Supporting Information

Polymeric PEG-based bioorthogonal triggers for prodrug activation in breast cancer

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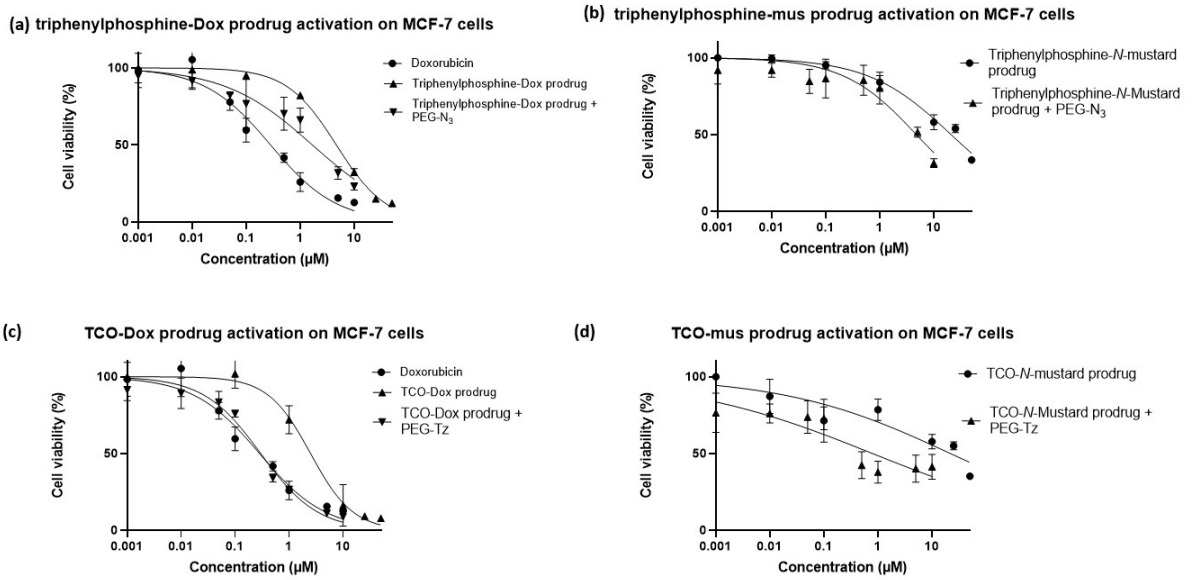


Figure *S*1: a) Cytotoxicity profile of Dox **12**, triphenylphosphine-Dox prodrug **13** and prodrug **13** after activation by PEG-azide **4** against MCF-7 cells. b) Cytotoxicity profile of triphenylphosphine-*N*-mustard prodrug **11** and prodrug **11** after activation by PEG-azide **4** against MCF-7 cells. c) Cytotoxicity profile of Dox **12,** TCO-Dox prodrug **16** and prodrug **16** after activation by PEG-Tz **3** against MCF-7 cells. d) Cytotoxicity profile of TCO-*N*-mustard prodrug **15** and prodrug **15** after activation by PEG-Tz **3** against MCF-7 cells. Data are presented as mean ± SEM (n=3).

