cancers

MDPI

Article

Antibody-Drug Conjugate Using Ionized Cys-Linker-MMAE as the Potent Payload Shows Optimal Therapeutic Safety

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Supplementary Materials

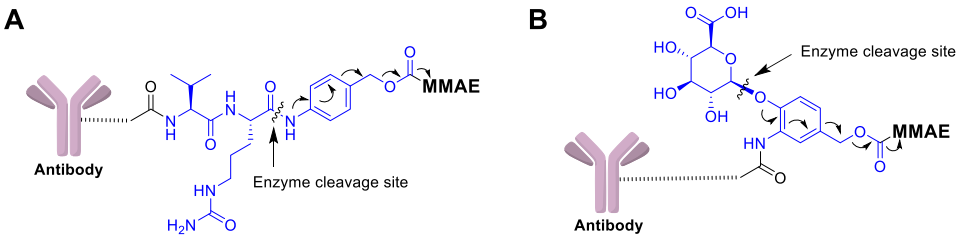

Figure S1. Structures of cleavable linker systems for current MMAE-based ADCs. (**A**) Structure of the cathepsin B-cleavable ADCs and its drug release pattern. (**B**) Structure of the β-glucuronidase-cleavable ADCs and its drug release pattern.

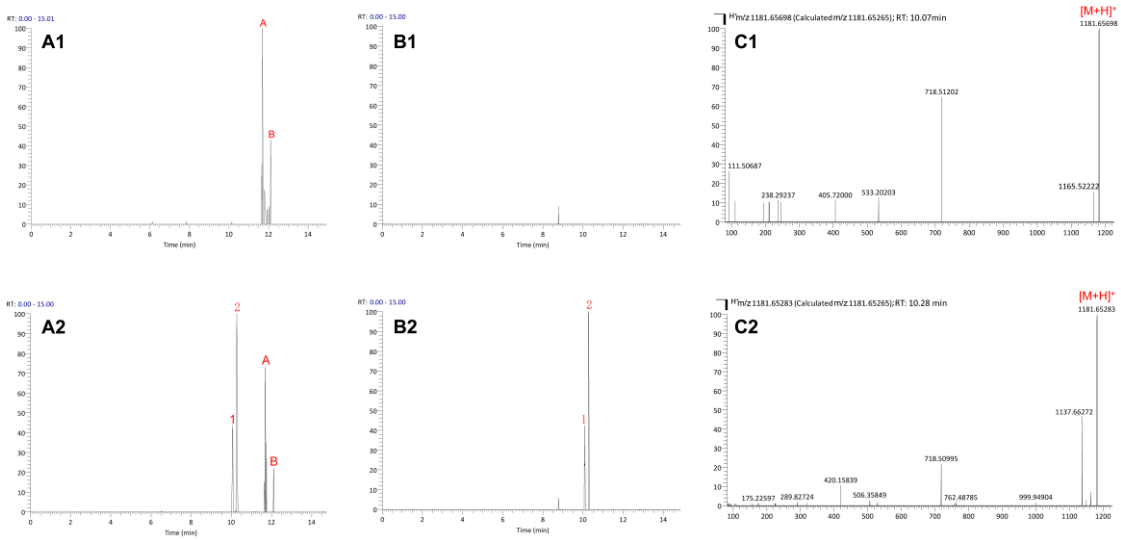

Figure S2. Drug release study of the Cys-linker-MMAE-based ADC at the cellular level. (**A1**) The XIC of full MS/ddMS2 scan of the vehicle in BT-474 cells; (**A2**) The XIC of full MS/ddMS2 scan of the metabolites in BT-474 cells; (**B1**) The XIC of full MS/ddMS2 scan of the vehicle in NCI-N87 cells; (**B2**) The XIC of full MS/ddMS2 scan of the metabolites in NCI-N87 cells; (**C1**) The MS2 fragmentation of M1 (R.T. = 10.07 min); (**C2**) The MS2 fragmentation of M2 (R.T. = 10.28 min). The compound A (m/z 591.32976, R.T=11.69 min) and B (m/z 591.32976, R.T = 12.11 min) could also be detected in the control group of BT-474 cells (Figure A1 and A2), meaning that they are not related to the ADC being administered. Each dosing group included two replicates. R.T.: retention time.

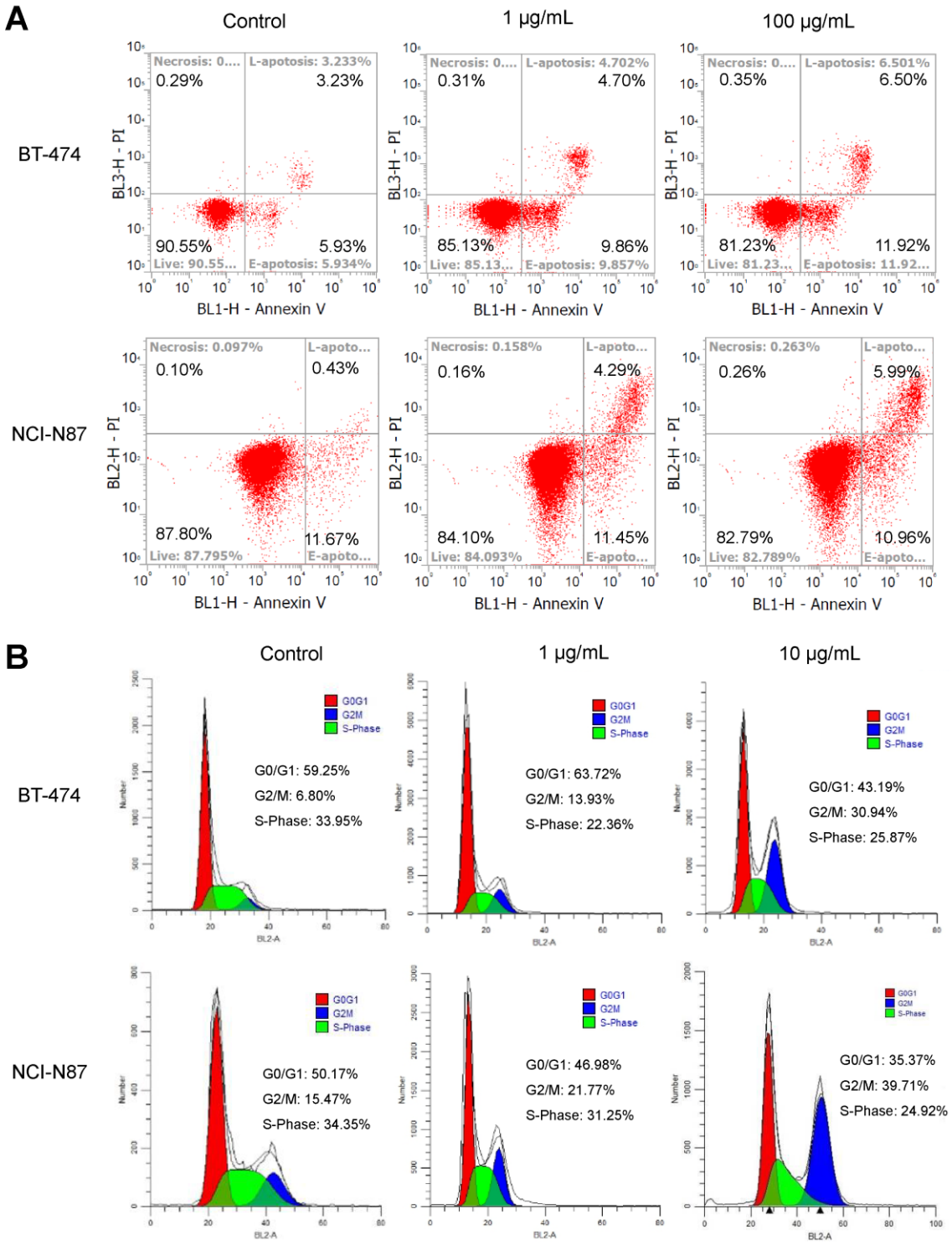

Figure S3. Flow cytometry for apoptosis and cell cycle arrest analysis. (**A**) The induction of apoptosis in BT-474 and NCI-N87 cells was detected by flow cytometry; the cells were treated with mil40-**15** for 24 h. (**B**) Cell cycle arrest analysis in the BT-474 and NCI-N87 cells was detected by flow cytometry; the cells were treated with mil40-**15** for 24 h.

