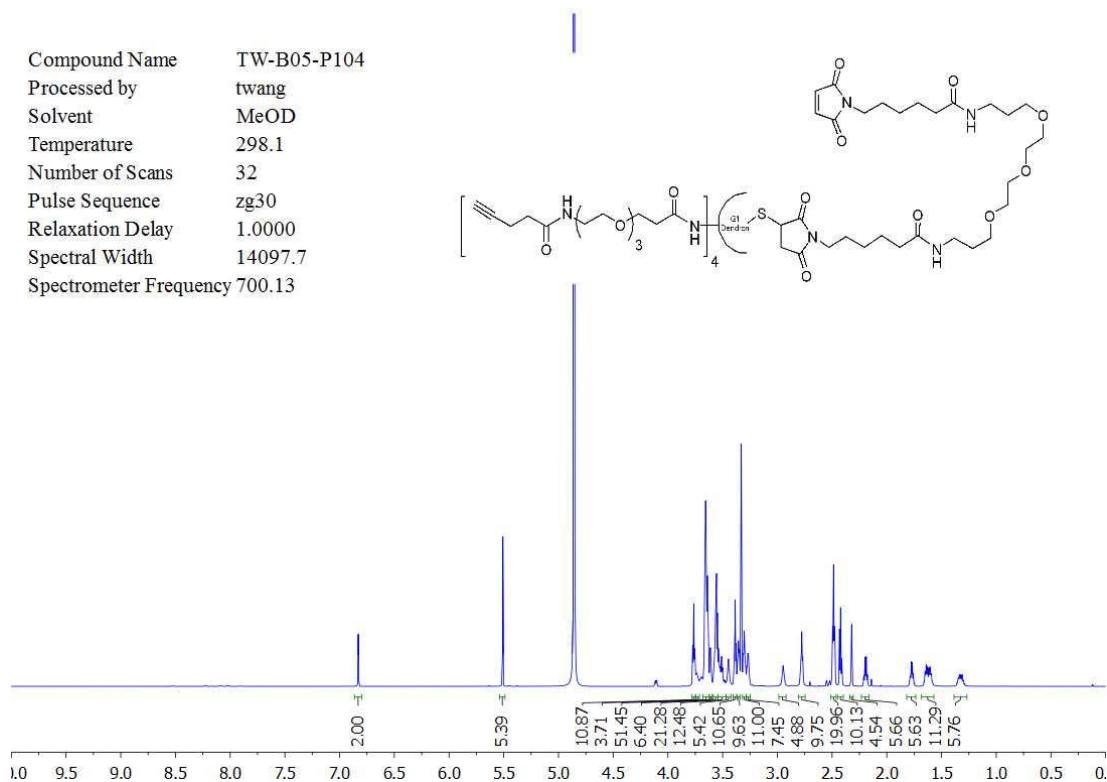


Figure S17. ^1H NMR spectrum of **12**

Compound Name TW-B05-P104
 Processed by twang
 Solvent MeOD
 Temperature 298.1
 Number of Scans 3582
 Pulse Sequence zgpg30
 Relaxation Delay 2.0000
 Spectral Width 41666.7
 Spectrometer Frequency 176.07

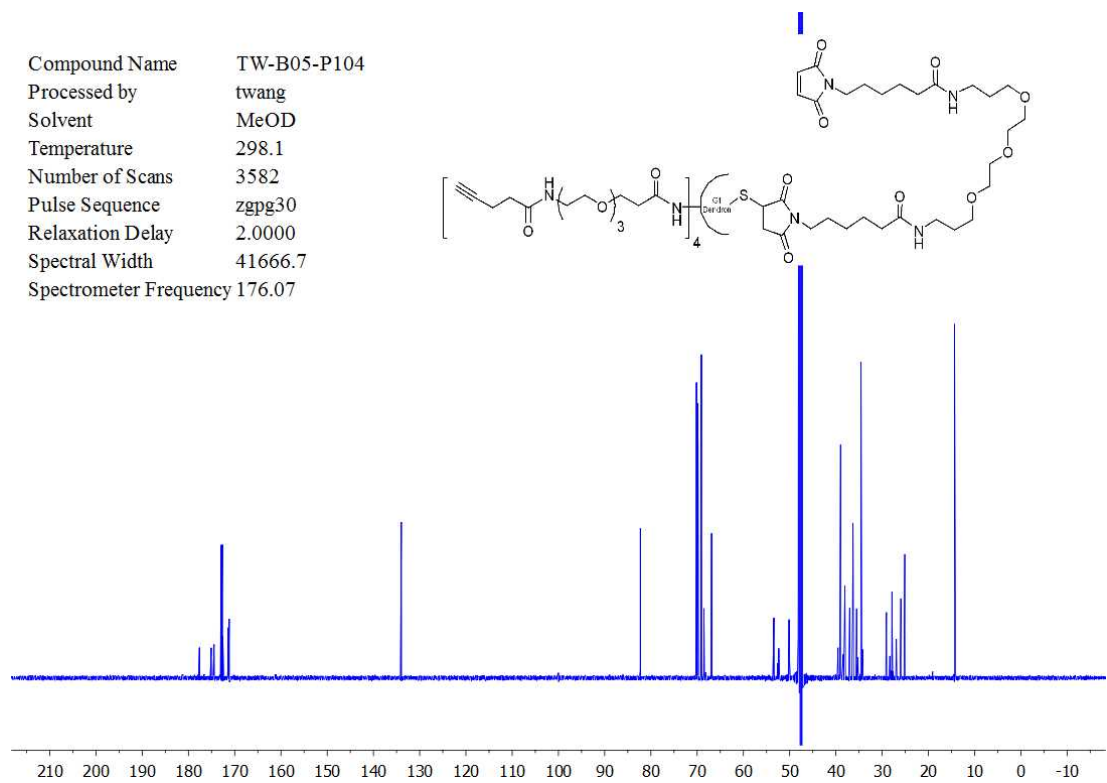


Figure S18. ^{13}C NMR spectrum of **12**

Compound Name TW-B05-P25
 Processed by twang
 Solvent CDCl3
 Temperature 298.2
 Number of Scans 16
 Pulse Sequence zg30
 Relaxation Delay 1.0000
 Spectral Width 10000.0
 Spectrometer Frequency 499.89

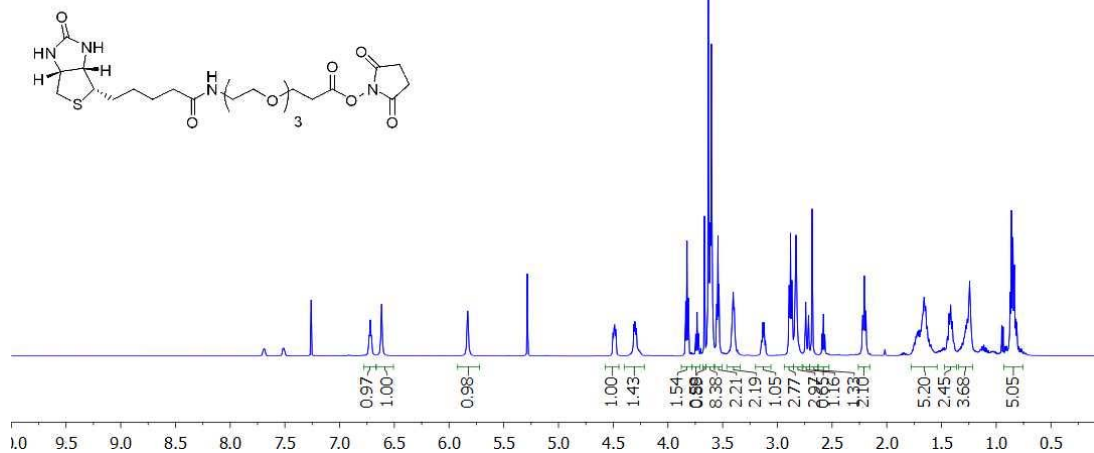


Figure S19. ^1H NMR spectrum of **14**

Compound Name TW-B05-P25
 Processed by twang
 Solvent CDCl3
 Temperature 298.2
 Number of Scans 203
 Pulse Sequence zgpg30
 Relaxation Delay 2.0000
 Spectral Width 29761.9
 Spectrometer Frequency 125.71

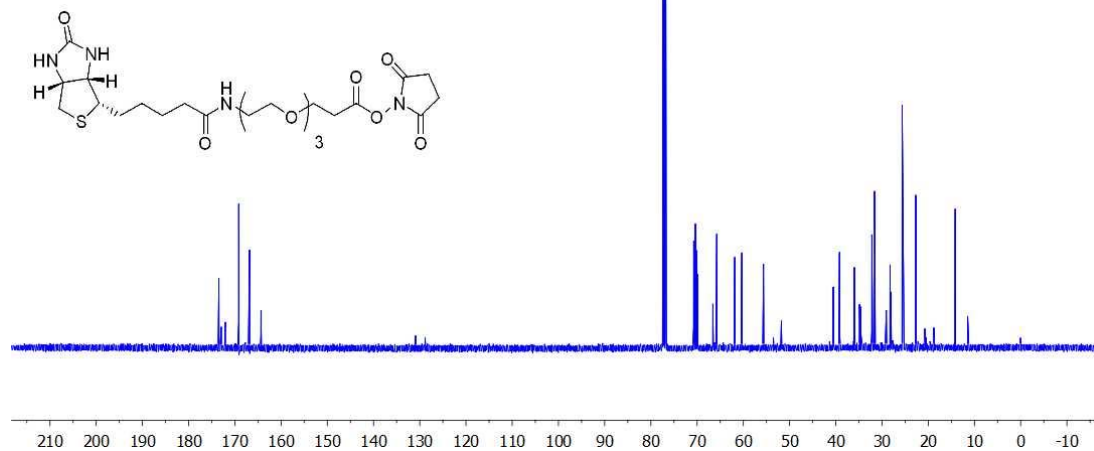


Figure S20. ^{13}C NMR spectrum of **14**

Compound Name TW-B07-P93-500MHz
 Processed by twang
 Solvent CDCl3
 Temperature 298.2
 Number of Scans 16
 Pulse Sequence zg30
 Relaxation Delay 1.0000
 Spectral Width 10000.0
 Spectrometer Frequency 499.89

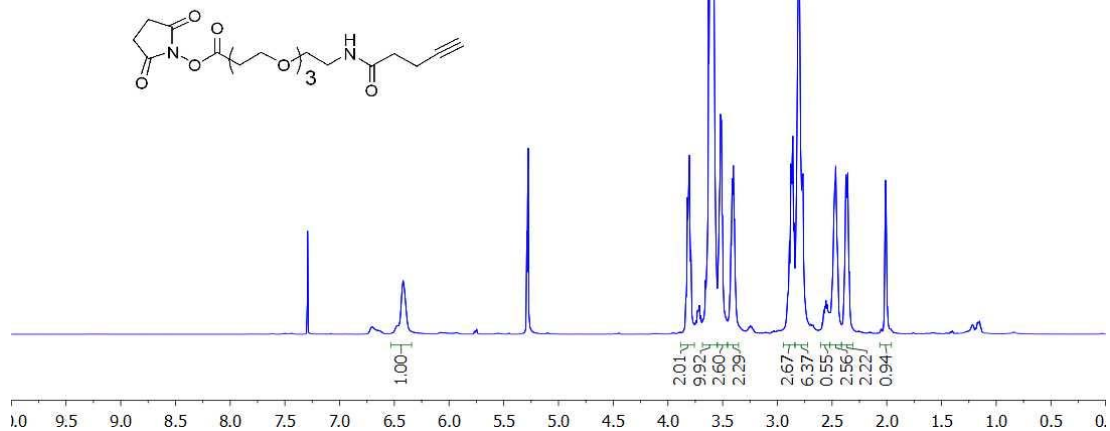


Figure S21 ^1H NMR spectrum of **16**

Compound Name TW-B07-P93-500MHz
 Processed by twang
 Solvent CDCl3
 Temperature 298.1
 Number of Scans 69
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 Relaxation Delay 2.0000
 Spectral Width 29761.9
 Spectrometer Frequency 125.71

