Supplementary Data

Synthesis and characterization of polyethylene terephthalate (PET) precursors and potential degradation products: Toxicity study and application in discovery of novel PETases

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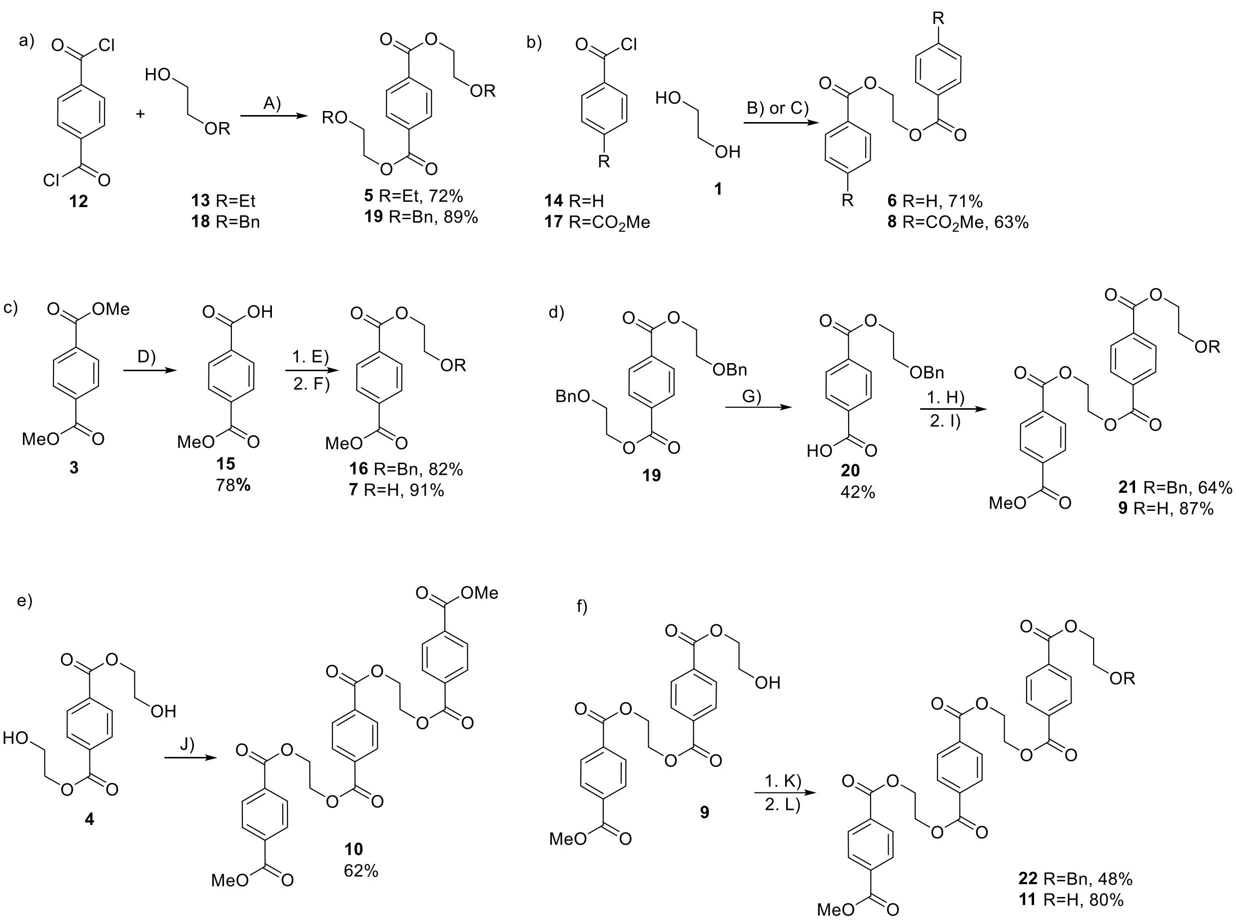
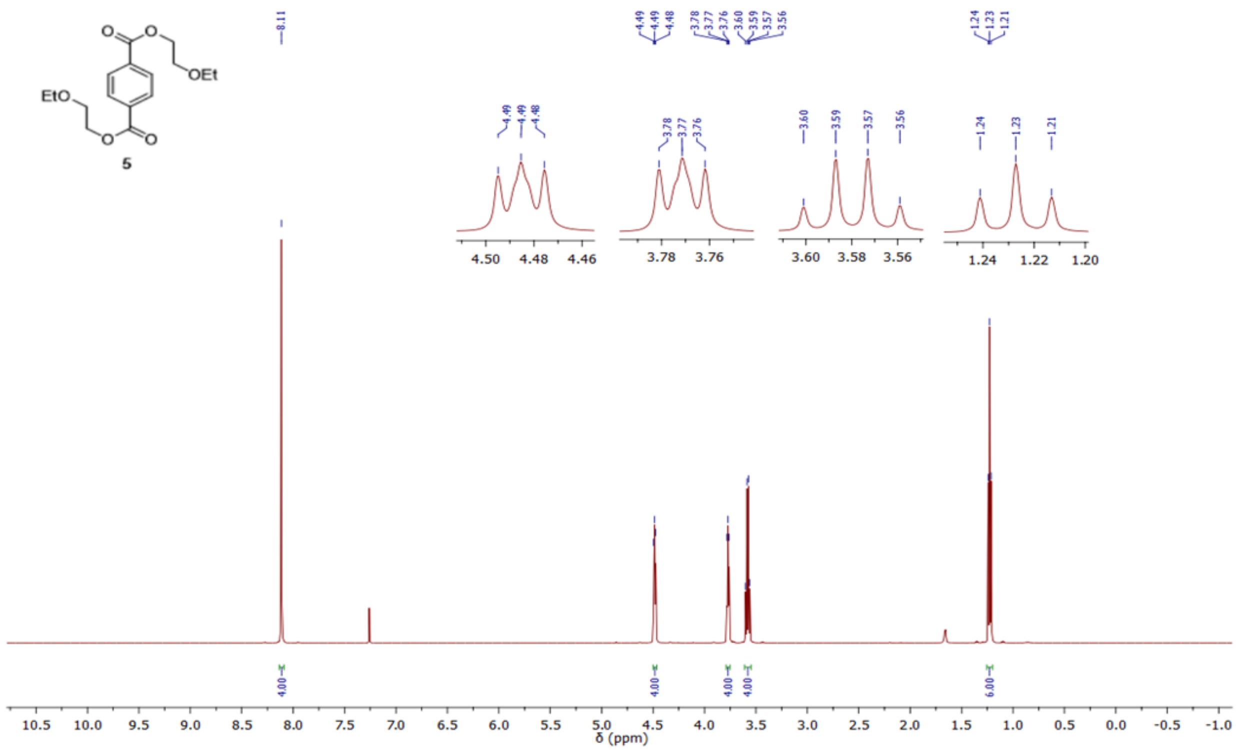


Figure S1. Methodological approach in synthesis of **1-11**: A) Pyridine, toluene, 60 °C, 5 h; B) pyridine, toluene, 120 °C, 4 h; C) Pyridine, CH_2Cl_2, r.t., 12 h; D) KOH, MeOH/toluene, 120 °C, 5 h; E) DCC/DMAP, CH_2Cl_2, r.t,. 4 h; F) H_2, Pd/C, EtOAc, 45 psi, r.t., 3 h; G) **18**, KOH, toluene, 120 °C, 3.5 h; H) **7**, DCC/DMAP, CH_2Cl_2, r.t., 20 h; I) H_2 (balloon), Pd/C, 1,4-dioxane, r.t., 4 h; J) **17**, pyridine, CH_2Cl_2, r.t., 12 h; K) **20**, DCC/DMAP, CH_2Cl_2, r.t., 24 h; L) H_2 (balloon), Pd/C, 1,4-dioxane, r.t., 6 h.