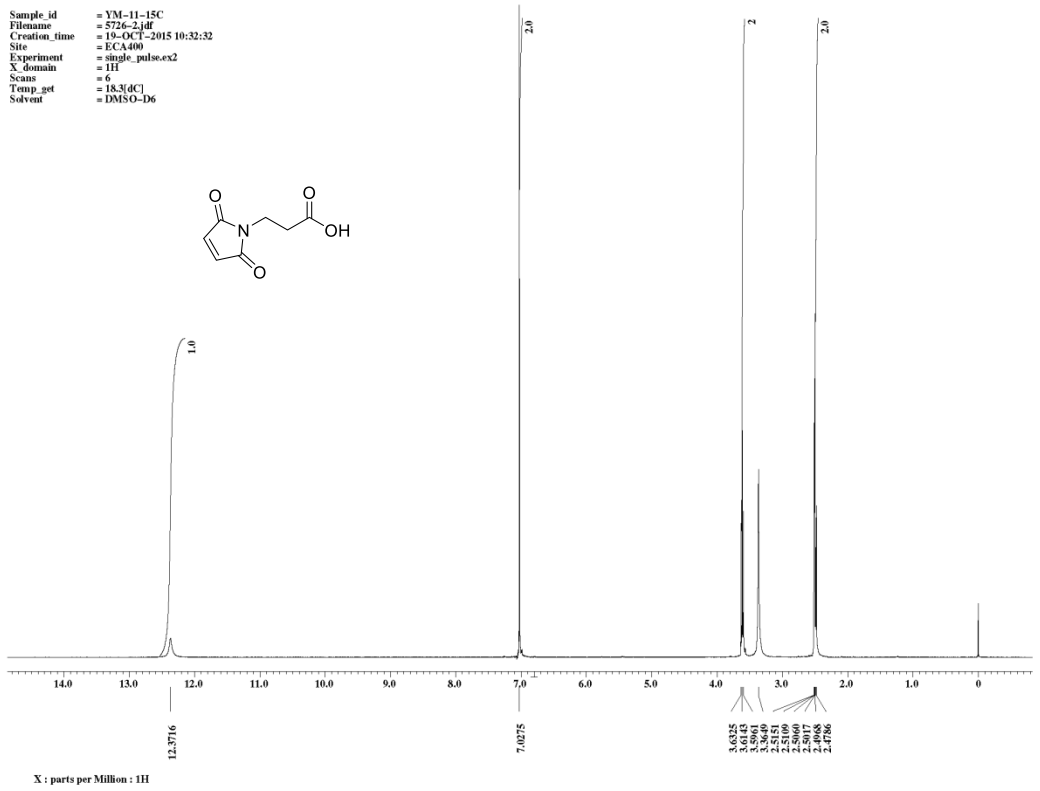

Figure S4. The ^{1}H-NMR spectrum of compound **4.**

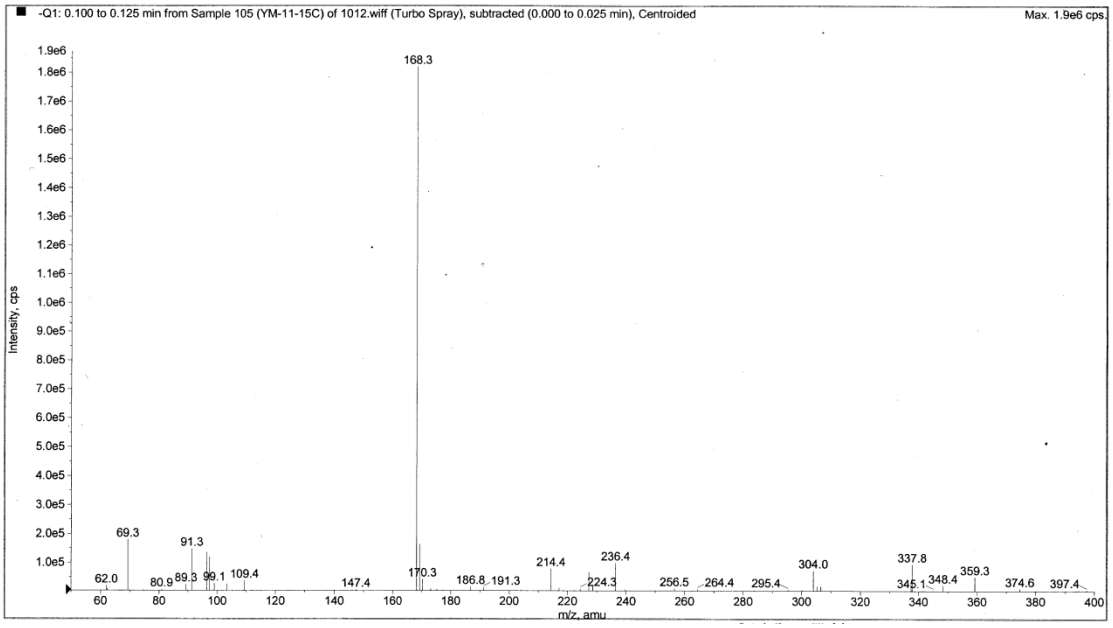

Figure S5. The ESI-MS spectrum of compound **4**.

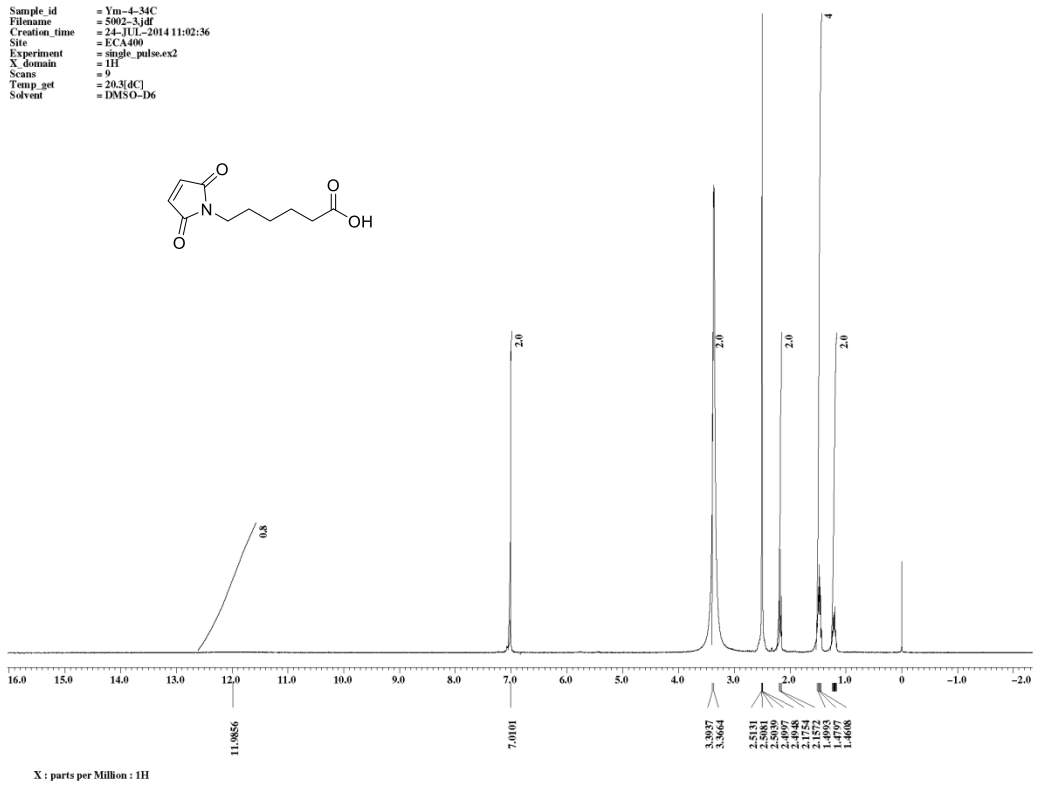

Figure S6. The ^{1}H-NMR spectrum of compound **5**.

