

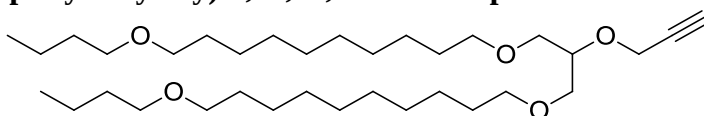
# Double Conjugation Strategy to Incorporate Lipid Adjuvants into Multiantigenic Vaccines

Waleed M. Hussein, Tzu-Yu Liu, Pirashanthini Maruthayanar, Saori Mukaida, Peter M. Moyle, James W Wells, Istvan Toth,\* and Mariusz Skwarczynski\*

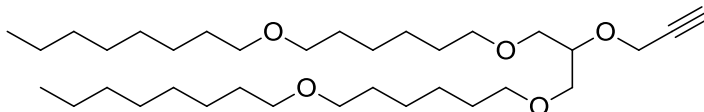
## Supporting Information

Structure,  $^1\text{H}$  and  $\text{C}^{13}$  NMR for lipid derivatives

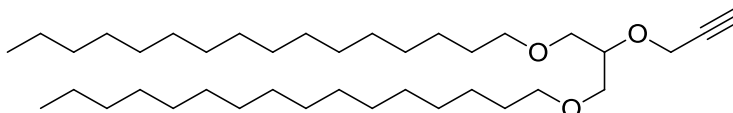
Structure of 18-(prop-2-yn-1-yloxy)-5,16,20,31-tetraoxapentatriacontane or lipoalkyne (1)



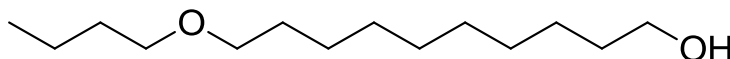
Structure of 18-(prop-2-yn-1-yloxy)-9,16,20,27-tetraoxapentatriacontane or lipoalkyne (2)



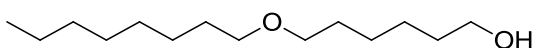
Structure of 1-(3-(hexadecyloxy)-2-(prop-2-yn-1-yloxy)propoxy)hexadecane or lipoalkyne (3)



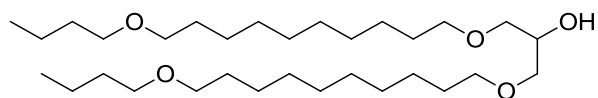
Structure of 10-Butoxydecan-1-ol (6)



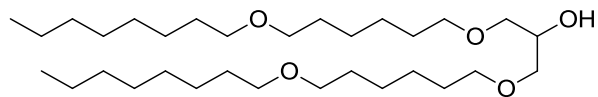
Structure of 6-(octyloxy)hexan-1-ol (7)



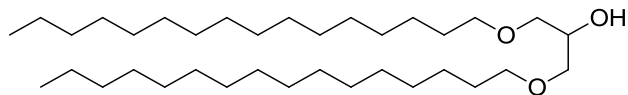
Structure of 5,16,20,32-tetraoxahexatriacontan-18-ol (9)

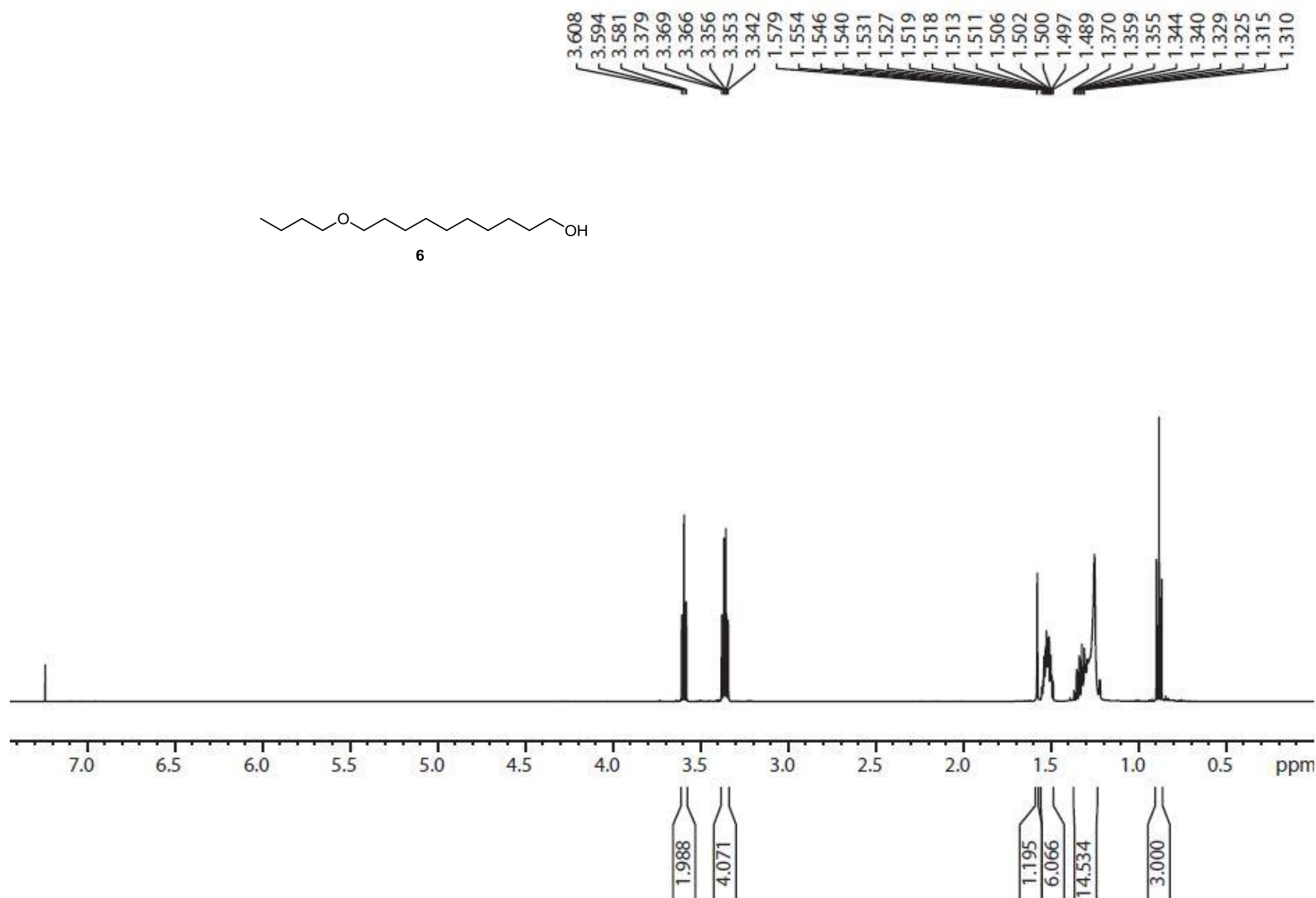


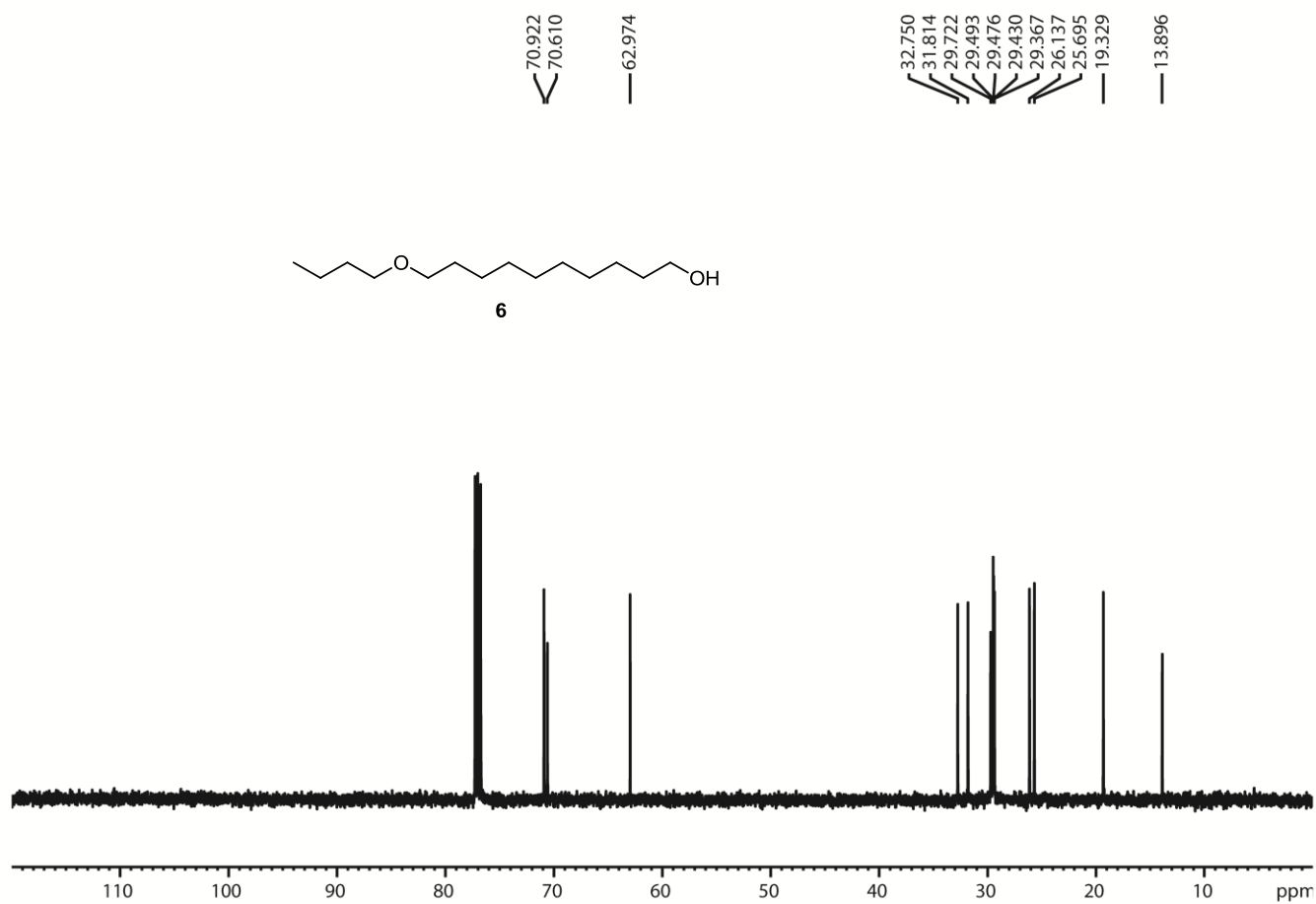
**Structure of 9,16,20,27-tetraoxapentatriacontan-18-ol (10)**

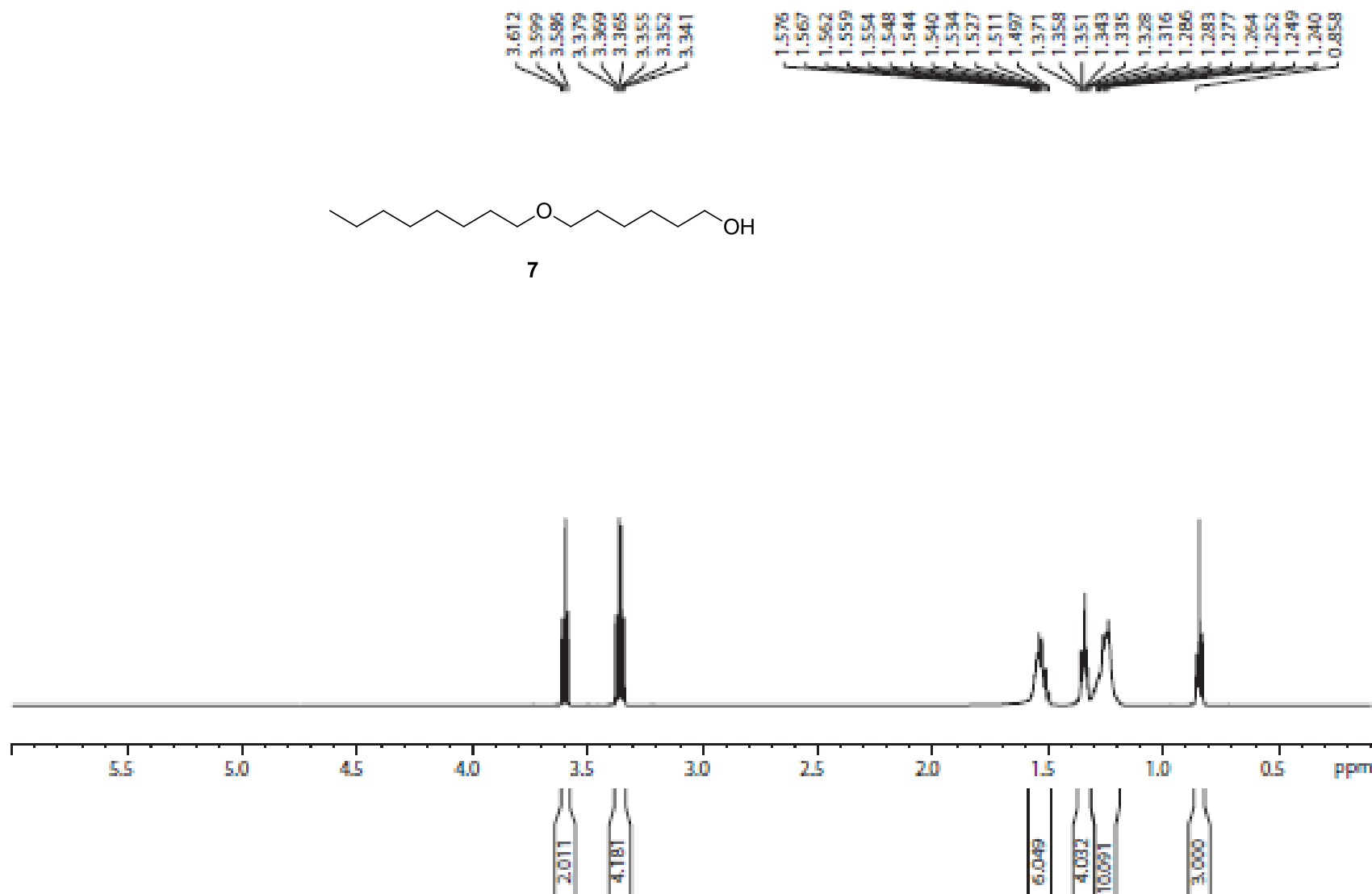


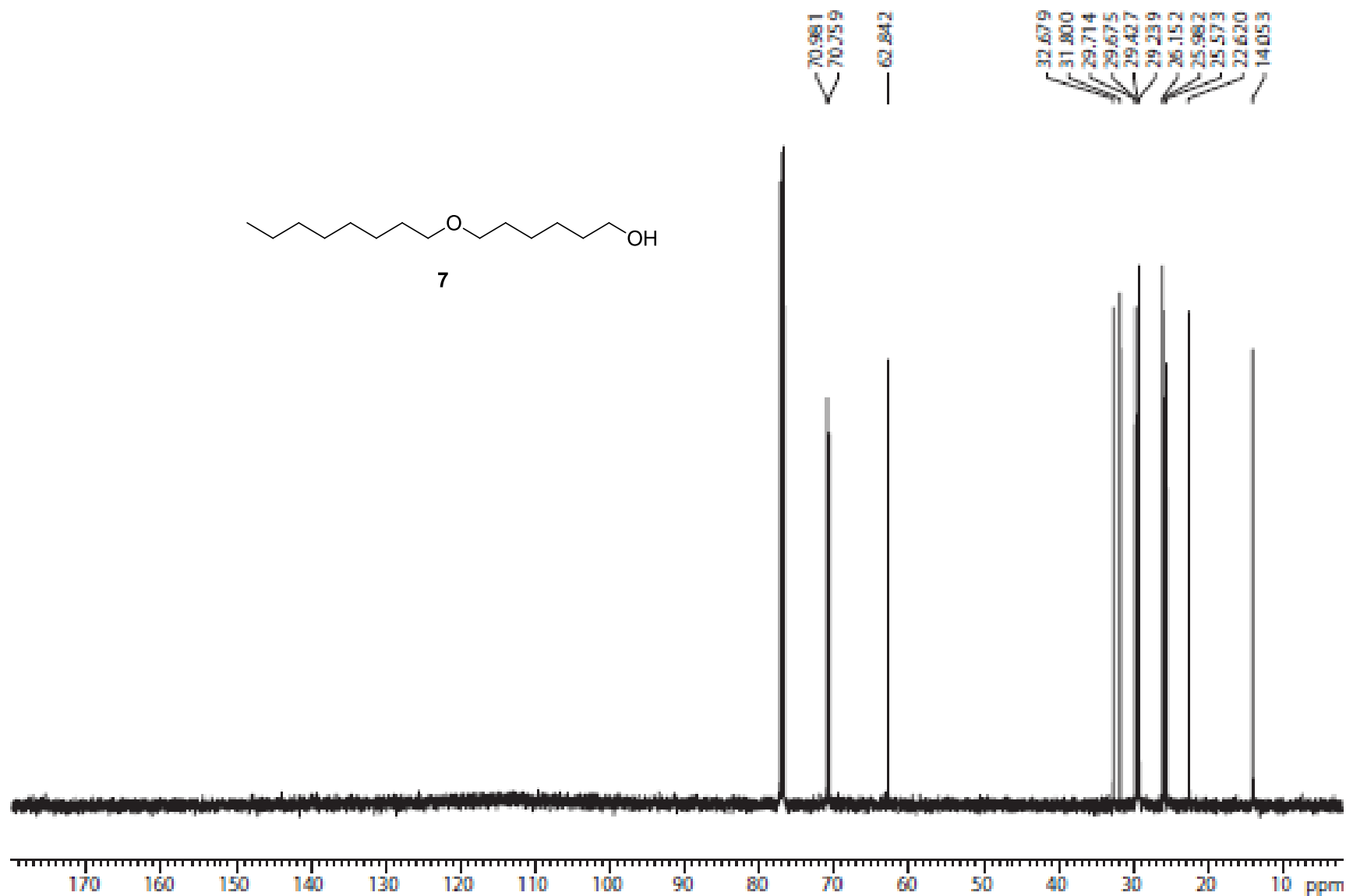
**Structure of 1,3-bis(hexadecyloxy)propan-2-ol (11)**