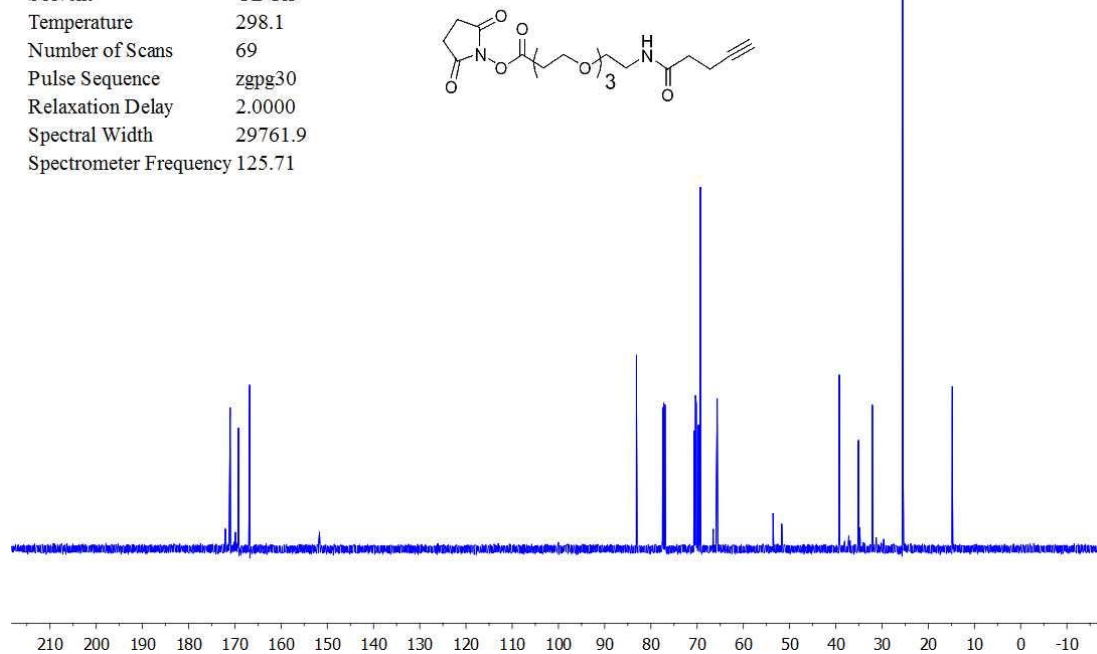


Figure S22. ^{13}C NMR spectrum of **16**

Compound Name TW-B05-P100
Processed by twang
Solvent CDCl3
Temperature 298.1
Number of Scans 16
Pulse Sequence zg30
Relaxation Delay 1.0000
Spectral Width 10000.0
Spectrometer Frequency 499.89

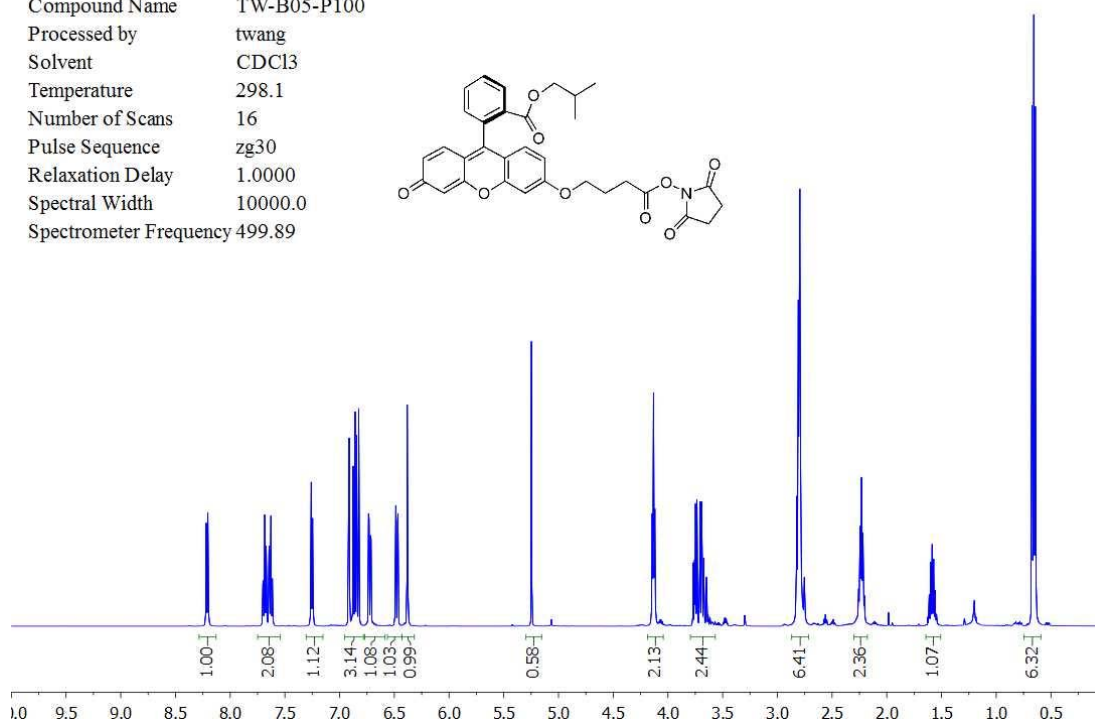
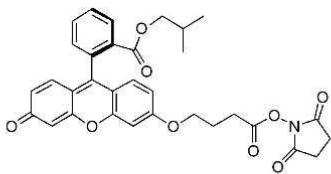


Figure S25. ¹H NMR spectrum of 20

Compound Name TW-B05-P100
Processed by twang
Solvent CDCl3
Temperature 298.2
Number of Scans 138
Pulse Sequence zgpg30
Relaxation Delay 2.0000
Spectral Width 29761.9
Spectrometer Frequency 125.71

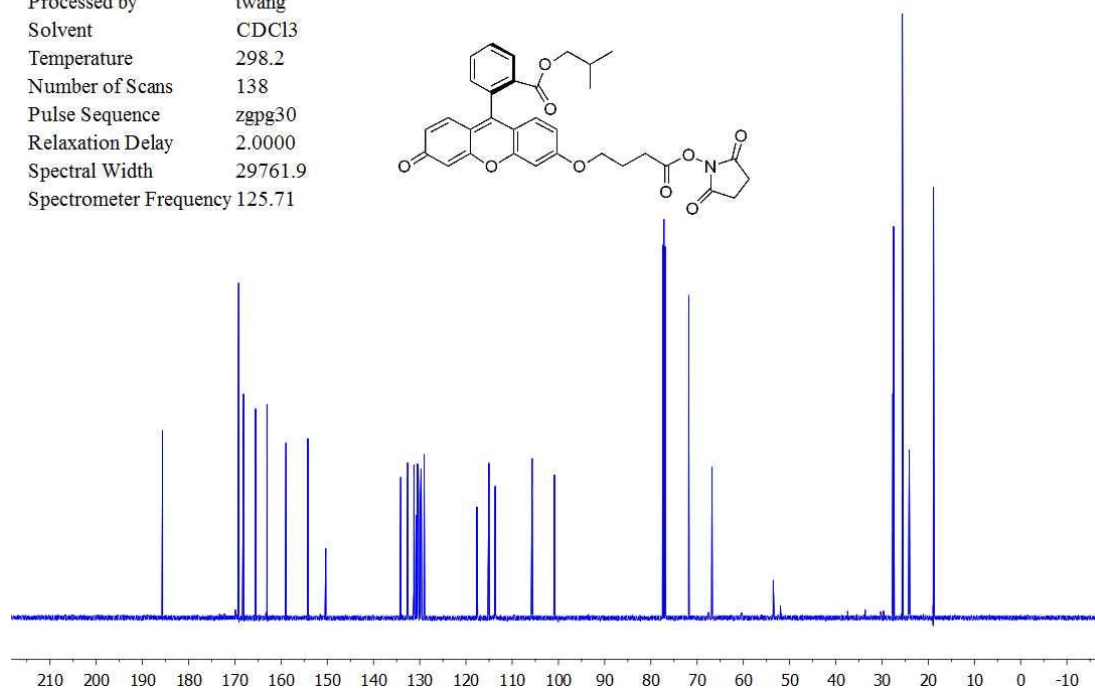
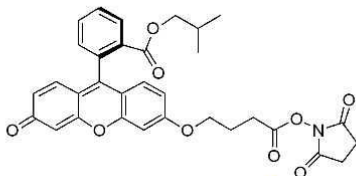


Figure S26. ¹³C NMR spectrum of 20

Compound Name TW-B05-P61
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 Solvent CDCl3
 Temperature 298.3
 Number of Scans 32
 Pulse Sequence zg30
 Relaxation Delay 1.0000
 Spectral Width 10000.0
 Spectrometer Frequency 499.89

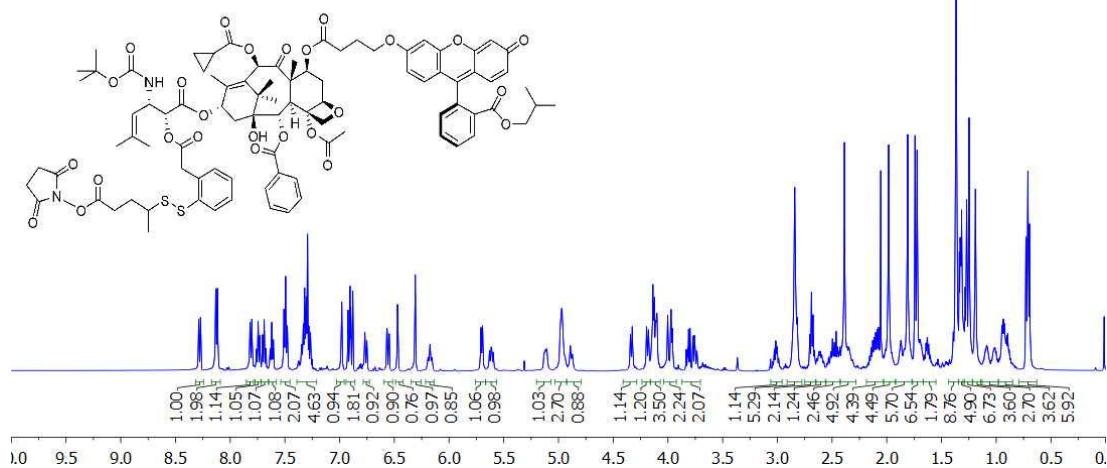


Figure S27. ^1H NMR spectrum of **22**

Compound Name TW-B05-P61
 Processed by twang
 Solvent CDCl3
 Temperature 298.3
 Number of Scans 951
 Pulse Sequence zgpg30
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 Spectral Width 29761.9
 Spectrometer Frequency 125.71