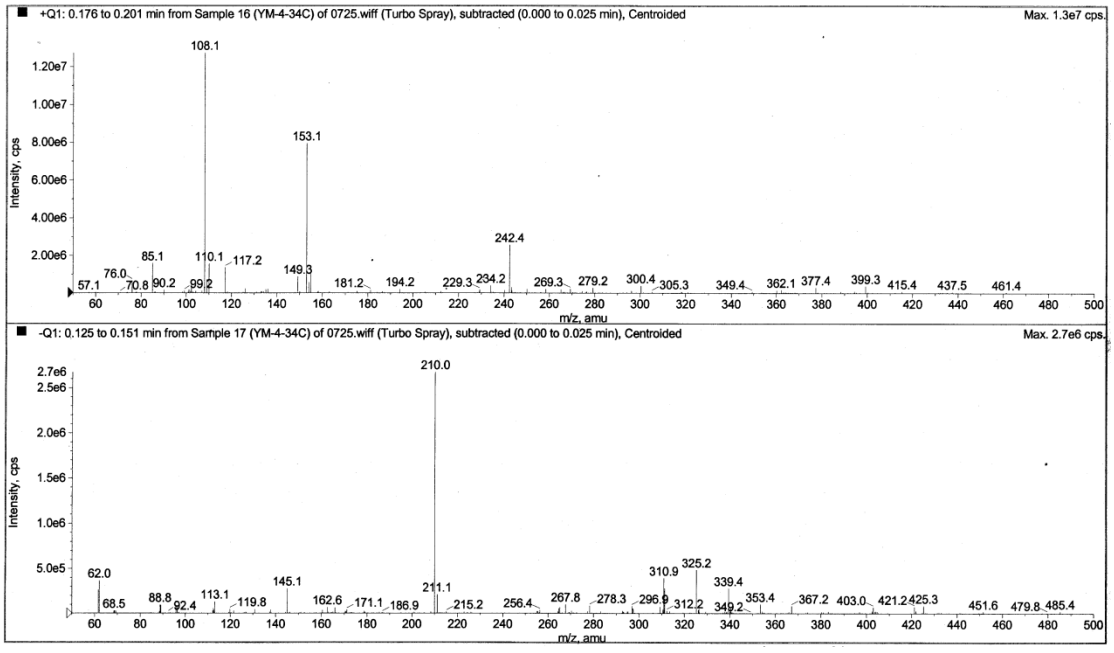

Figure S7. The ESI-MS spectrum of compound **5**.

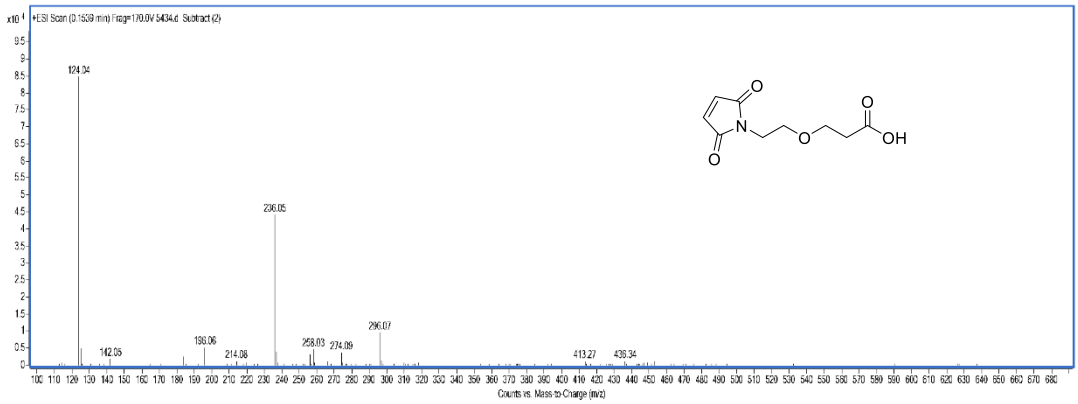

Figure S8. The ESI-MS spectrum of compound **6**.

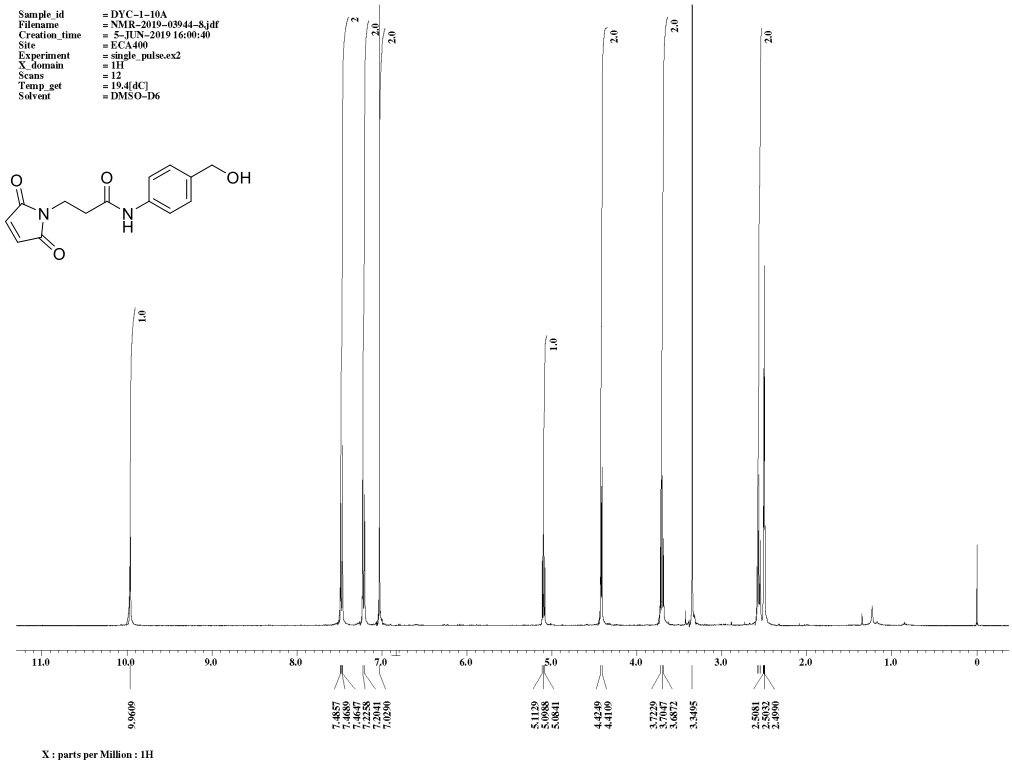

Figure S9. The ^{1}H-NMR spectrum of compound **7**.

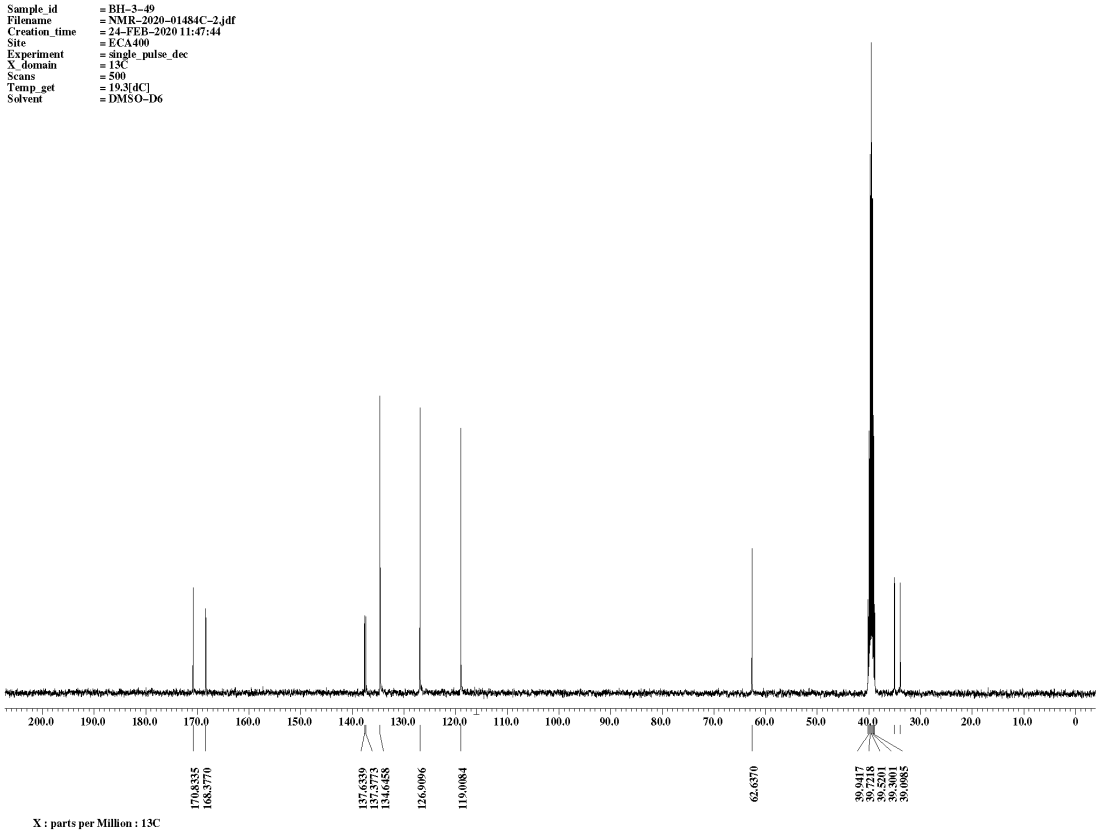

Figure S10. The ^{13}C-NMR spectrum of compound **7**.