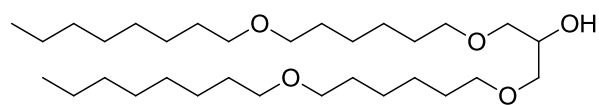






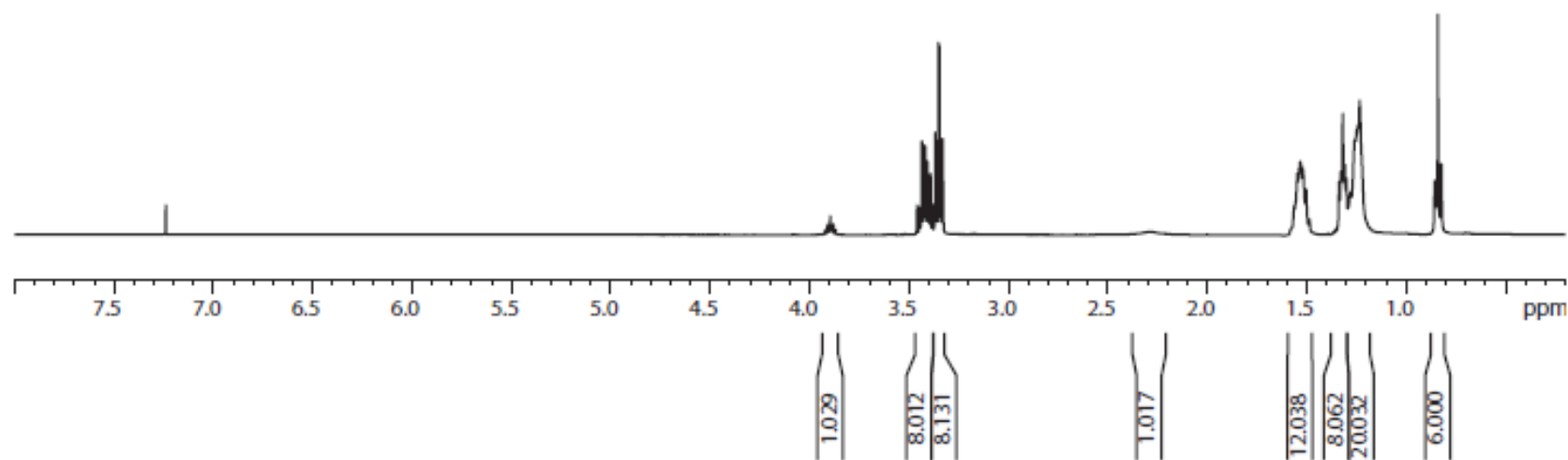


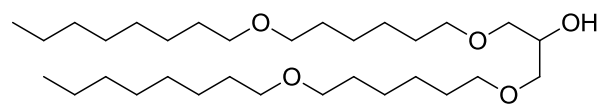




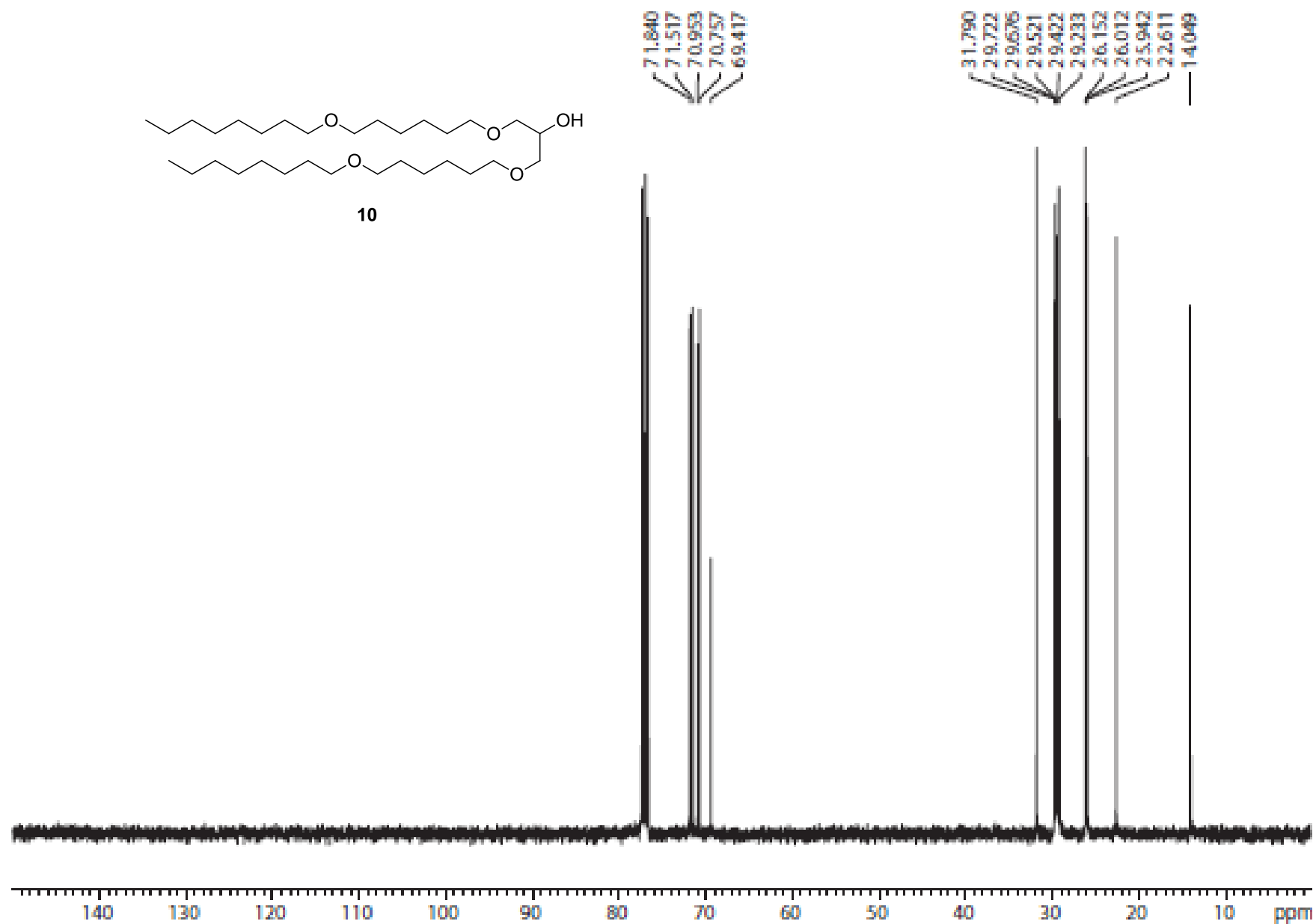
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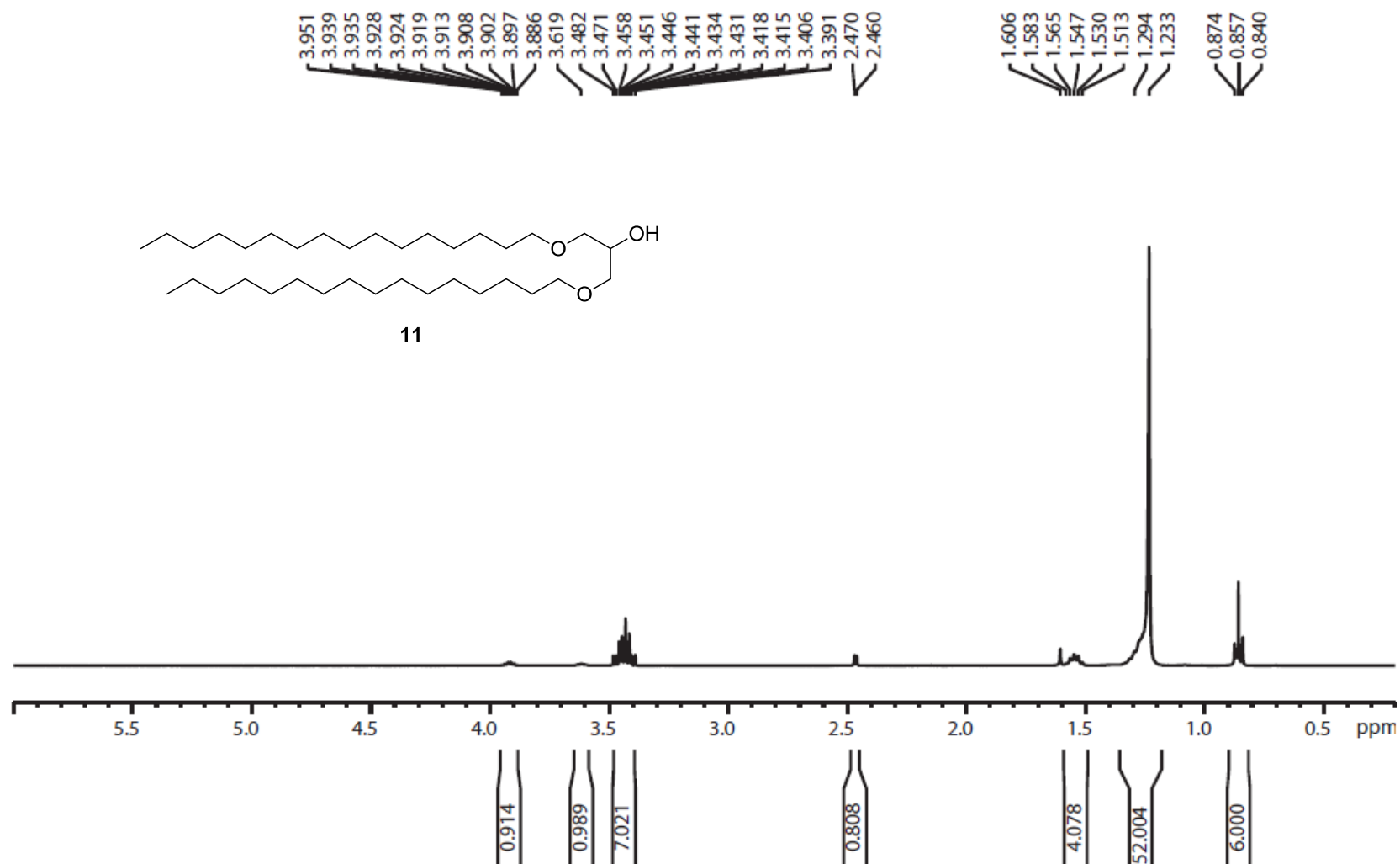
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3.450  
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3.437  
3.426  
3.423  
3.419  
3.410  
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1.282

