Electronic Supplementary Material (ESI) for Faraday Discussions.

High-throughput selection of (new) enzymes: phage display-mediated isolation of alkylhalide hydrolases from a library of active-site mutated epoxide hydrolases

Marija Blazic, Candice Gautier, Thomas Norberg and Mikael Widersten,*

Department of Chemistry – BMC, Uppsala University, Box 576, SE-751 23 Uppsala, Sweden

* mikael.widersten@kemi.uu.se

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Table S1 Oligonucleotides used in the construction of mutant cDNA library

Name	Sequence 5'→ 3'	Ratios in PCR	Mutation(s)	Note
Fp_PUC	CCCAGTCACGACGTTGTAAAACG		-	Fragment 1, forward primer
Rp_HYX1	TATAGCCTTTAGCCCCTCAAC		-	Fragment 1, reverse primer
Fp_ HYN1A	GCTAAAGGCTATATATGGGGAAGATNDTNDTATATCGAGATTTCAGGTACCGG	144	H153X, Y154X	Fragment A, forward primer
	GCTAAAGGCTATATATGGGGAAGATNDTVMAATATCGAGATTTCAGGTACCGG	72		
	GCTAAAGGCTATATATGGGGAAGATNDTATGATATCGAGATTTCAGGTACCGG	12		
	GCTAAAGGCTATATATGGGGAAGATNDTTGGATATCGAGATTTCAGGTACCGG	12		
Fp_HYV1B	GCTAAAGGCTATATATGGGGAAGATVMAVMAATATCGAGATTTCAGGTACCGG	36		
	GCTAAAGGCTATATATGGGGAAGATVMANDTATATCGAGATTTCAGGTACCGG	72		
	GCTAAAGGCTATATATGGGGAAGATVMAATGATATCGAGATTTCAGGTACCGG	6		
	GCTAAAGGCTATATATGGGGAAGATVMATGGATATCGAGATTTCAGGTACCGG	6		
Fp_HYA1C	GCTAAAGGCTATATATGGGGAAGATATGATGATATCGAGATTTCAGGTACCGG	1		
	GCTAAAGGCTATATATGGGGAAGATATGNDTATATCGAGATTTCAGGTACCGG	12		
	GCTAAAGGCTATATATGGGGAAGATATGVMAATATCGAGATTTCAGGTACCGG	6		
	GCTAAAGGCTATATATGGGGAAGATATGTGGATATCGAGATTTCAGGTACCGG	1		
Fp_HYT1D	GCTAAAGGCTATATATGGGGAAGATTGGTGGATATCGAGATTTCAGGTACCGG	1		
	GCTAAAGGCTATATATGGGGAAGATTGGNDTATATCGAGATTTCAGGTACCGG	12		
	GCTAAAGGCTATATATGGGGAAGATTGGVMAATATCGAGATTTCAGGTACCGG	6		
	GCTAAAGGCTATATATGGGGAAGATTGGATGATATCGAGATTTCAGGTACCGG	1		
Rp_PF1	CTGTATGGCGCTAGGAC			Fragment A, reverse primer
Fp_PFN1A	ACATACCGCGATCCTGCANDTNDTTATTTTCCTAAAGGCAAAGGCC	144	P188X, F189X	Fragment B, forward primer
	ACATACCGCGATCCTGCANDTVMATATTTTCCTAAAGGCAAAGGCC	72		
	ACATACCGCGATCCTGCANDTATGTATTTTCCTAAAGGCAAAGGCC	12		
	ACATACCGCGATCCTGCANDTTGGTATTTTCCTAAAGGCAAAGGCC	12		
Fp_PFV1B	ACATACCGCGATCCTGCAVMAVMATATTTTCCTAAAGGCAAAGGCC	36		