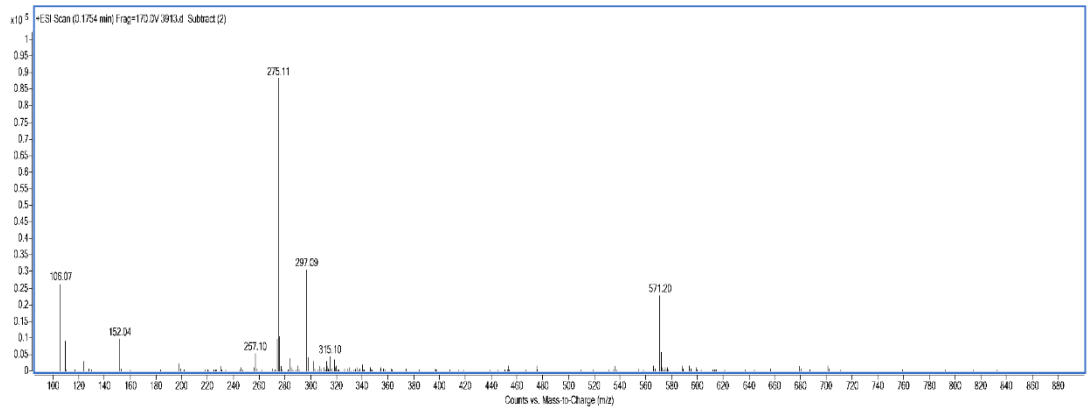

Figure S11. The ESI-MS spectrum of compound **7**.

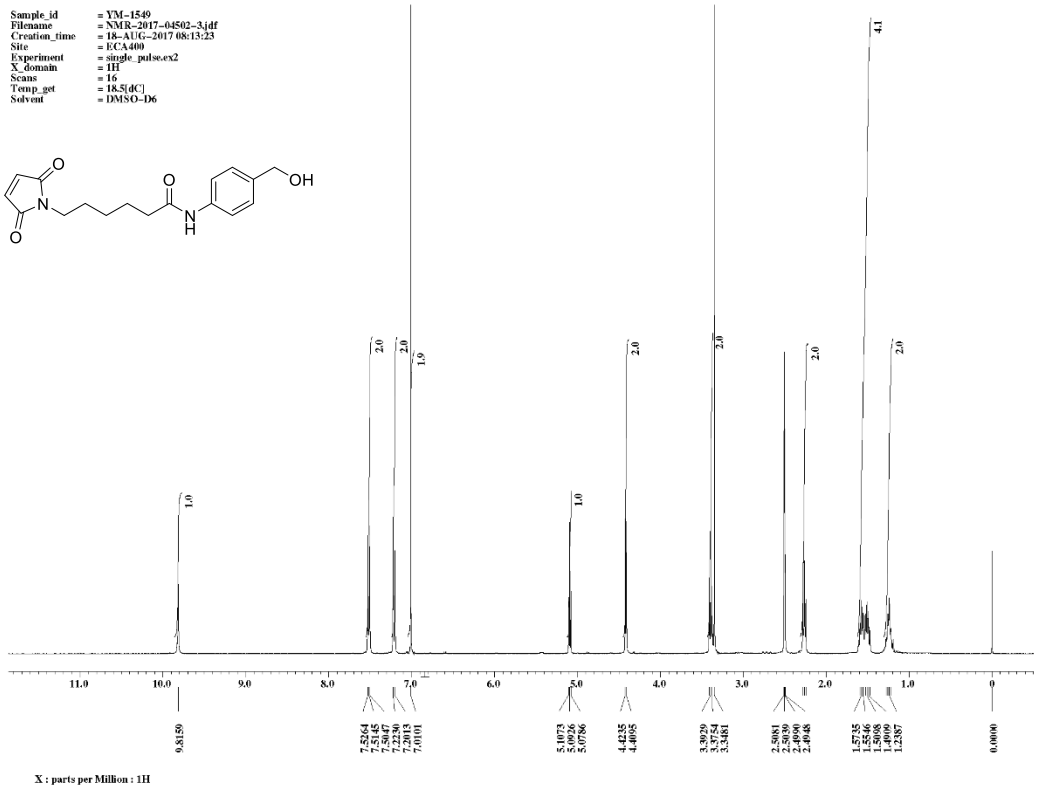

Figure S12. The ^{1}H-NMR spectrum of compound **8**.

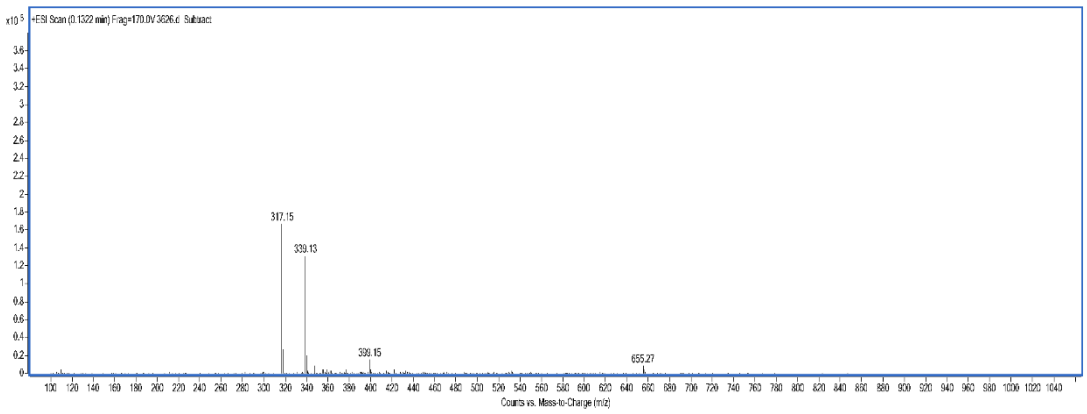

Figure S13. The ESI-MS spectrum of compound **8**.