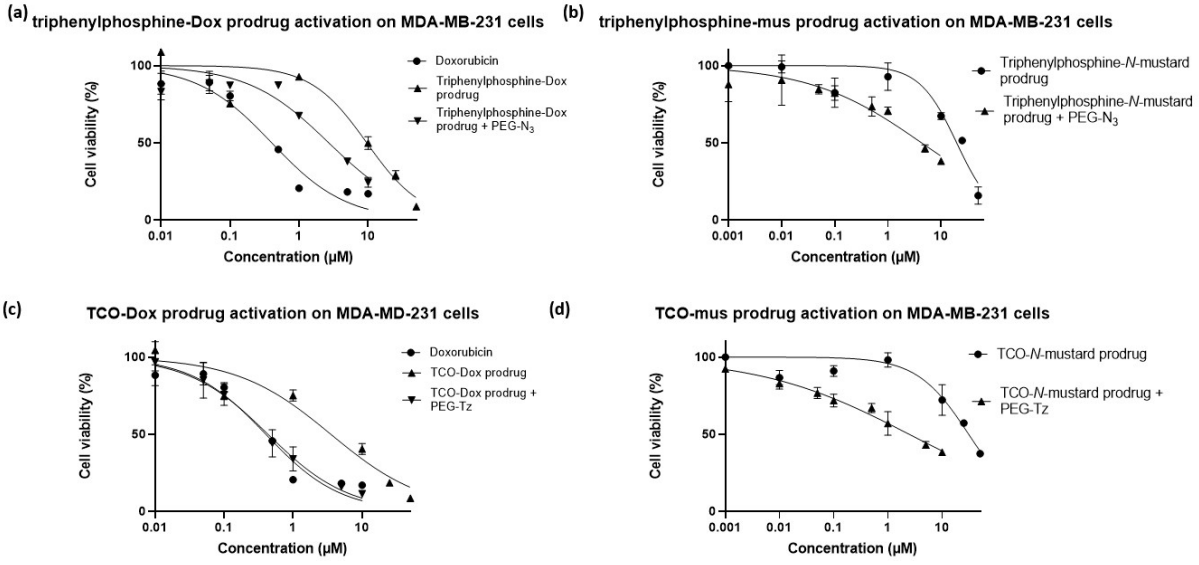


Figure *S*2: a) Cytotoxicity profile of Dox **12**, triphenylphosphine-Dox prodrug **13** and prodrug **13** after activation by PEG-azide **4** against MDA-MB-231 cells. b) Cytotoxicity profile of triphenylphosphine-*N*-mustard prodrug **11** and prodrug **11** after activation by PEG-azide **4** against MDA-MB-231 cells. c) Cytotoxicity profile of Dox **12,** TCO-Dox prodrug **16** and prodrug **16** after activation by PEG-Tz **3** against MDA-MB-231 cells. d) Cytotoxicity profile of TCO-*N*-mustard prodrug **15** and prodrug **15** after activation by PEG-Tz **3** against MDA-MB-231 cells. Data are presented as mean ± SEM (n=3).