	ACATACCGCGATCCTGCAVMANDTTATTTTCCTAAAGGCAAAGGCC	72		
	ACATACCGCGATCCTGCAVMAATGTATTTTCCTAAAGGCAAAGGCC	6		
	ACATACCGCGATCCTGCAVMATGGTATTTTCCTAAAGGCAAAGGCC	6		
Fp_PFA1C	ACATACCGCGATCCTGCAATGATGTATTTTCCTAAAGGCAAAGGCC	1		
	ACATACCGCGATCCTGCAATGTGGTATTTTCCTAAAGGCAAAGGCC	12		
	ACATACCGCGATCCTGCAATGVMATATTTTCCTAAAGGCAAAGGCC	6		
	ACATACCGCGATCCTGCAATGTGGTATTTTCCTAAAGGCAAAGGCC	1		
Fp_PFT1D	ACATACCGCGATCCTGCATGGTGGTATTTTCCTAAAGGCAAAGGCC	1		
	ACATACCGCGATCCTGCATGGNDTTATTTTCCTAAAGGCAAAGGCC	12		
	ACATACCGCGATCCTGCATGGVMATATTTTCCTAAAGGCAAAGGCC	6		
	ACATACCGCGATCCTGCATGGATGTATTTTCCTAAAGGCAAAGGCC	1		
Rp_TYR1	GTTAACTGCACCAGTGAAACC			Fragment B, reverse primer
Fp_TYRN1A	GTTTCACTGGTGCAGTTAACTATNDTCGTGCTTTACCCATAAACTGG	6	Y235X	Fragment C, forward primer
Fp_TYRV1B	GTTTCACTGGTGCAGTTAACTATVMACGTGCTTTACCCATAAACTGG	3		
Fp_TYRA1C	GTTTCACTGGTGCAGTTAACTATATGCGTGCTTTACCCATAAACTGG	0.5		
Fp_TYRT1D	GTTTCACTGGTGCAGTTAACTATTGGCGTGCTTTACCCATAAACTGG	0.5		
Rp_PUC	AGCGGATAACAATTTCACACAGG			Fragment C, reverse primer
Fp_PUC_EH	CTGAAATGAGCTGTTGACAATTAATCATCGGCTCGTATAATG			Fragment 1A, forward primer
Rp-BamHI	CAGGATCCCGGTATGTCAATATTTTCTTAAGAACAGACTTAGCACC			Fragment 1A, reverse primer
Fp_BamHI	GGTGCTAAGTCTGTTCTTAAGAAAATATTGACATACCGGGATCCTG		R184 silent mutation,	Fragment BC, forward primer, introducing *Bam*HI site
Rp_PUC_EH	GACCATGATTACGCCAAGCTTGCATGC			Fragment BC, reverse primer
Fp_PCEH	CGGATAACAATTTCACACAGG			Forward primer, StEH
Rp_BCEH	GGAAACAGCTATGACCATG			Reverse primer, StEH
NcoI_fp	AATCCTCTTCATCCACGGCTTCCCTGAACT		H31H, silent mutation	Forward primer, removing one *Nco*I site
PauI_fp	CGACTGGGGCGCGCTAATTGCTTGGCATTT		L109L, silent mutation	Forward primer, introducing *Pau*I site
fp_D105A	GTGTTTGTCGTTGCGCACGCCTGGGGCGCGTTAATTGCTTGG			Forward primer for D105A mutagenesis
rp_D105A	CCAAGCAATTAACGCGCCCCAGGCGTGCGCAACGACAAACAC			Reverse primer for D105A mutagenesis

Table S2 Deduced amino acid sequences of selected protein variants

	Amino acid position					
Clone #	H153	Y154	P188	F189	Y235	Comments
HYPFV-2_1, -2_2, -3_1, -3_2, -3_3	○[a]	○	○	○	V (gtt)	
HYPFI-2, -3_1, -3_2, -3_3	○	○	○	○	I (att)	
HYPFR-1_1	○	○	○	○	R (cgt)	
HYPFR-1_2	○	○	○	○	R (cgt)	E198D (gag→gac)
HYPSE-2, -3	○	○	○	S (agt)	E (gaa)	
HYPFC-1, -3	○	○	○	○	C (tgt)	
HYPFD-1	○	○	○	○	D (gat)	
HYPFG-1	○	○	○	○	G (ggt)	
HYPFH-2	○	○	○	○	H (cat)	
VEPFR-1	V (gtt)	E (gaa)	○	○	R (cgt)	S311N (agc→aac)
GYFPV-1	G (ggt)	○	F (ttt)	P (cca)	V (gtt)	
HYFAV-3	○	○	F (ttt)	A (gca)	V (gtt)	
YNPFH-2	Y (tat)	N (aat)	○	○	H (cat)	
HYACK-1	○	○	A (gca)	C (tgt)	K (aaa)	
HYFKY-2	○	○	F (ttt)	K (aaa)	○	
HWPFT-2	○	W (tgg)	○	○	T (aca)	
ISTMK-3	I (att)	S (agt)	T (aca)	M (atg)	K (aaa)	
QMLGY-3	Q (caa)	M (atg)	L (ctt)	G (ggt)	○	
HYMMY-3	○	○	M (atg)	M (atg)		
CEYFC-3	C (tgt)	E (gaa)	Y (tat)	○	C (tgt)	
VKPFY-3	V (gtt)	K (aaa)	○	○	○	
HYFHY-3	○	○	F (ttt)	H (cat)	○	
HYAKM-3	○	○	A (gca)	K (aaa)	M (atg)	
HYEWW-3	○	○	E (gaa)	W (tgg)	W(tgg)	
WAPFH-3	W (tgg)	A (gca)	○	○	H (cat)	
HYYAW-3	○	○	Y (tat)	A (gca)	W (tgg)	
HYQQL-3	○	○	Q (caa)	Q (caa)	L (ctt)	
EAPFY-3	E (gaa)	A (gca)	○	○	○	
EQPQL-3	E (gaa)	Q (caa)	○	Q (caa)	V (gtt)	
KKMAW-3	K (aaa)	K (aaa)	M (atg)	A (gca)	W (tgg)	

[a] ○, no substitution. Naming is according to amino acid substitution in the linear sequence. The number following the one-letter sequence refers to the round of phage display selection from which the clone was isolated.