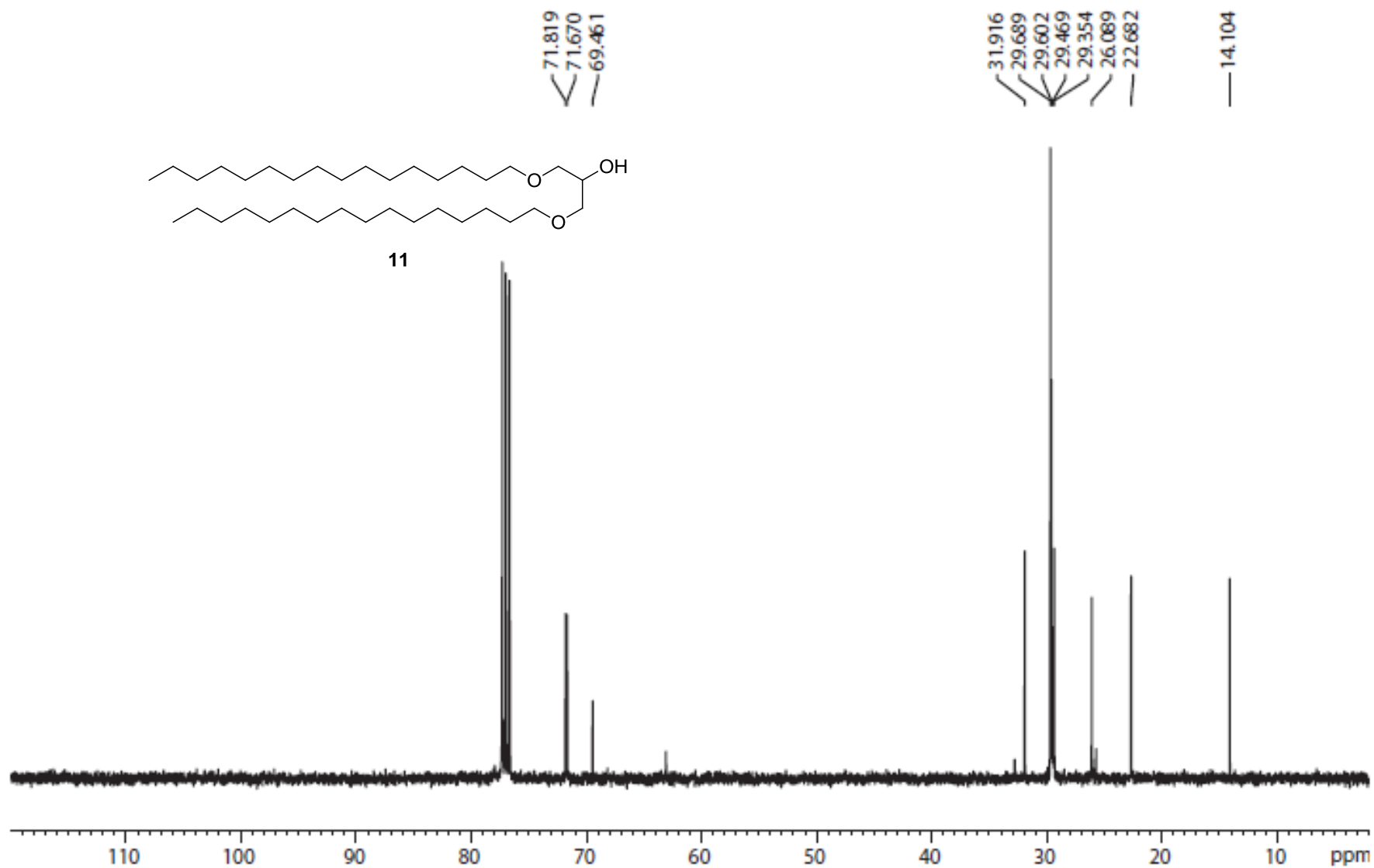


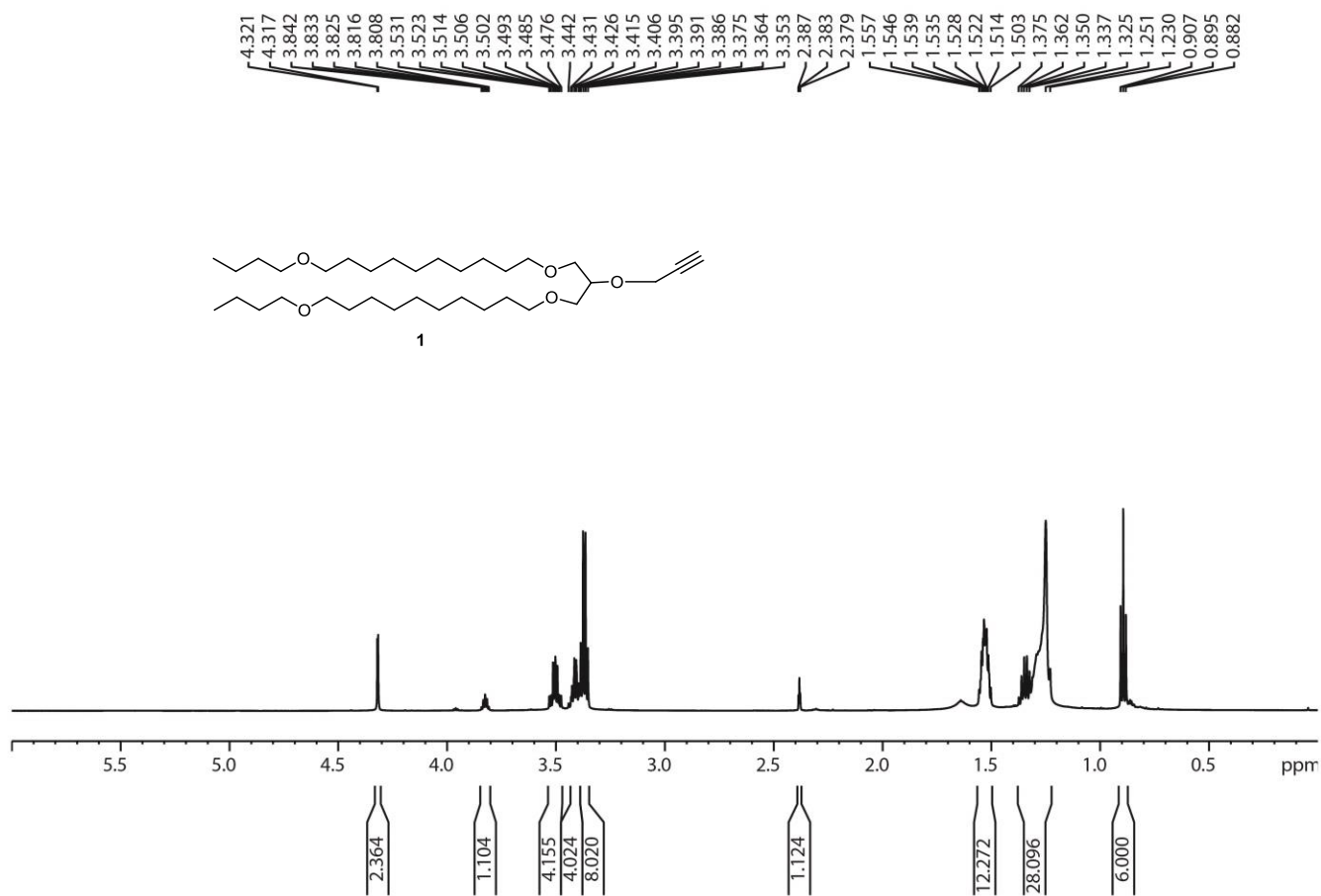


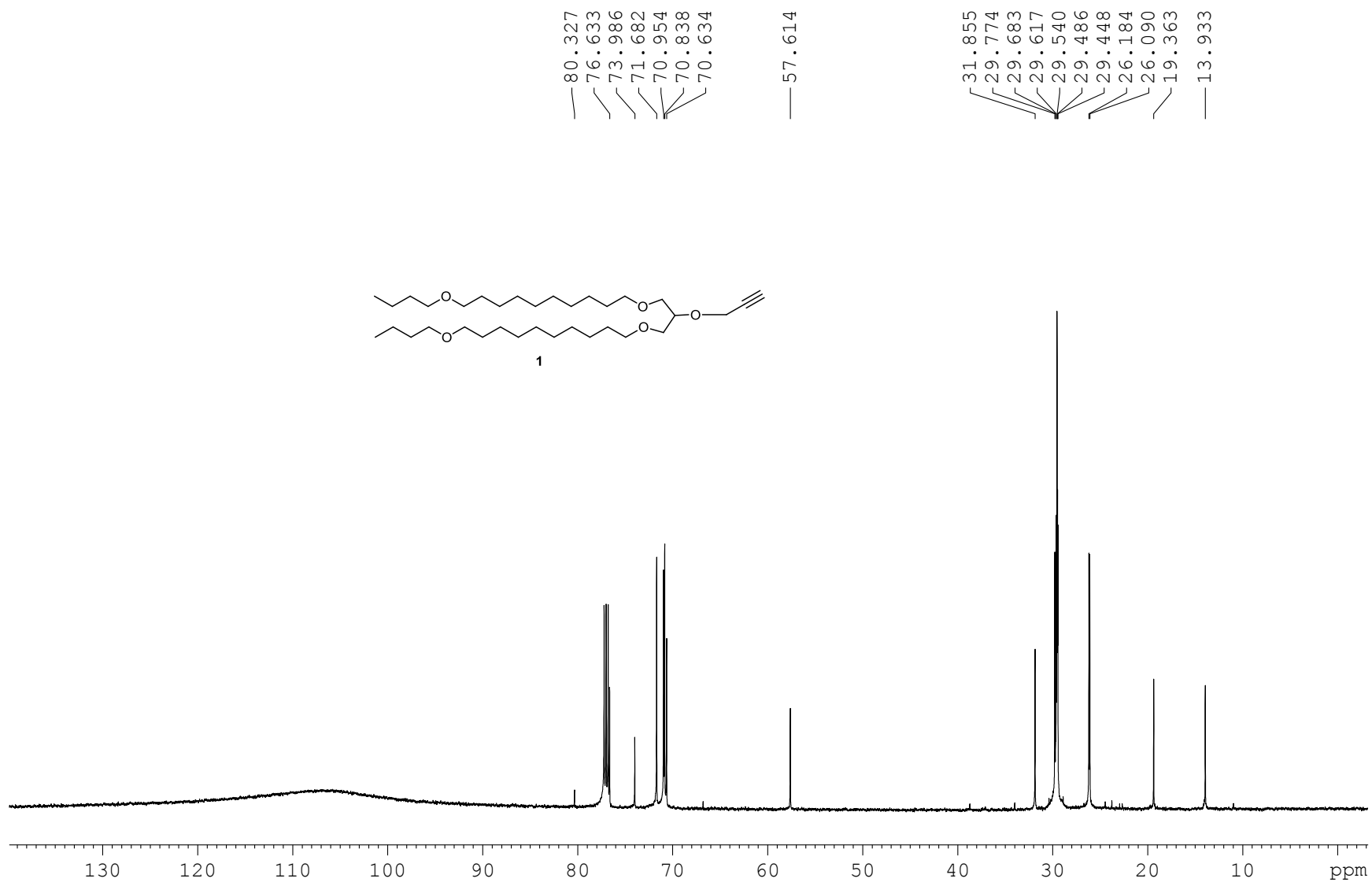
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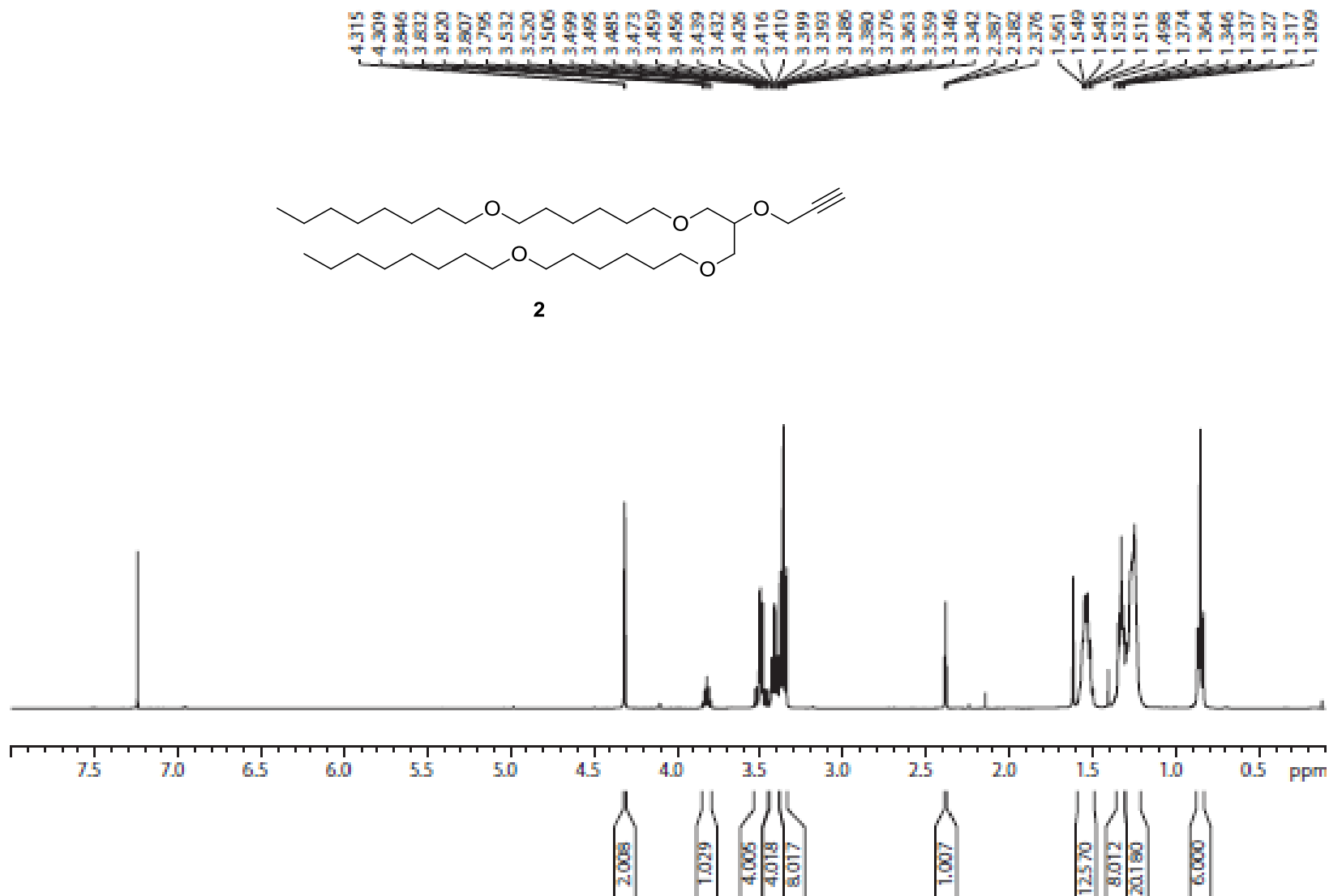
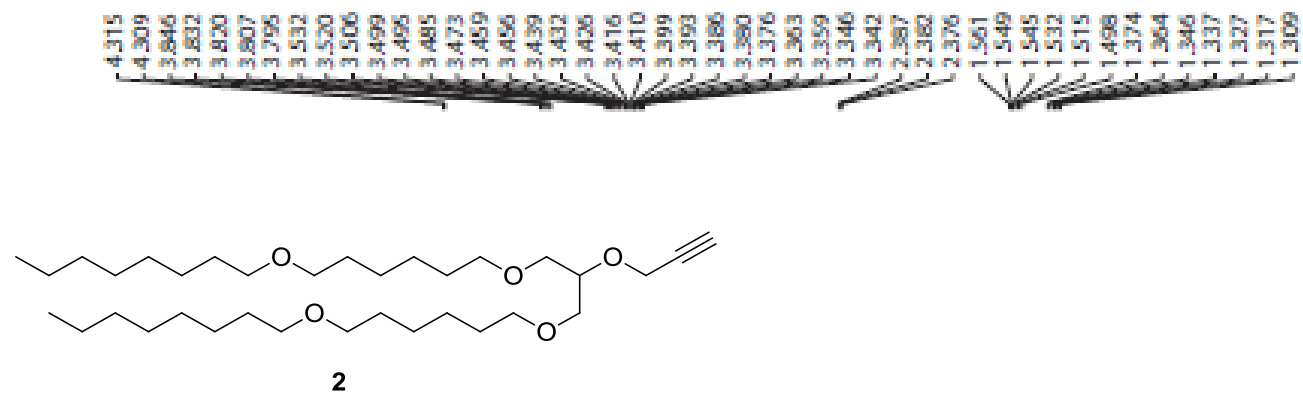


