SUPPORTING INFORMATION

PDE6D inhibitors with a new design principle selectively block K-Ras activity

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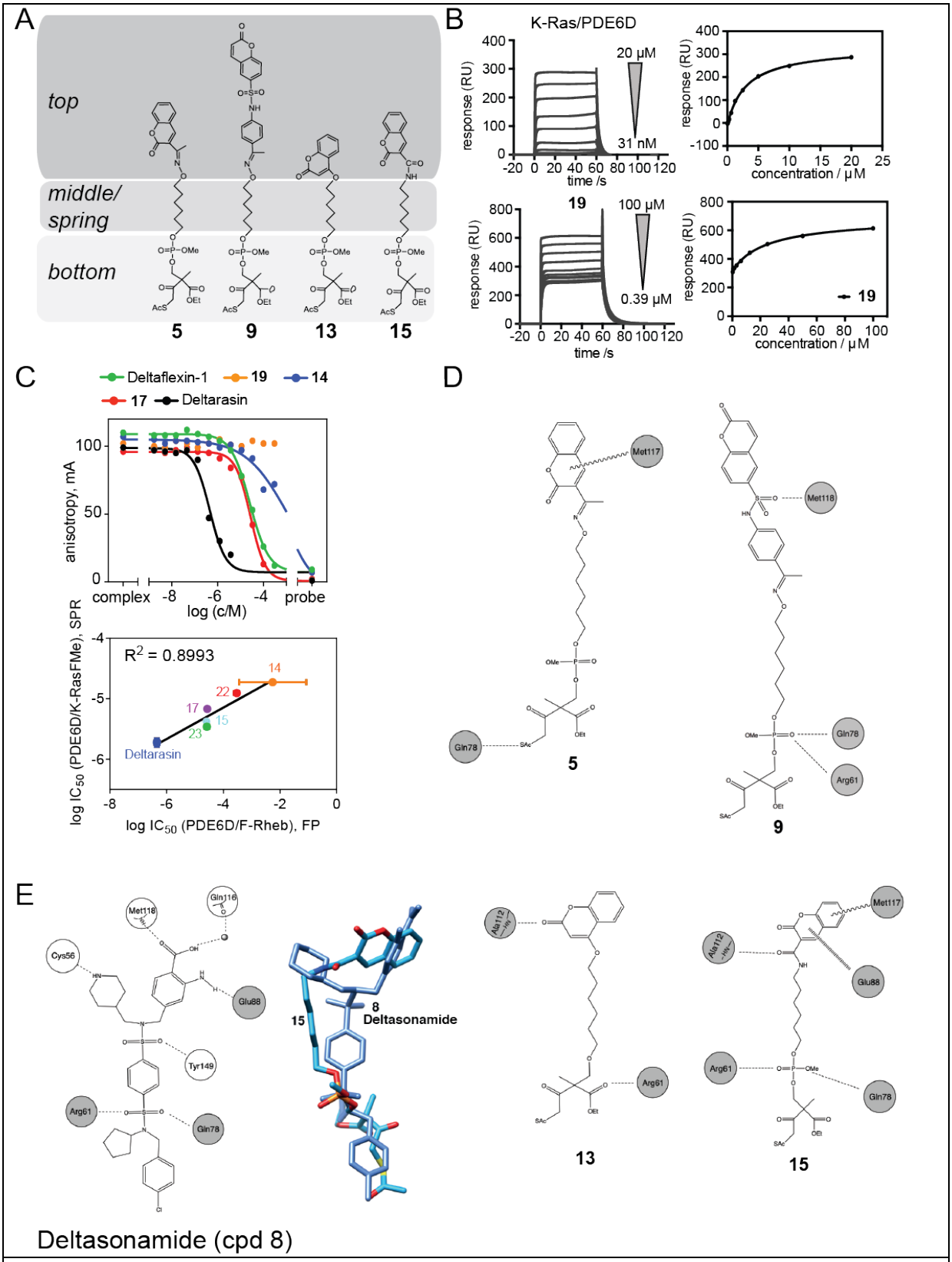
Supplementary Table 1. Affinity of Deltaflexin compounds and Deltarasin determined in a fluorescence anisotropy assay (data from Figures S1C and S4A)

Compound	FP (PDE6D/ F-Rheb)	
	IC_{50} (95% CI) /μM	K_d /μM
Deltarasin	0.45 (0.26 – 0.9)	0.13
15 (Deltaflexin-1)	25.4 (22 – 29)	7.27
19	No binding	
14	855 (337 – 19540)	244.2
17	27.1 (22 – 33)	7.73
22	234 (180 – 336)	68.5
23 (Deltaflexin-2)	25.1 (17 – 37)	7.17