Supporting Figures

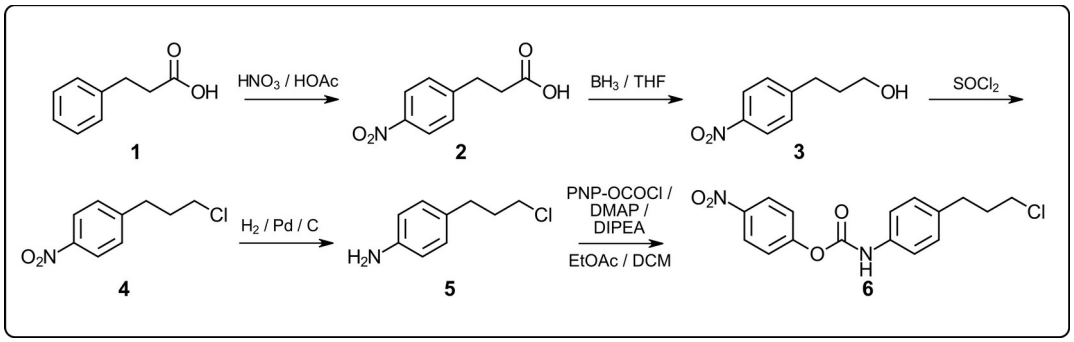

Fig. S1 Synthesis of building block **6** (see **Figs. S4**−**S7** for NMR spectra).

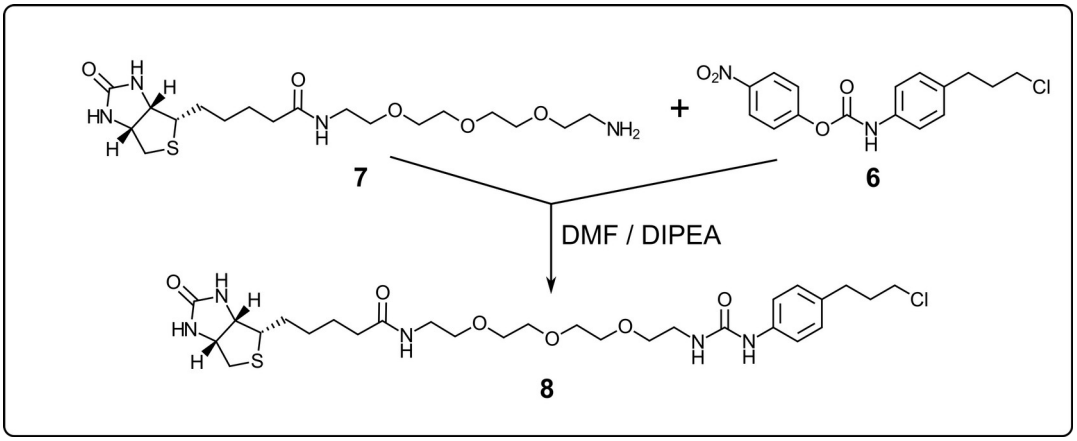

Fig. S2 Block synthesis of bait substrate (**8**) used in phage display selection (see **Fig. S8** for NMR spectra).