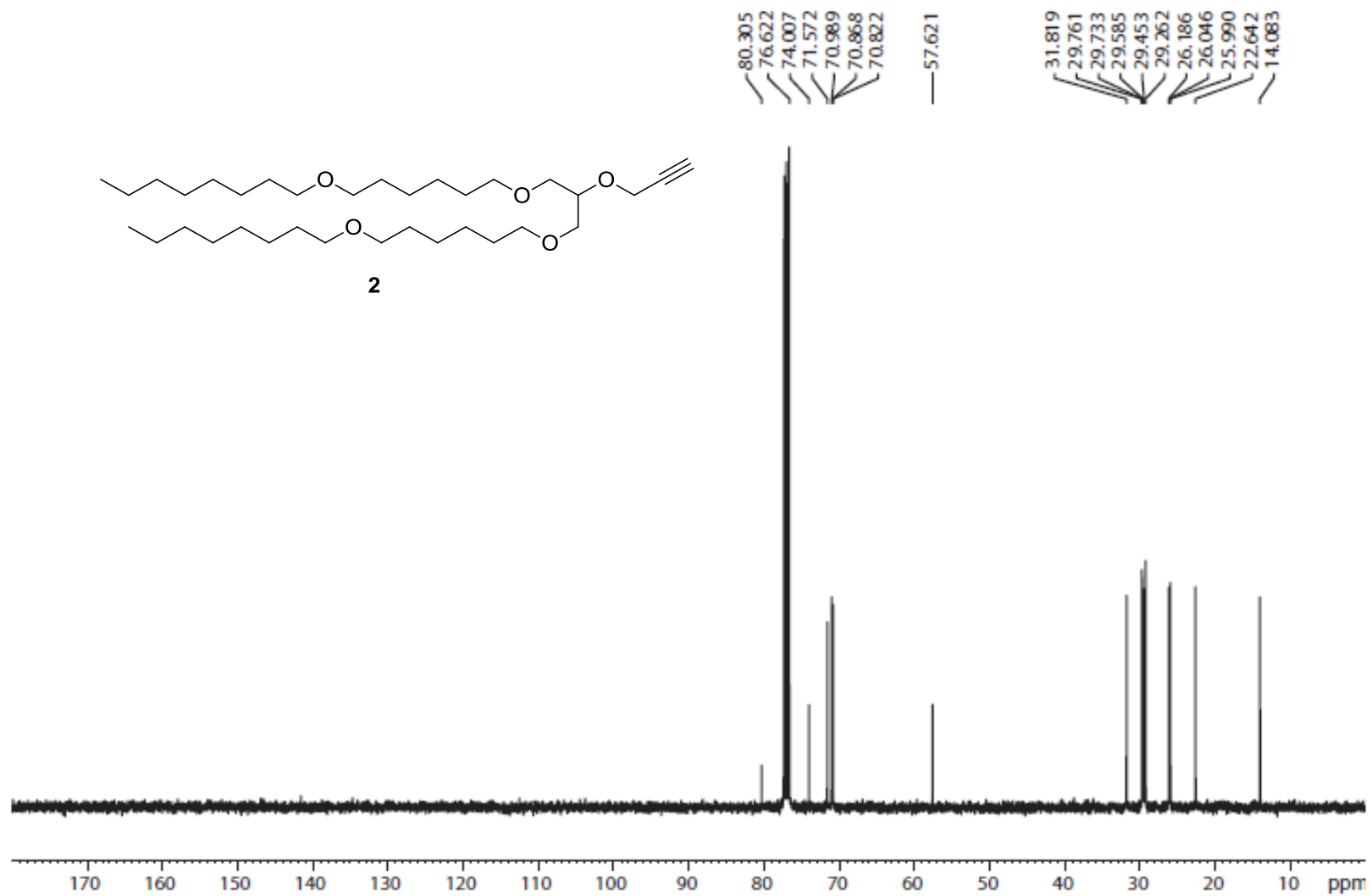


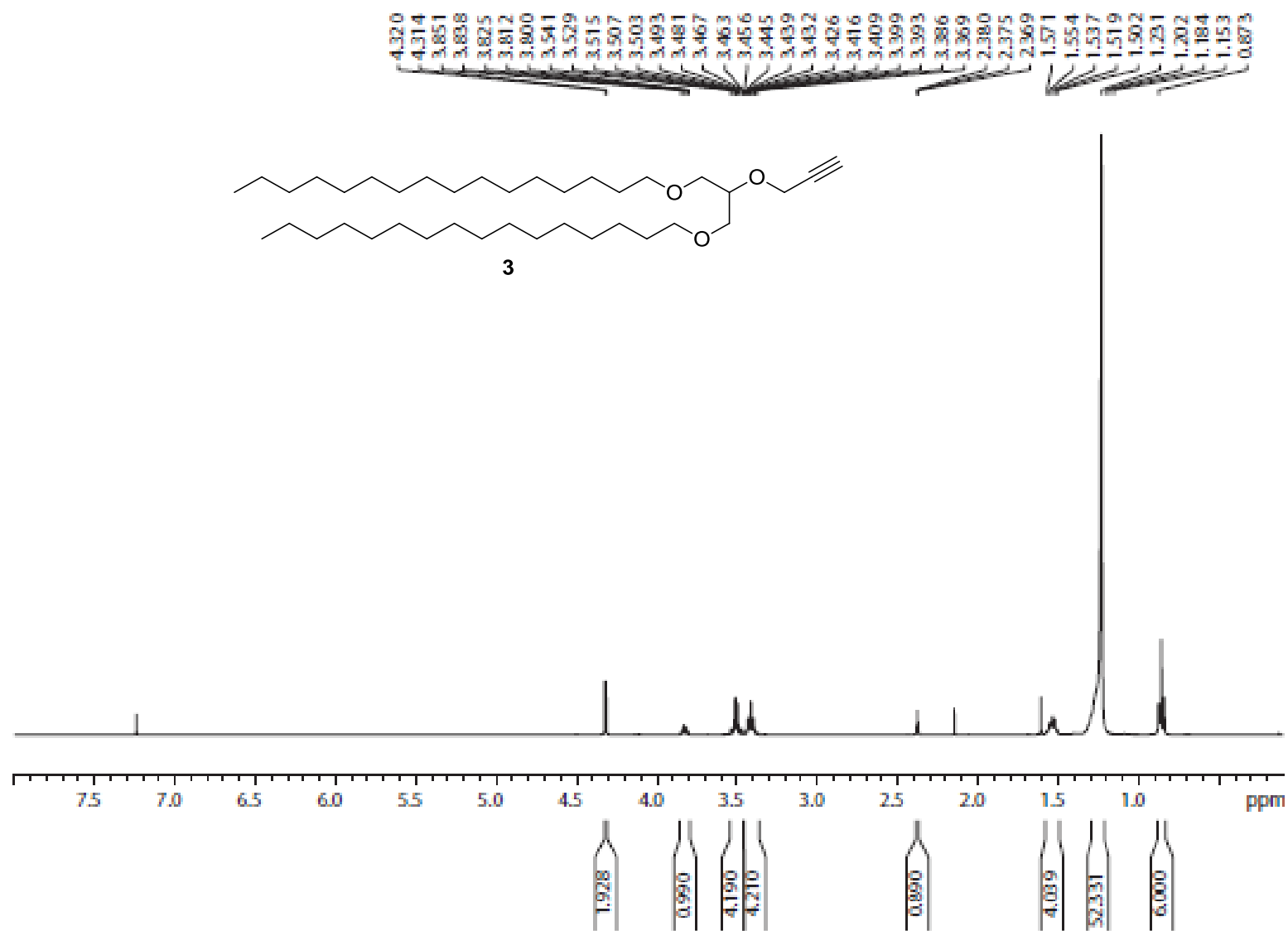


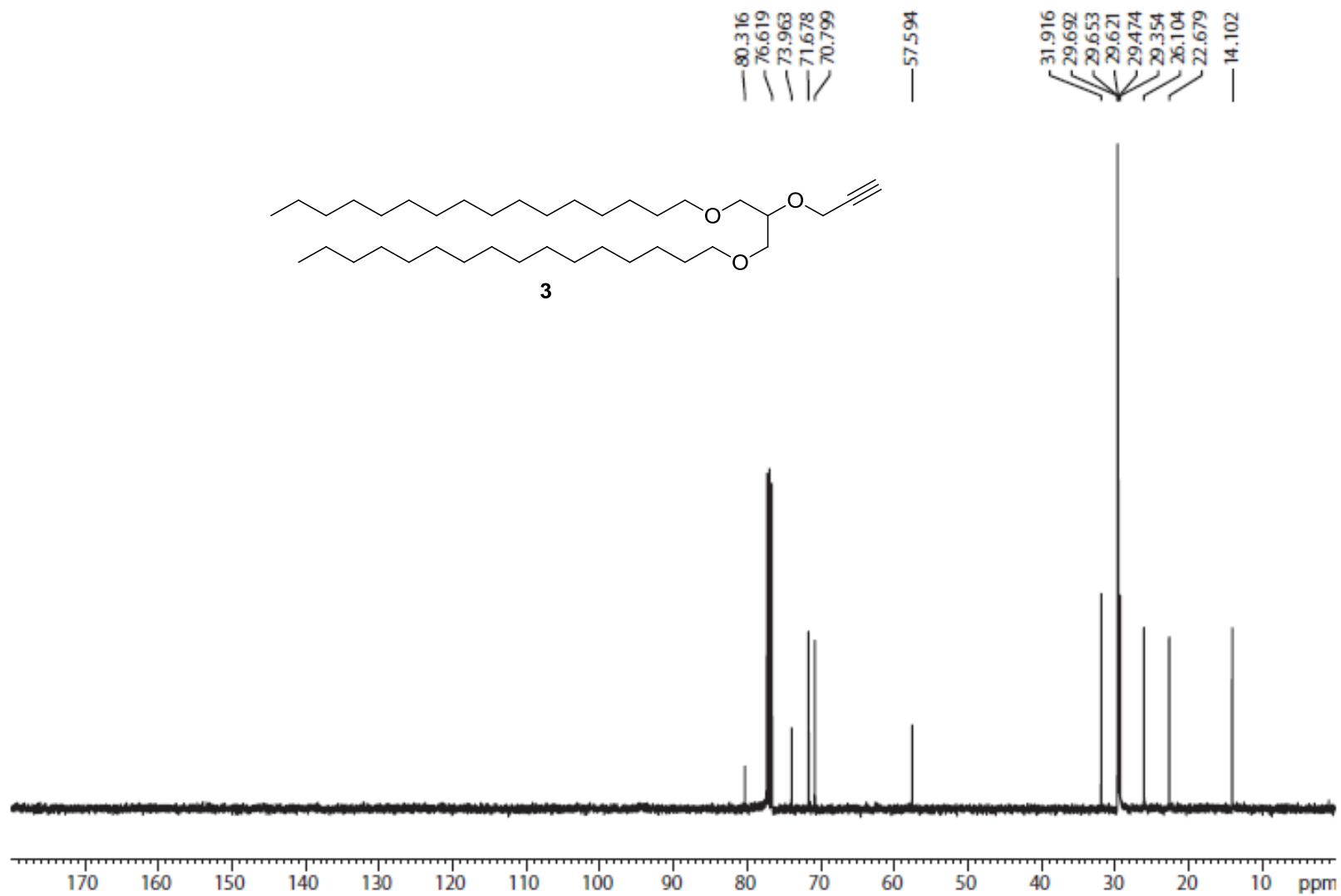






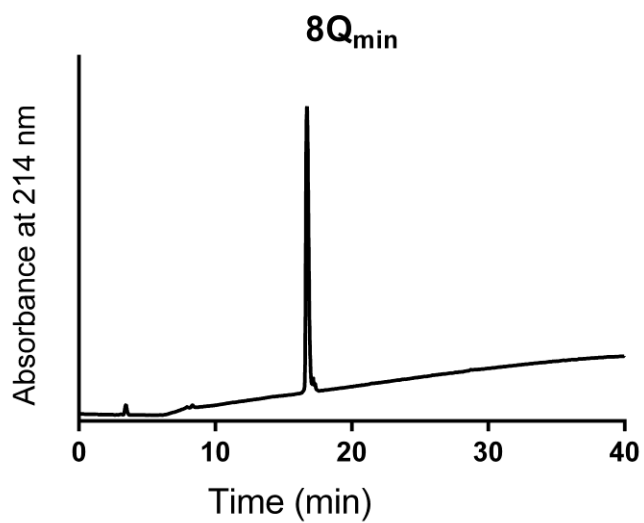
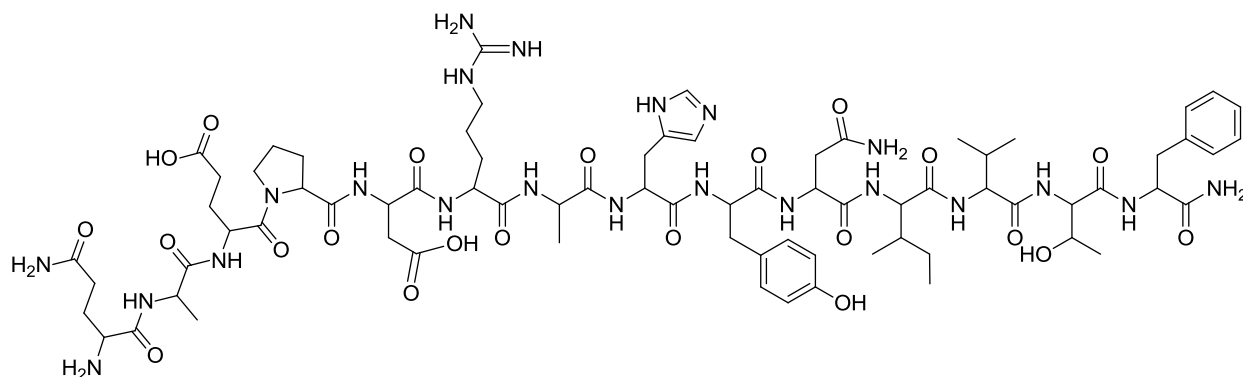




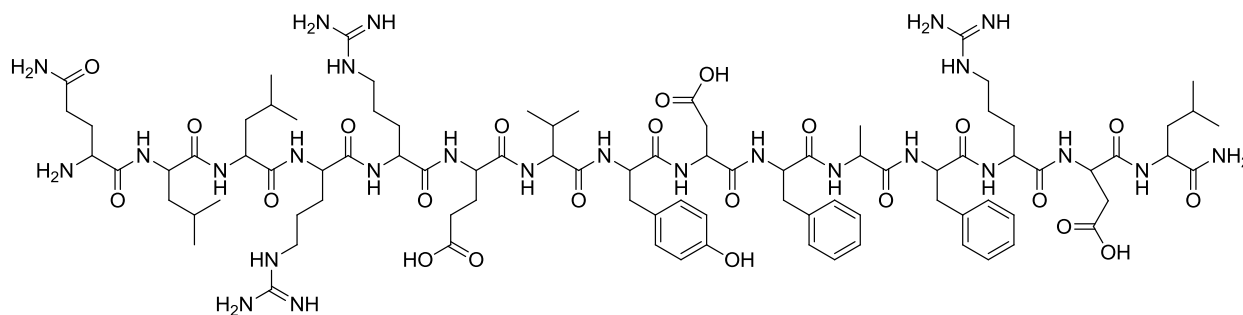


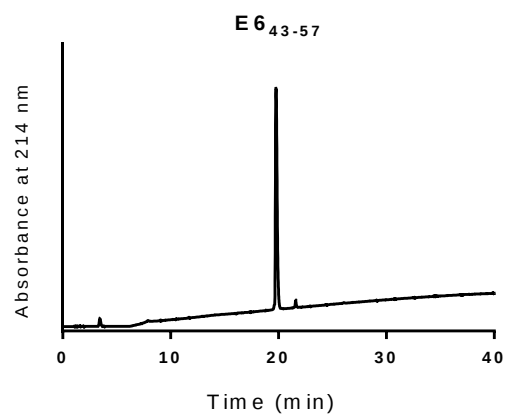
## Structure and HPLC for Known compounds and structure, HPLC and mass spec for novel compounds

### Structure of 8Q<sub>min</sub> Peptide.

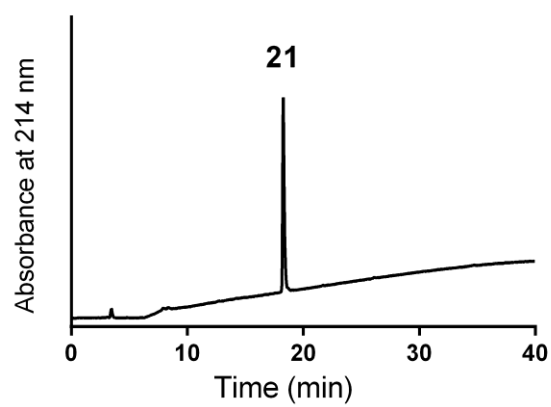
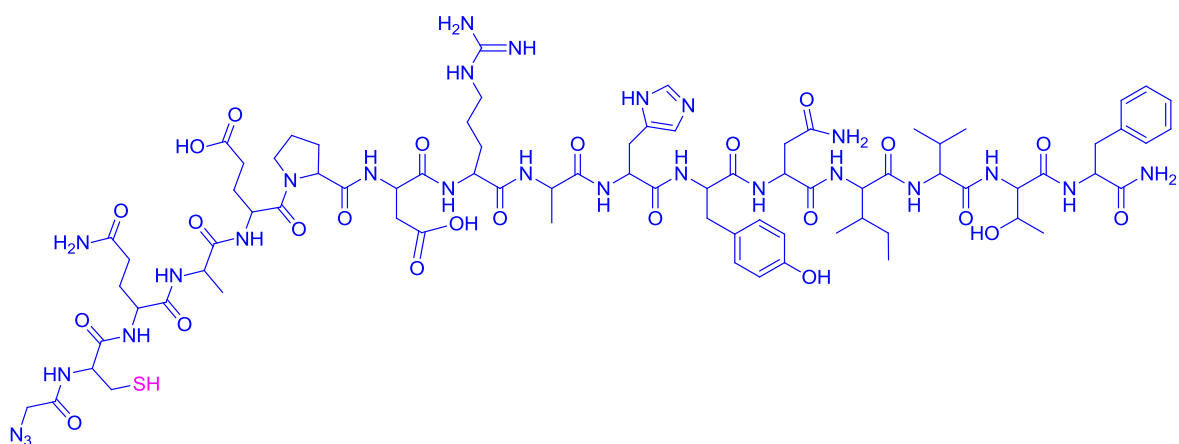


### Structure of E6<sub>43-57</sub>.

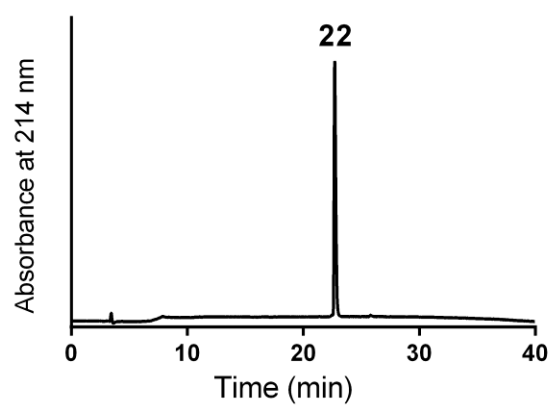
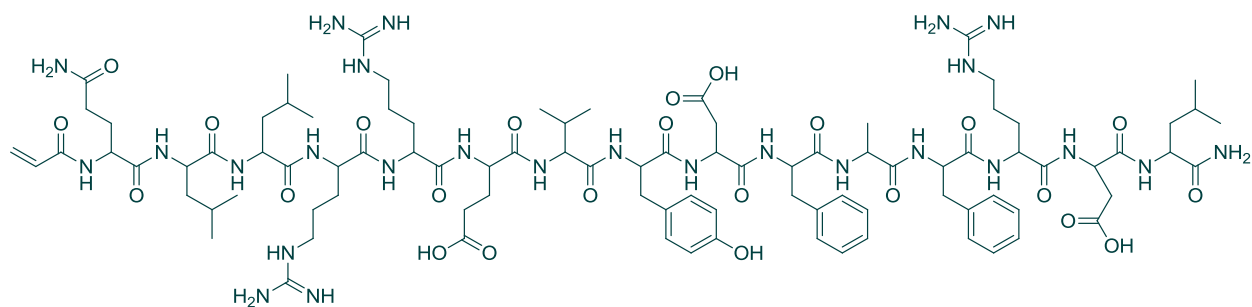