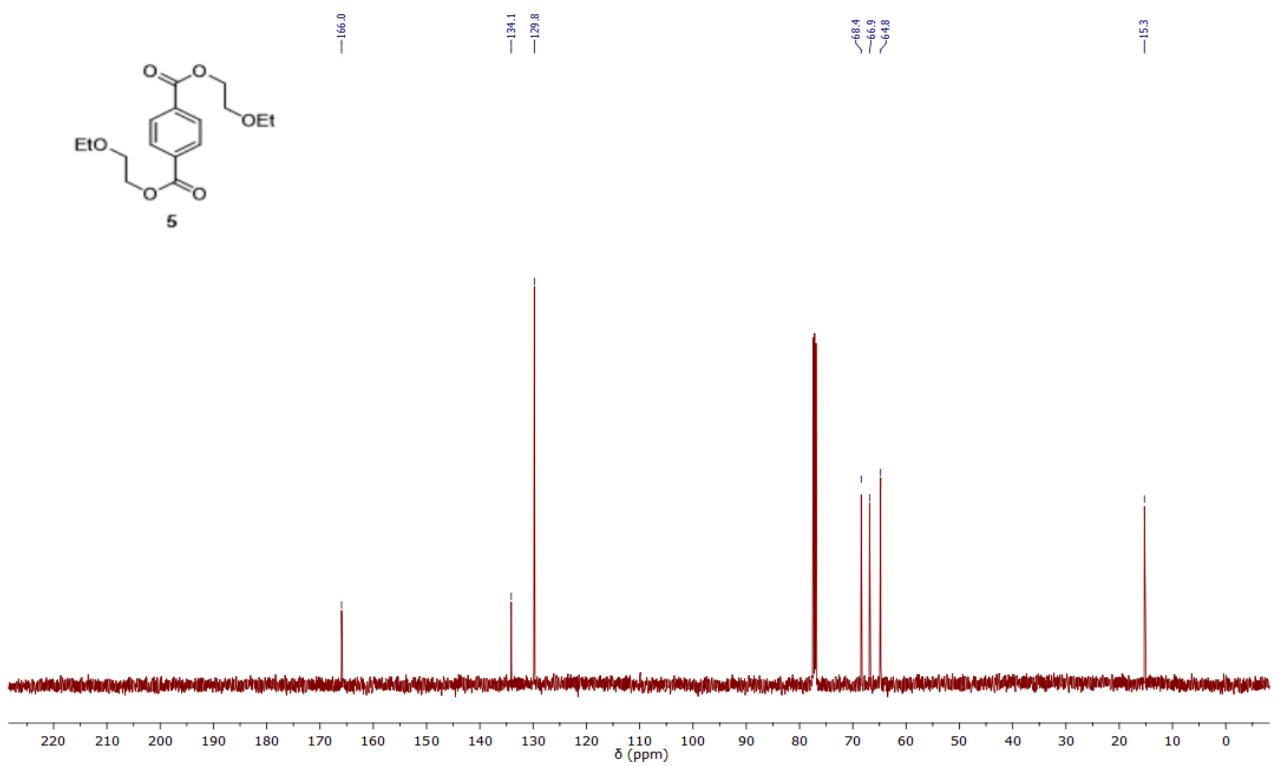
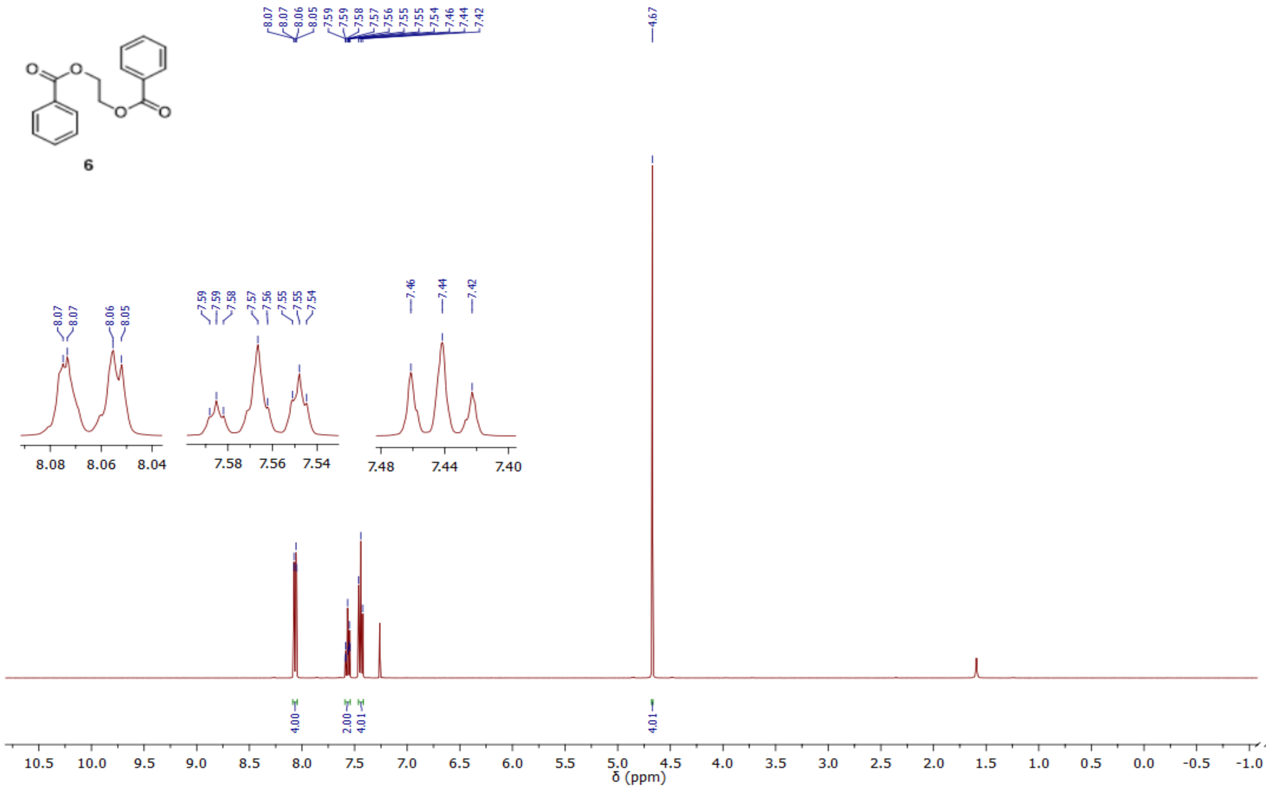


Figure S2. [1]H NMR spectrum (500 MHz) of **5** recorded in $CDCl_3$.[13]C NMR spectrum (125 MHz) of **5** recorded in $CDCl_3$.



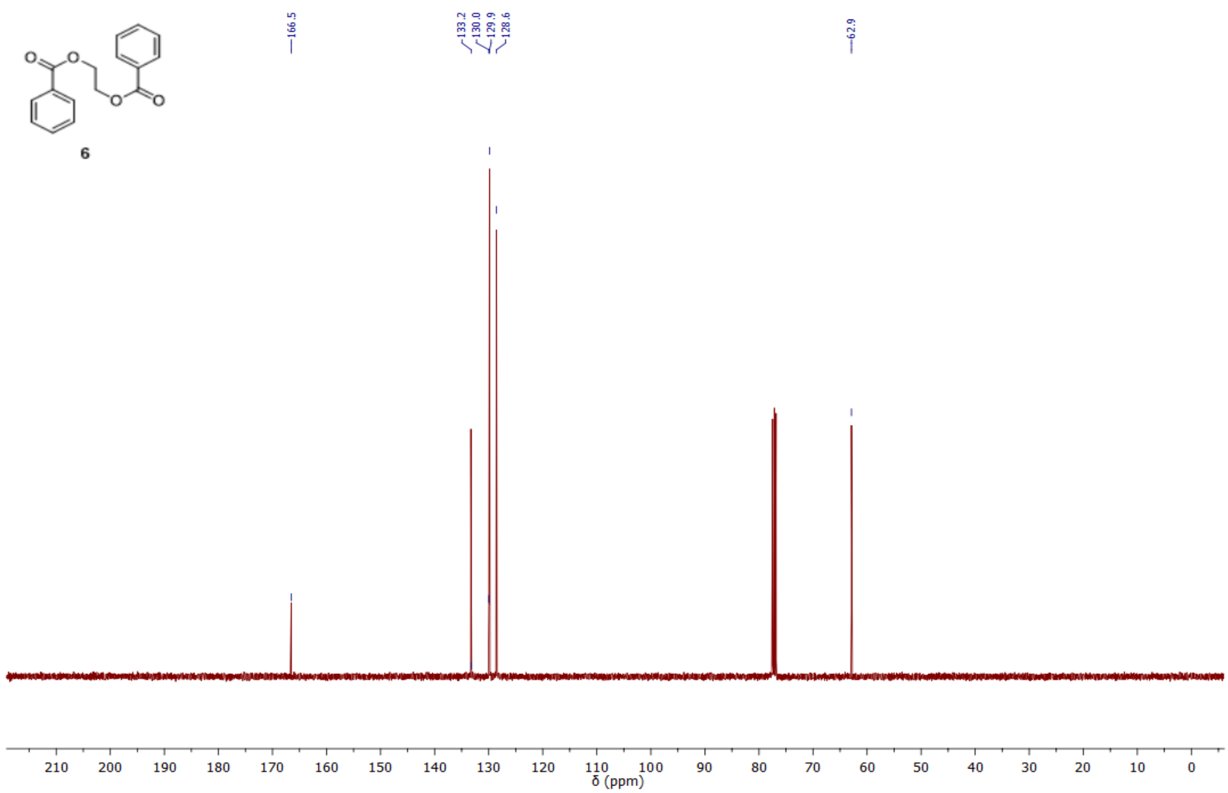
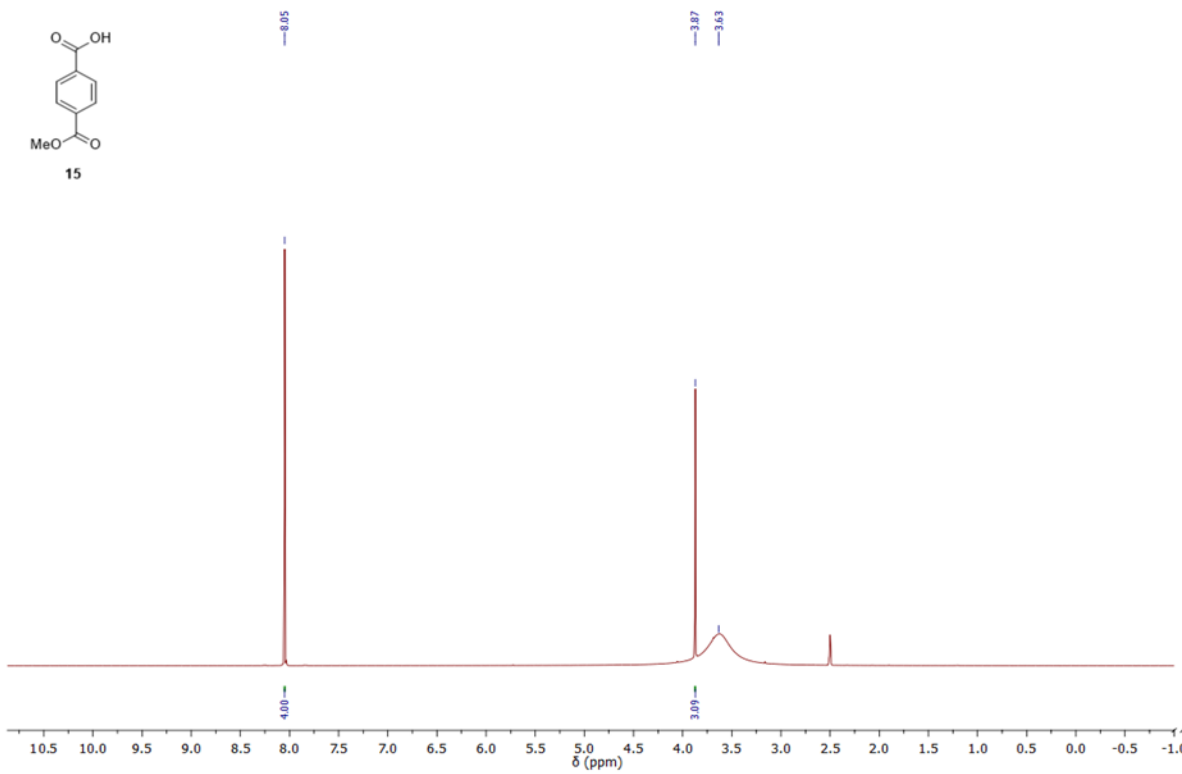


Figure S3. ^{1}H NMR spectrum (400 MHz) of **6** recorded in $CDCl_3$.^{13}C NMR spectrum (100 MHz) of **6** recorded in $CDCl_3$.