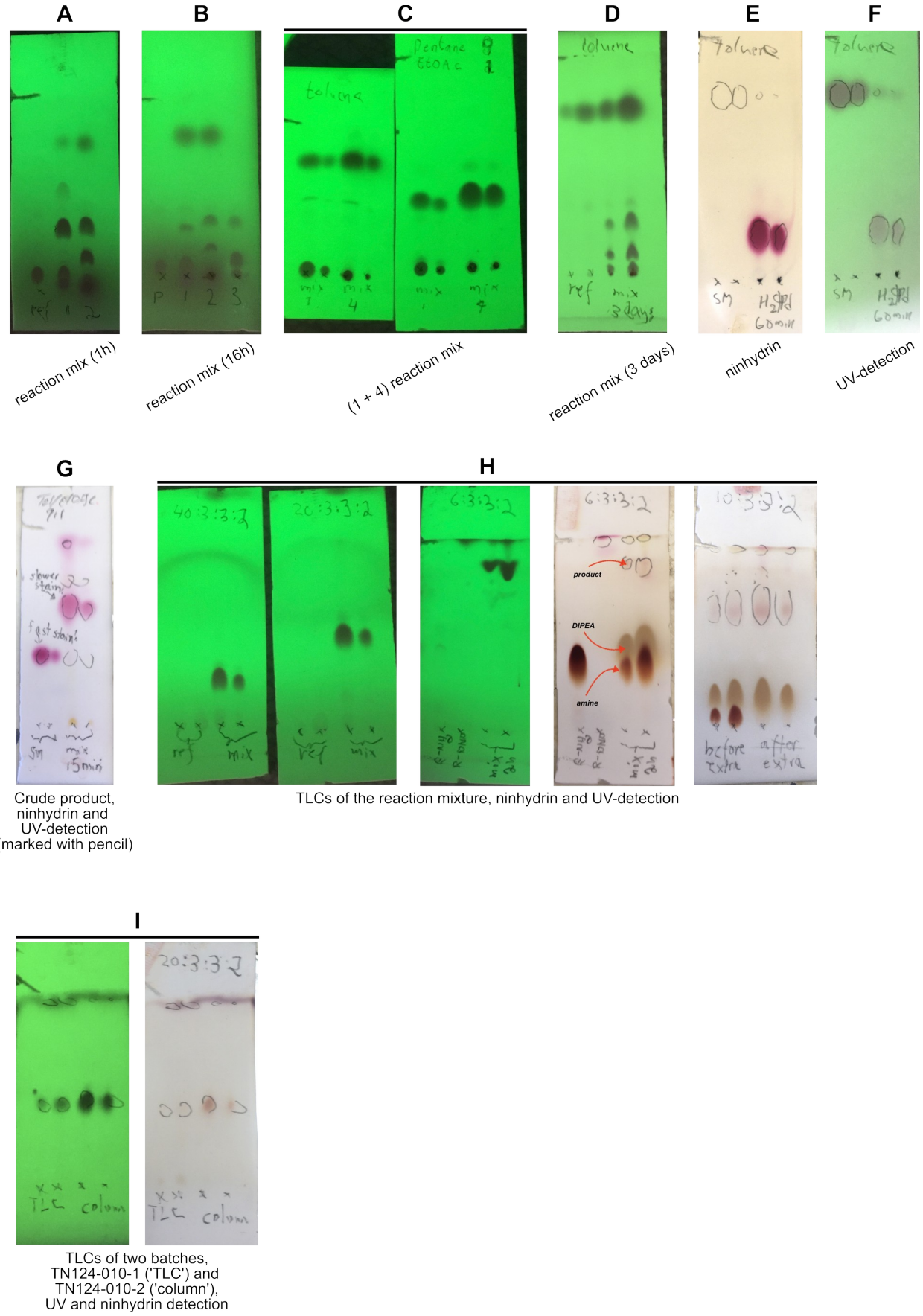

Fig. S3 TLC chromatograms of synthesis intermediates leading to compound **8**.