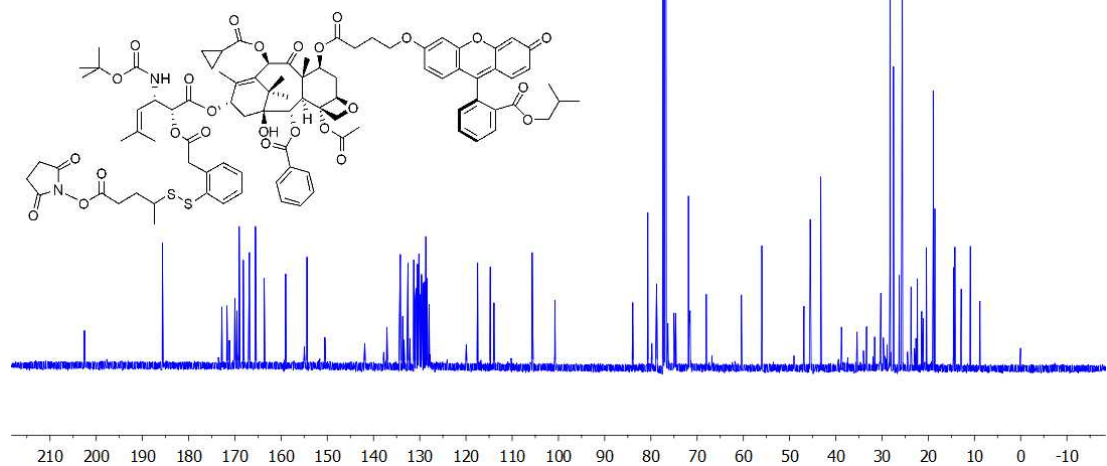


Figure S28. ^{13}C NMR spectrum of **22**

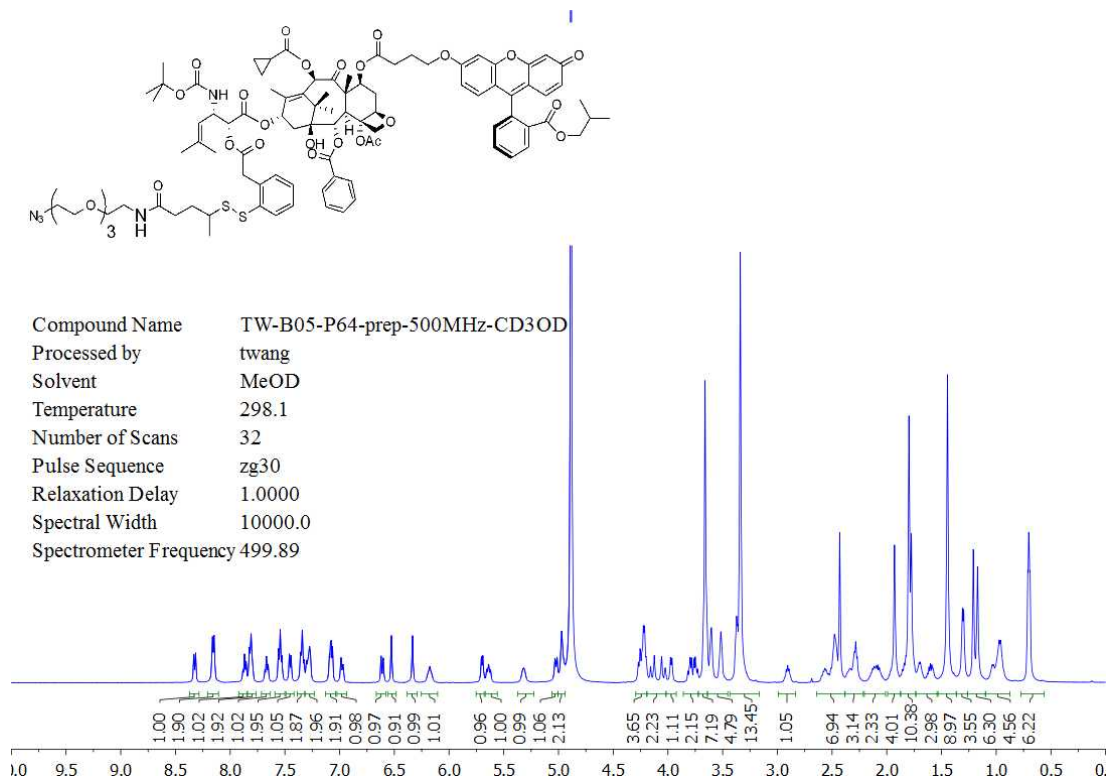


Figure S29. ¹H NMR spectrum of 24

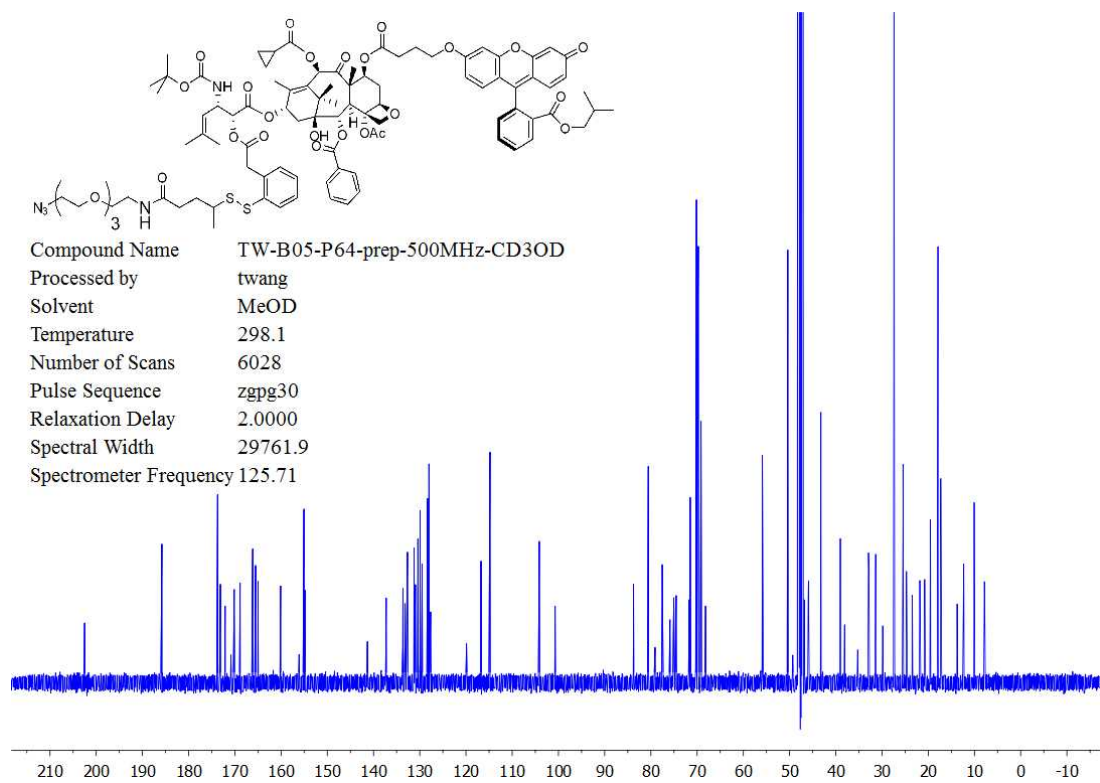


Figure S30. ¹³C NMR spectrum of 24

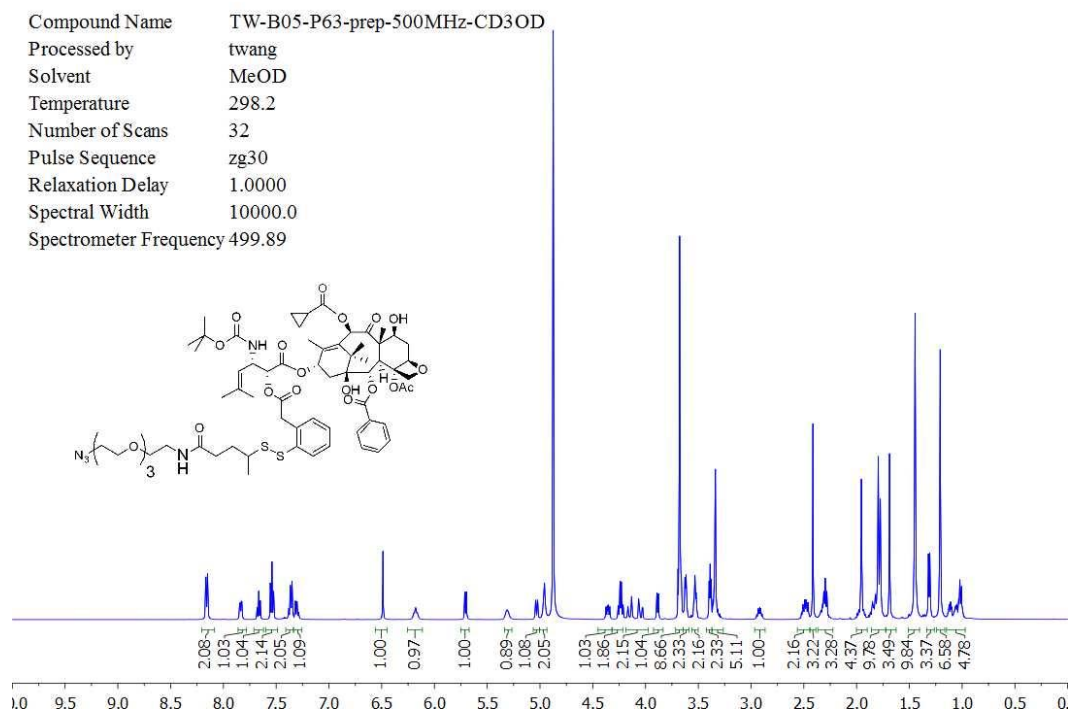


Figure S31. ^1H NMR spectrum of **25**

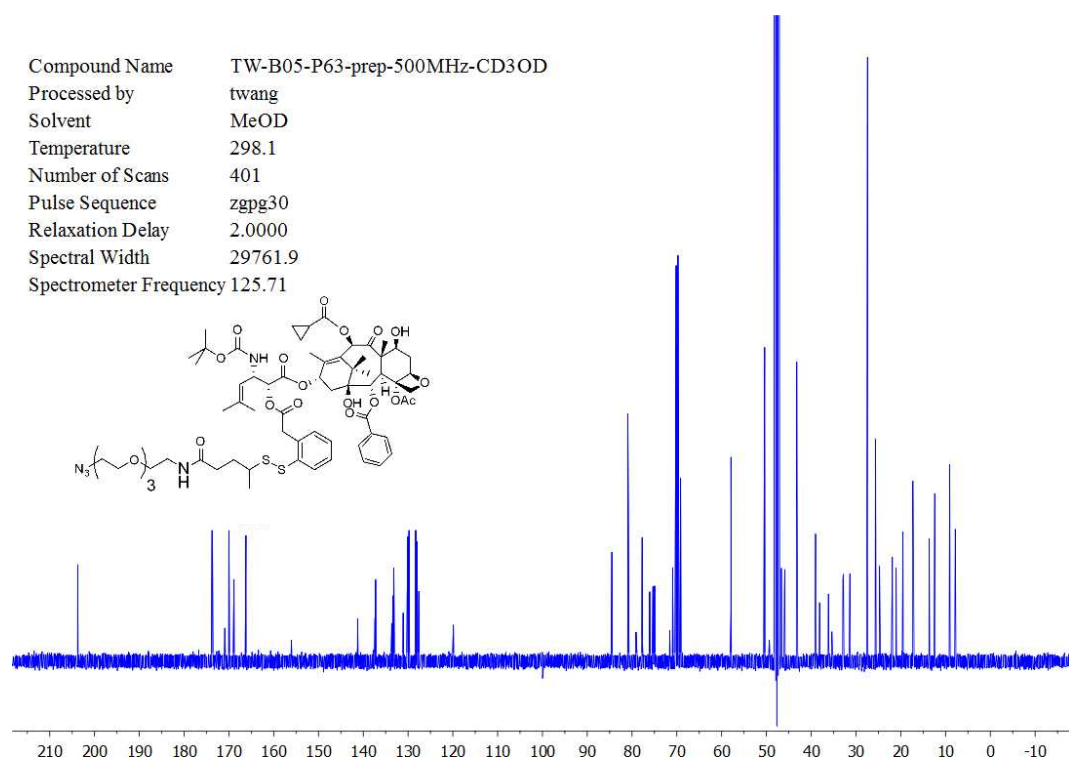


Figure S32. ^{13}C NMR spectrum of **25**

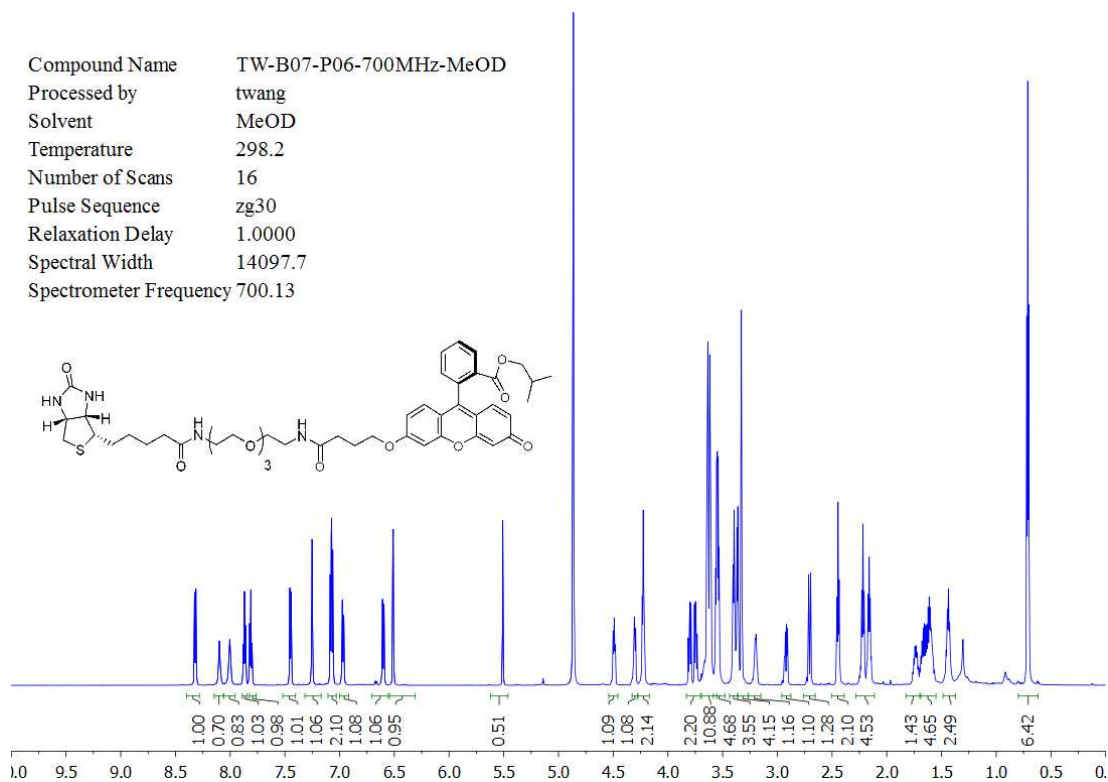