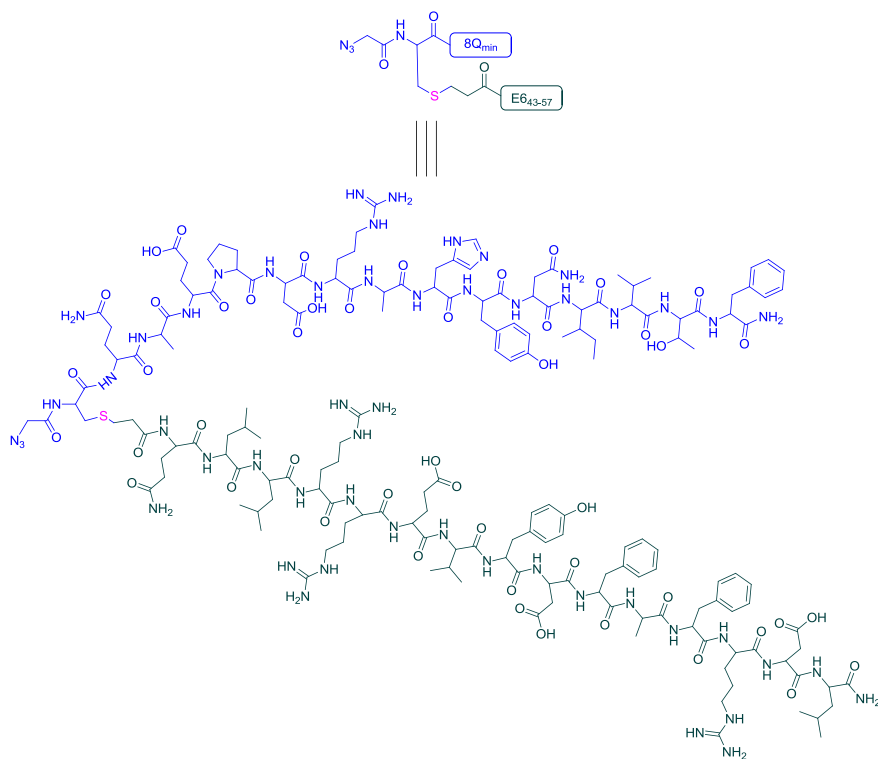
**Structure of N-terminus 8Q<sub>min</sub> mercapto-azide (21).**

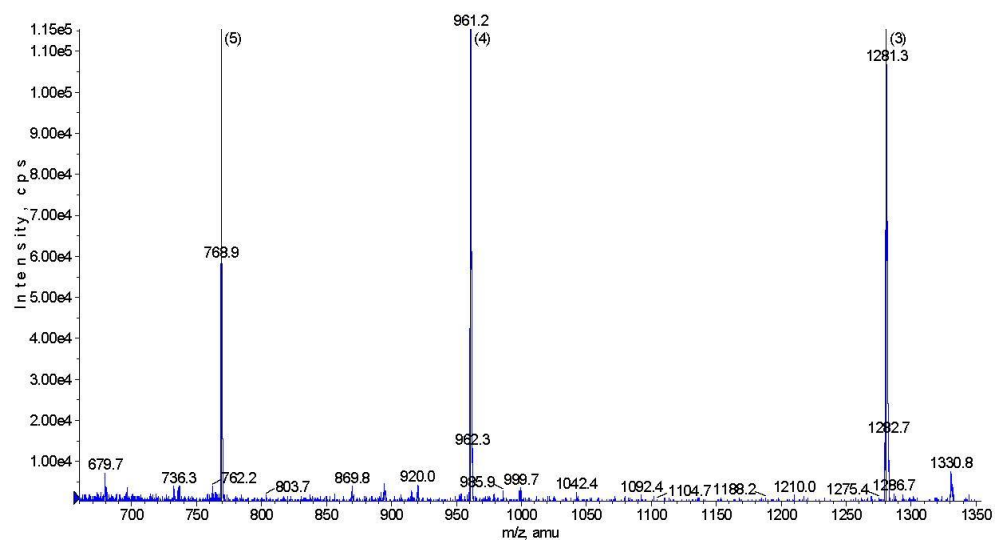
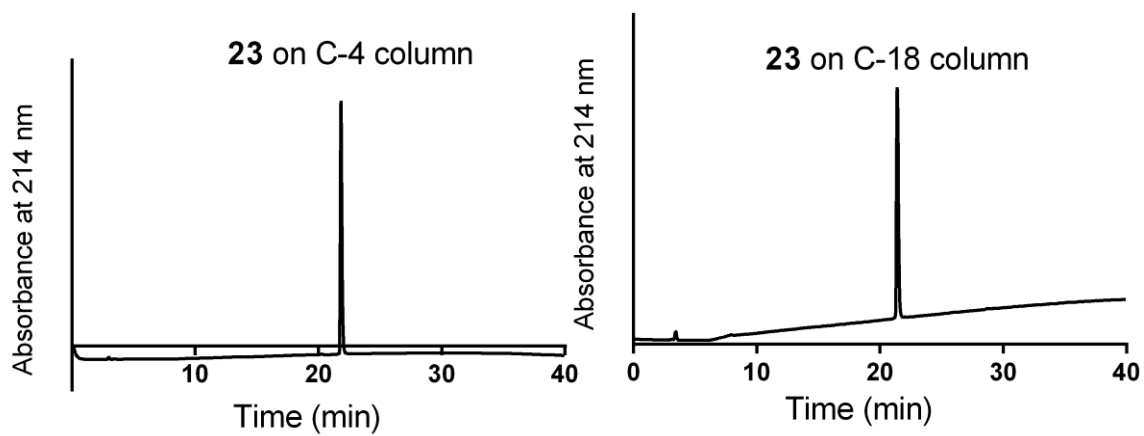


**Structure of N-terminal acryloyl E6<sub>43-57</sub> (22).**



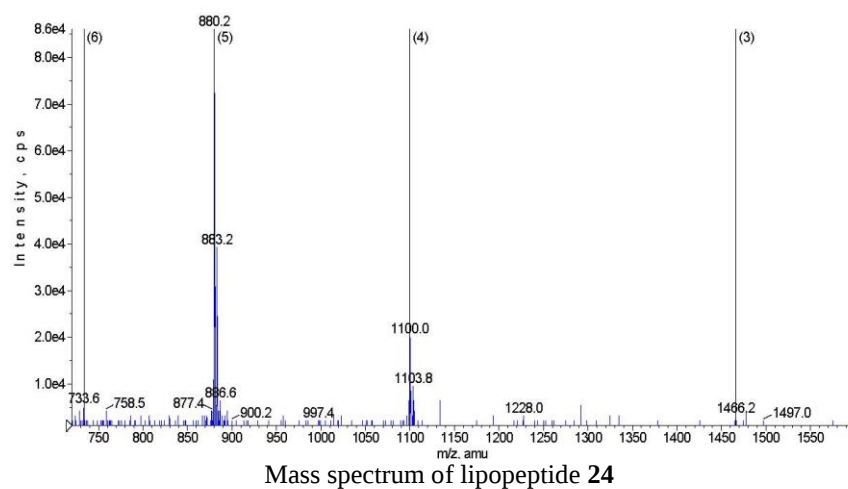
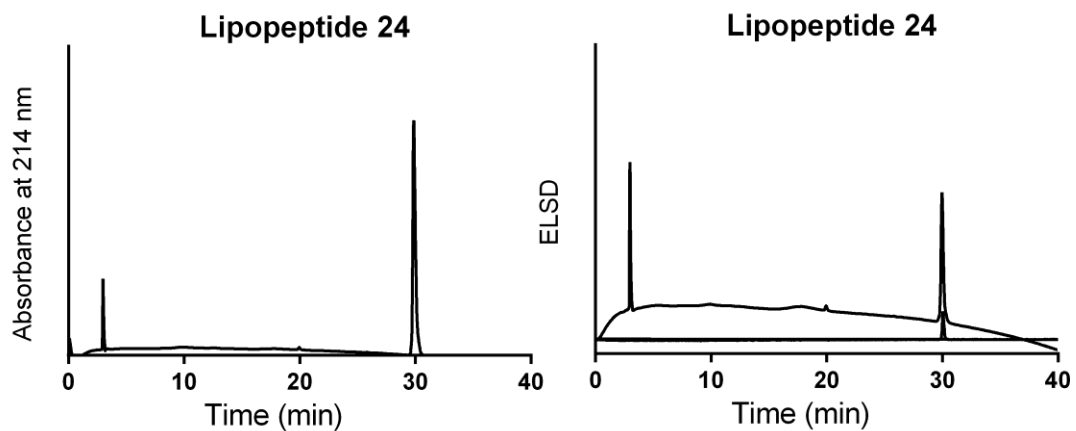
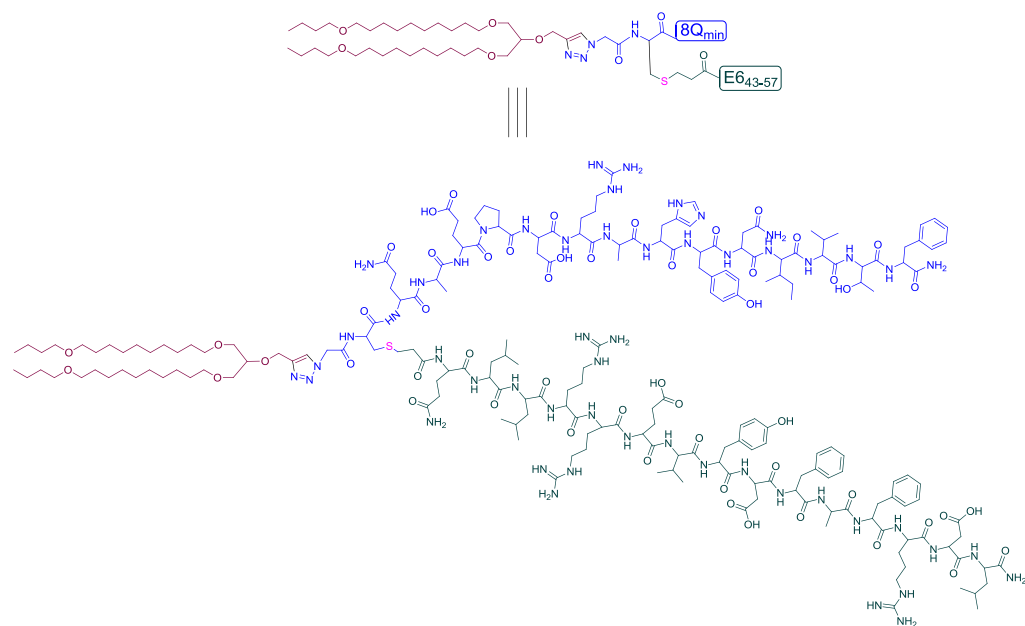
### Structure of multiantigenic peptide azide (23)



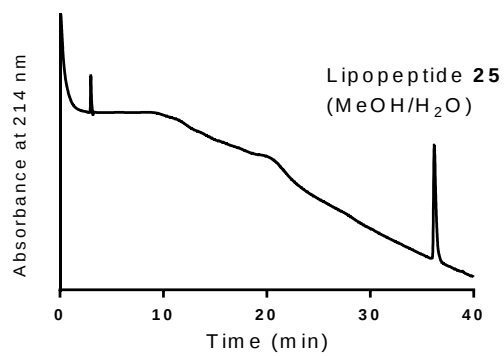
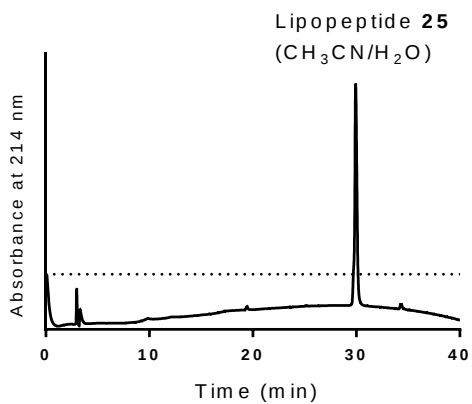
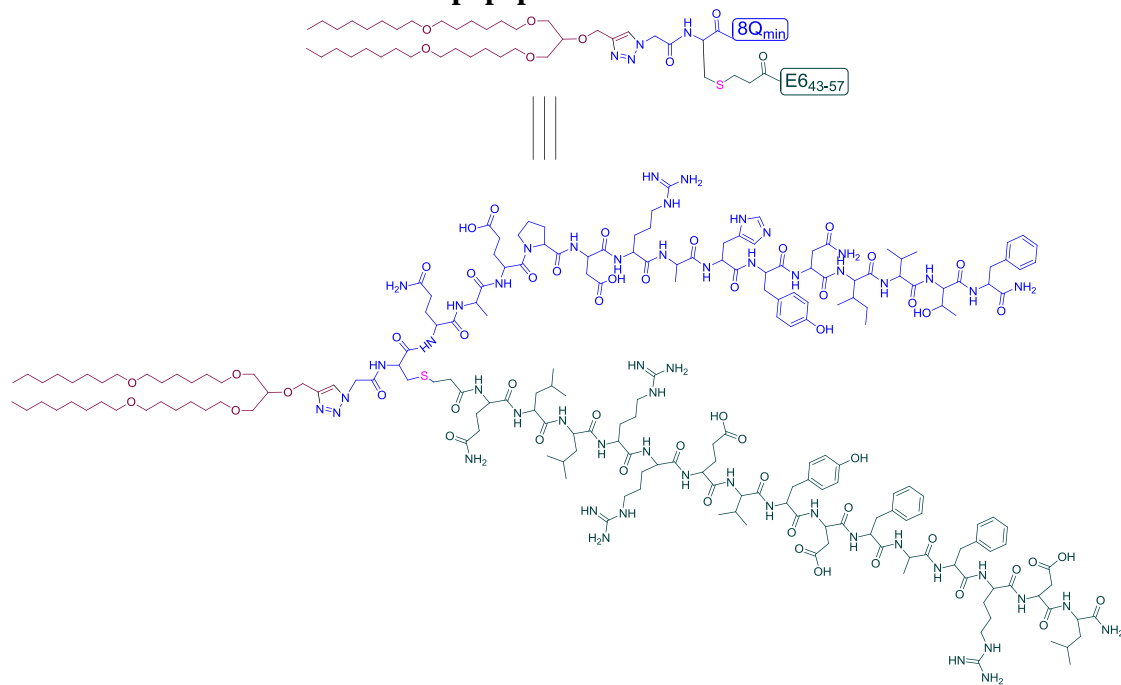


Mass spectrum of azide derivative (23)