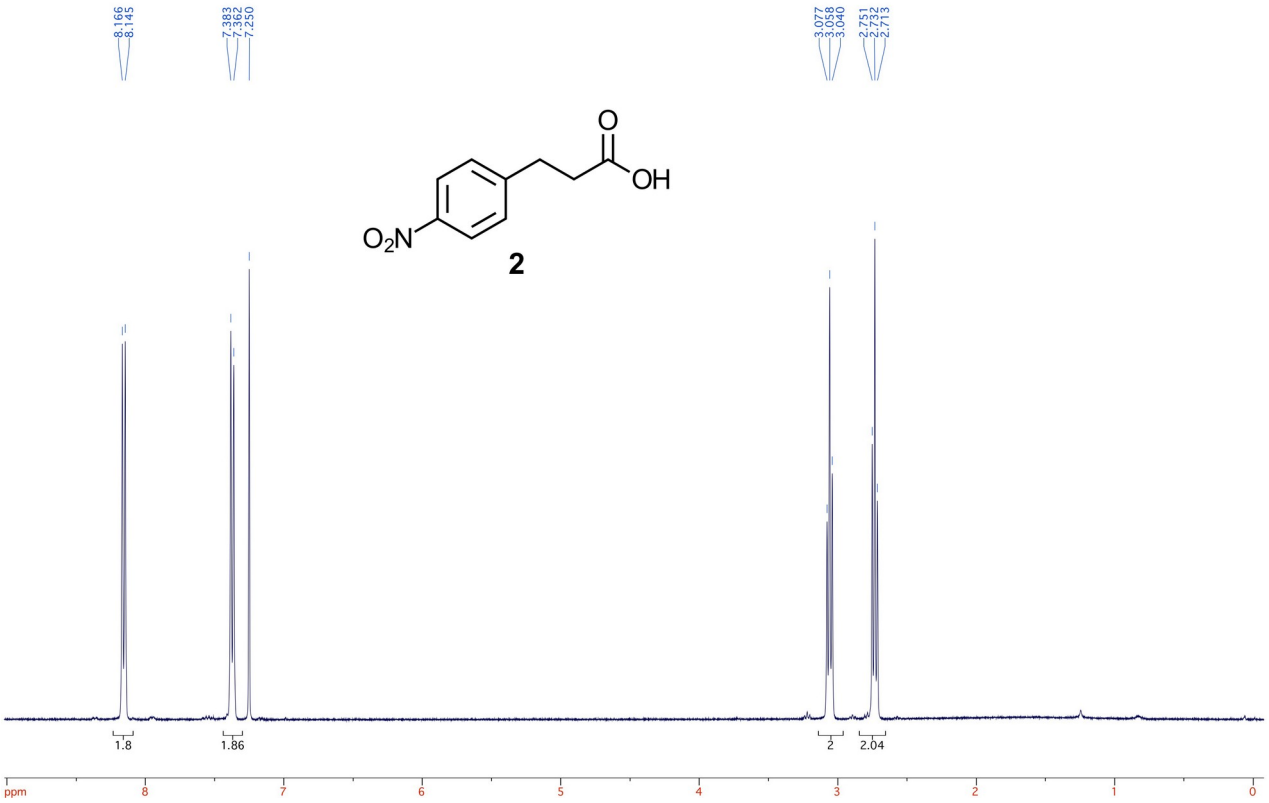

Fig. S4 ^{1}H-NMR spectrum of compound **2**.