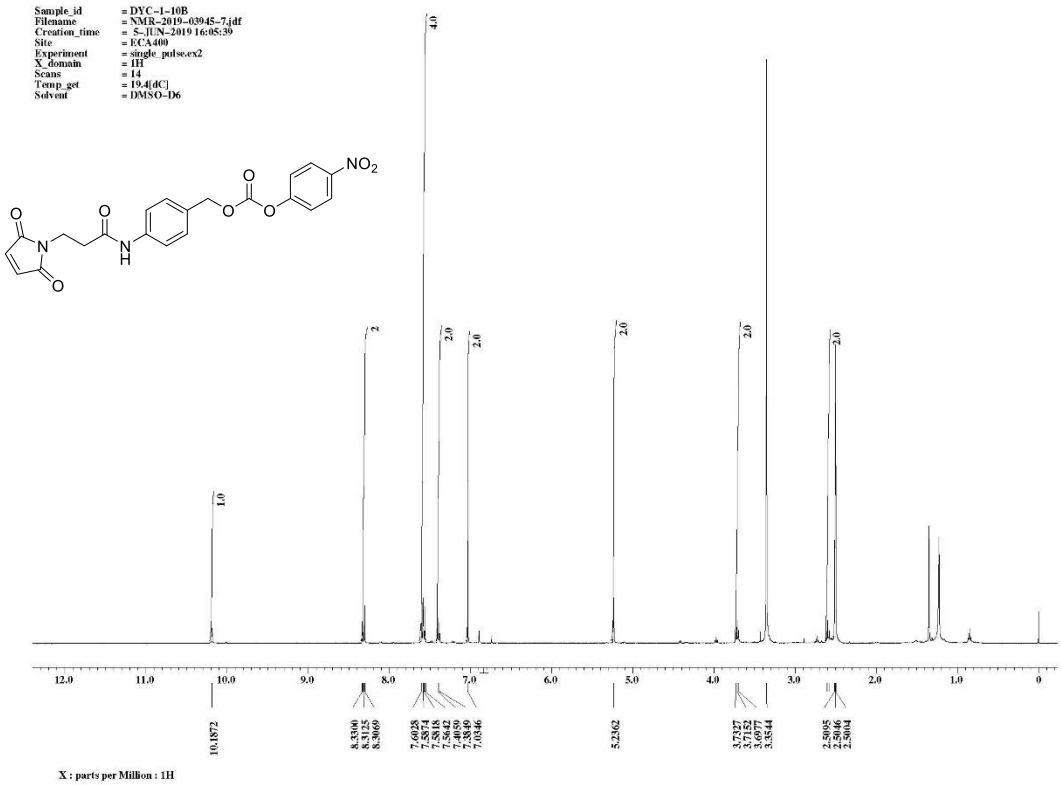

Figure S14. The ^{1}H-NMR spectrum of compound **9**.

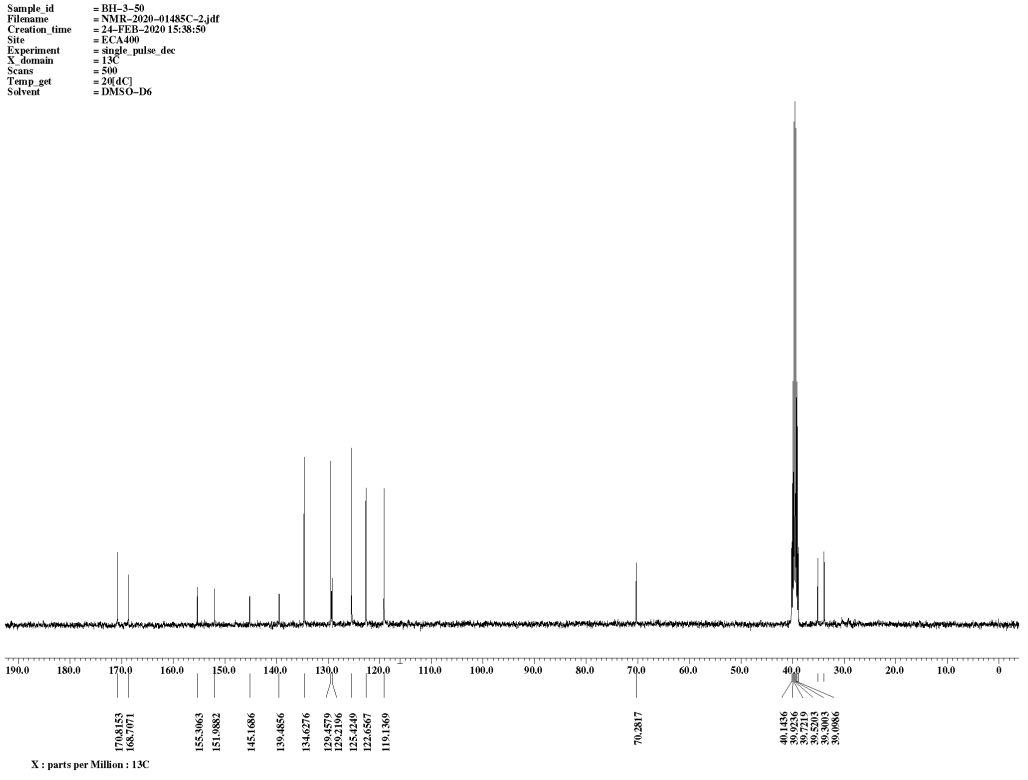

Figure S15. The ^{13}C-NMR spectrum of compound **9**.

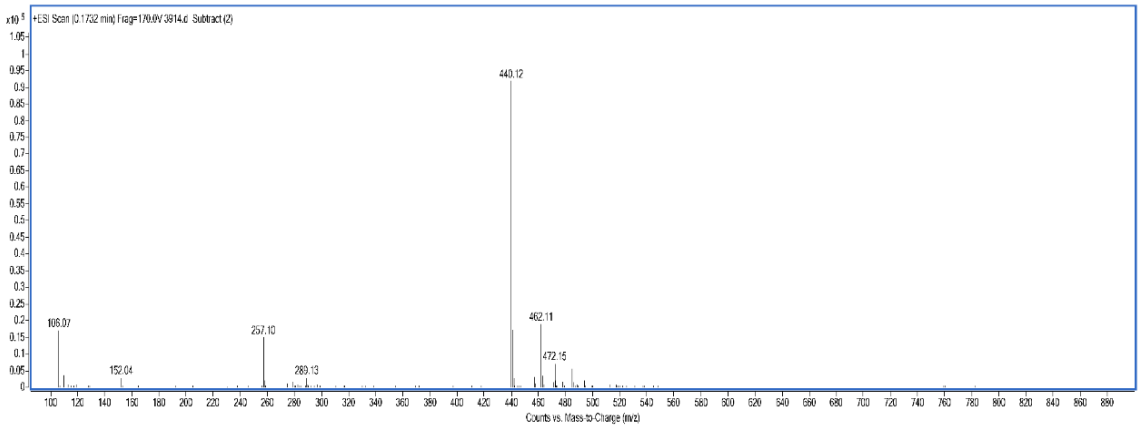

Figure S16. The ESI-MS spectrum of compound **9**.

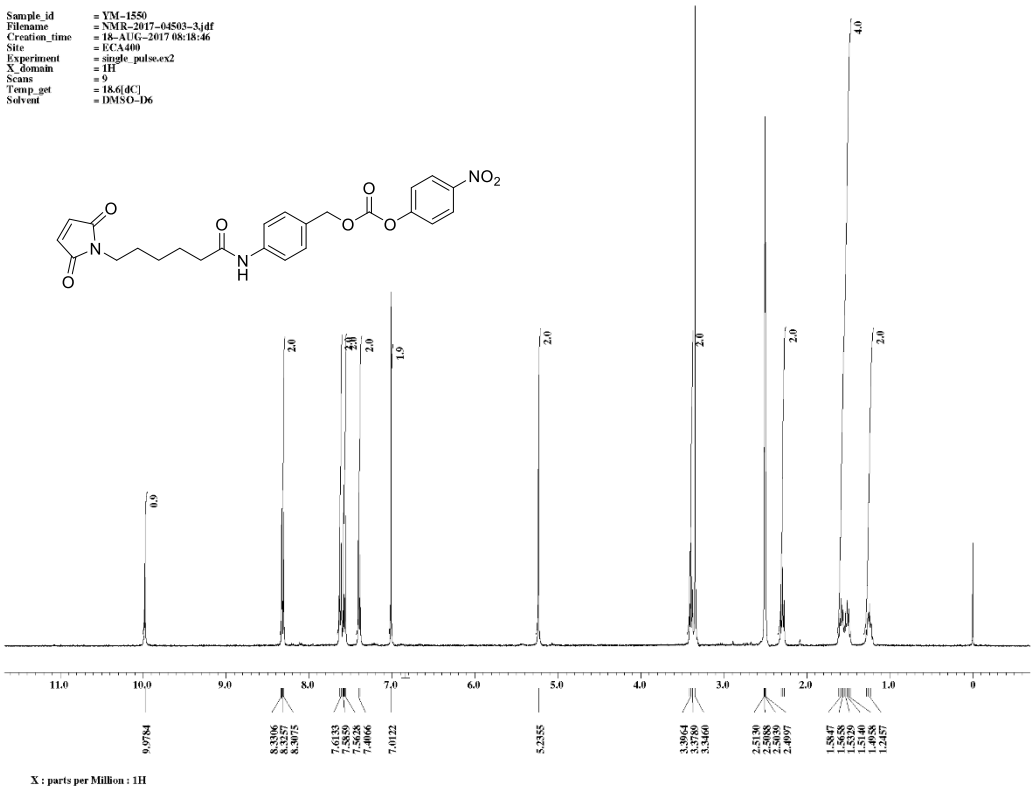

Figure S17. The ^{1}H-NMR spectrum of compound **10**.