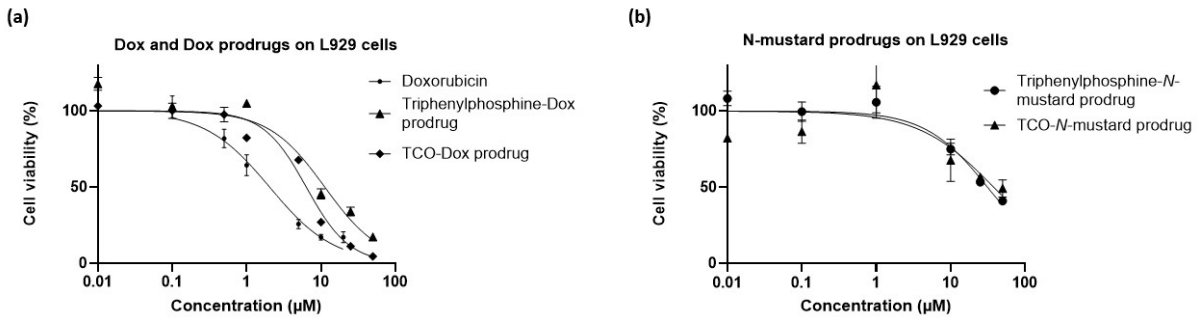
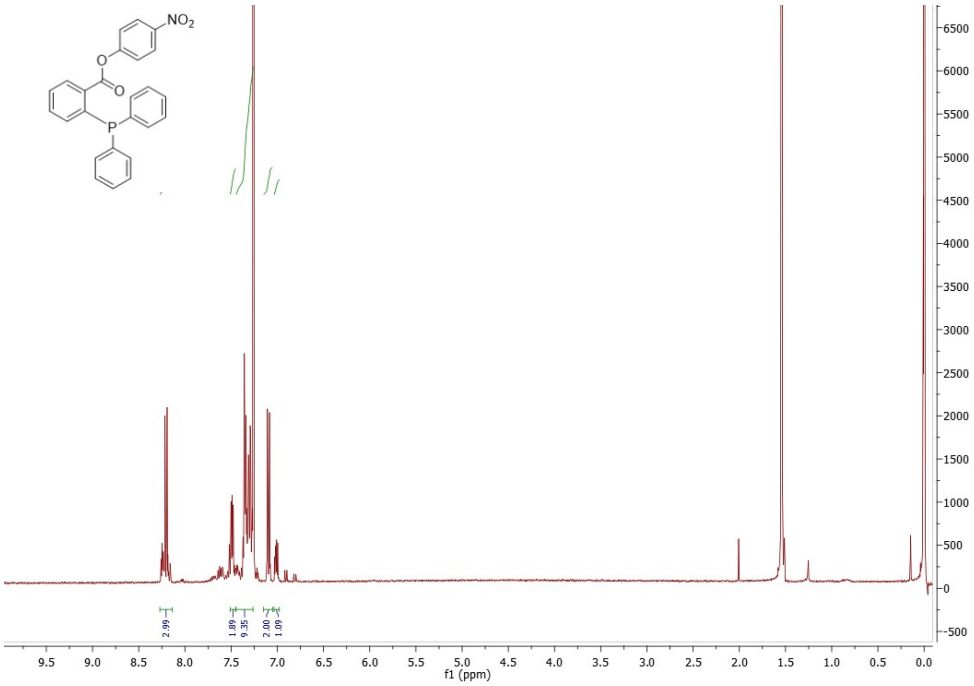
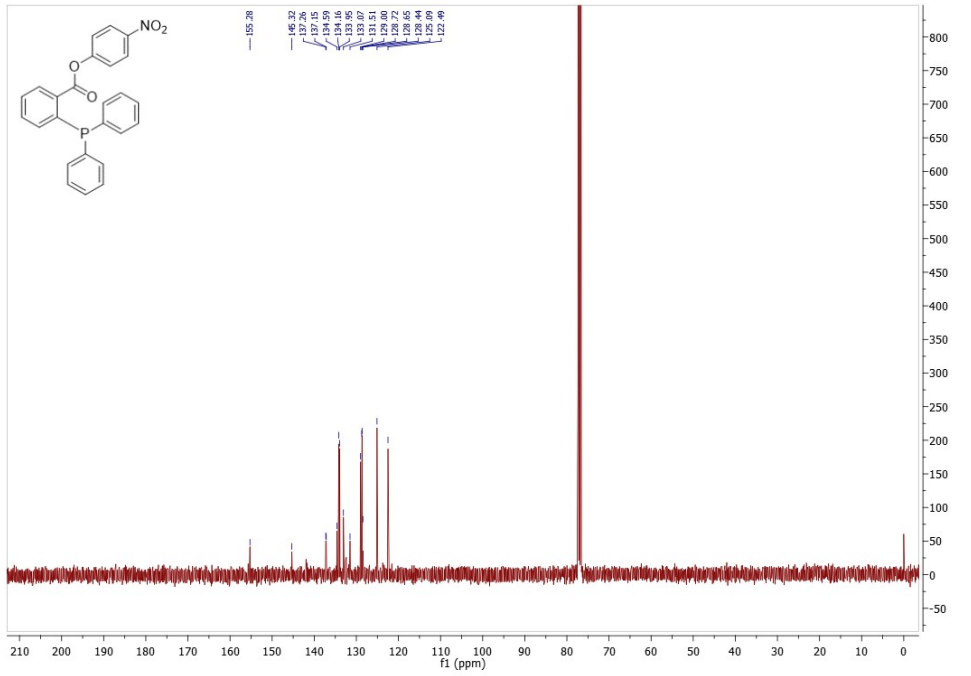


Figure *S*3: a) Cytotoxicity profile of Dox **12**, triphenylphosphine-Dox prodrug **13** and TCO-Dox prodrug **16** against L929 cells. b) Cytotoxicity profile of triphenylphosphine-*N*-mustard prodrug **11** and TCO-*N*-mustard prodrug **15** against L929 cells. Data are presented as mean ± SEM (n=3).