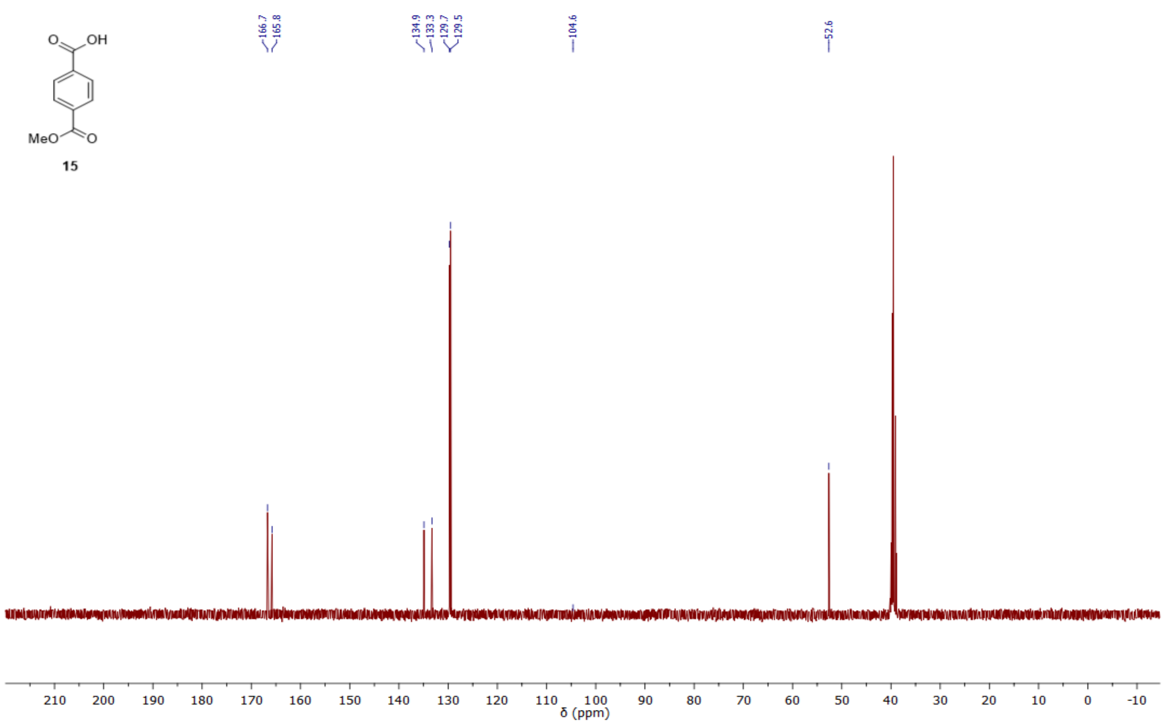


Figure S4. ^{1}H NMR spectrum (400 MHz) of **15** recorded in DMSO-d6 and ^{13}C NMR spectrum (100 MHz) of **15** recorded in DMSO-d6.

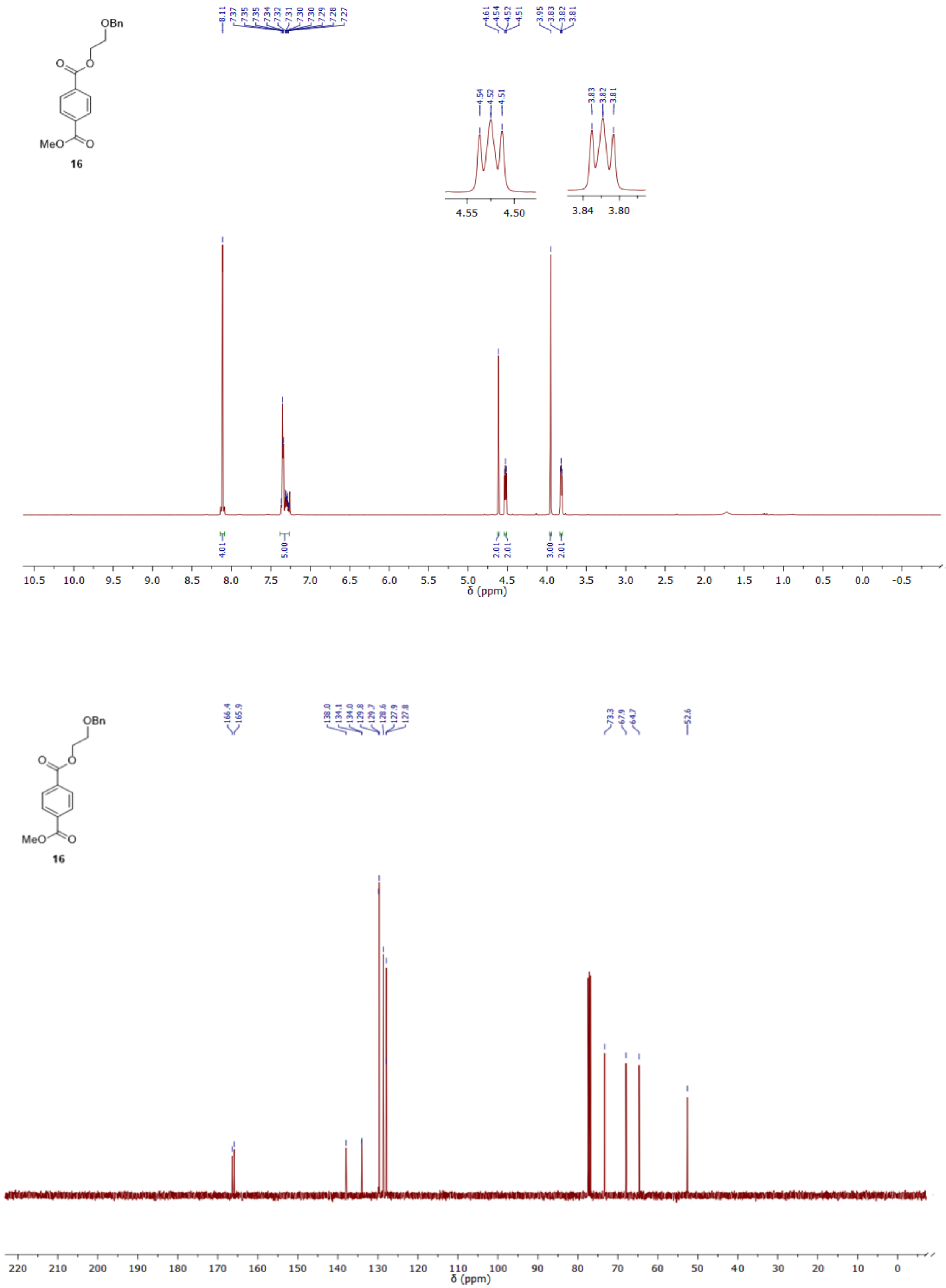
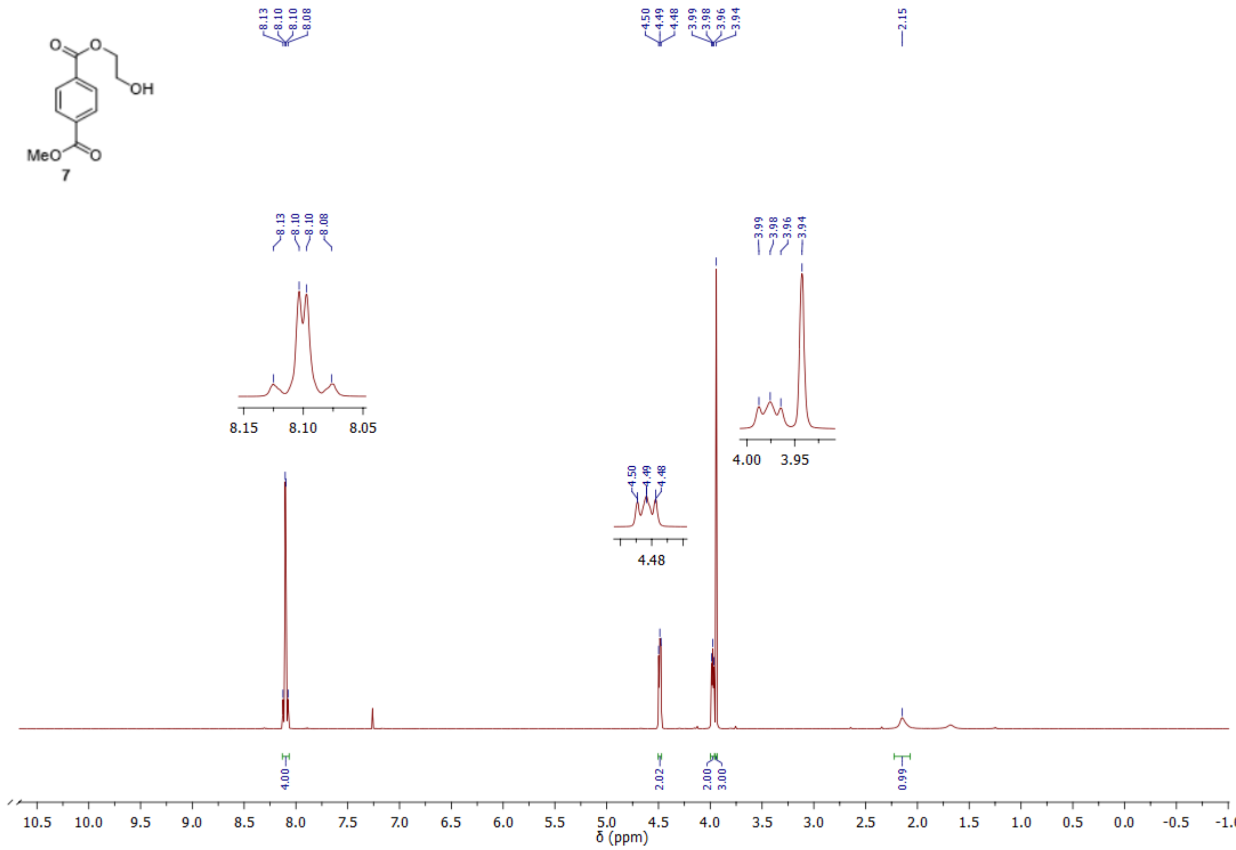


Figure S5. ^{1}H NMR spectrum (400 MHz) of **16** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **16** recorded in $CDCl_3$.