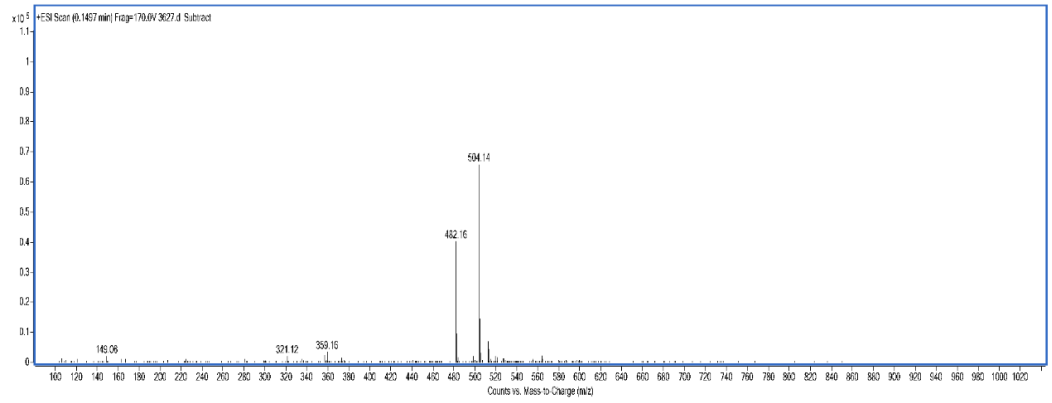

Figure S18. The ESI-MS spectrum of compound **10**.

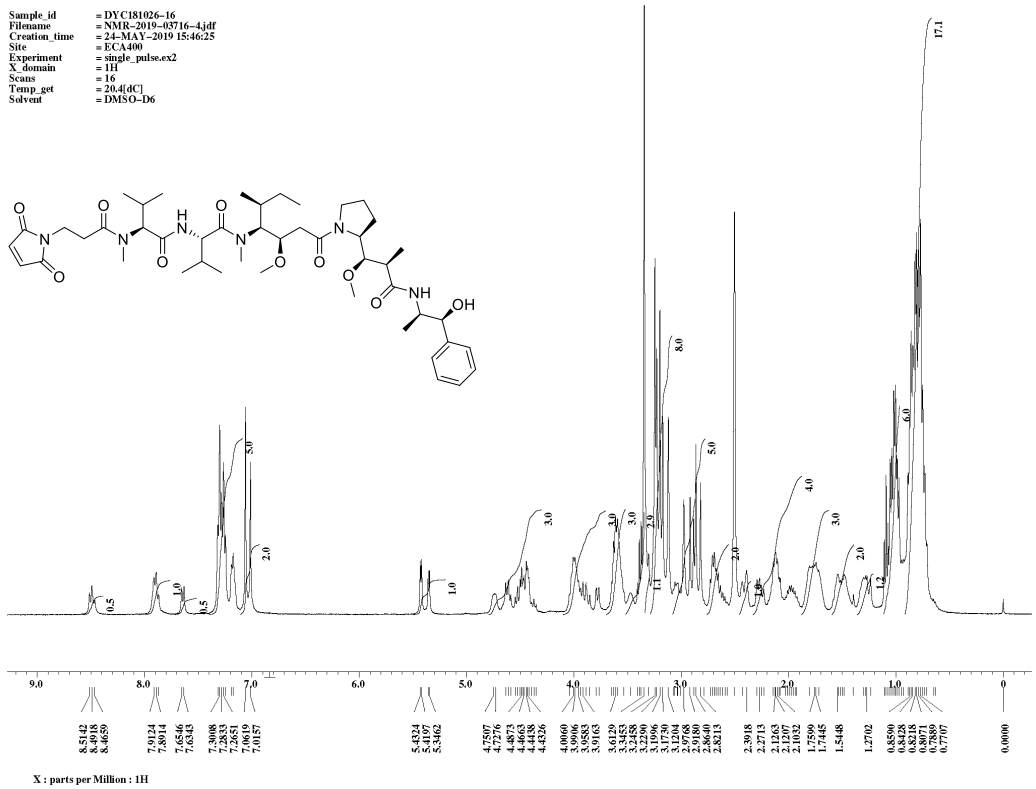

Figure S19. The ^{1}H-NMR spectrum of compound **11**.

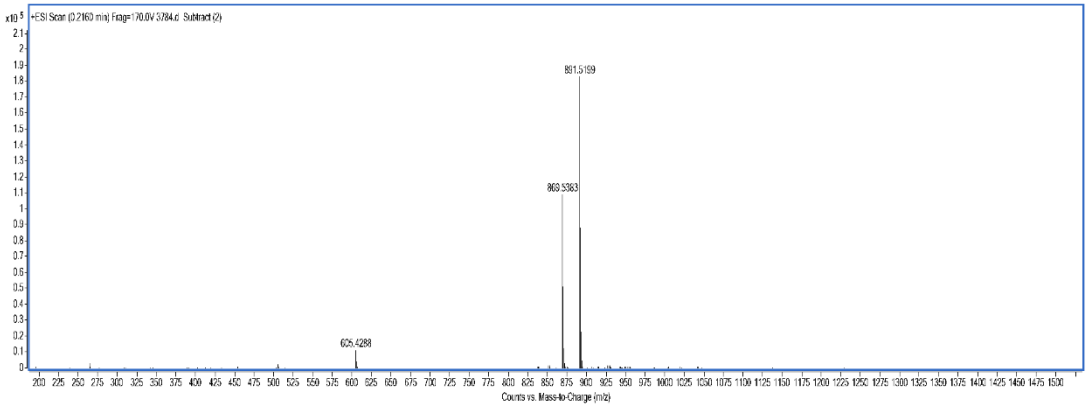

Figure S20. The HRMS spectrum of compound **11**.

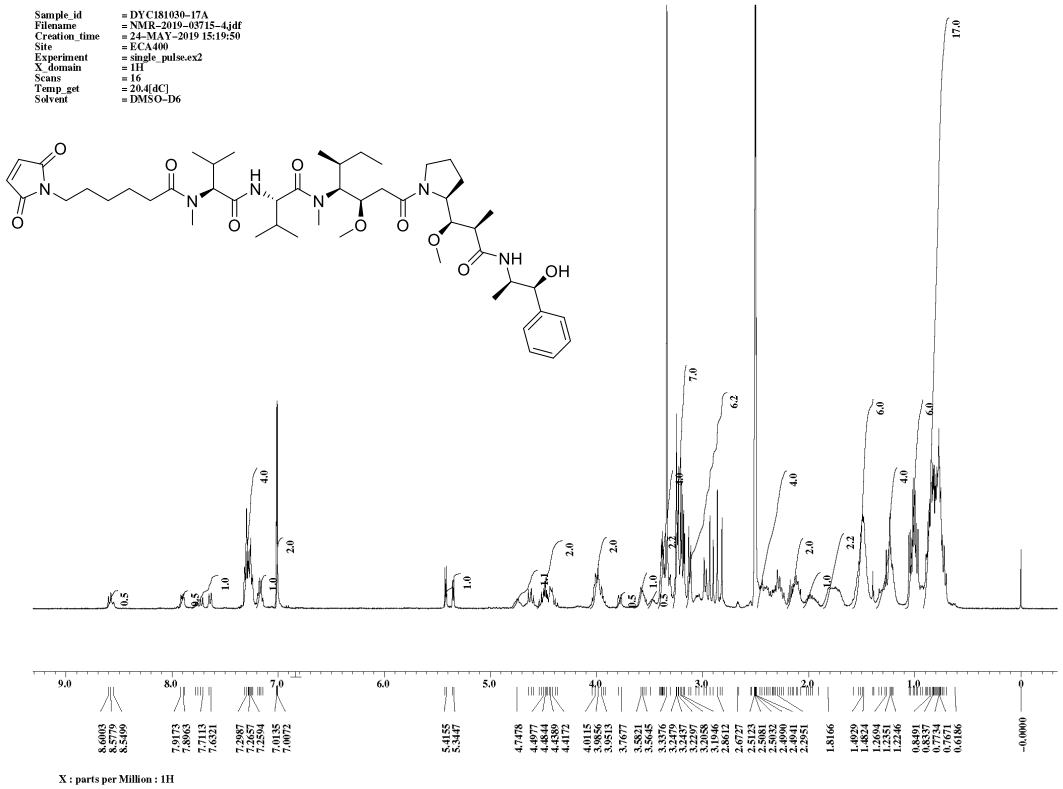

Figure S21. The ^{1}H-NMR spectrum of compound **12**.