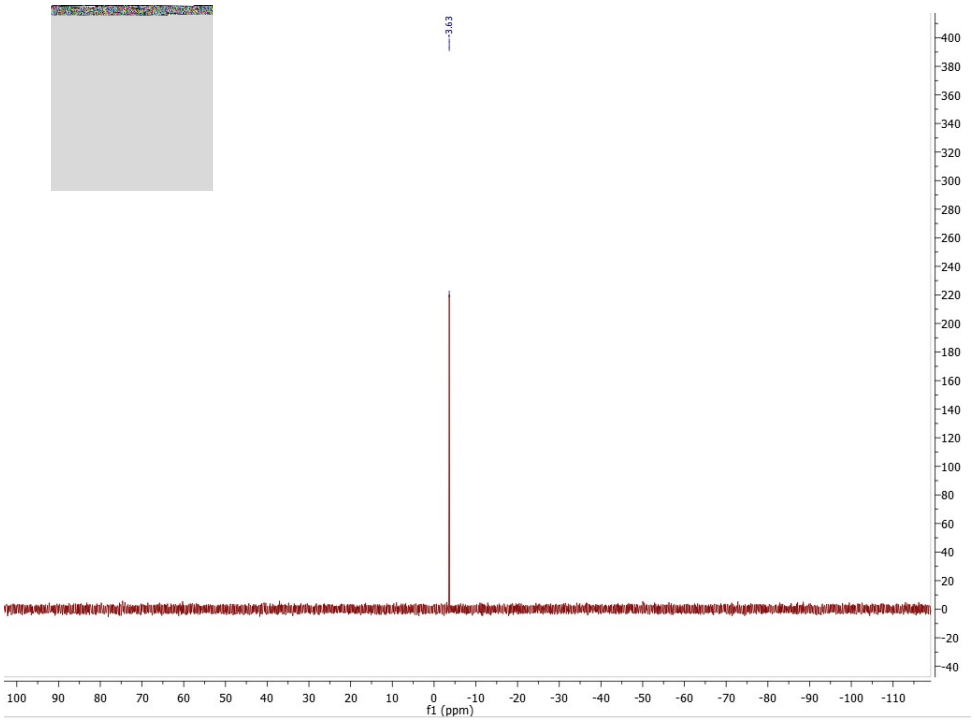
^{1}H and ^{13}C NMR Spectra



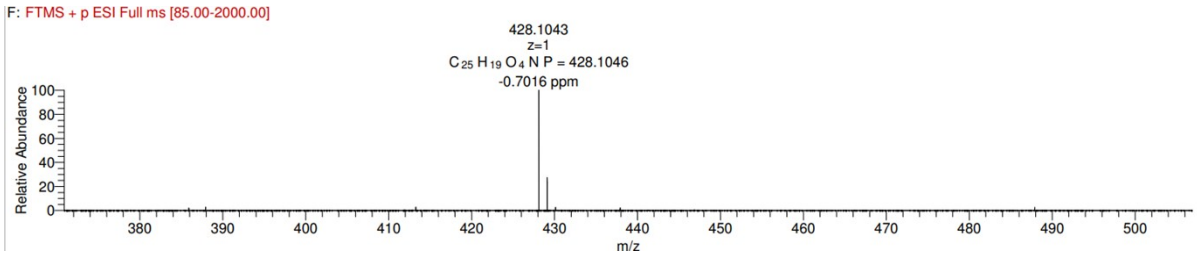
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **6**