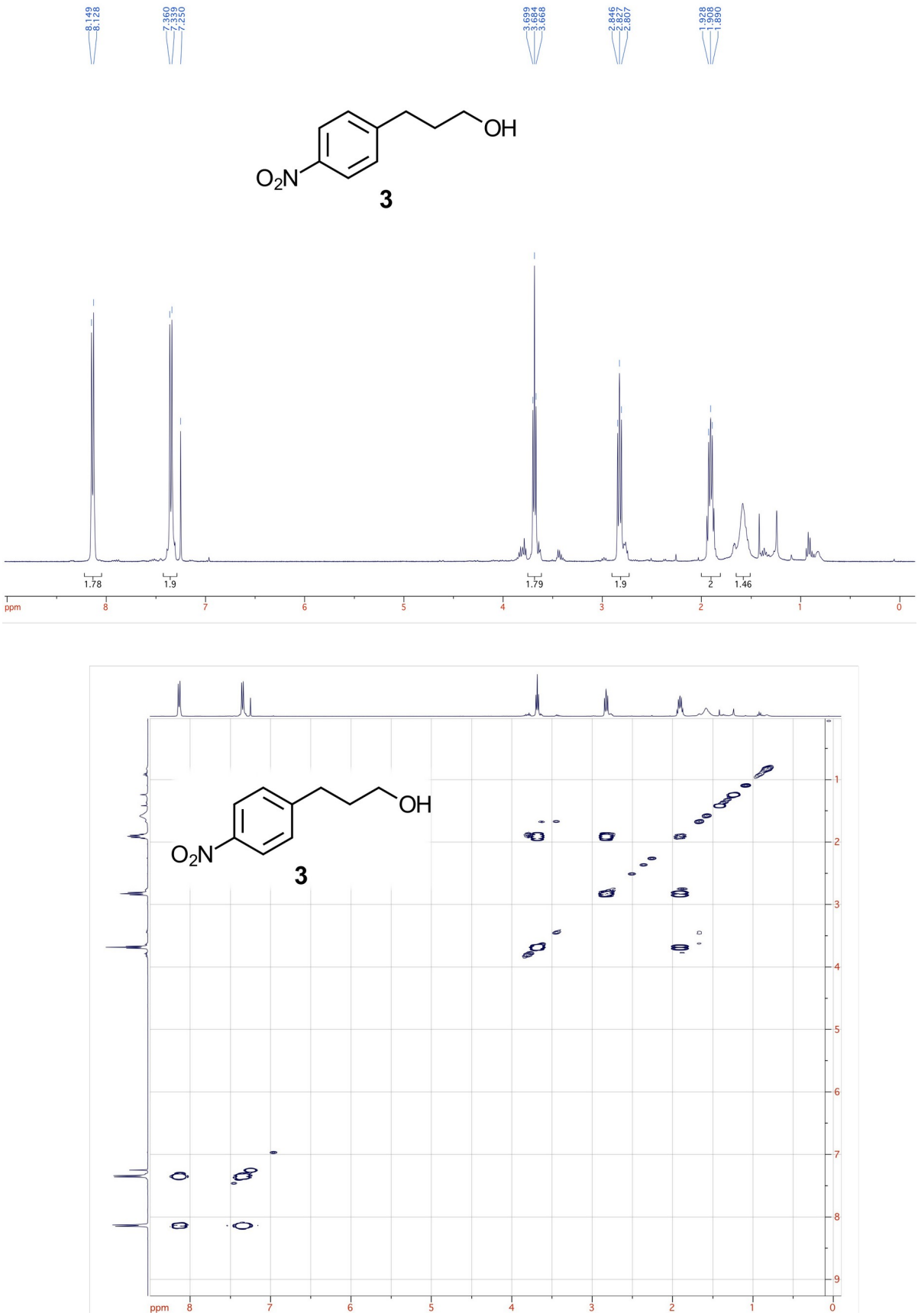

Fig. S5 ^{1}H-NMR (top) and COESY (bottom) spectra of compound **3**.

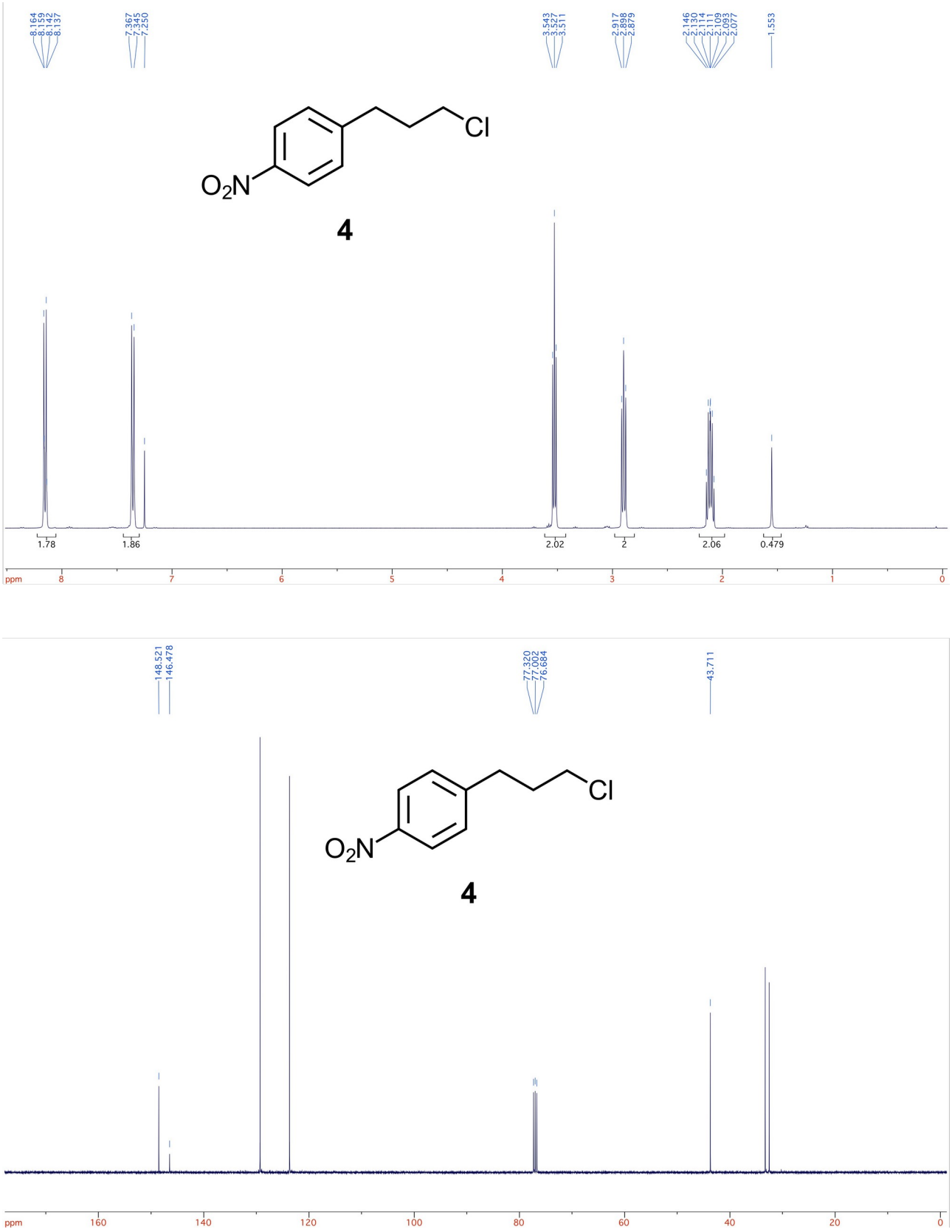

Fig. S6 ^{1}H- (top) and ^{13}C-NMR (bottom) spectra of compound **4**.