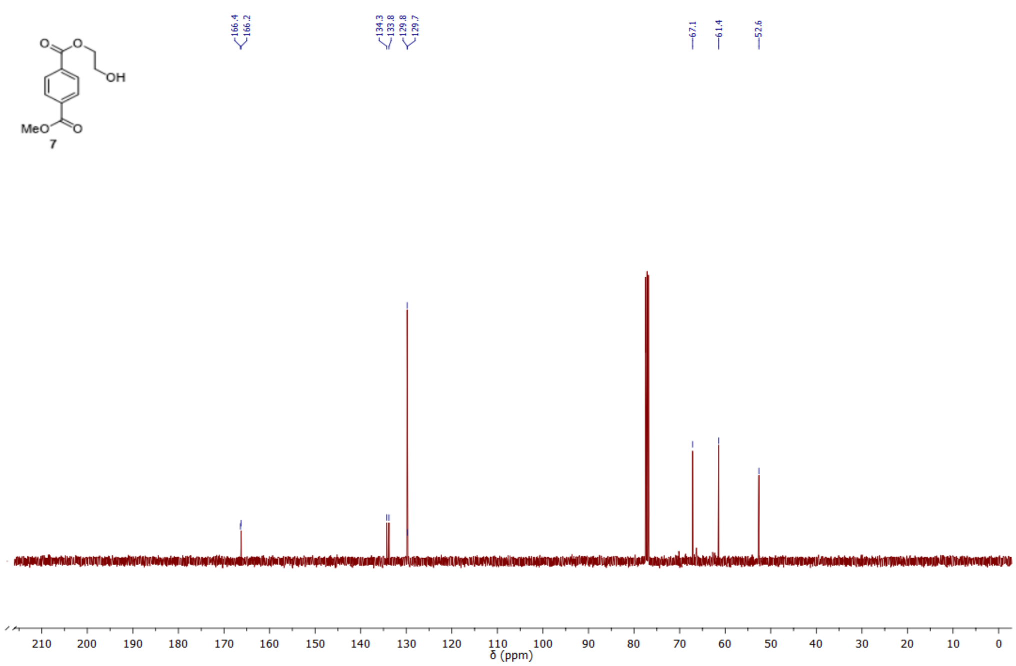
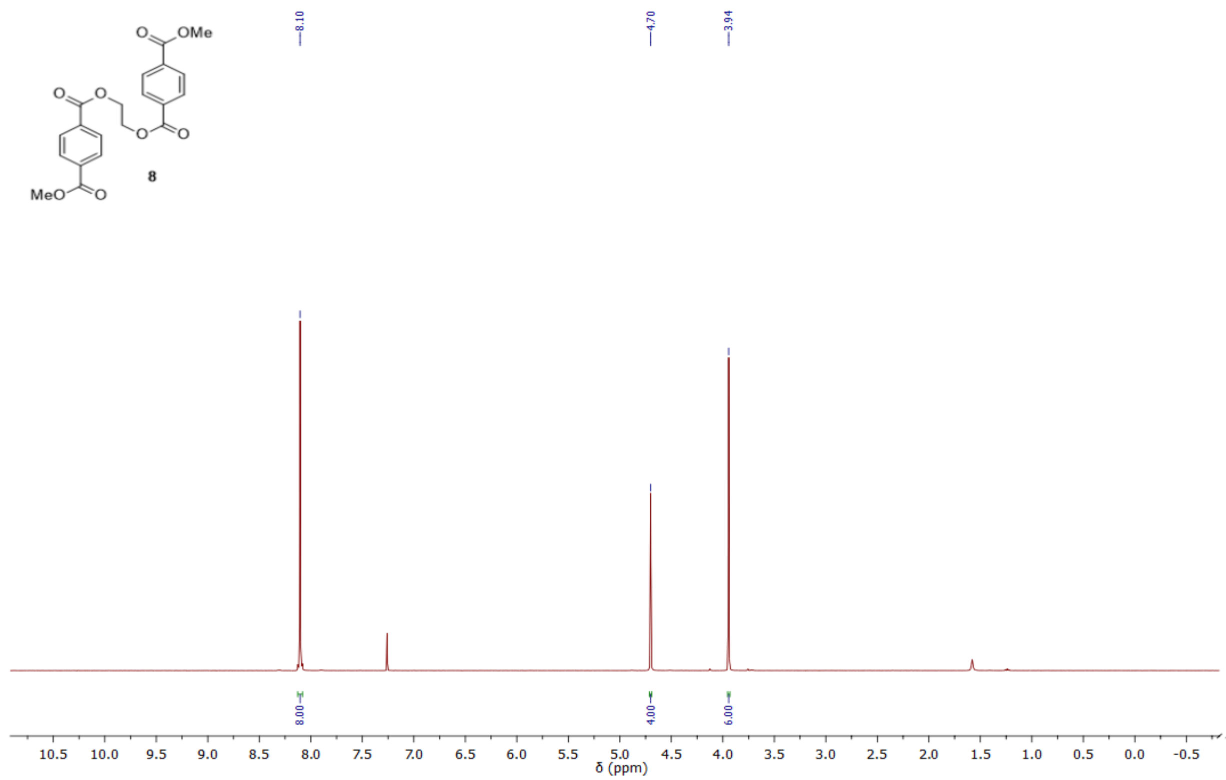


Figure S6. ^{1}H NMR spectrum (400 MHz) of **7** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **7** recorded in $CDCl_3$.



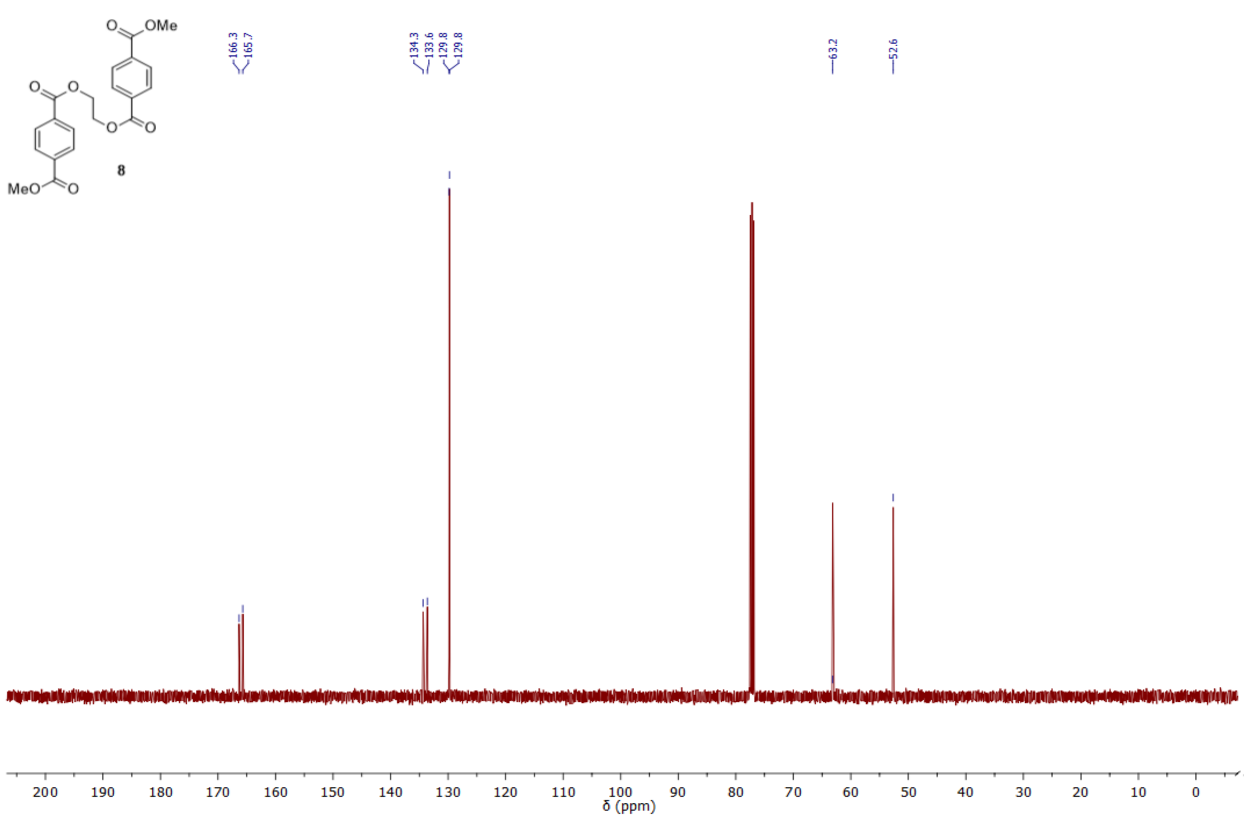
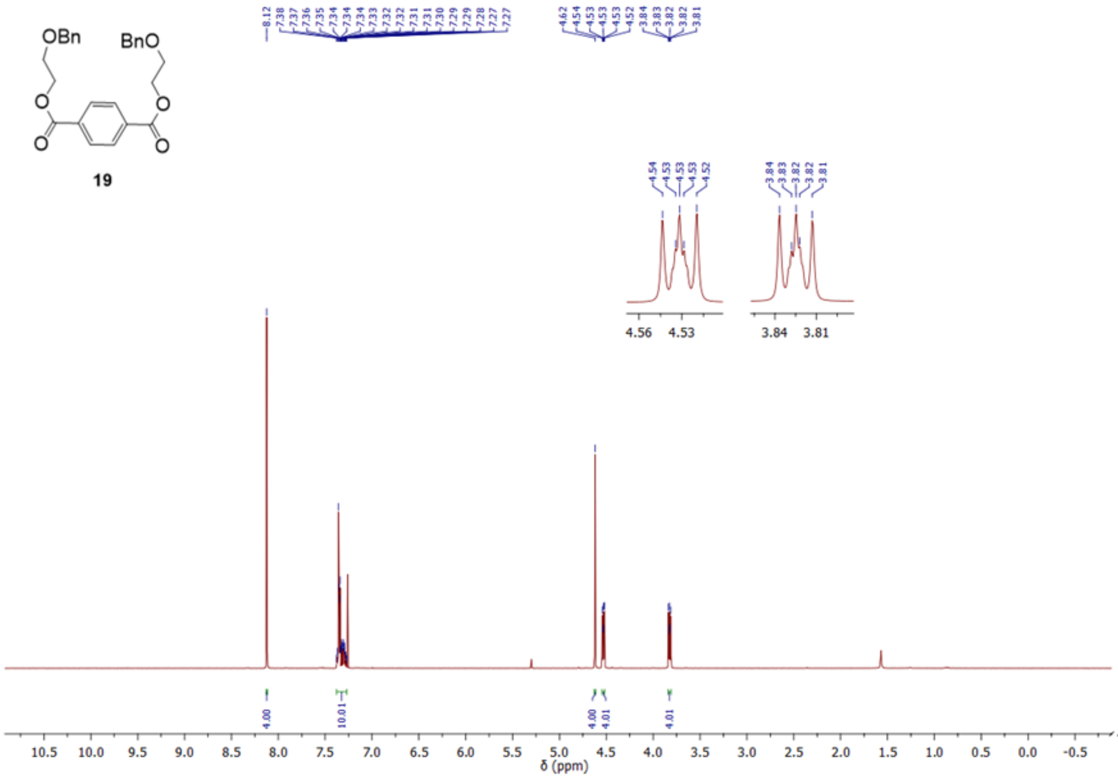


Figure S7. ^{1}H NMR spectrum (400 MHz) of **8** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **8** recorded in $CDCl_3$.