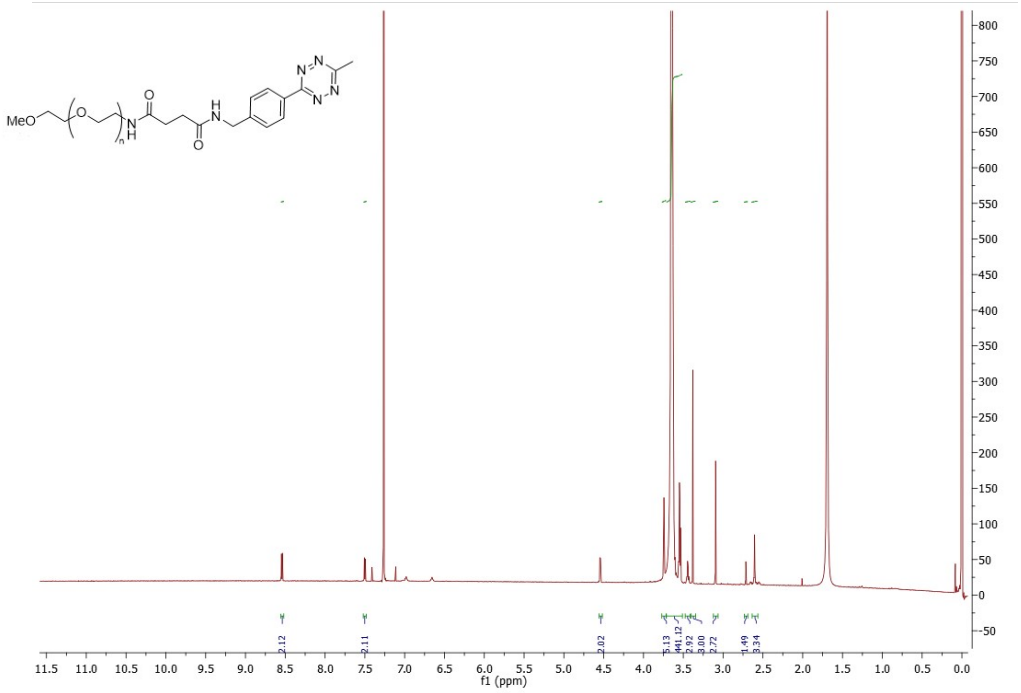
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **6**



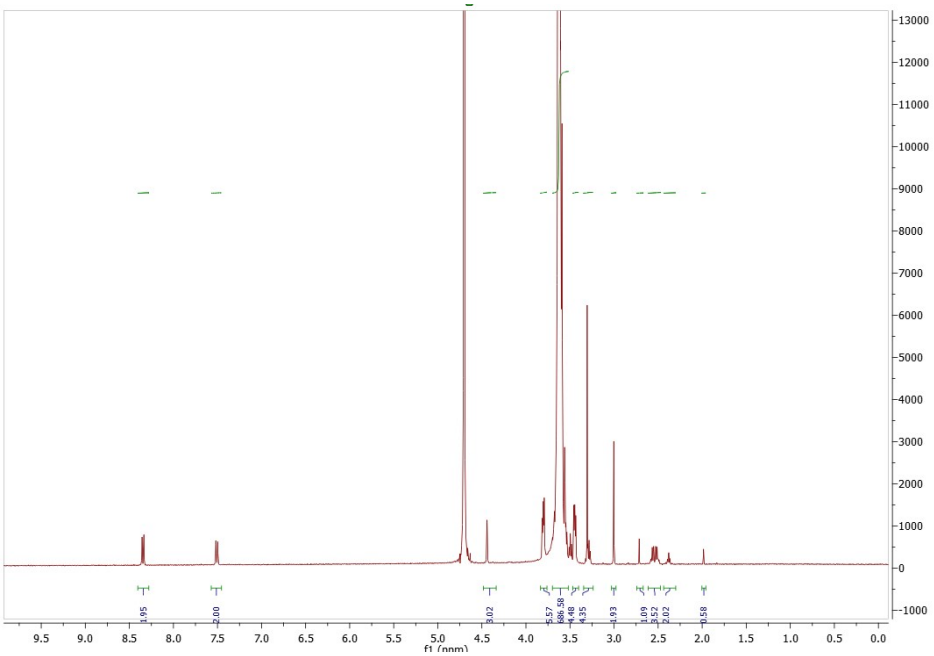
^{31}P NMR spectrum (162 MHz, $CDCl_3$) of compound **6**