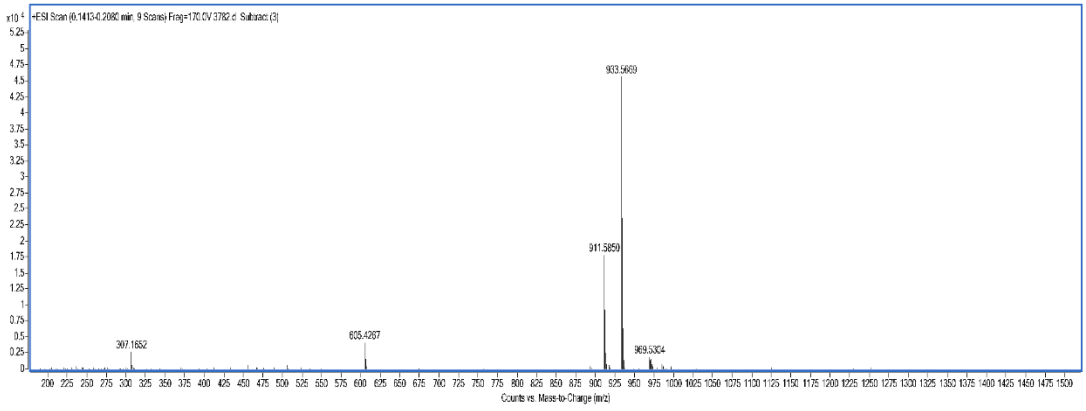

Figure S22. The HRMS spectrum of compound **12**.

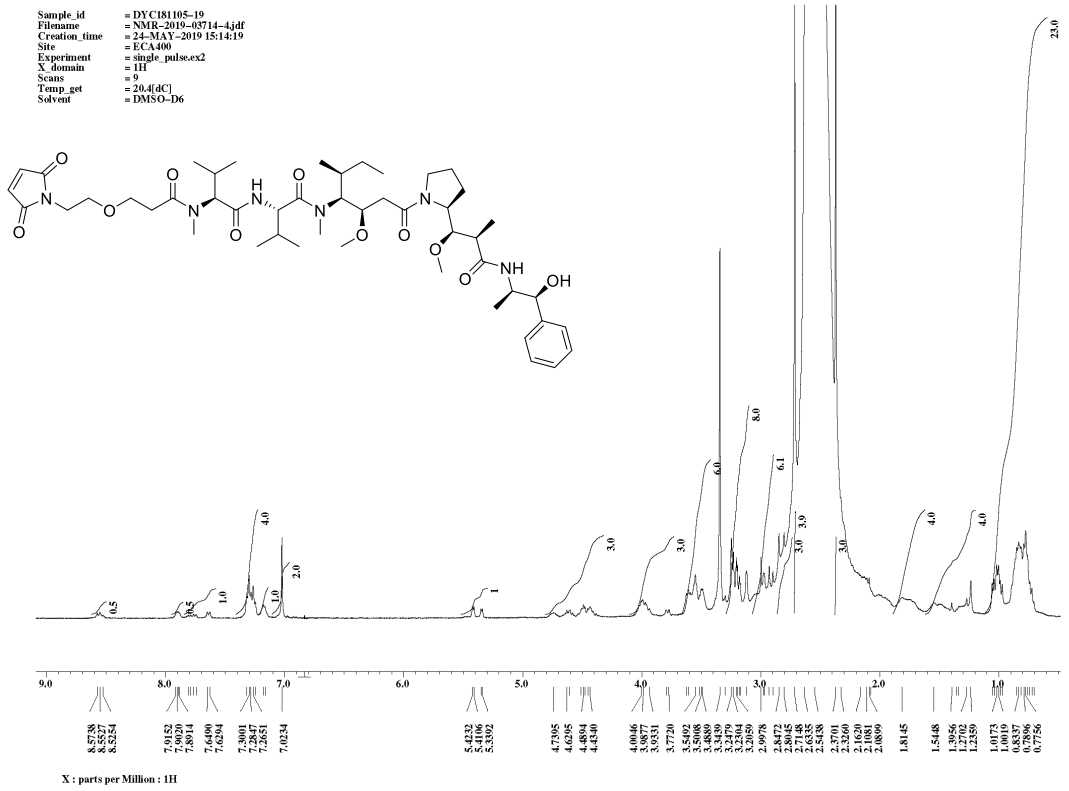

Figure S23. The ^{1}H-NMR spectrum of compound **13**.

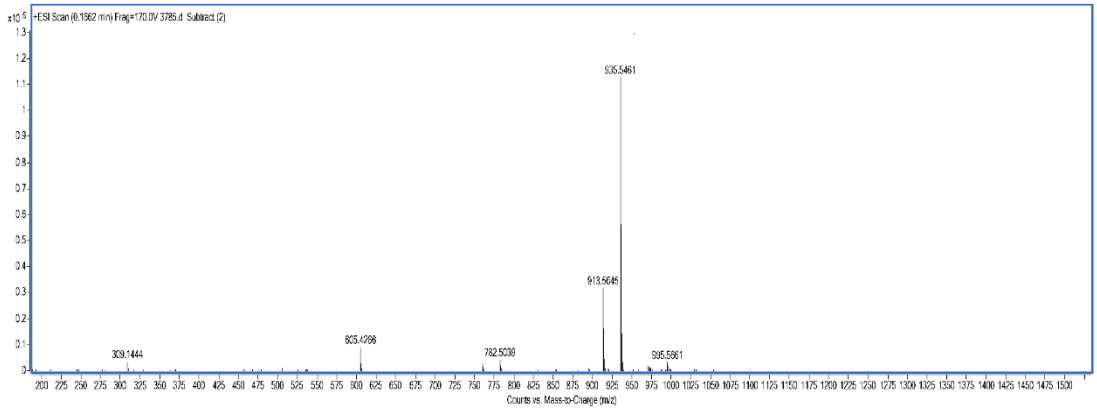

Figure S24. The HRMS spectrum of compound **13**.

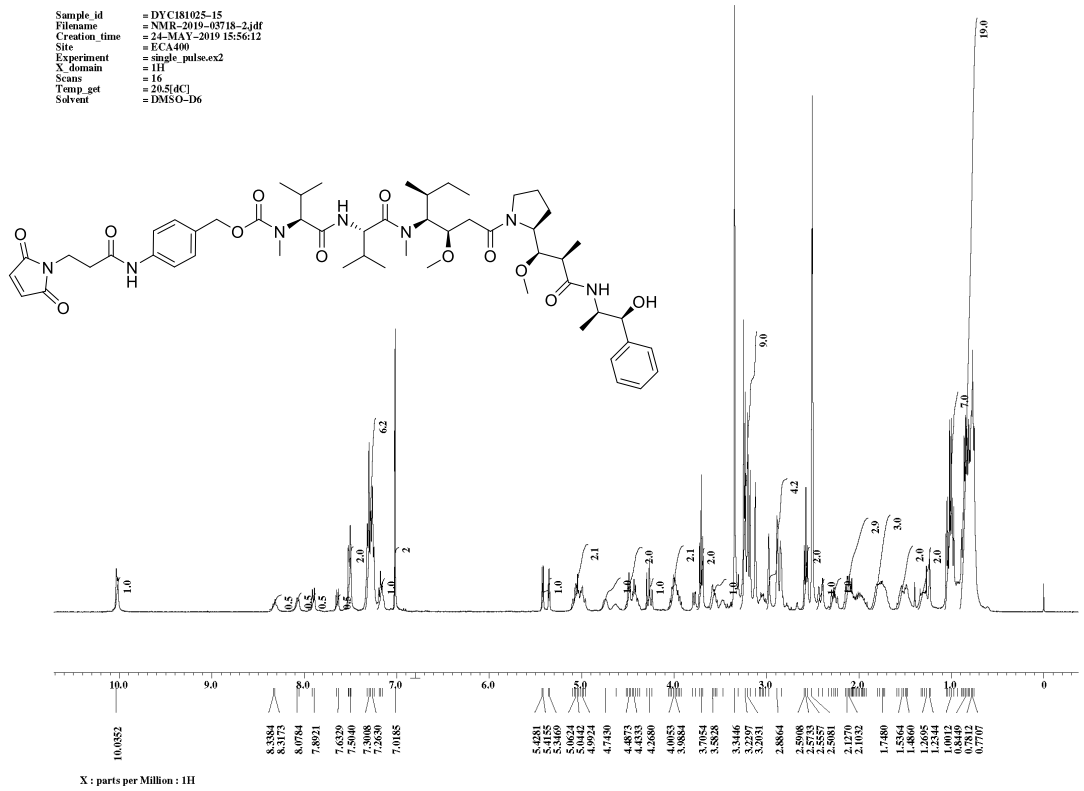

Figure S25. The ^{1}H-NMR spectrum of compound **14**.