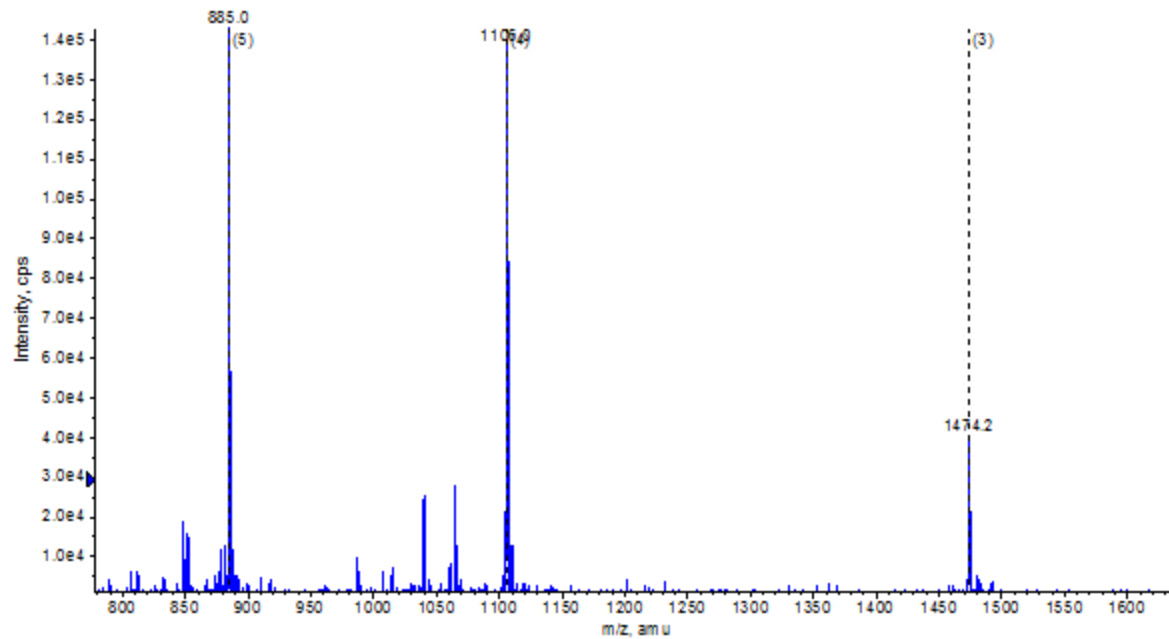
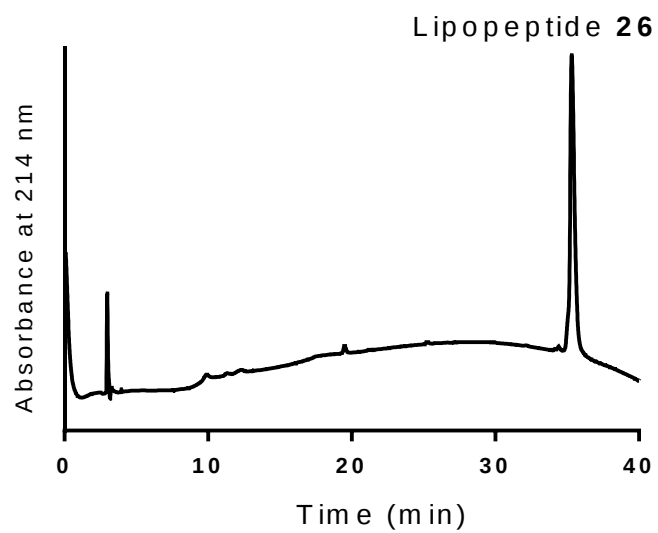
## Structure of vaccine candidate lipopeptide 24



## Structure of vaccine candidate lipopeptide 25

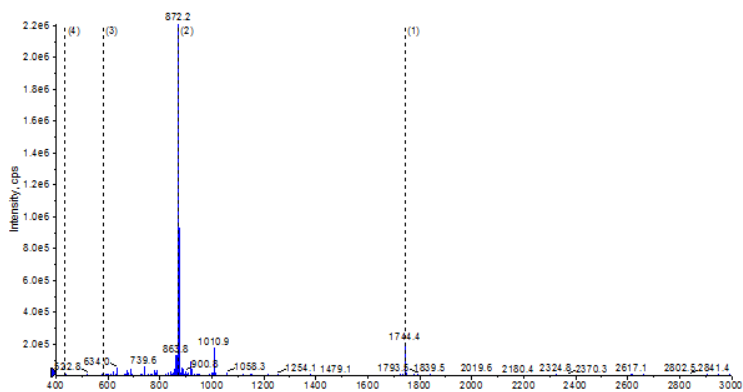
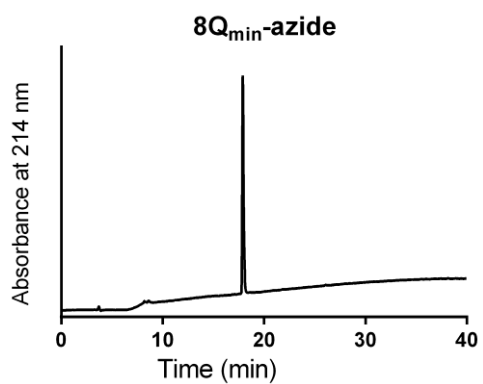
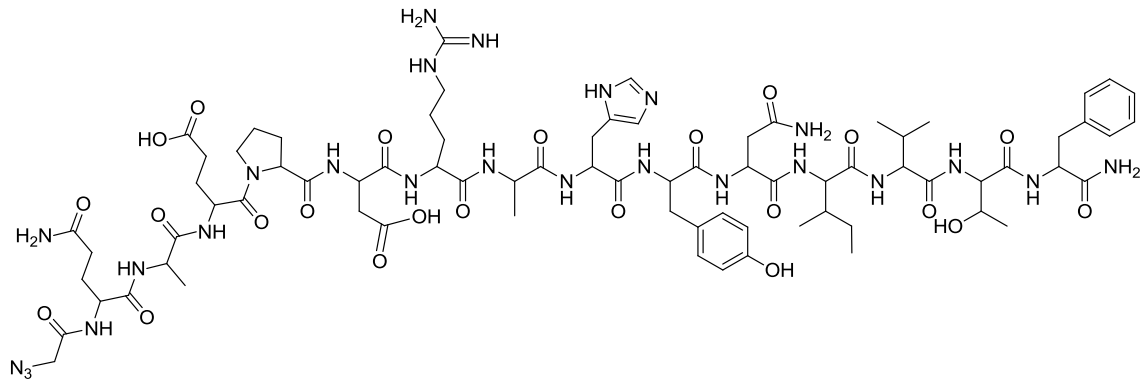






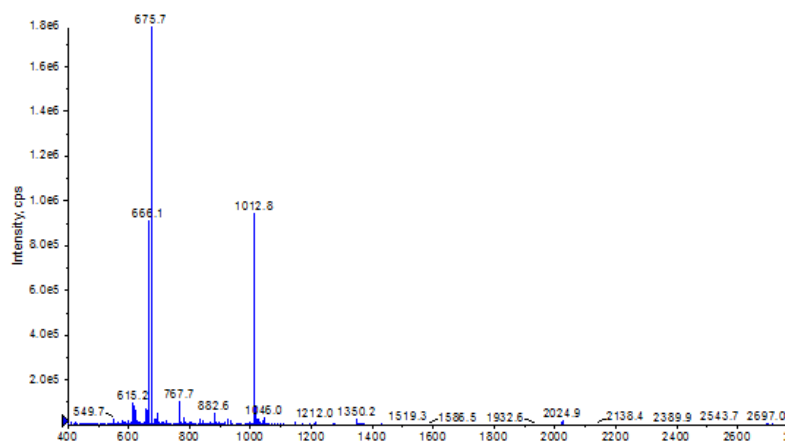
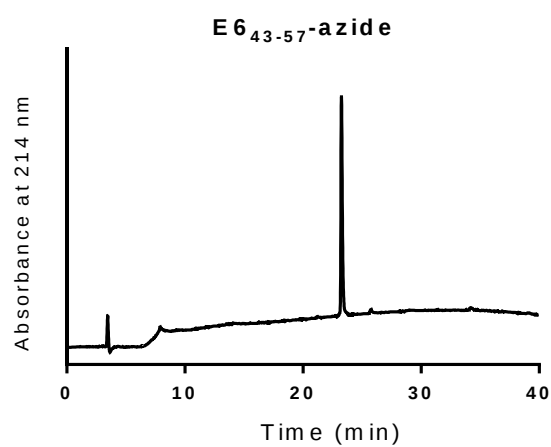
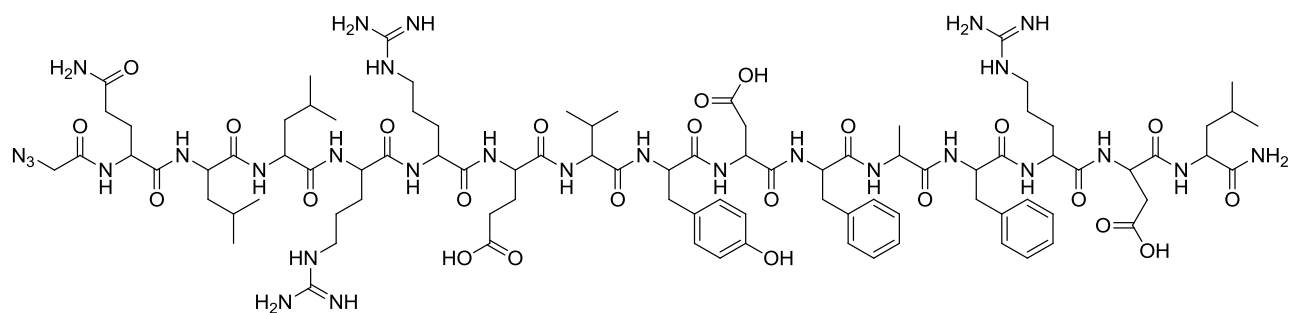
Mass spectrum of lipopeptide 26

## Structure of N-terminus 8Q<sub>min</sub>-azide.



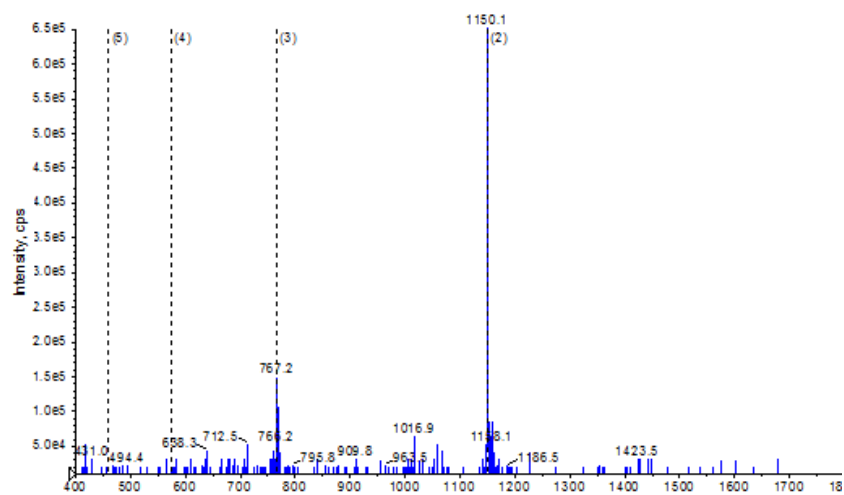
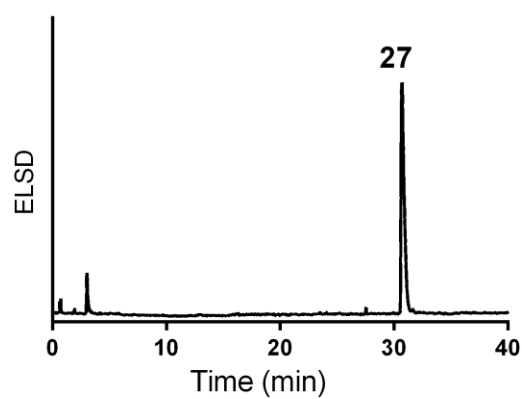
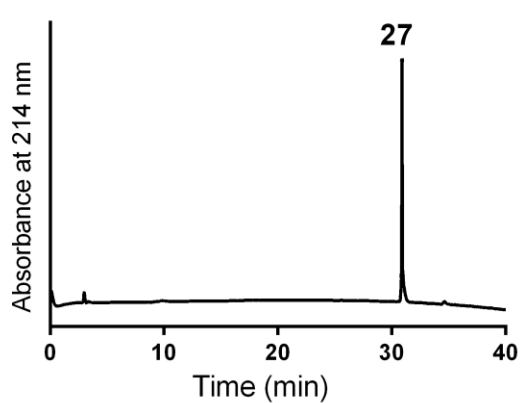
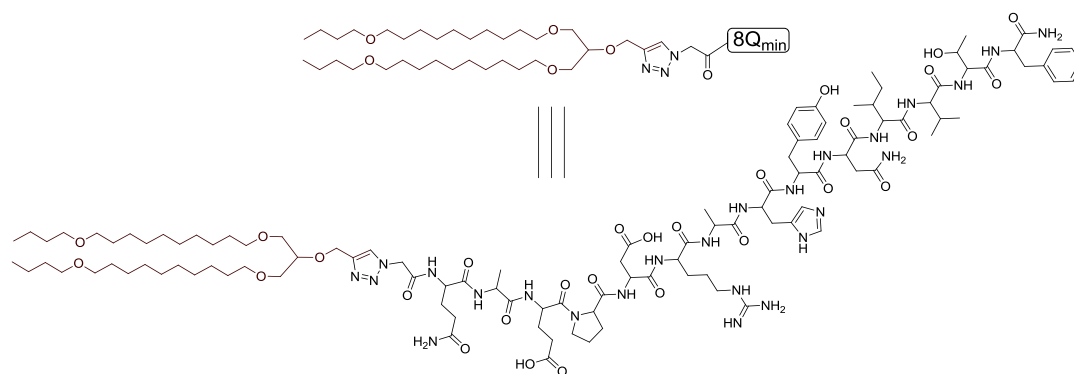
Mass spectrum of 8Q<sub>min</sub>-azide

## Structure of N-terminus E6<sub>43-57</sub>-azide.



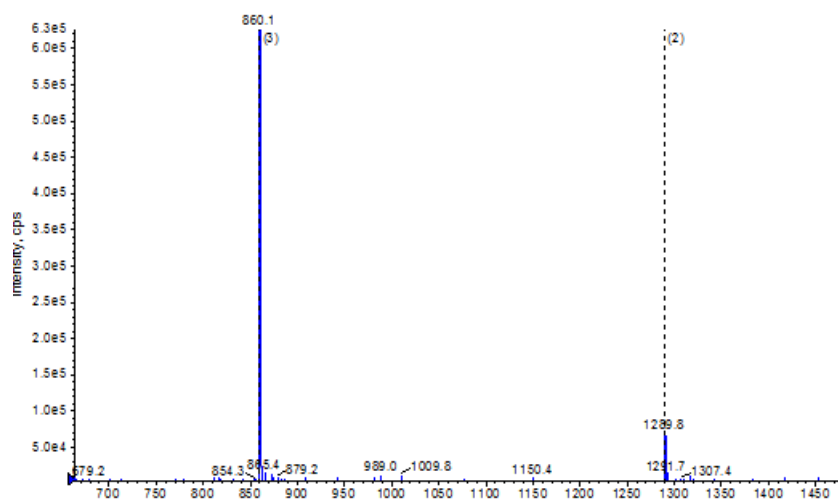
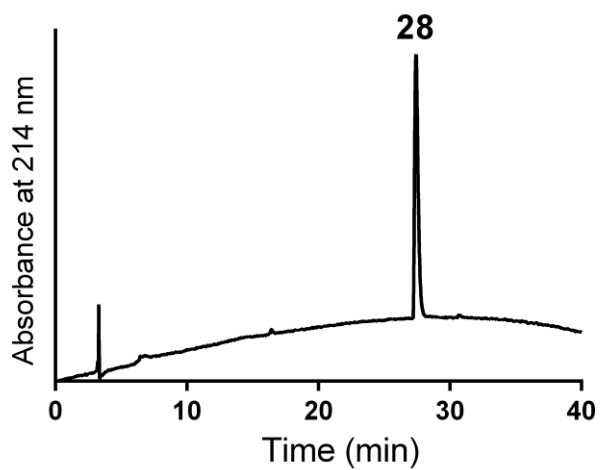
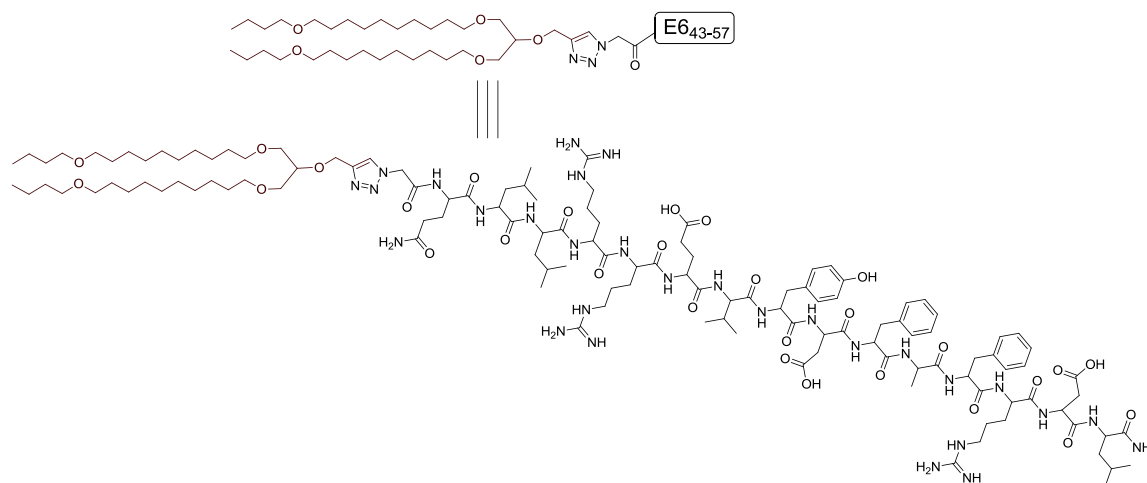
Mass spectrum of E6<sub>43-57</sub>-azide