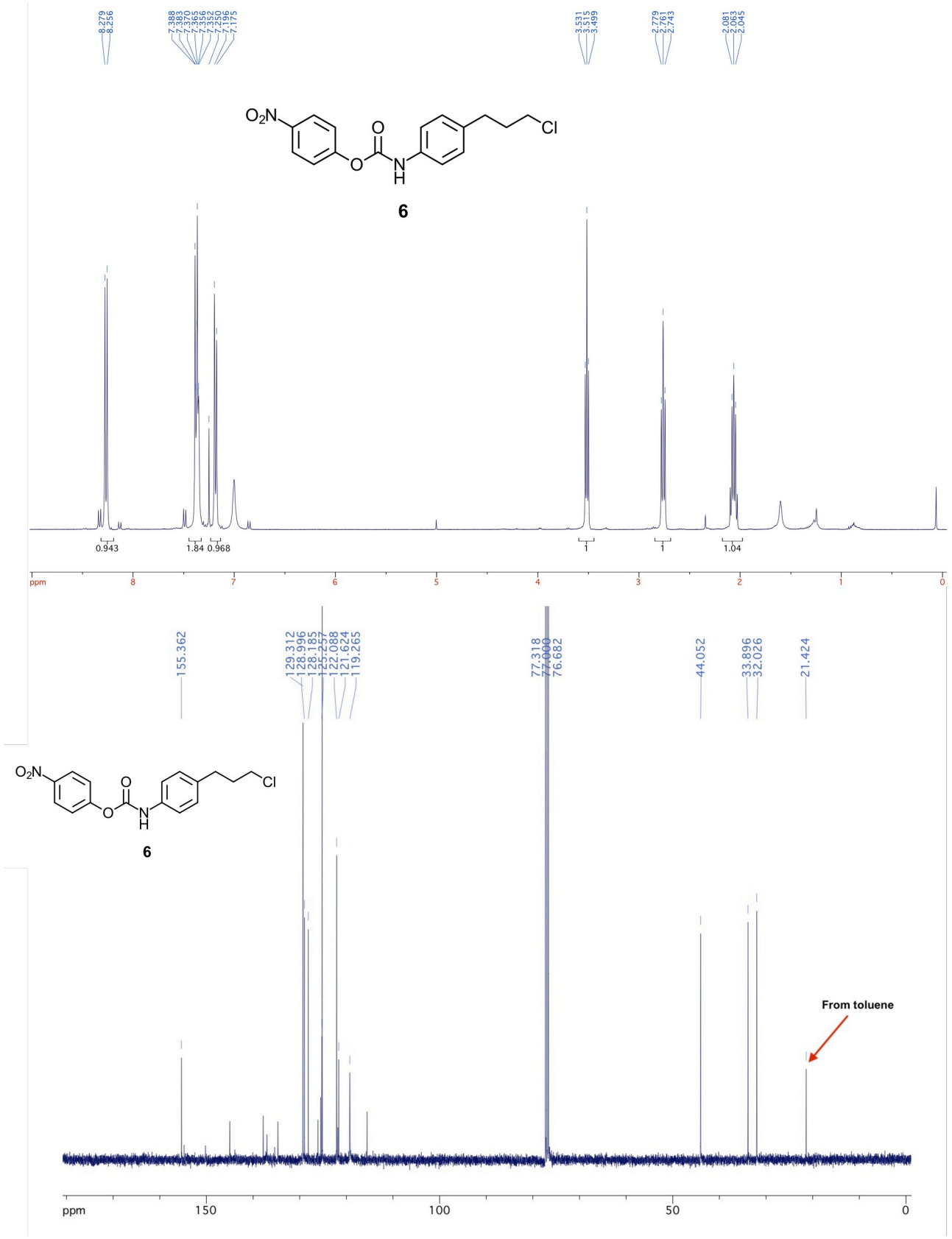

Fig. S7 ^{1}H- (top) and ^{13}C-NMR (bottom) spectra of compound **6**.