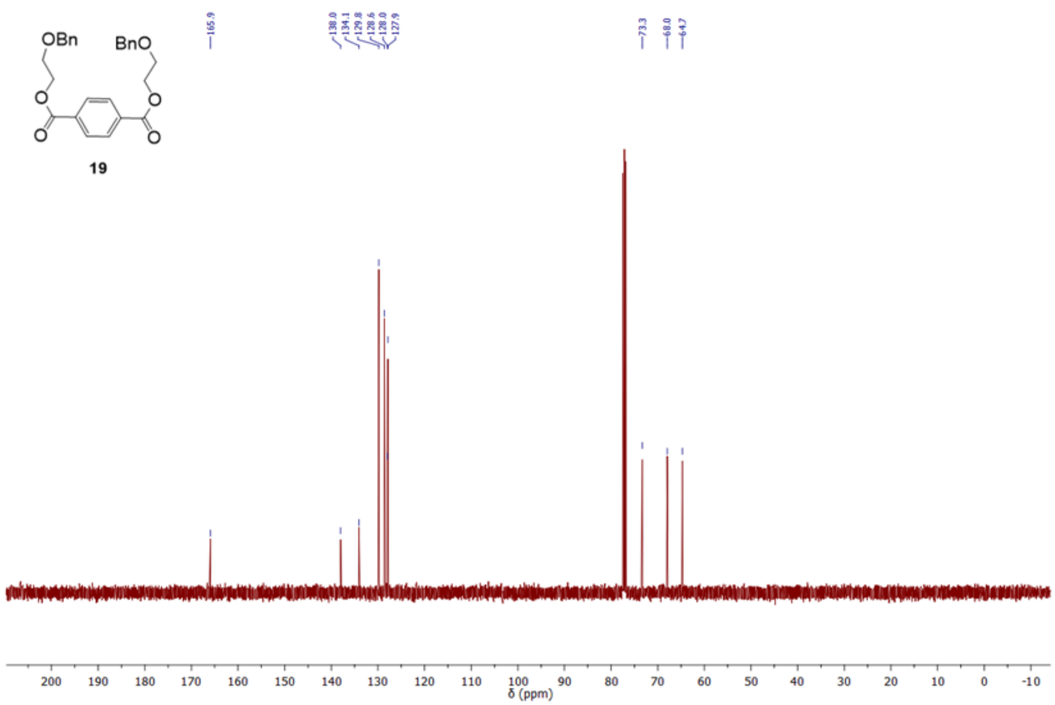
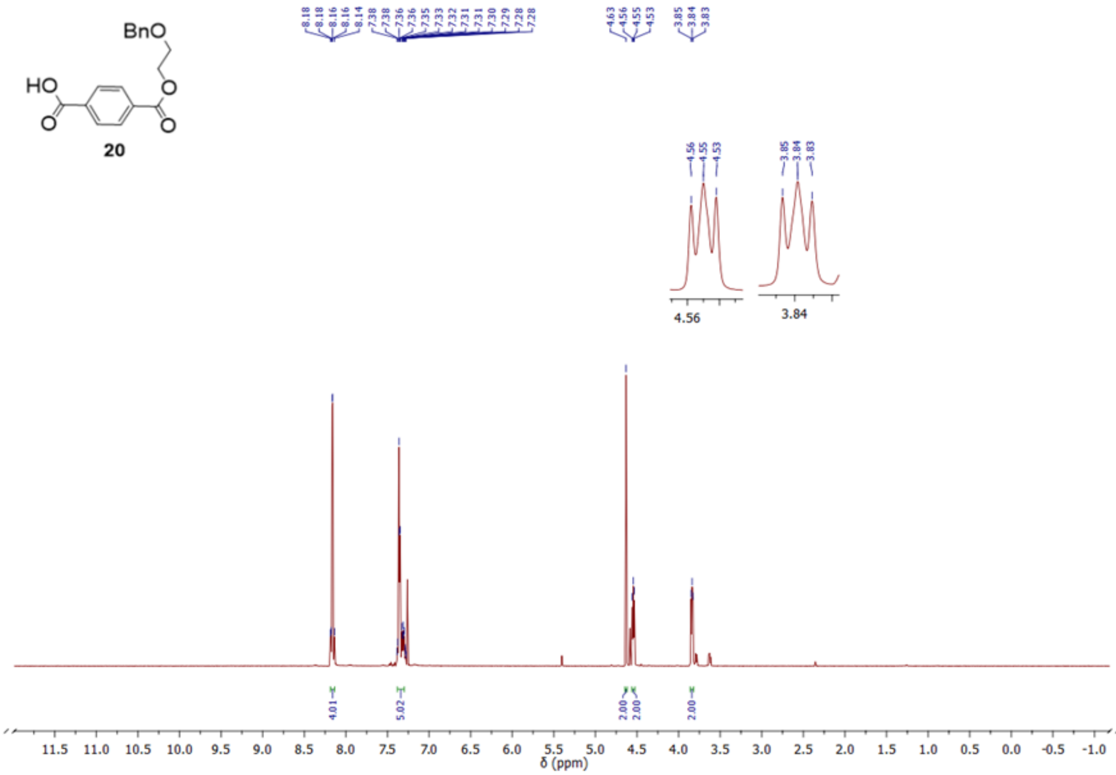


Figure S8. ^{1}H NMR spectrum (400 MHz) of **19** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **19** recorded in $CDCl_3$.



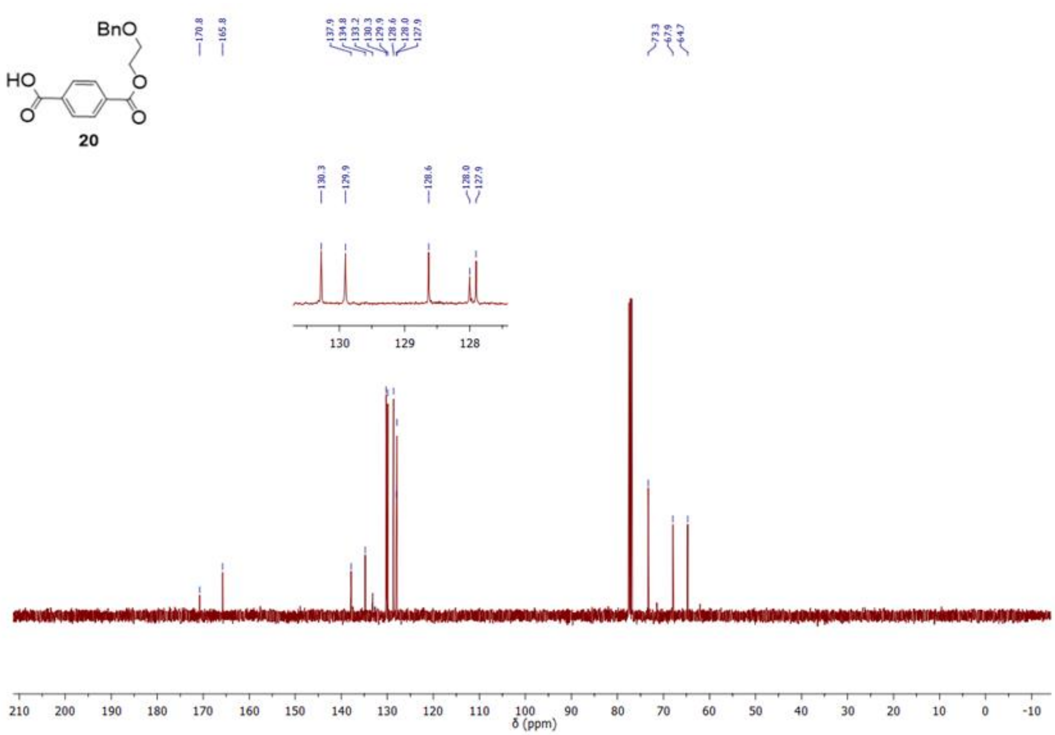
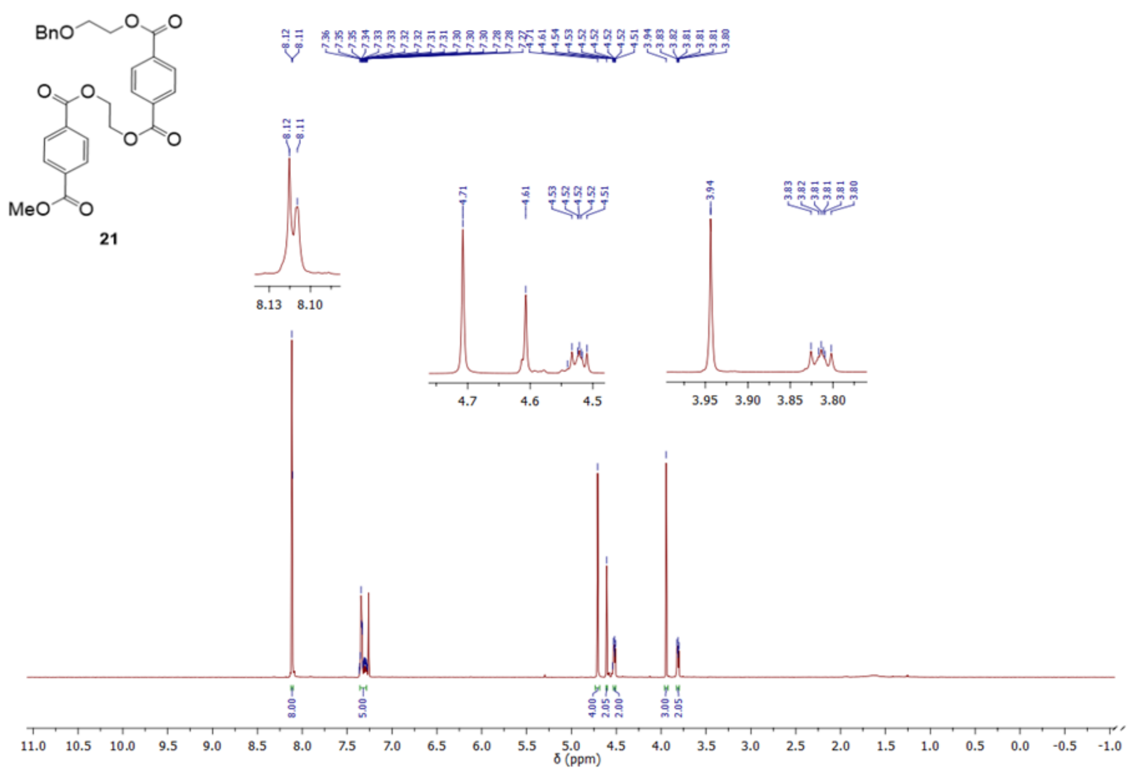


Figure S9. ^{1}H NMR spectrum (400 MHz) of **20** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **20** recorded in $CDCl_3$.