## Structure of lipid 1 conjugated with 8Q<sub>min</sub> 27



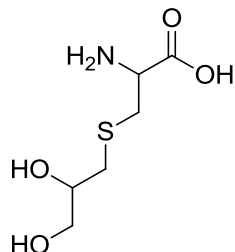
Mass spectrum of lipid 1 conjugated with 8Q<sub>min</sub> (27)

## Structure of lipid 1 conjugated with E6<sub>43-57</sub> (28)

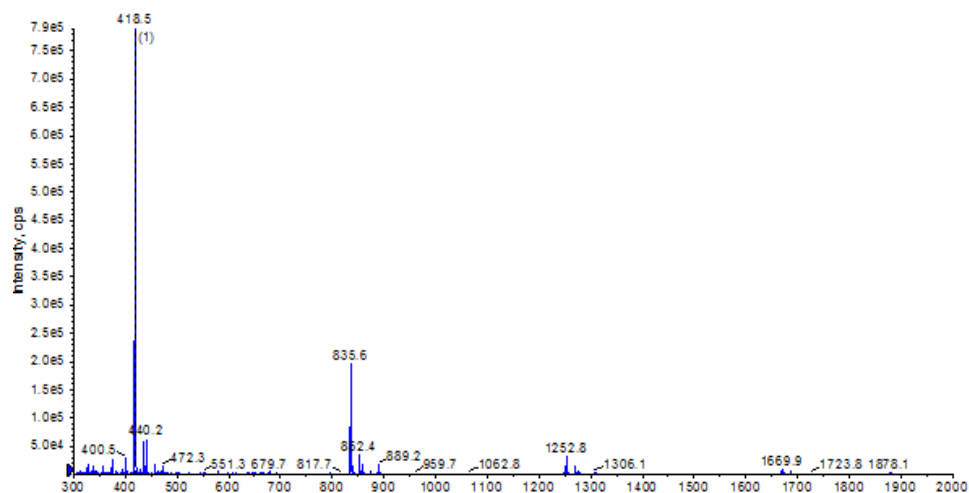
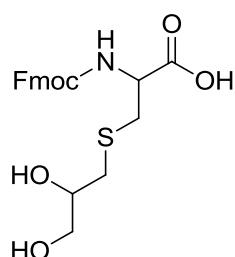


Mass spectrum of lipid 1 conjugated with E6<sub>43-57</sub> (28)

### Structure of S-(2,3-dihydroxypropyl) Cysteine (Dhc-OH)

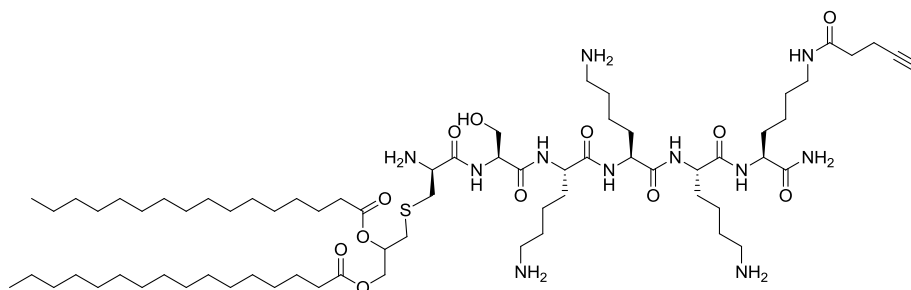


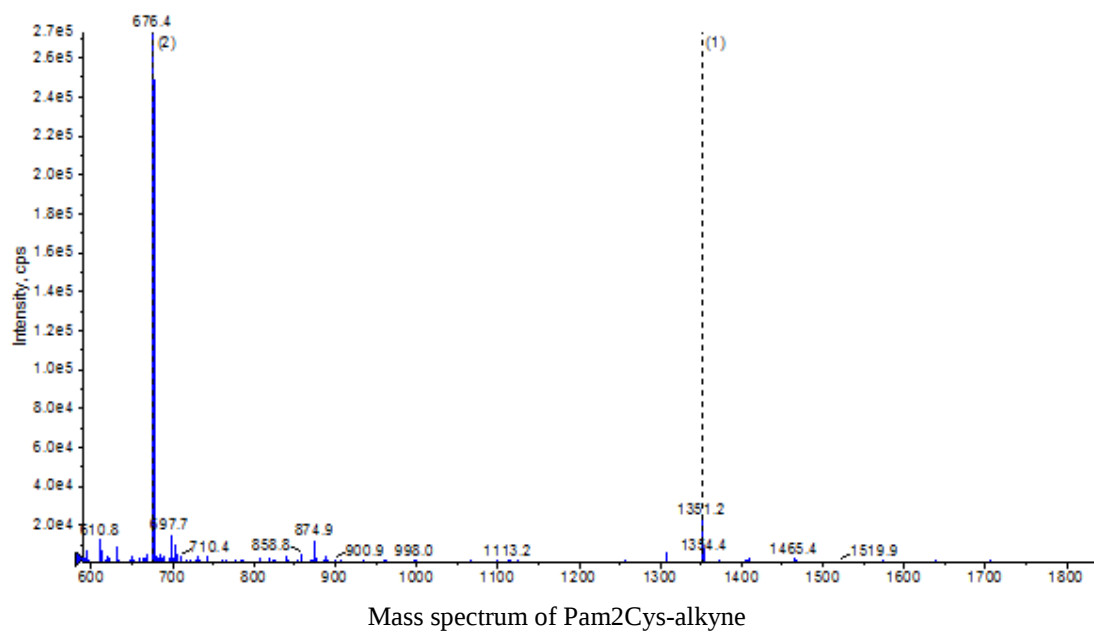
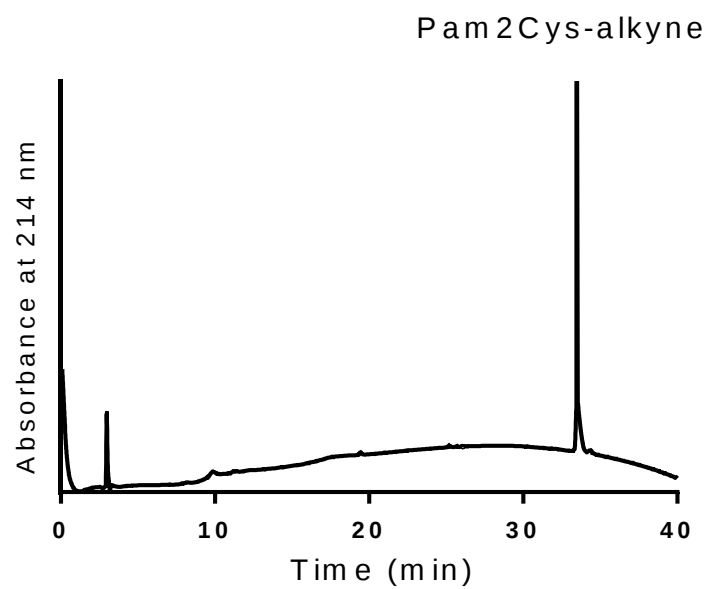
### Structure of N-fluorenylmethoxycarbonyl-S-(2,3-dihydroxypropyl) cysteine (Fmoc-Dhc-OH)



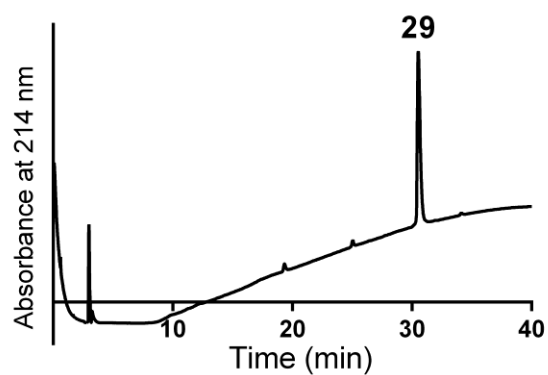
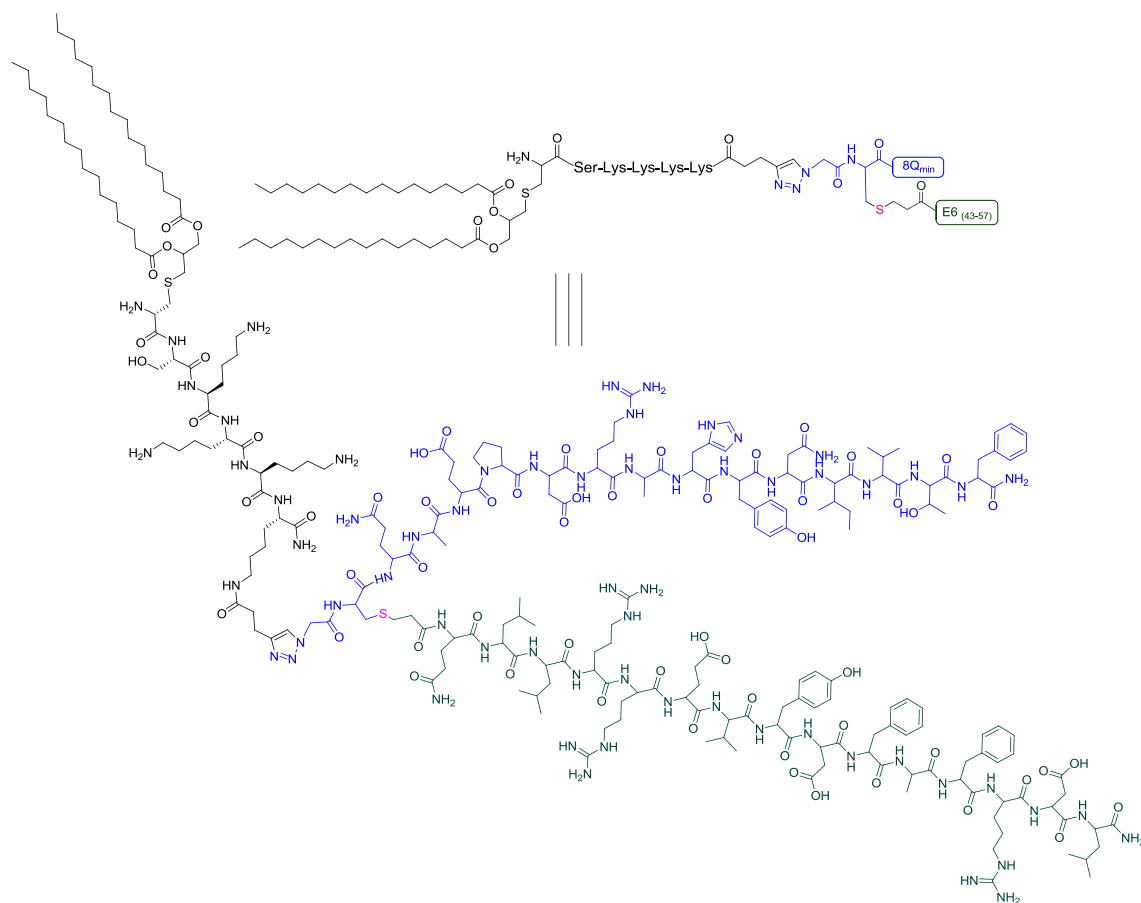
Mass spectrum of Fmoc-Dhc-OH

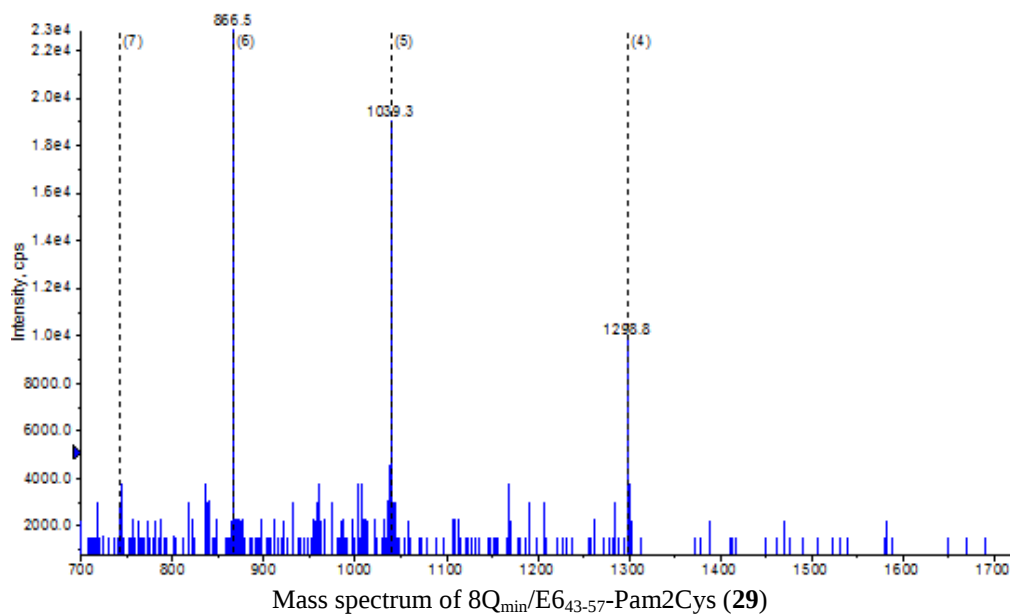
### Structure of Pam2Cys-alkyne





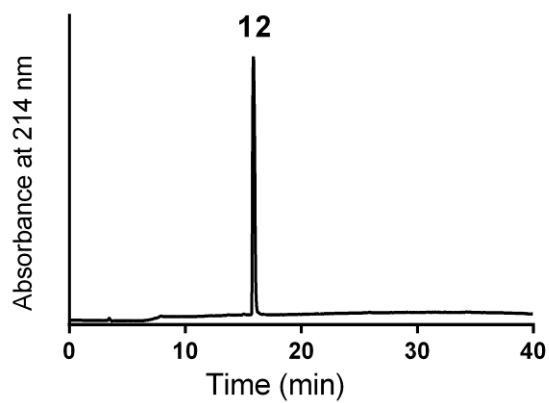
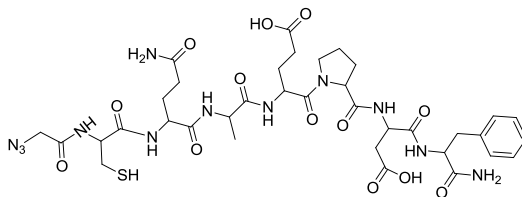
## Structure of 8Q<sub>min</sub>/E6<sub>43-57</sub>-Pam2Cys (29)



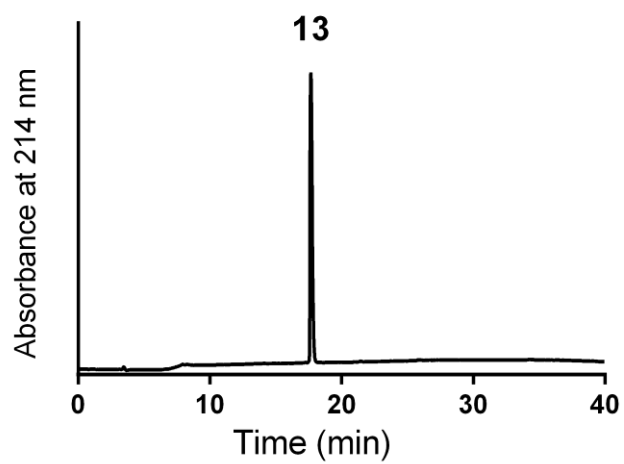
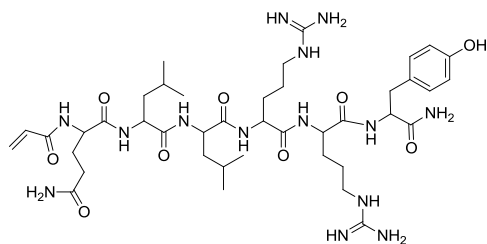


### Model Compounds

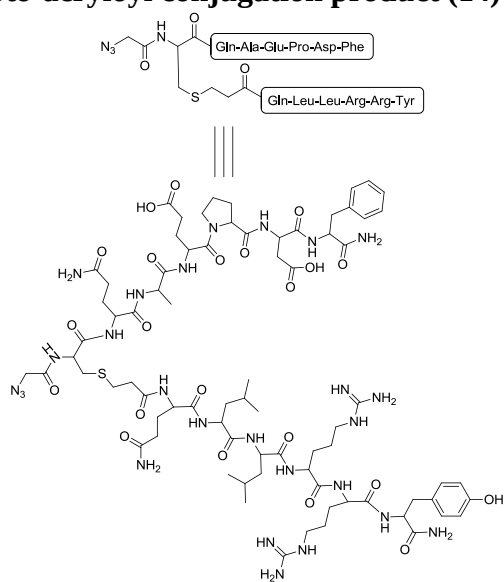
Structure of N-terminal Model mercapto-azide (12).