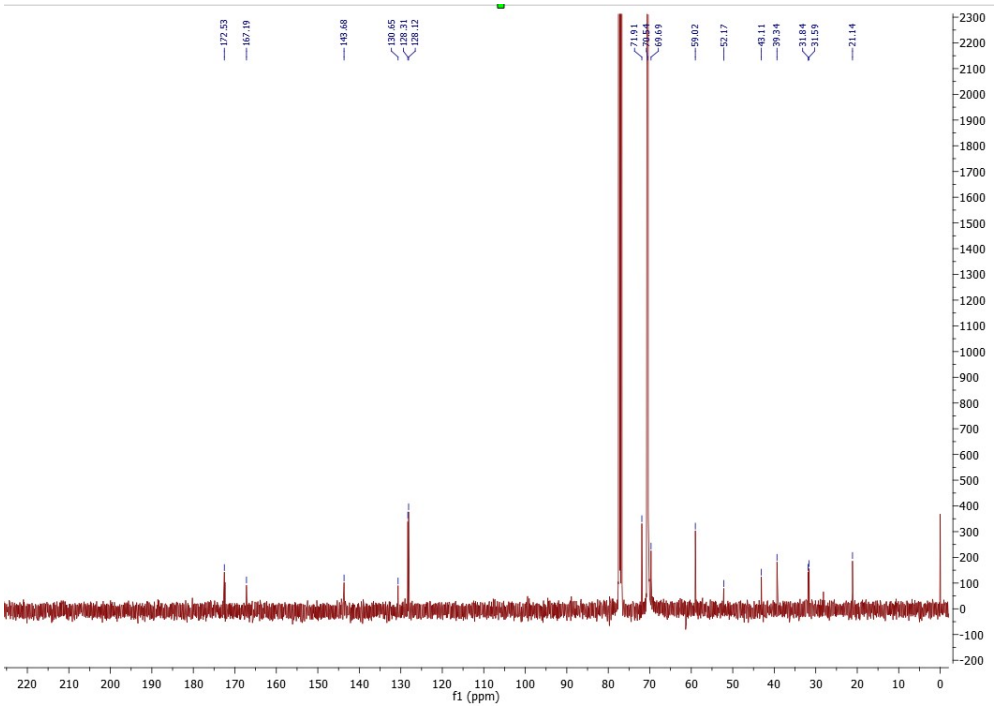
Mass spectrum of compound **6**



^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **3**



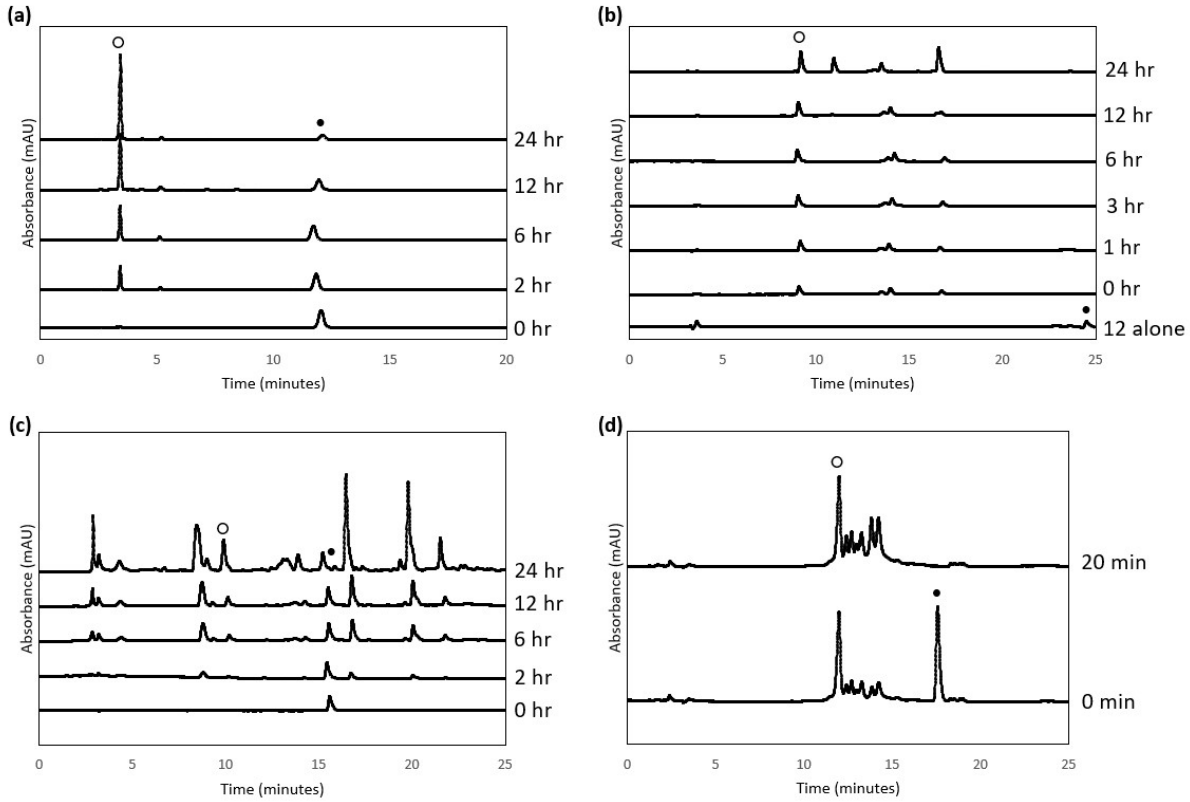
^{1}H NMR spectrum (400 MHz, D_2O) of compound **3**



^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **3**

Data for Compounds **11, 13, 15, 16** have been previously reported by ourselves within M. M. A. Mitry, S. Y. Boateng, F. Greco and H. M. I. Osborn, *RSC Med. Chem*., 2023, **14**, 1537–1548; M. M. A. Mitry, M. L. Dallas, S. Y. Boateng, F. Greco and H.M.I. Osborn, *Bioorg. Chem*., 2024, **147**, 107304.

HPLC chromatograms of release profile of 4-nitrophenol and Doxorubicin



a) HPLC chromatograph of 4-nitrophenol release from triphenylphosphine ester model prodrug **6** by PEG-azide (10 KDa) **4** at 37 °C in aqueous MeCN (1:1) as a function of time. Legend: •: triphenylphosphine ester model prodrug **6**; ○: 4-nitrophenol.

b) HPLC chromatograph of 4-nitrophenol release from TCO carbonate model prodrug **14** by PEG-Tz (10 KDa) **3** at 37 °C in aqueous MeCN (1:1) as a function of time. Legend: •: TCO carbonate model prodrug **14**; ○: 4-nitrophenol.

c) HPLC chromatograph of doxorubicin **12** release from triphenylphosphine-Dox prodrug **13** by PEG-azide (10 KDa) **4** at 37 °C in aqueous MeCN (1:1) as a function of time. Legend: •: triphenylphosphine-Dox prodrug **13**; ○: doxorubicin **12**.

d) HPLC chromatograph of doxorubicin **12** release from TCO-Dox prodrug **16** by PEG-Tz **3** at 37 °C in aqueous MeCN (1:1) as a function of time. Legend: •: TCO-Dox prodrug **16**; ○: doxorubicin **12**