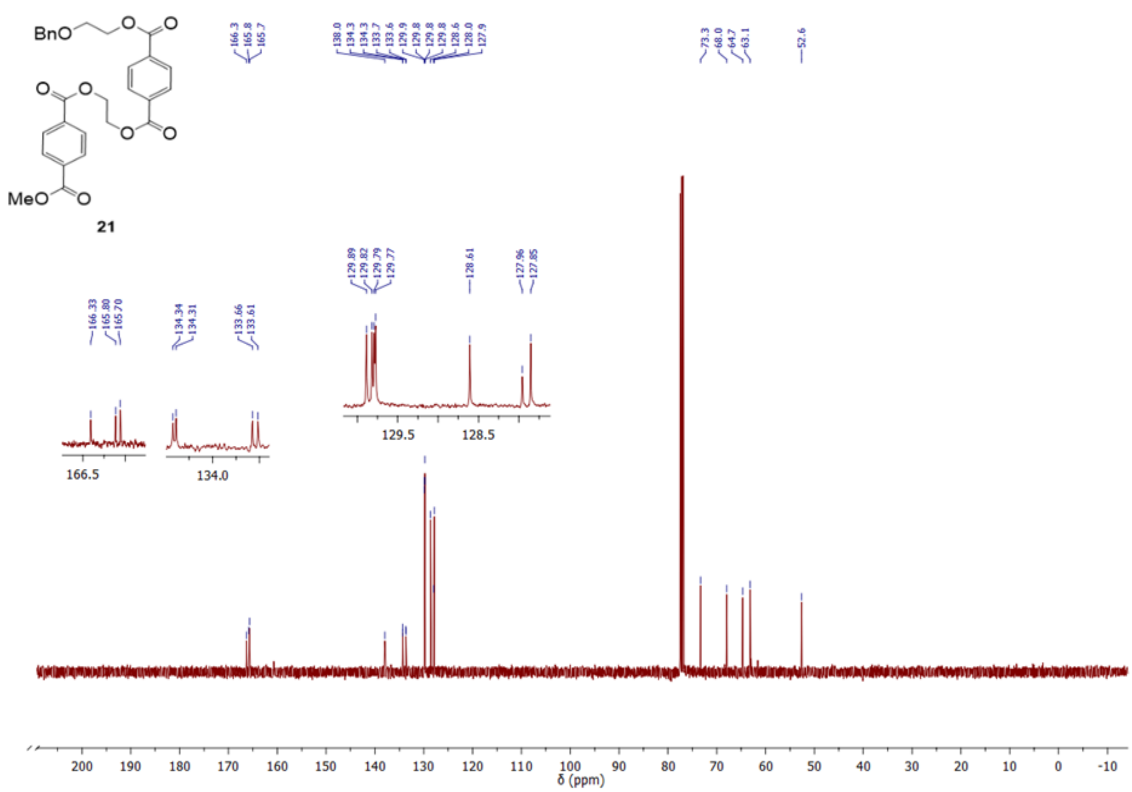
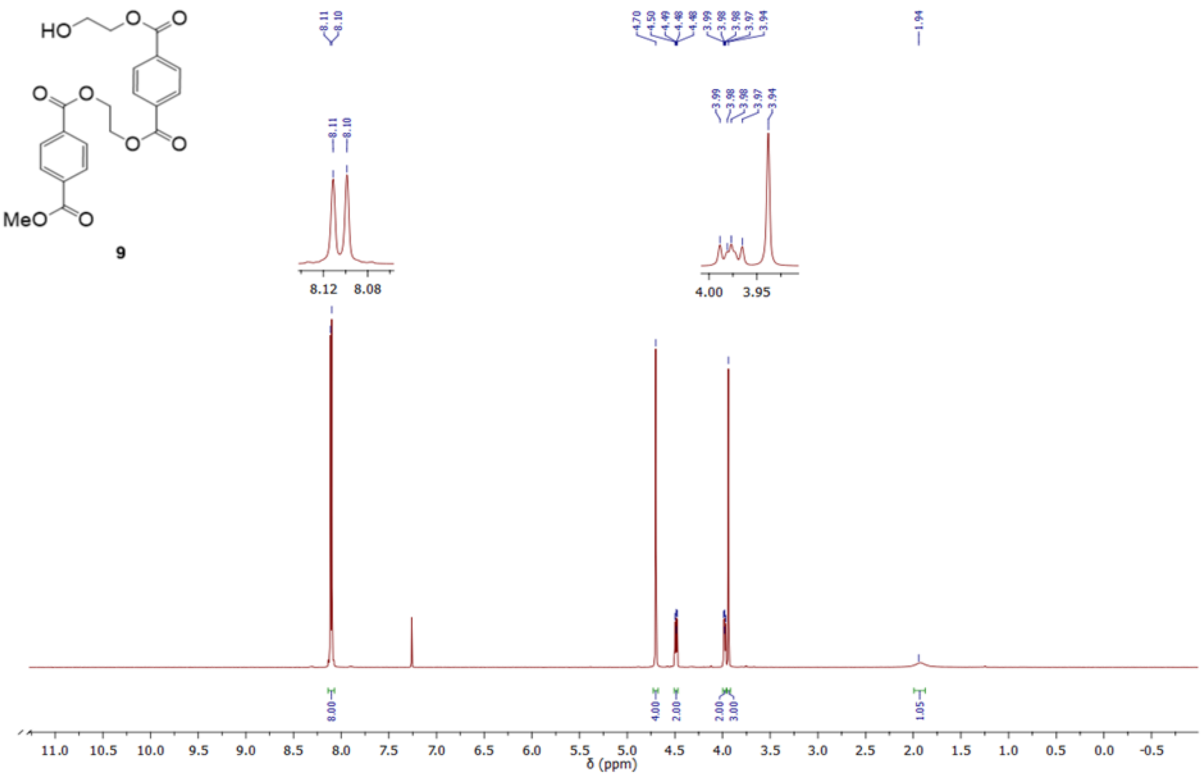


Figure S10. ^{1}H NMR spectrum (400 MHz) of **21** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **21** recorded in $CDCl_3$.



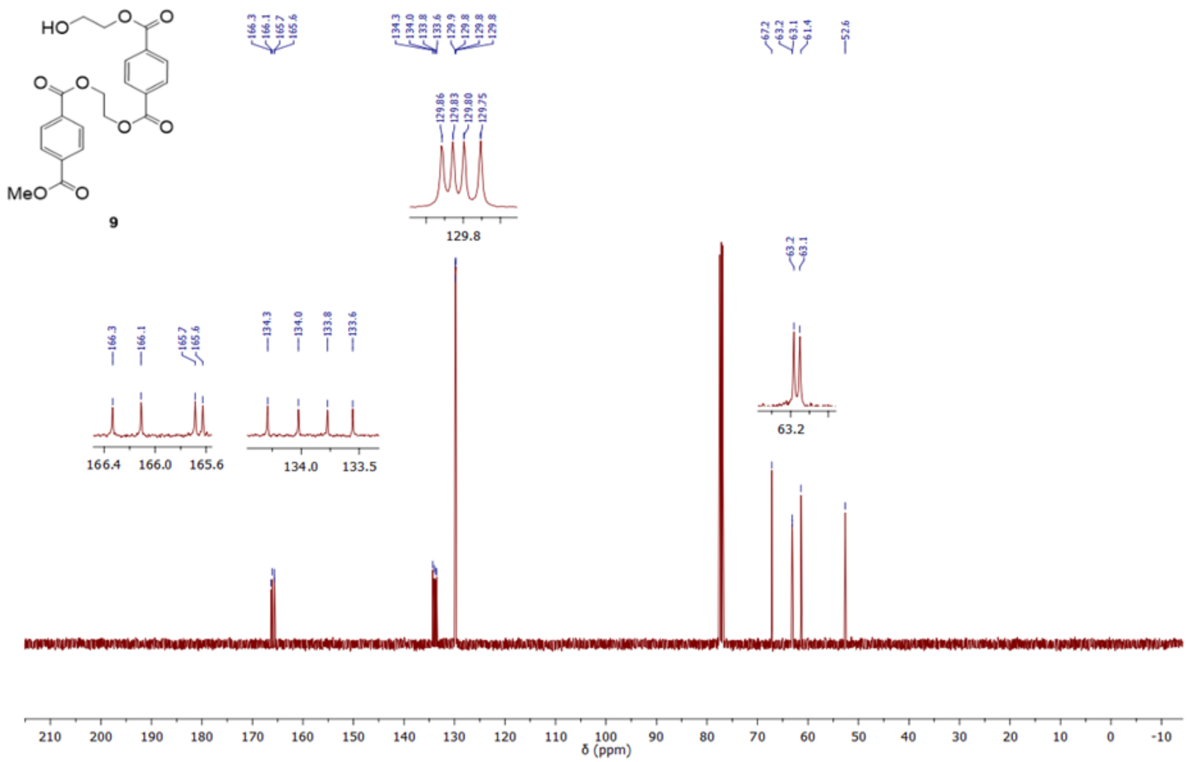


Figure S11. ^{1}H NMR spectrum (400 MHz) of **9** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **9** recorded in $CDCl_3$.