Figure S33. ^1H NMR spectrum of **28**

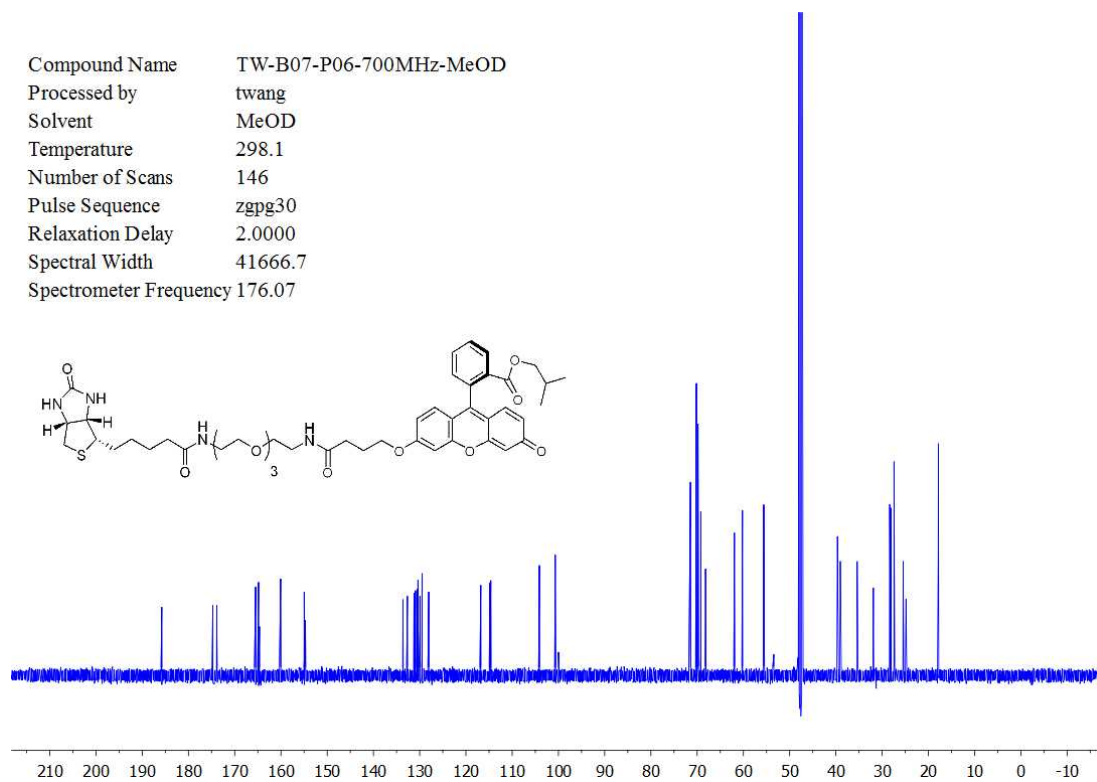


Figure S34. ^{13}C NMR spectrum of **28**

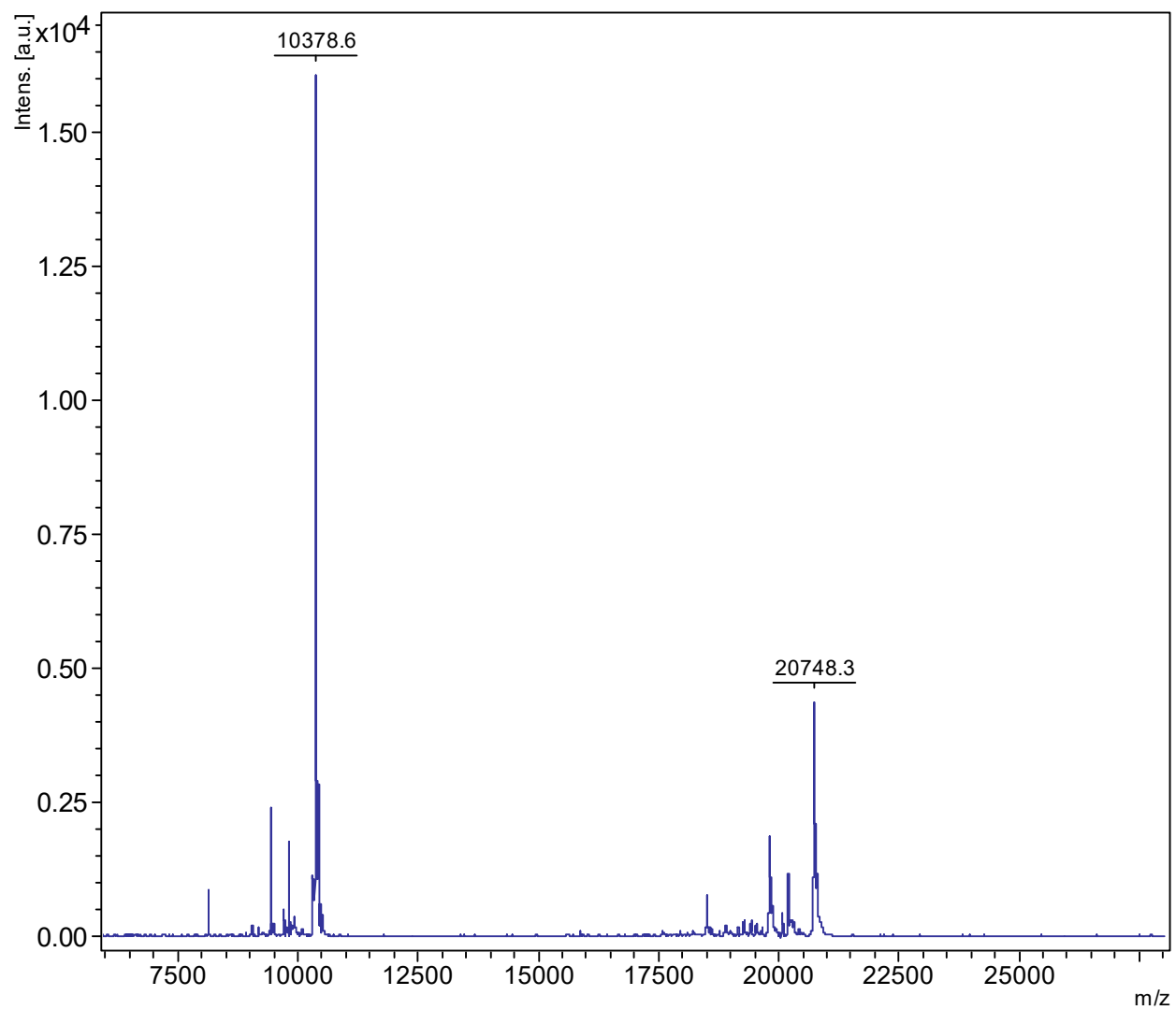


Figure S35. MALDI-TOF analysis of fully biotinylated G3 PAMAM dendrimer 7.

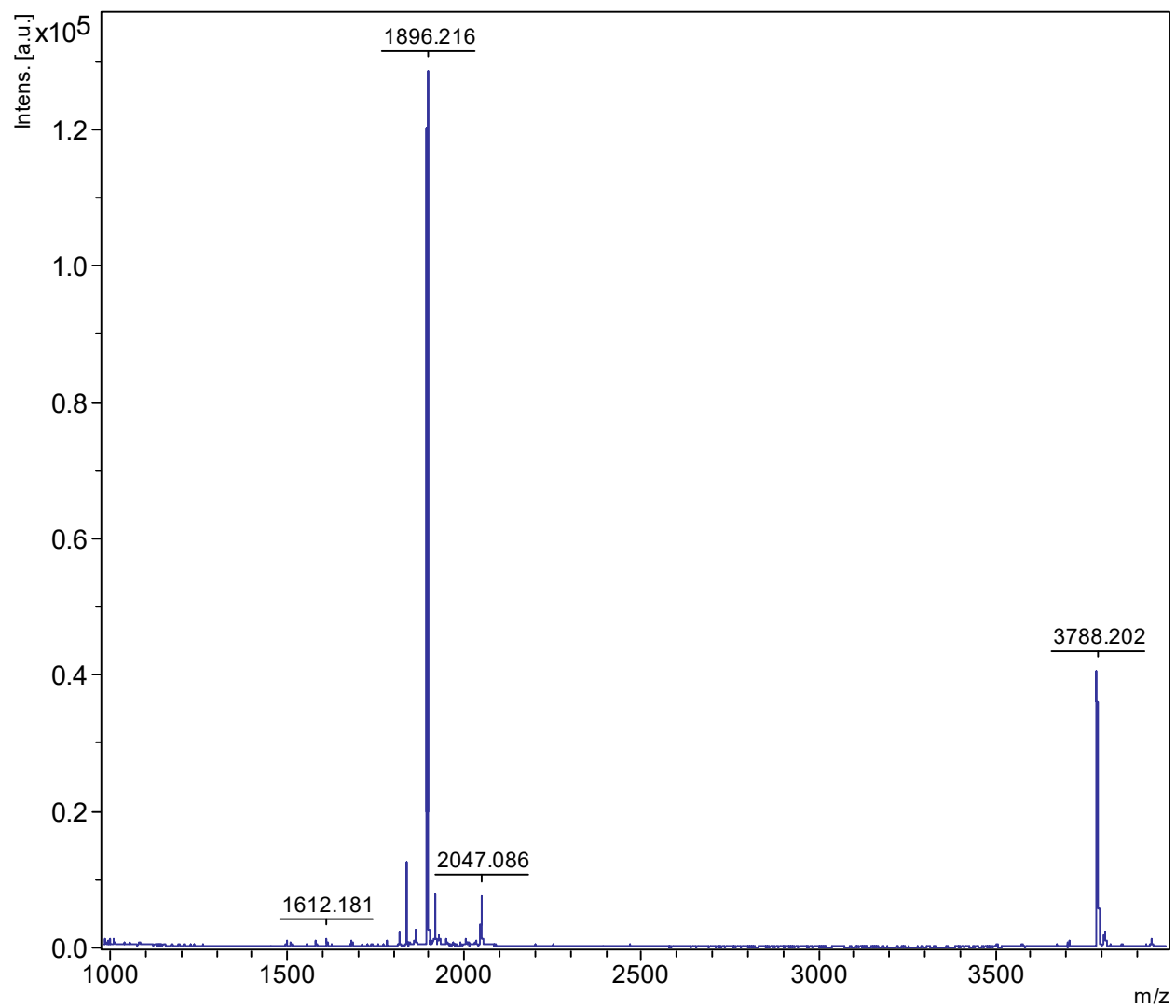


Figure S36. MALDI-TOF analysis of fully alkynylated G1-PAMAM dendrimer **8**.

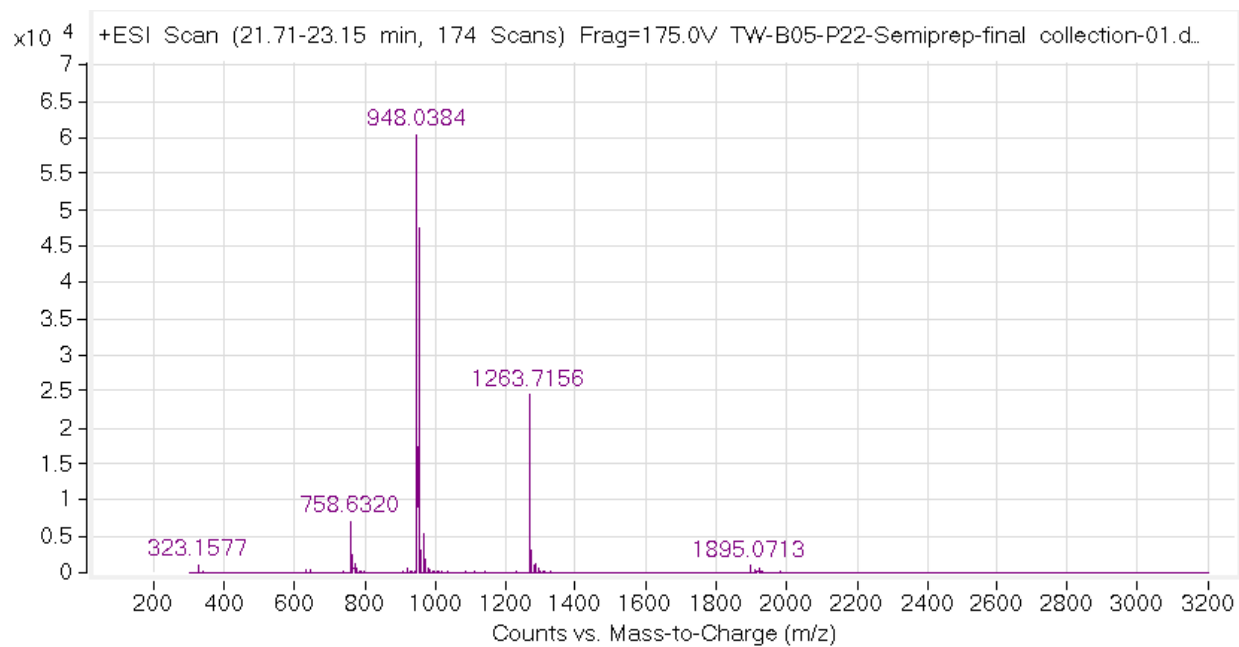


Figure S37. LC-UV-TOF analysis of fully alkynylated G1-PAMAM dendrimer **8**.