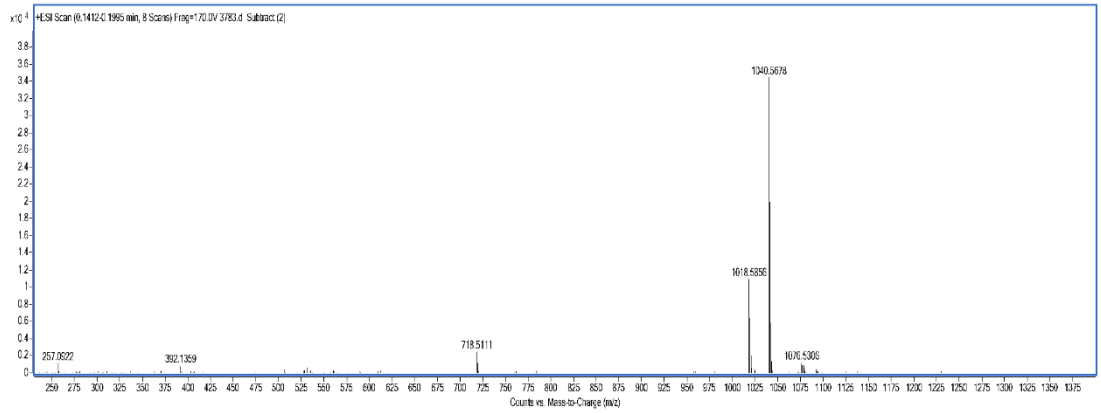

Figure S26. The HRMS spectrum of compound **14**.

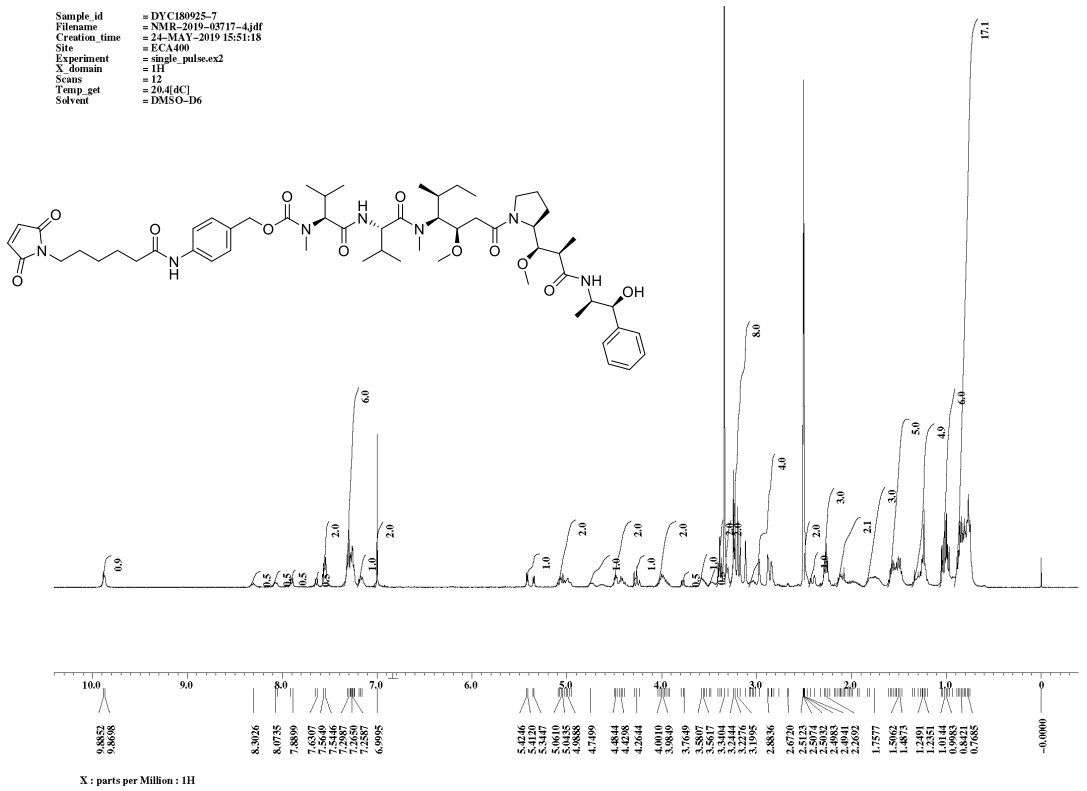

Figure S27. The ^{1}H-NMR spectrum of compound **15**.

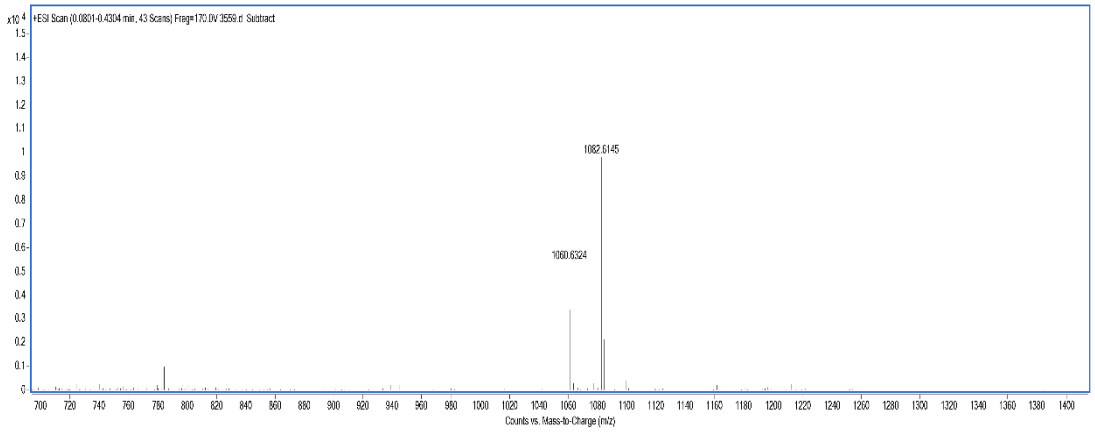

Figure S28. The HRMS spectrum of compound **15**.

Table S1. Permeability prediction of MMAE and Cys-linker-MMAE conjugates.

Compound	MlogP	S + Peff (cm/s × 10^4)	S + MDCK (cm/s × 10^7)	Perm Cornea (cm/s × 10^7)
Cys-**11**	−2.931	0.176	5.747	10.399
Cys-**12**	−2.464	0.190	4.902	11.843
Cys-**13**	−3.299	0.168	4.906	10.573
Cys-**14**	−2.533	0.162	5.019	12.262
Cys-**15**	−2.093	0.181	4.795	13.842
MMAE	1.191	0.353	21.818	63.090

MlogP: moriguchi model of octanol-water partition coefficient, larger logP values indicate higher lipophilicity; S + Peff: human effective jejunal permeability, larger S + Peff values indicate greater permeability and the predicted value for membrane permeable molecules is usually >0.25; S + MDCK: apparent MDCK COS permeability, larger S + MDCK values also indicate greater permeability and the predicted value for membrane permeable molecules is usually >20; Perm_Cornea: permeability through rabbit cornea, larger Perm_Cornea values indicate greater membrane permeability.