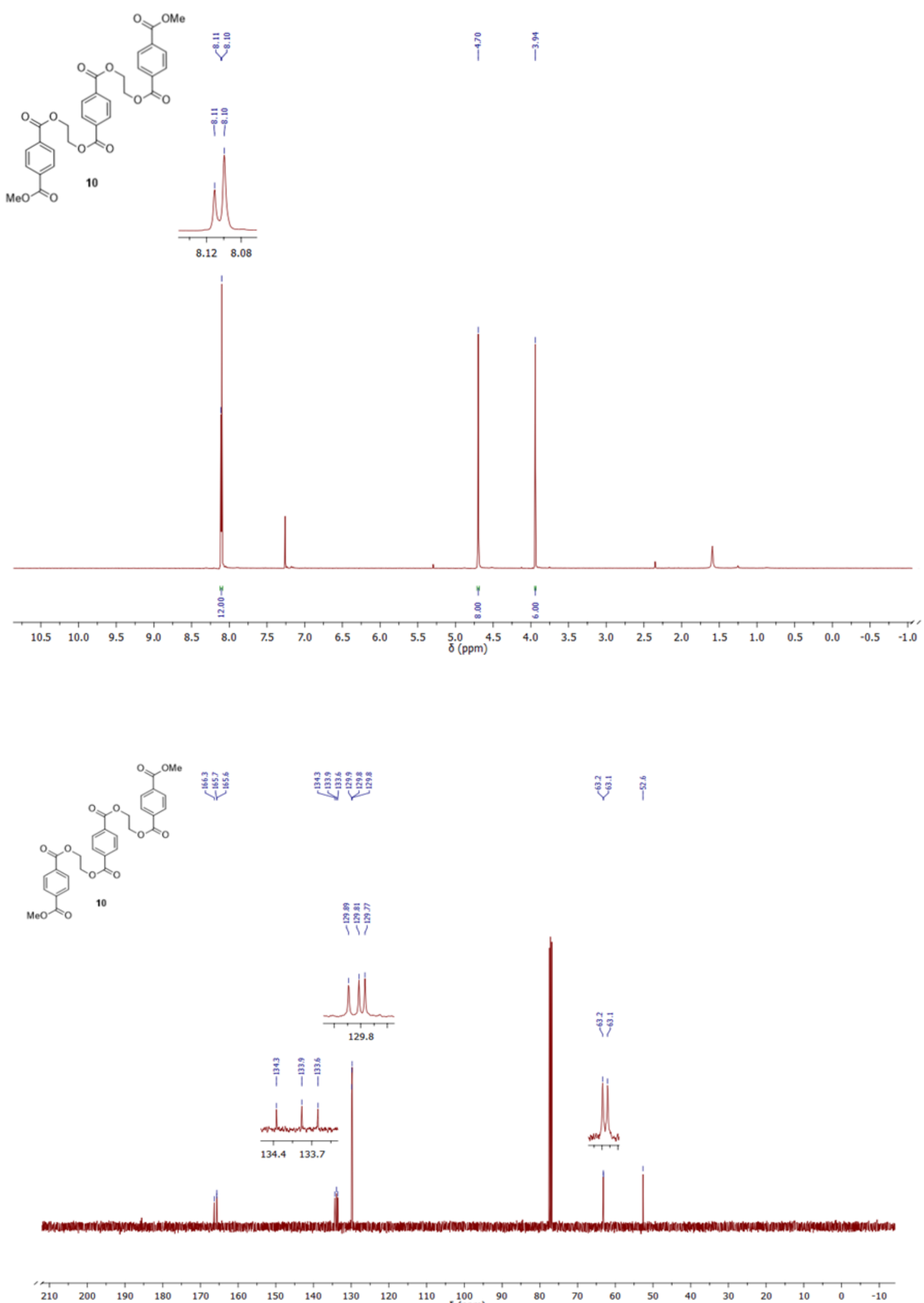
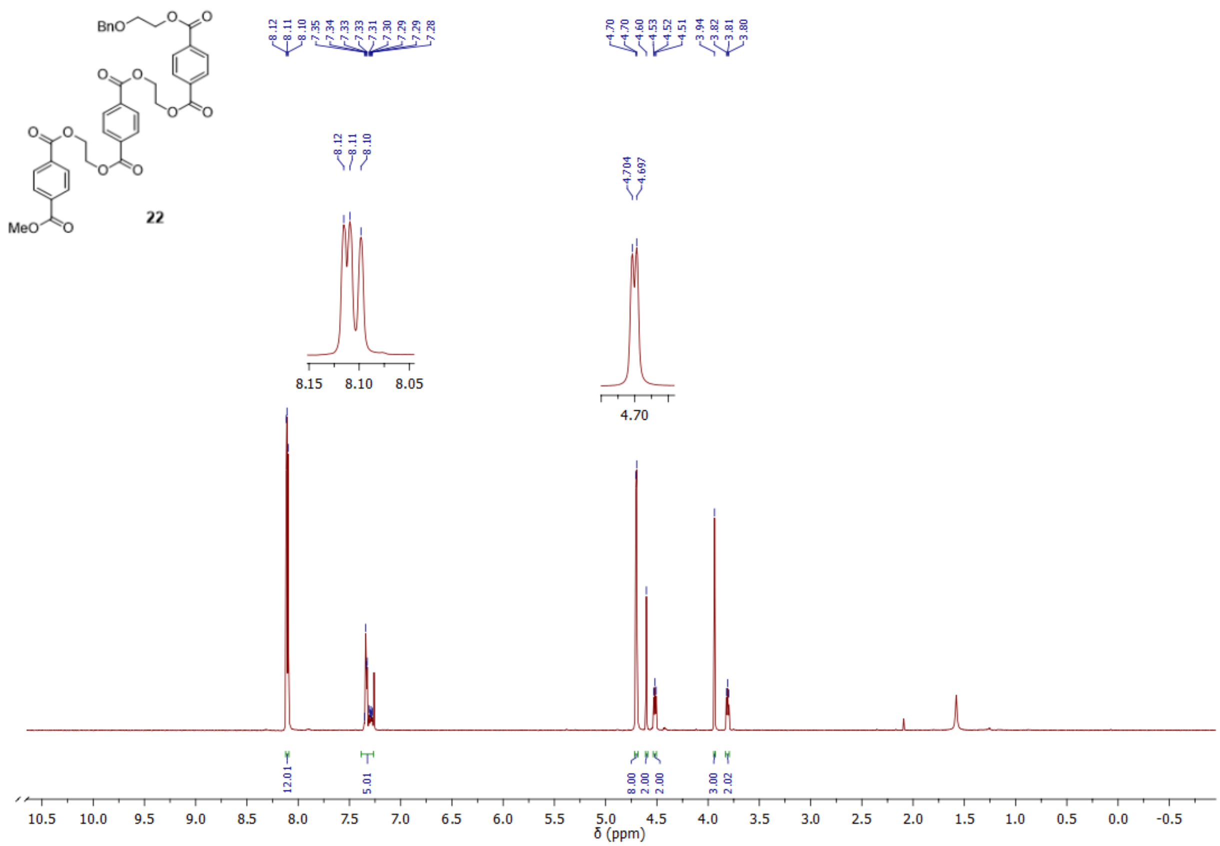


Figure S12. ^{1}H NMR spectrum (400 MHz) of **10** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **10** recorded in $CDCl_3$.



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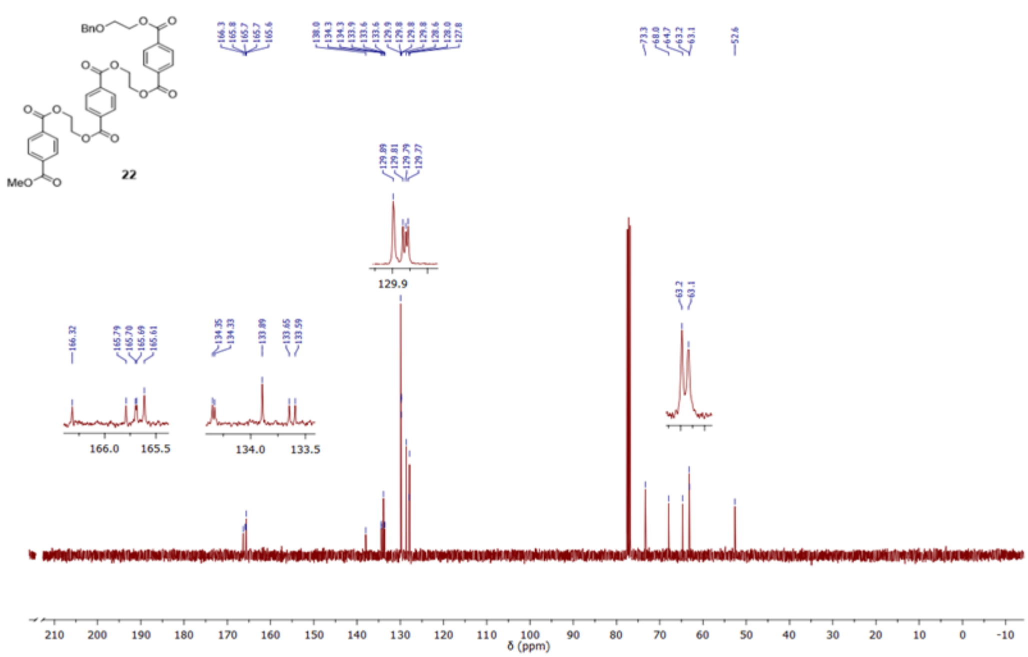