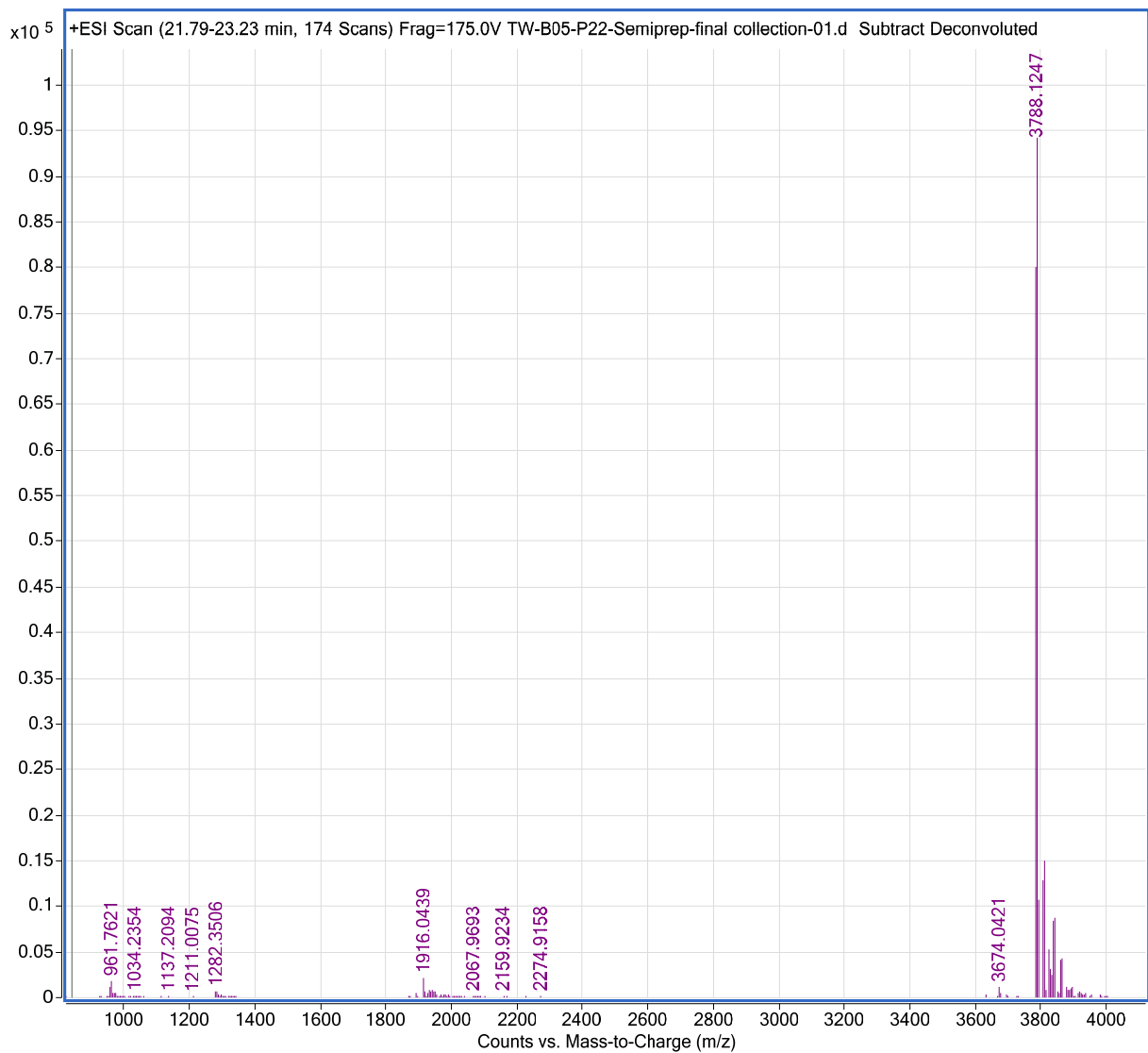


Figure S38. LC-TOF deconvolution result for fully alkynylated G1-PAMAM dendrimer **8**.

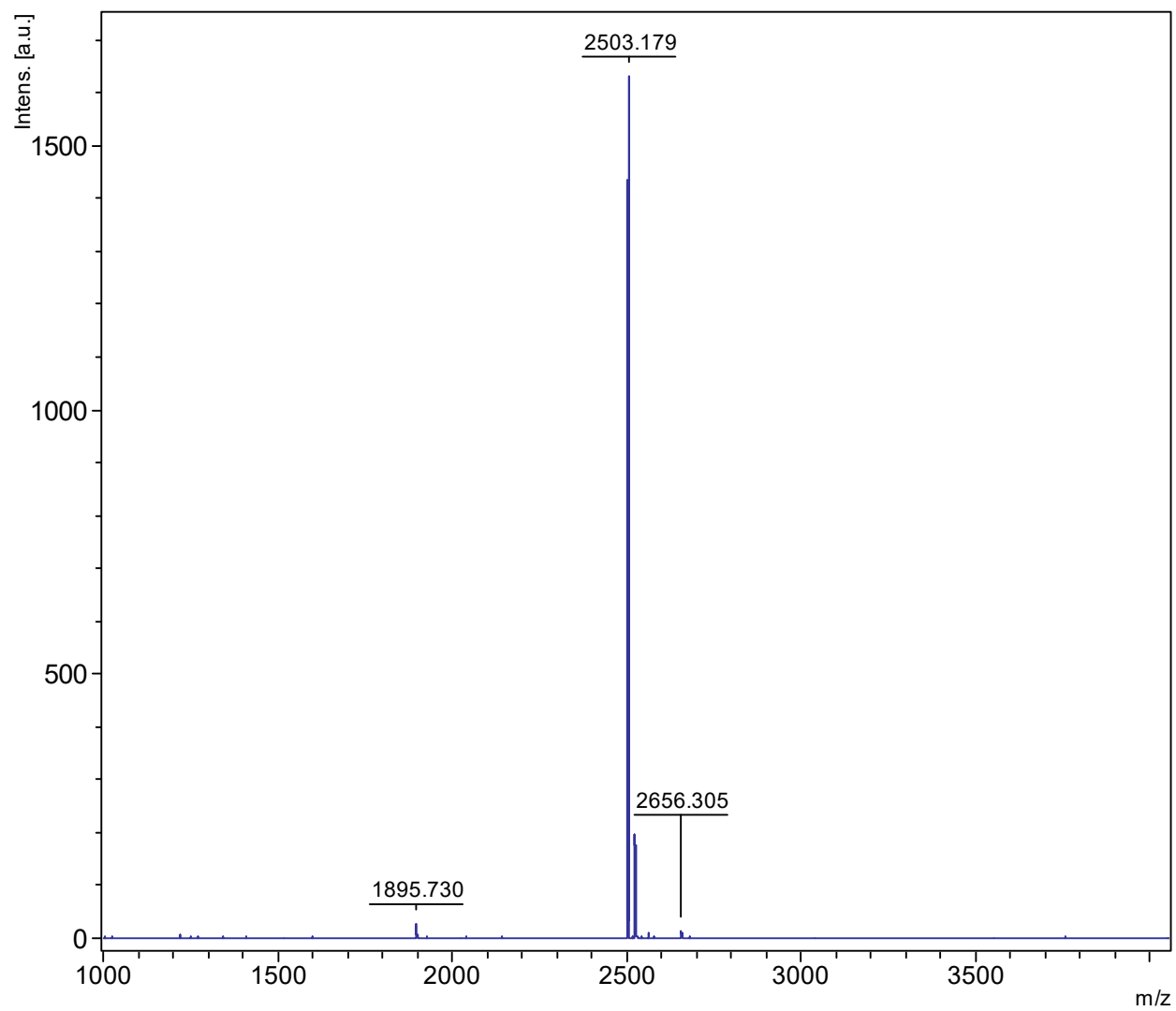


Figure S39. MALDI-TOF analysis of G1-PAMAM half-dendron-linker **12**.

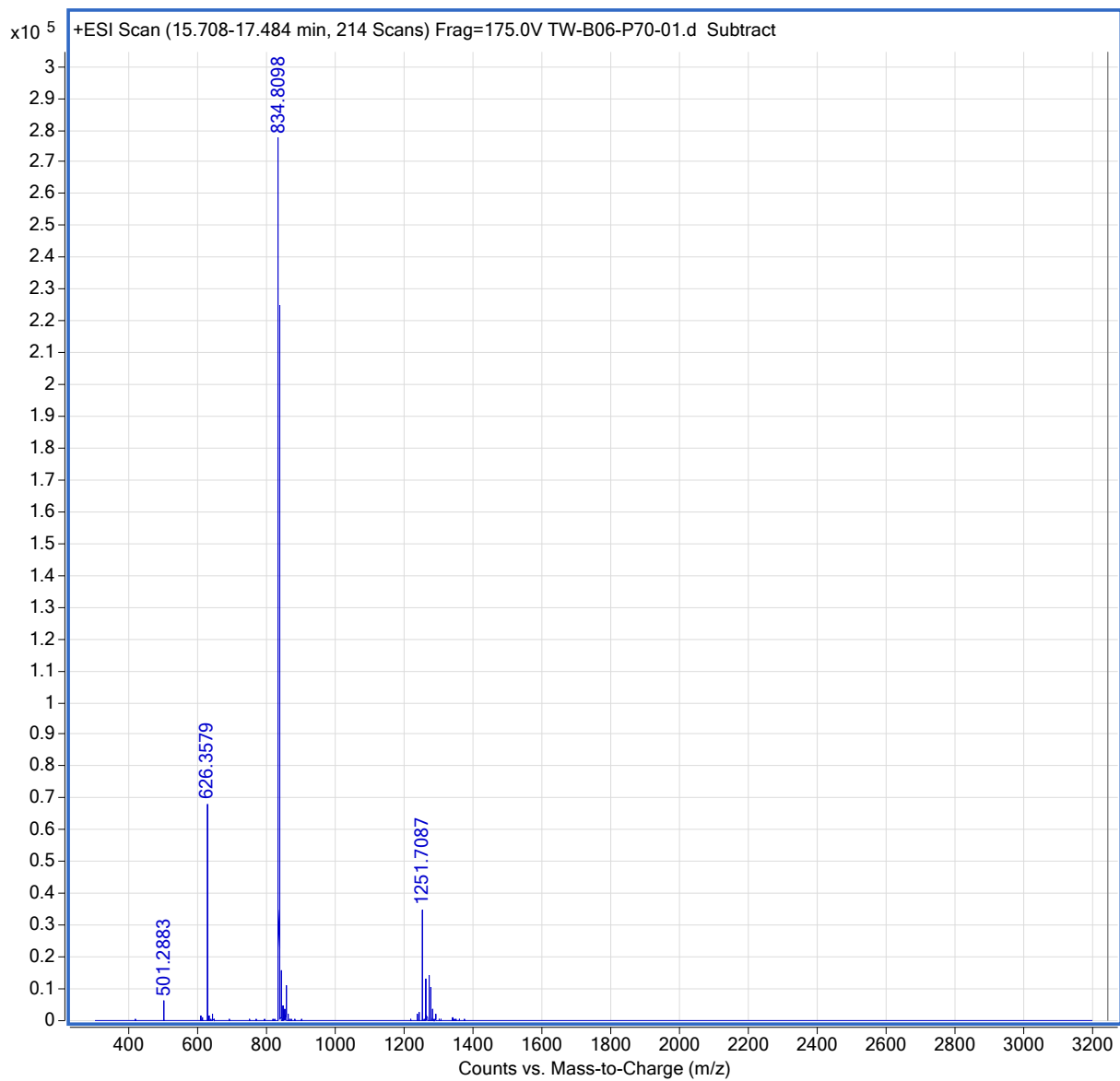


Figure S40. LC-UV-TOF analysis of G1-PAMAM half-dendron-linker **12**.