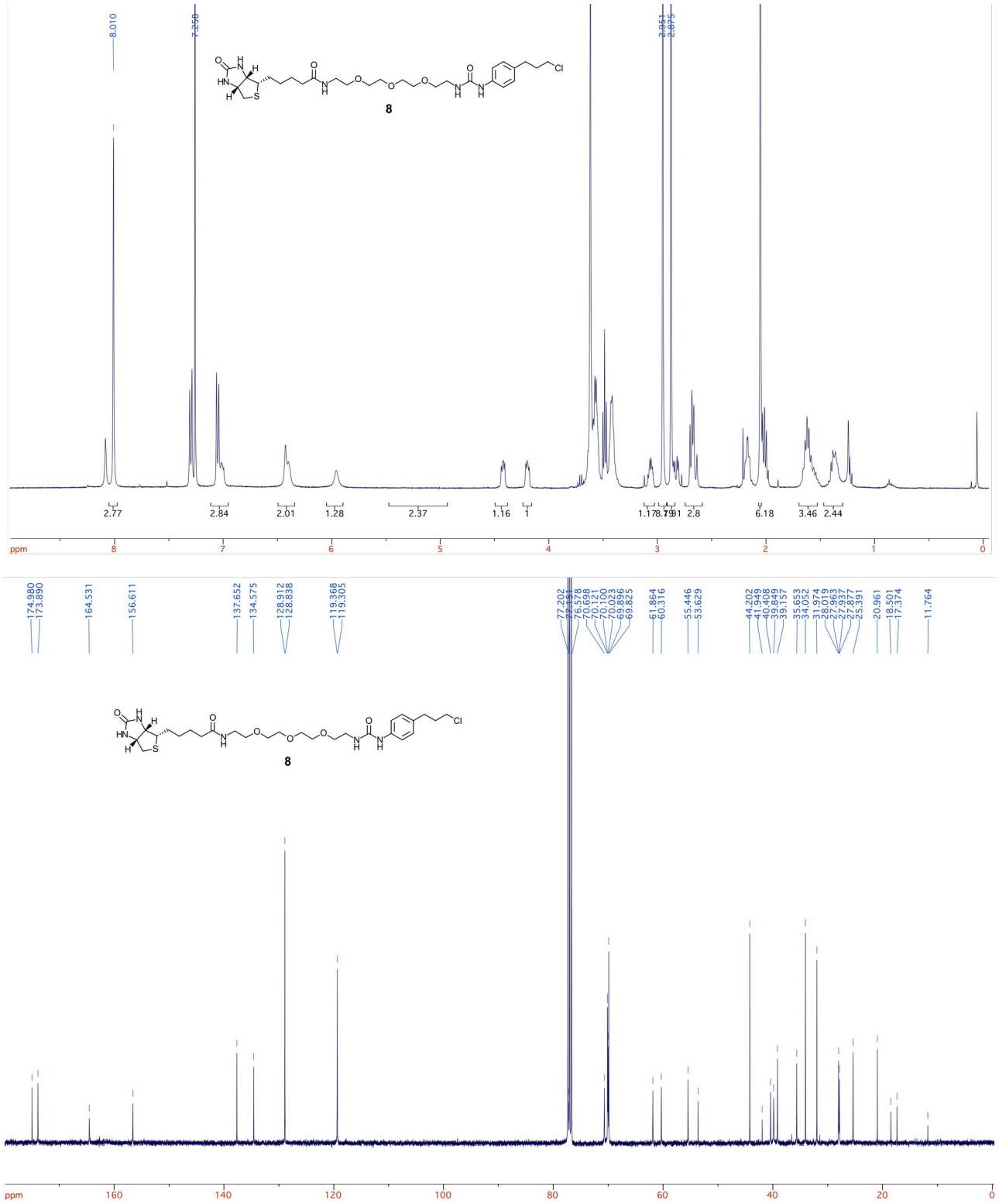

Fig. S8 ^{1}H- (top) and ^{13}C-NMR (bottom) spectra of compound **8**.

```
    30      32              107     109
... ATC CAC GGC ... // ...GGC GCG CTA ...
     I   H   G               G   A   L
       NcoI                    PauI
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Fig. S9 Silent mutations (in yellow) in the StEH1 cDNA that removes an *Nco*I site at codons 30 – 32 and introduces a unique *Pau*I site at codons 107 – 109.

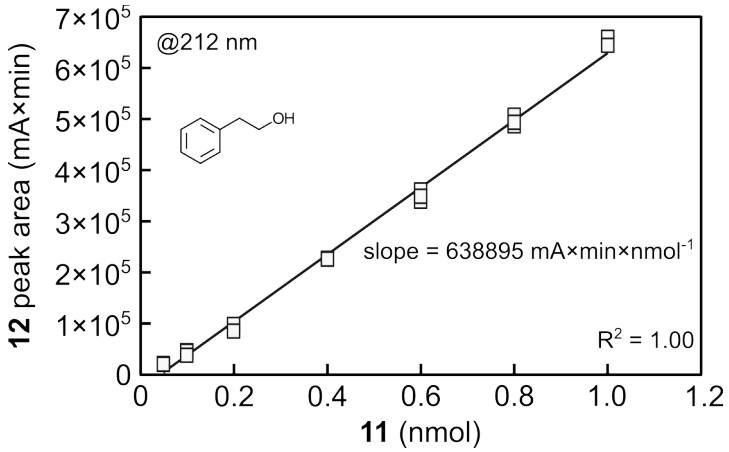

Fig. S10 Standard curve of peak area as a function of amount of alcohol **11**.