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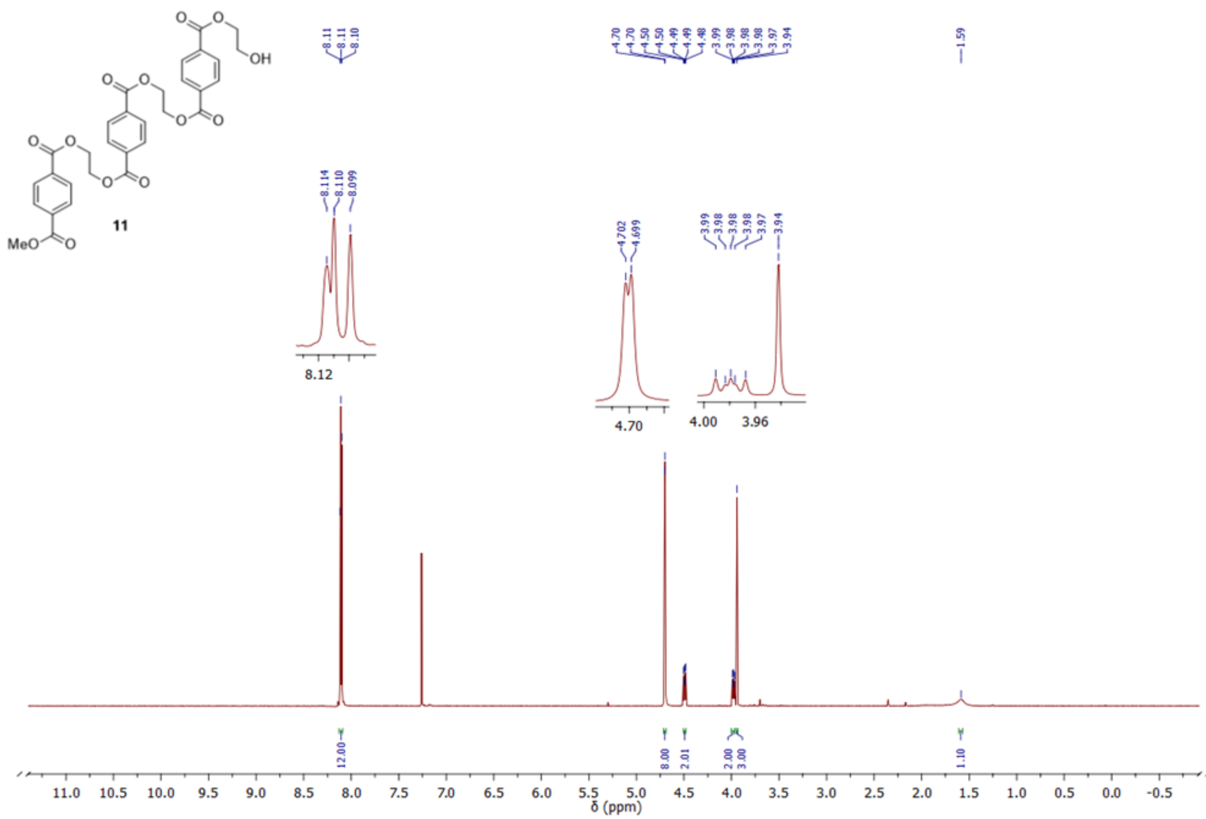
Figure S13. ^{1}H NMR spectrum (400 MHz) of **22** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **22** recorded in $CDCl_3$.



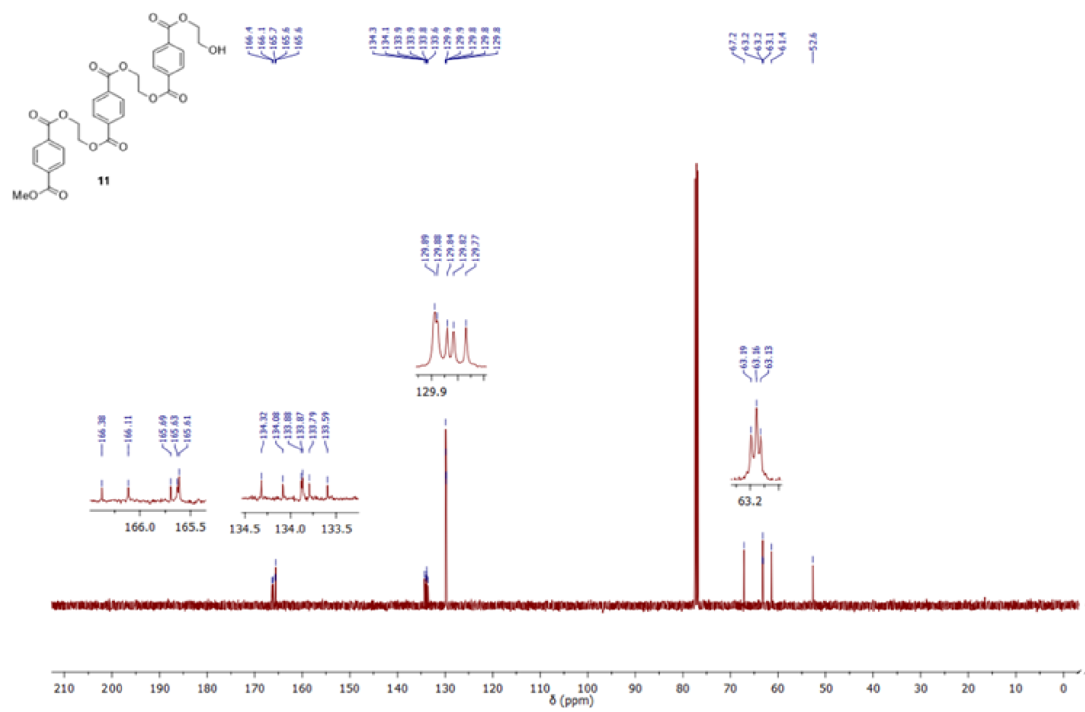


Figure S14. ^{1}H NMR spectrum (400 MHz) of **11** recorded in $CDCl_3$ and ^{13}C NMR spectrum (100 MHz) of **11** recorded in $CDCl_3$.

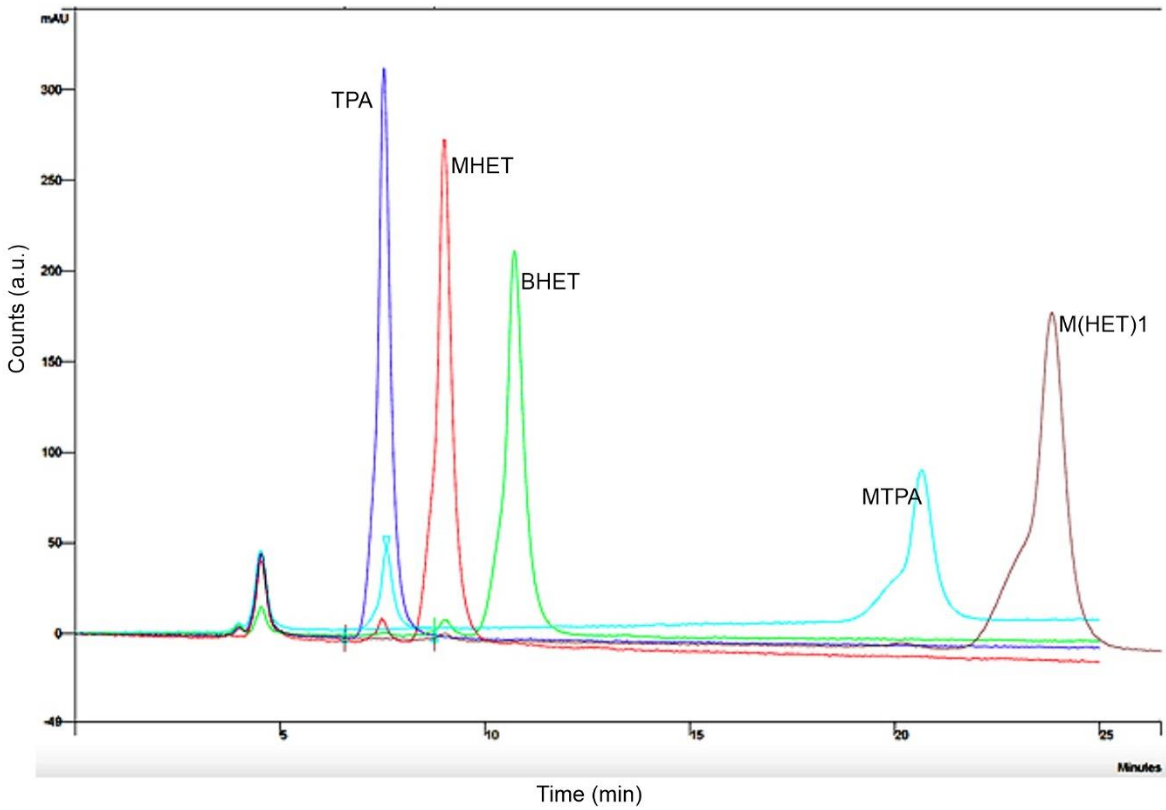


Figure S15. HPLC spectrum of standard compounds used for identification and quantification enzymatic hydrolysis products. Blue: TPA, red: MHET, green: BHET, turquoise: MTPA and brown: M(HET)1.

Table S1. Calculated effective concentrations causing 50% and 20% bioluminescence inhibition (EC_{50} and EC_{20} values) and the respective 95% confidence intervals obtained after 15 min of *A. fischeri* exposure to PET compounds **1-11**

PET compound	**EC_{50} (μg/mL)**	**EC_{20} (μg/mL)**
1 (EG)	>125	>125
2 (TPA)	27.12 (22.87-31.37)	13.64 (9.05-18.23)
3 (DMTP)	133.11 (129-.2-137.02)	32.64 (28.99-36.29)
4 (BHET)	>125	>125
5 (BEET)	43.06 (41.63-44.49)	13.10 (11.64-14.56)
6 (EGDB)	<7.81	<7.81
7 (M(HET)1)	>125	>125
8 (M2(HET)1.5)	>125	>125
9 (M(HET)2)	>125	80.64 (75.96-85.32)
10 (M2(HET)2.5)	>125	>125
11 (M(HET)3)	8.95 (7.16-10.74)	2.25 (0.22-4.28)

Table S2. Concentration of each product derived from the hydrolysis of model compounds **8** (M2(HET)1.5), **9** (M(HET)2) and **10** (M2(HET)2.5) by HiC after 24 h at either 30 or 60 °C.

Substrate	**TPA (μM)**	**methyl TPA (μM)**	**MHET (μM)**	**PET-J (μM)**	**BHET (μM)**
8 (M2(HET)1.5) 60 °C control	16.3±0.5	14.5±0.4	7.2±0.4	0.0±0.0	0.0±0.0
8 (M2(HET)1.5) 60 °C	31.4±4.0	26.1±0.1	8.4±0.2	0.0±0.0	0.0±0.0
8 (M2(HET)1.5) 30 °C[a]	4.1±0.6	278.6±30.0	32.9±1.9	0.0±0.0	0.0±0.0
9 (M(HET)2) 60 °C control	259.9±1.4	190.6±0.8	194.6±1.2	8.0±0.1	9.7±0.2
9 (M(HET)2) 60 °C	497.4±1.1	342.2±9.1	326.7±7.3	10.2±1.0	13.6±0.2
9 (M(HET)2) 30 °C[a]	41.7±3.8	2325.1±84.3	1691.1±98.8	86.5±11.5	192.5±7.9
10 (M2(HET)2.5) 60 °C[a]	traces	traces	0.0±0.0	traces	0.0±0.0
10 (M2(HET)2.5) 30 °C[a]	0.0±0.0	0.0±0.0	traces	traces	0.0±0.0

[a] Corresponding control reactions showed no peaks