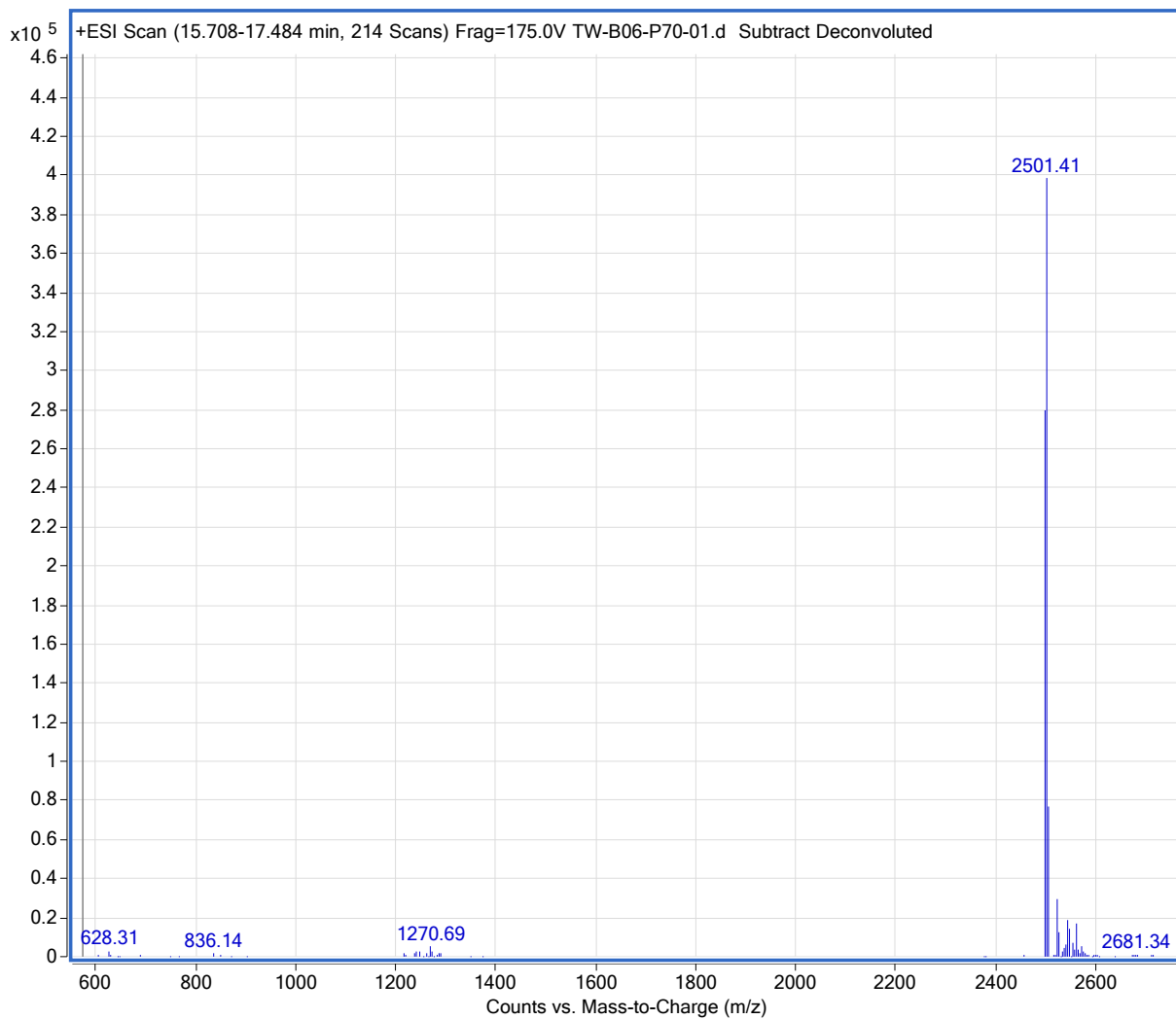


Figure S41. LC-UV-TOF deconvolution result for G1-PAMAM half-dendron-linker **12**.

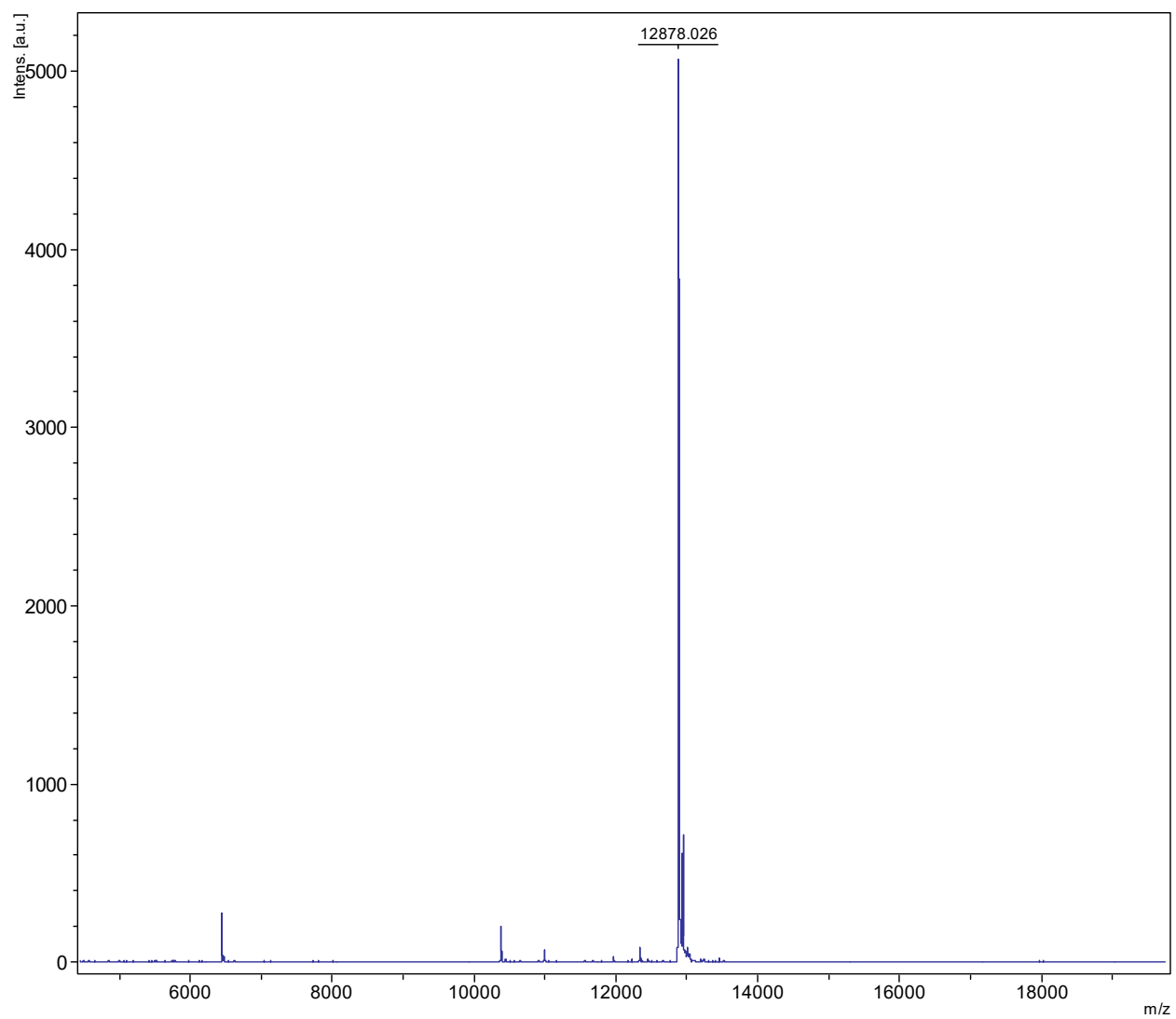


Figure S42. MALDI-TOF analysis of (G1 half-dendron)-linker-(G3 half-dendron) **6**.

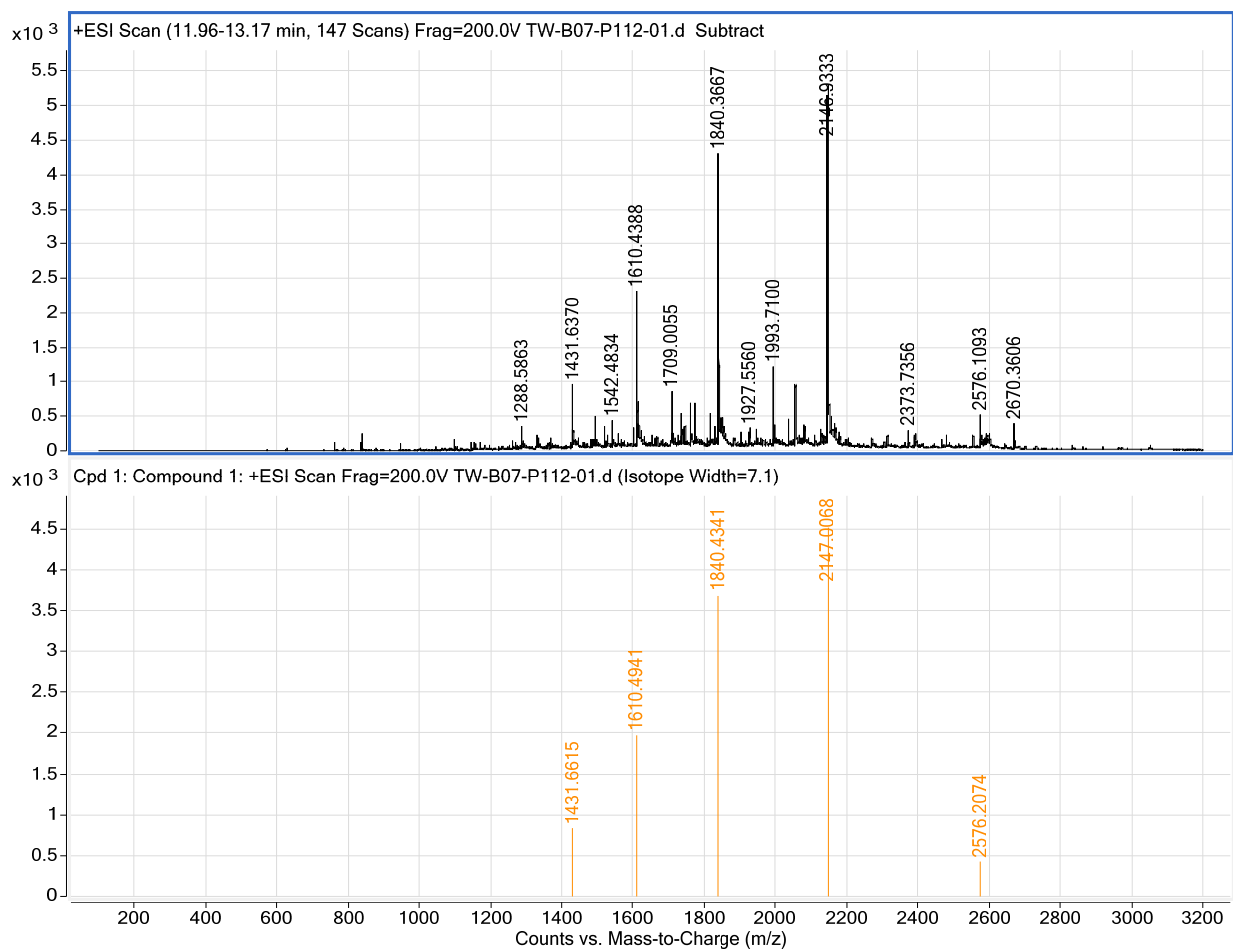


Figure S43. LC-UV-TOF mass analysis of (G1 half-dendron)-linker-(G3 half-dendron) **6**.