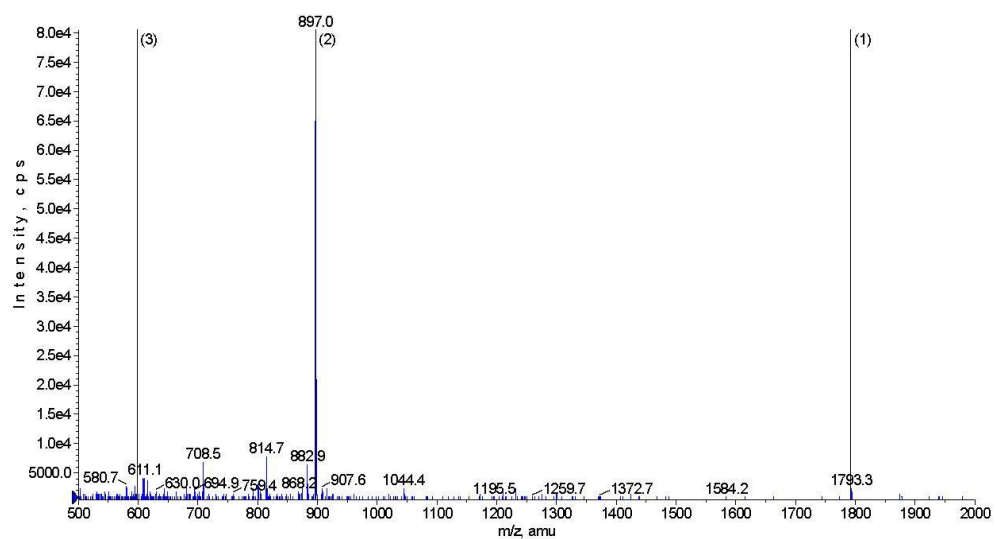
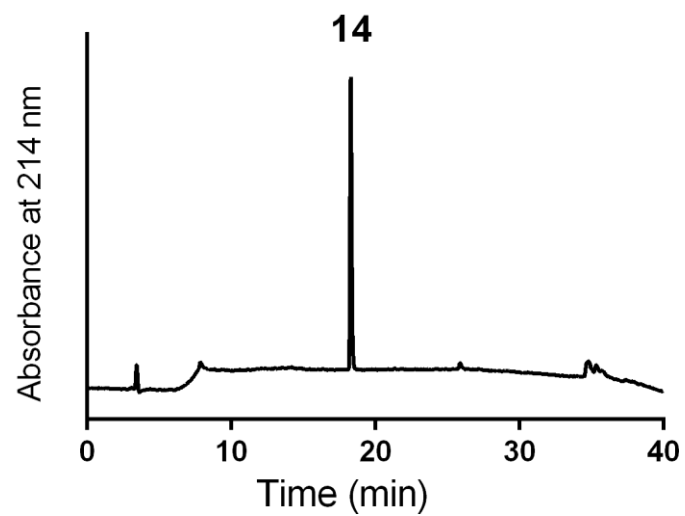


Structure of N-terminal acryloyl Model (13).



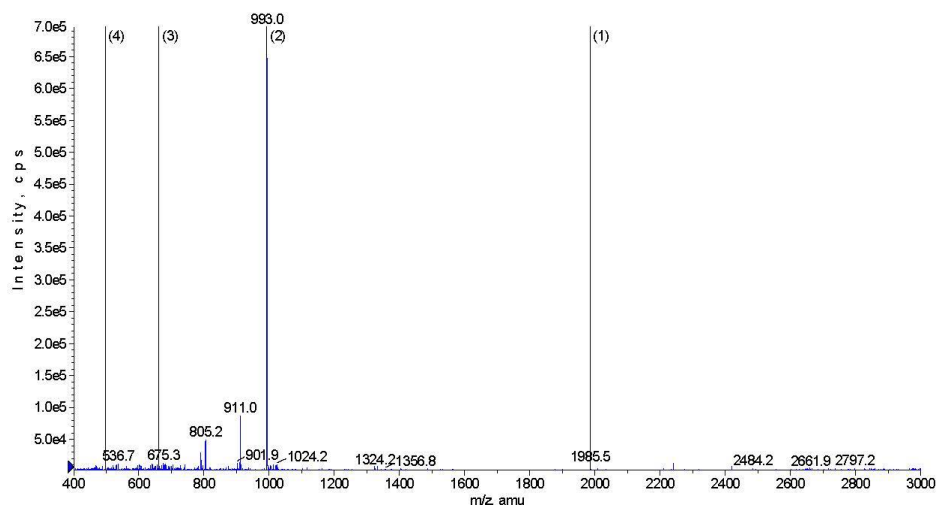
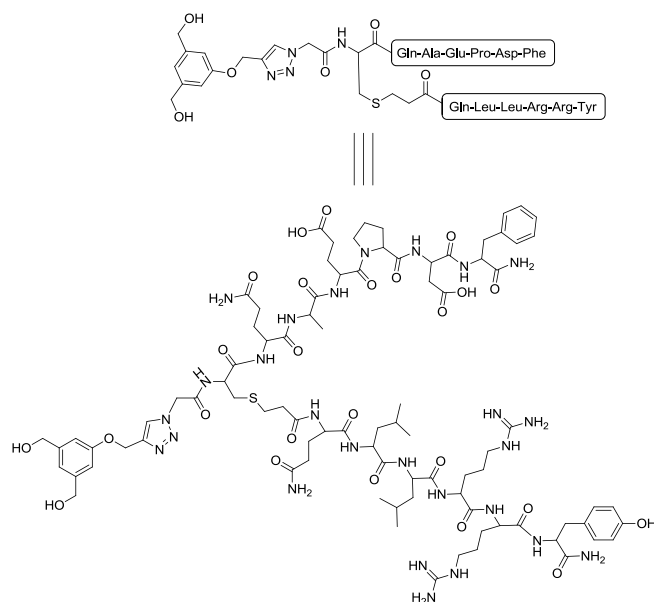
### Structure of Model mercapto-acryloyl conjugation product (14)





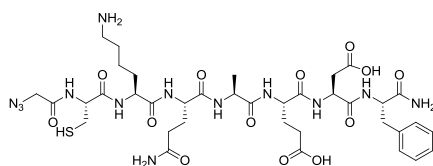
Mass spectrum of Model mercapto-acryloyl conjugation product (**14**)

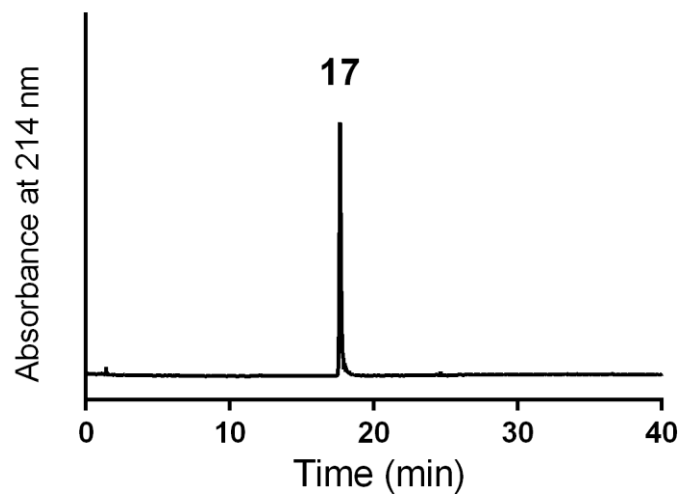
## Structure of compound 16.



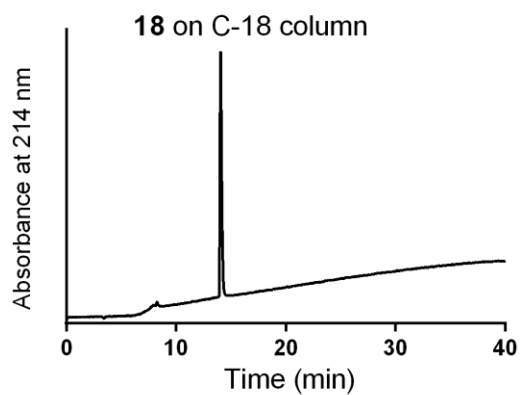
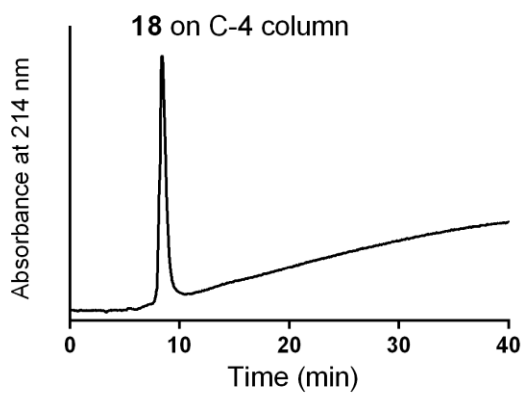
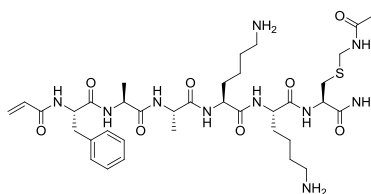
Mass spectrum of Model CuAAC product (16)

## Structure of N-terminal Model mercapto-azide (17).

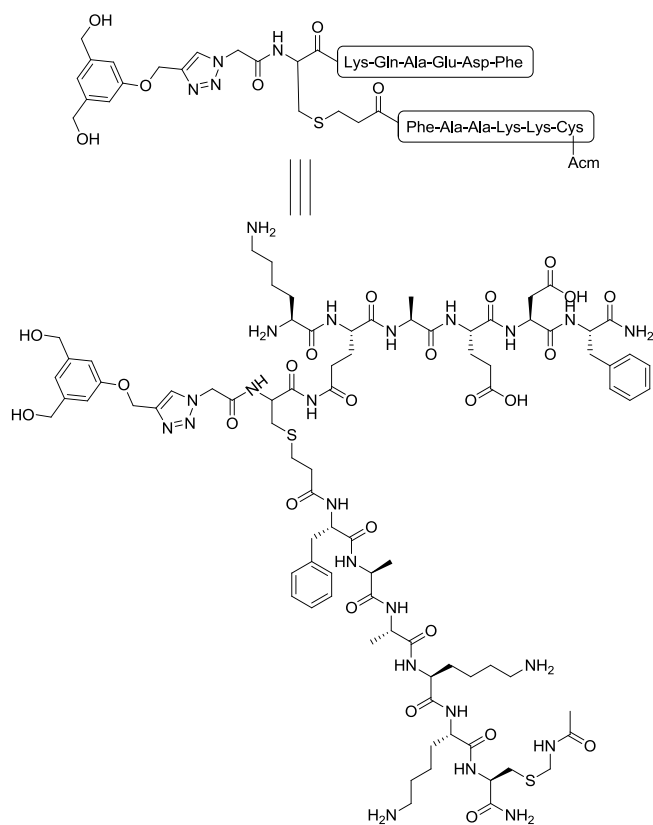


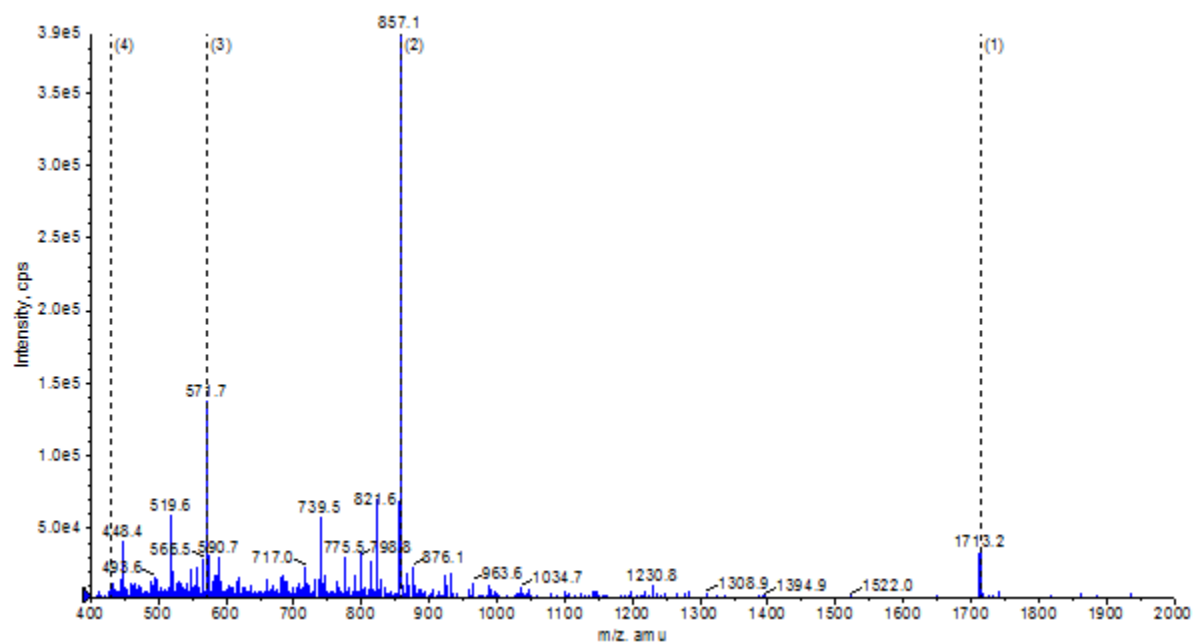


Structure of N-terminal acryloyl Model (18).

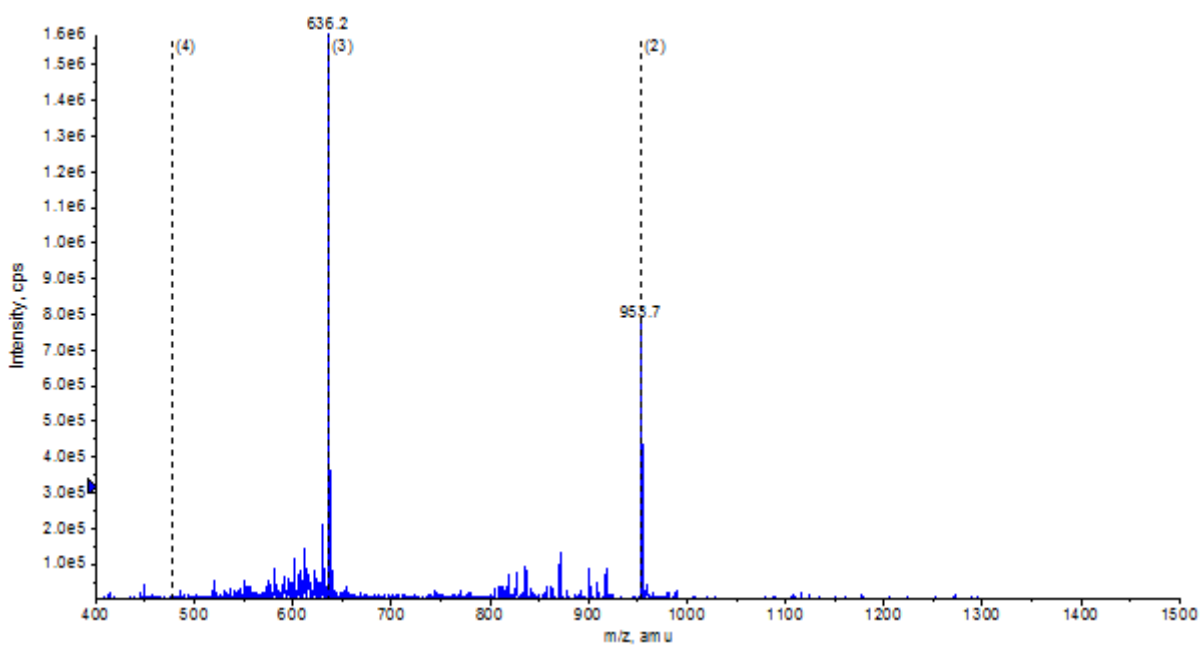


## Structure of 20





Mass spectrum of 19



Mass spectrum of 20