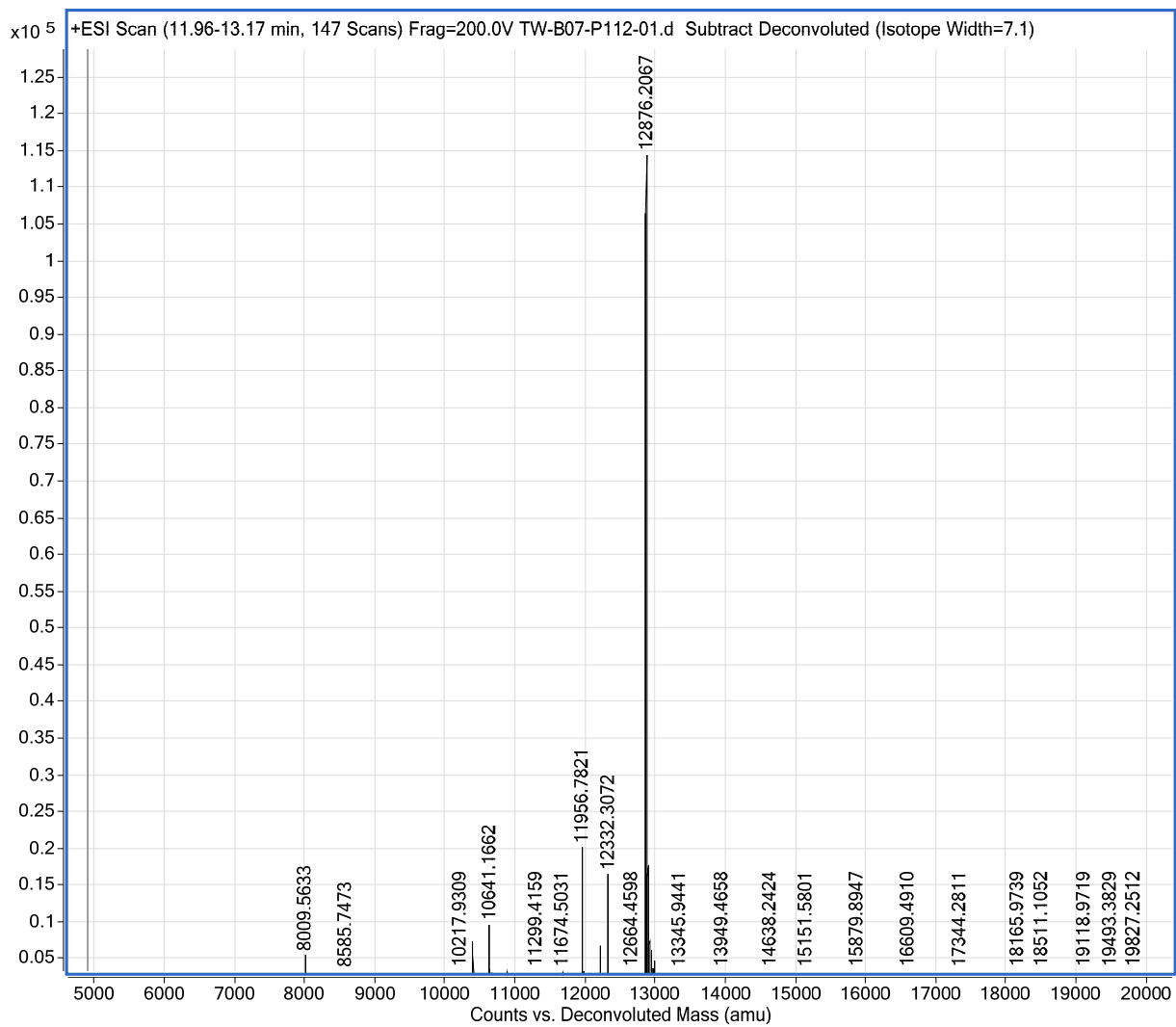
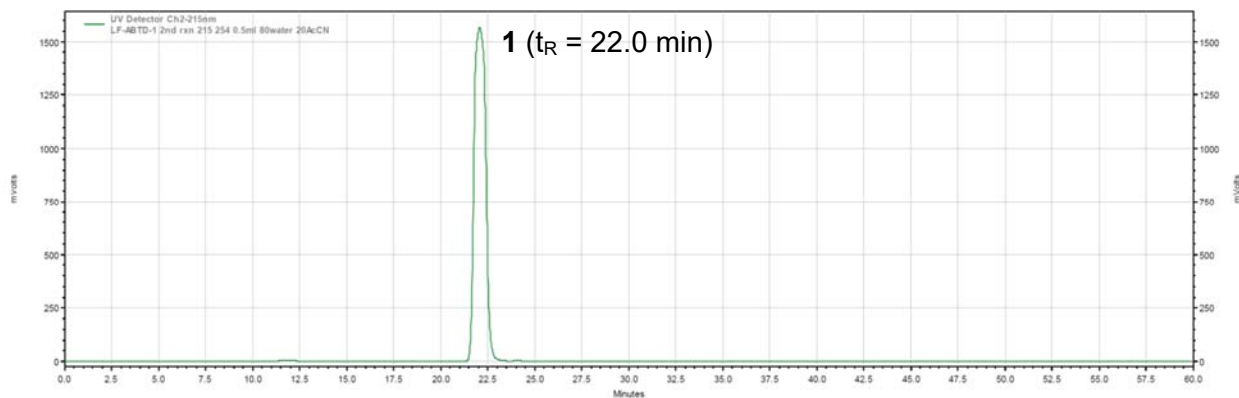


Figure S44. LC-UV-TOF deconvolution result of (G1 half-dendron)-linker-(G3 half-dendron) **6**.

(A)



(B)

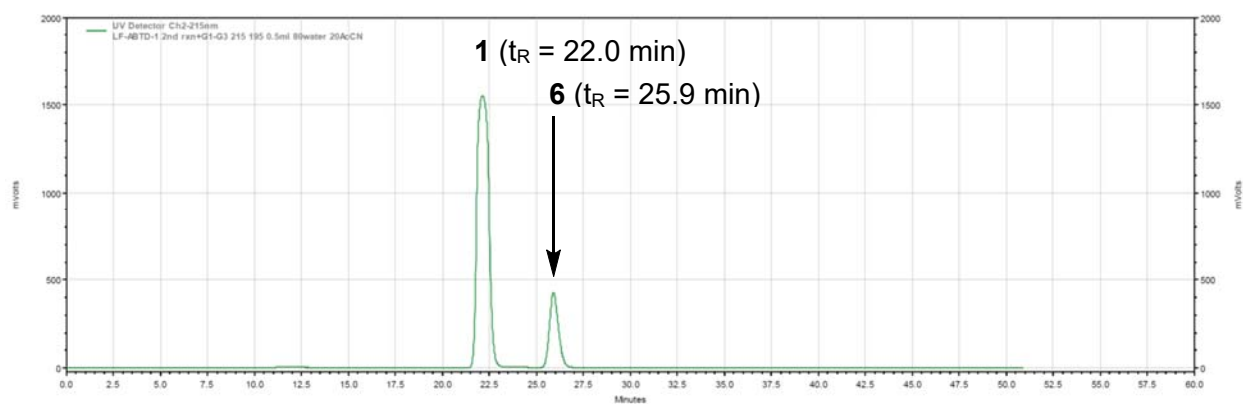


Figure S45. GPC analysis of (A) ABTD-TTC-1 (**1**) and (B) ABTD-TTC-1 (**1**) + click-ready ABTD **6**. Conditions: Ultrahydrogel™ 500 column (7.8 mm x 300 mm, 10 μ m); MeCN/H₂O (20/80); 0.5 mL/min; 55 μ M; UV detector at 215 nm.

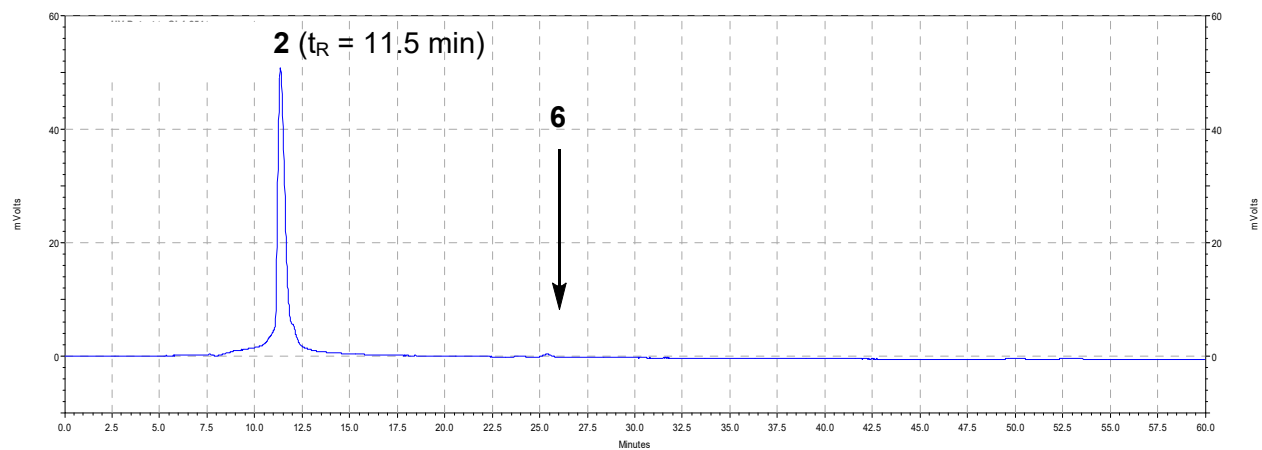
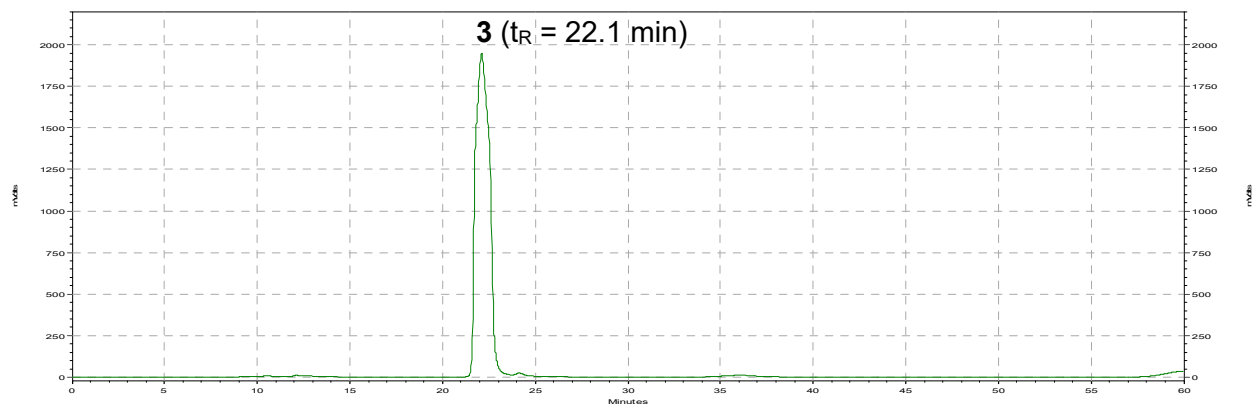


Figure S46. GPC analysis of ABTD-TTC-2 (**2**). Conditions: UltrahydrogelTM 500 column (7.8 mm x 300 mm, 10 μ m); MeCN/H₂O (20/80); 0.5 mL/min; 50 μ M; UV detector at 254 nm. The anticipated t_R of **6** is also shown.

(A)



(B)

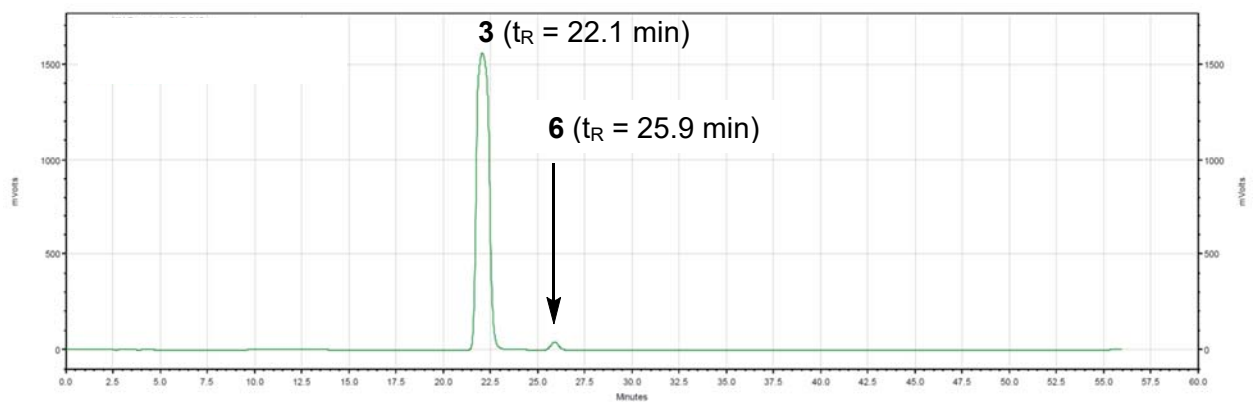
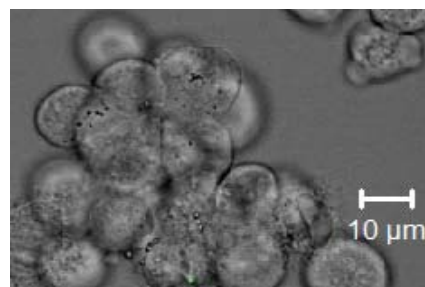
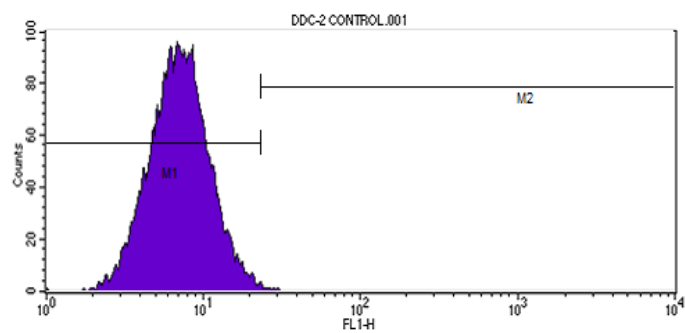


Figure S47. GPC analysis of (A) ABTD-TTC-3 (**3**) and (B) ABTD-TTC-2 (**2**) + click-ready ABTD **6**. Conditions: Ultrahydrogel™ 500 column (7.8 mm x 300 mm); MeCN/H₂O (20/80); 0.5 mL/min; 64 μM; UV detector at 215 nm.

(A)



(B)

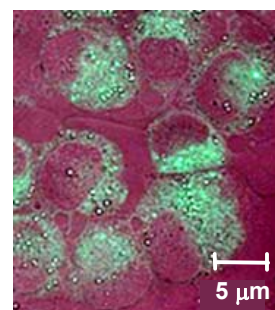
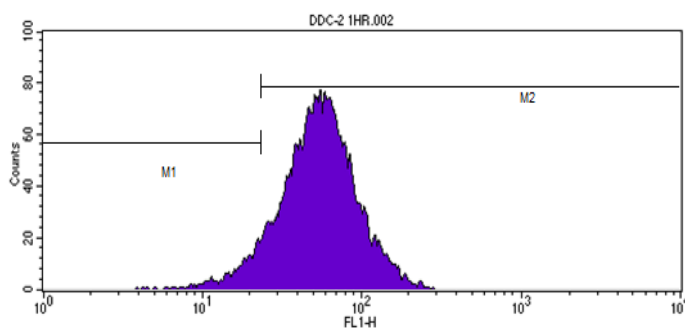


Figure S48. Internalization of fluorescent probe ABTD-TTC-2 (**2**) (20 μM) in ID-8 (ovarian cancer) cells by confocal fluorescence microscopy (CFM) and flow cytometry after 1 h of incubation at 37 $^{\circ}\text{C}$ (B) as compared to the control ID8 cells (A).

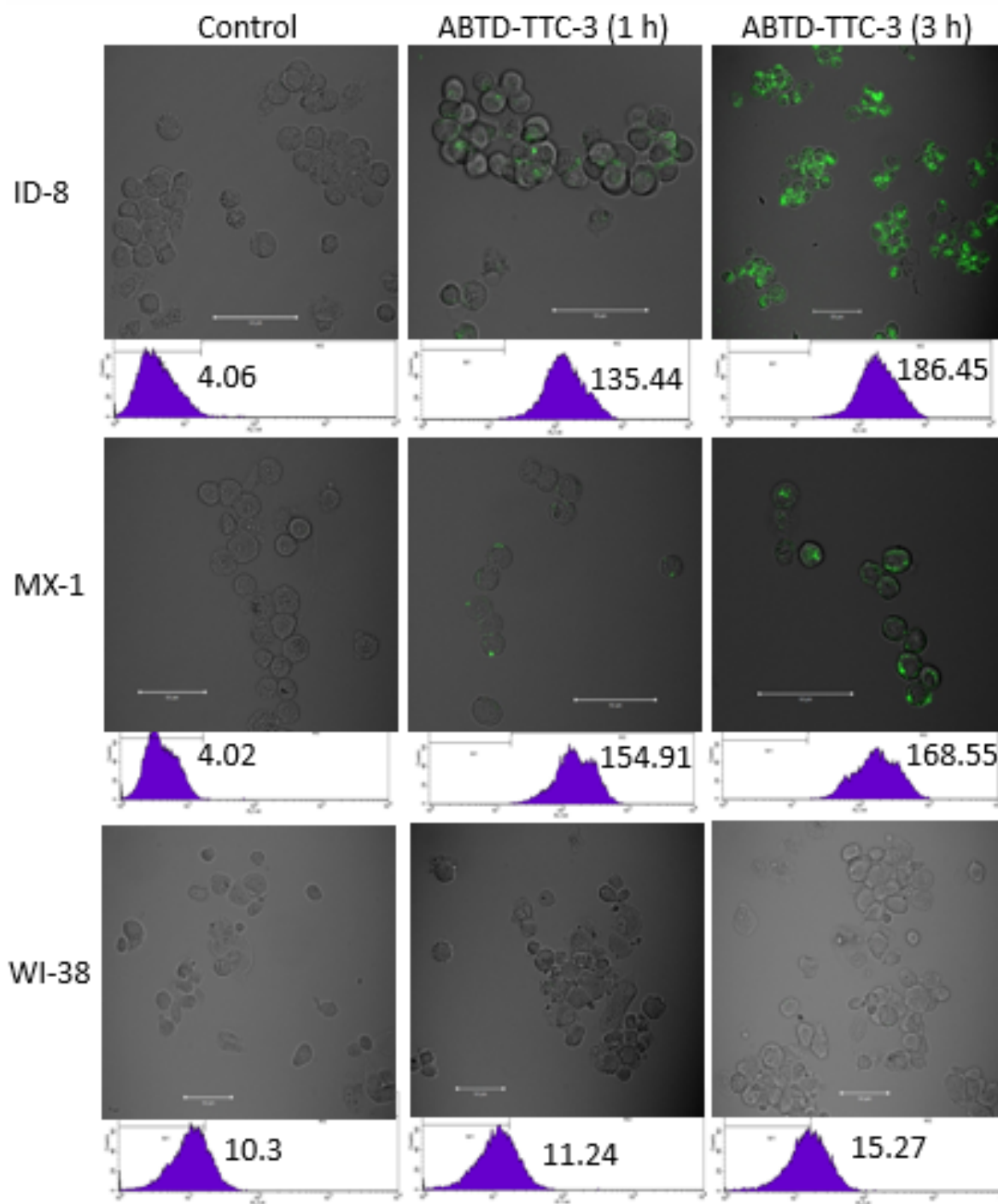


Figure S49. Assessment of the internalization of fluorescent probe ABTD-TTC-3 (**3**) (20 μ M) in ID-8 (ovarian cancer), MX-1 (breast cancer) and WI-38 (normal lung fibroblast) cells by confocal fluorescence microscopy (CFM) and flow cytometry at 0 h, 1 h, and 3 h periods, at 37 $^{\circ}$ C.

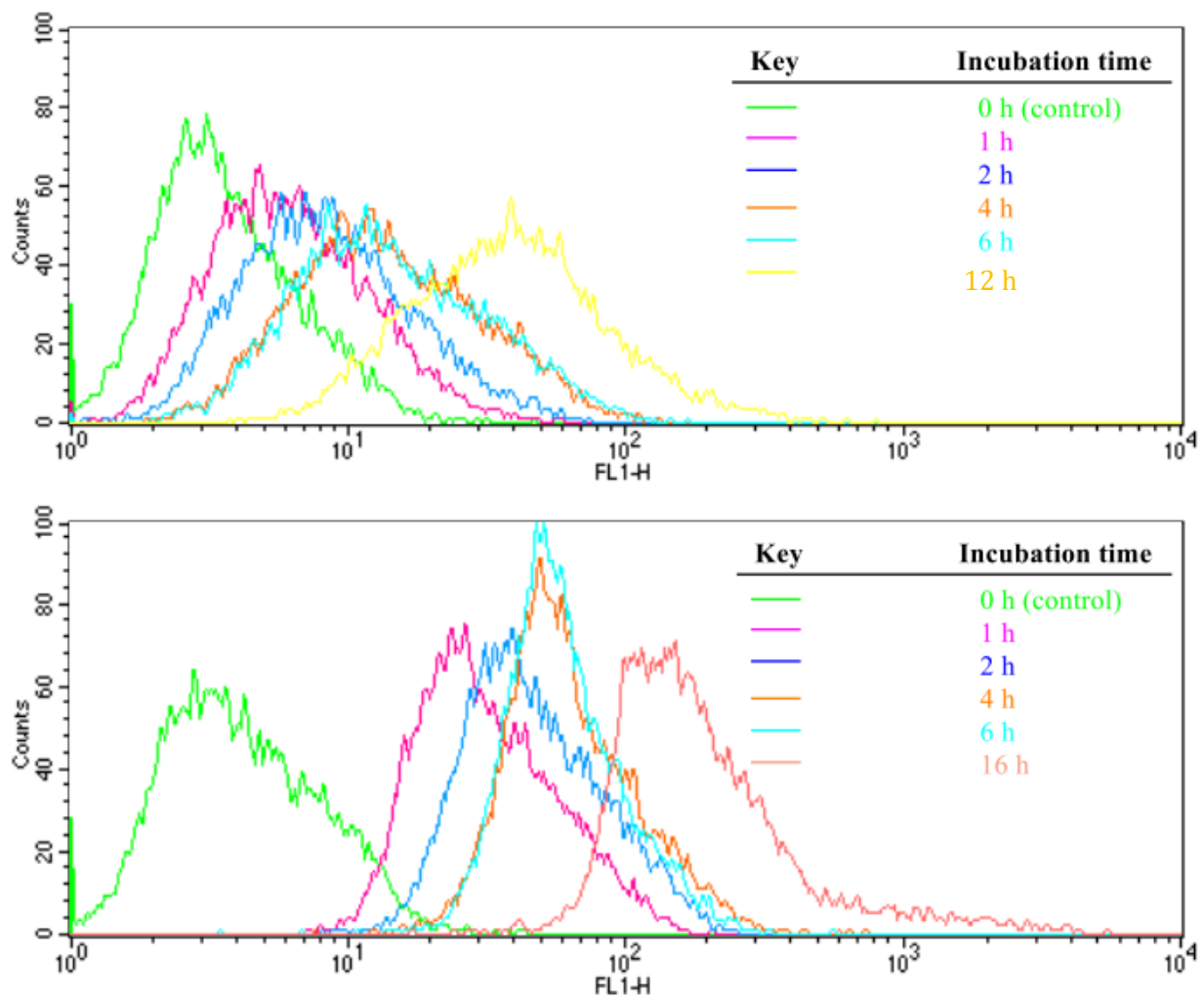


Figure S50. Assessment of the multi-binding effect by flow cytometry based on the internalization of fluorescent probe **28** (10 μ M, top) and ABTD-TTC-3 (**3**) (10 μ M, bottom) in ID-8 cells at 0, 1, 2, 4, 6 and 16 h periods, at 37 $^{\circ}$ C.

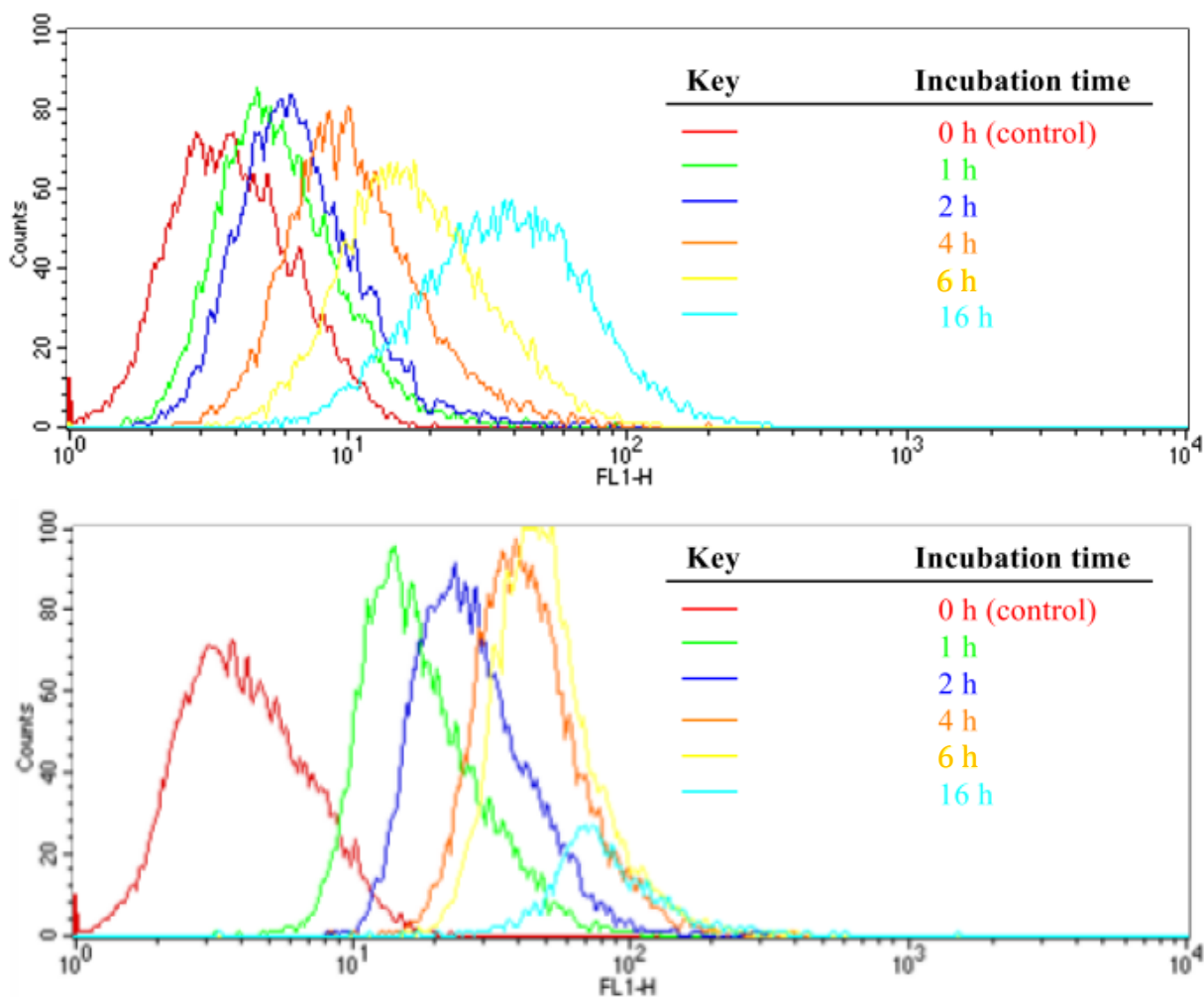


Figure S51. Assessment of the multi-binding effect by flow cytometry based on the internalization of fluorescent probe **28** (10 μ M, top) and ABTD-TTC-3 (**3**) (10 μ M, bottom) in MX-1 cells at 0, 1, 2, 4, 6 and 16 h periods, at 37 $^{\circ}$ C.