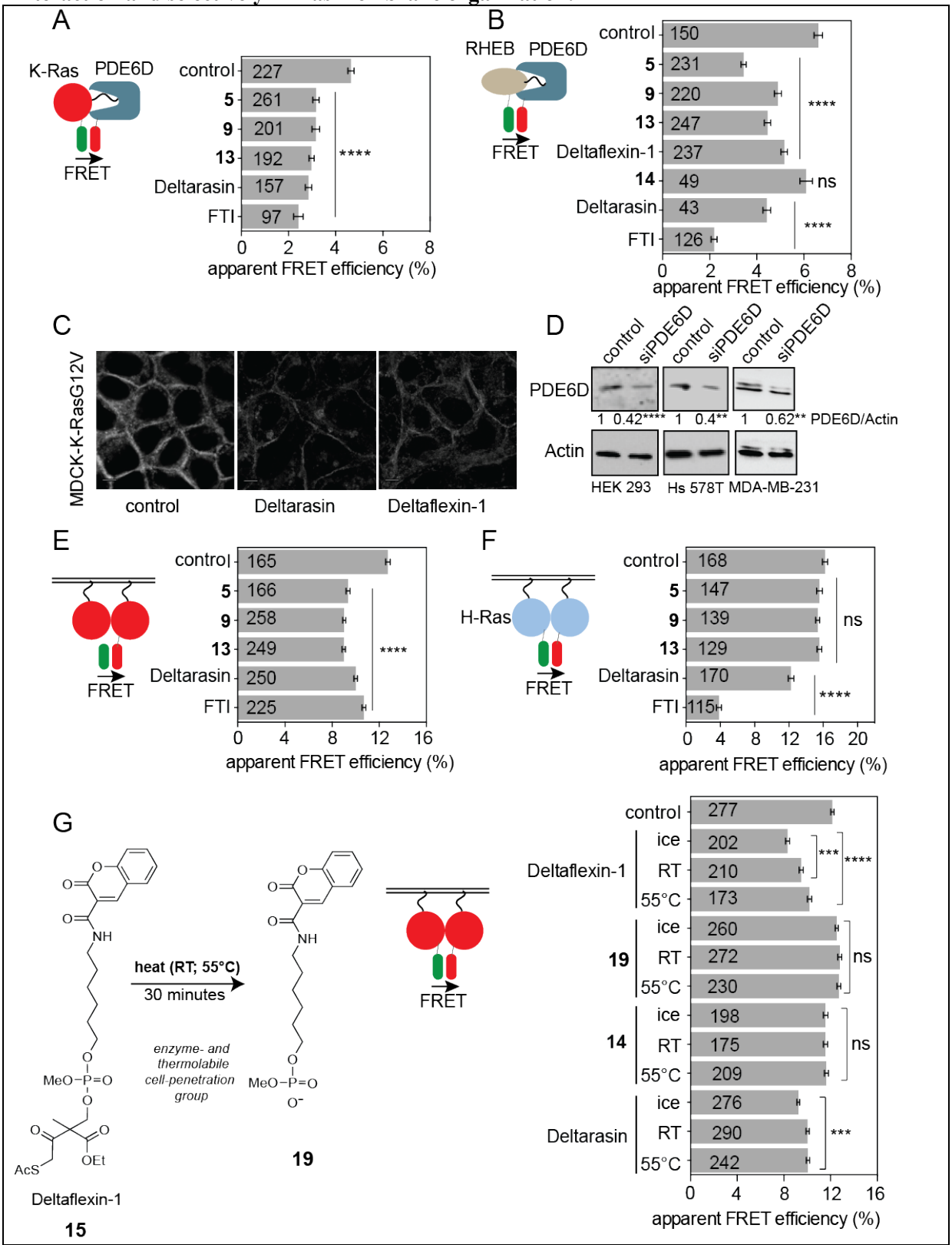
Supplementary Figure S1. Related to Figure 1 - Newly designed inhibitors compete with K-Ras for PDE6D in vitro.



(**A**) Chemical structures of synthesized and tested linkage derivatives **5**, **9**, **13** of the Deltaflexin-series, as compared to **15** (Delataflexin-1). (**B**) SPR binding kinetics of PDE6D

with N-terminal avi-tagged K-RasFMe captured on a neutravidin chip (top left) and SPR binding kinetics of compound **19** in competition with PDE6D/ K-RasFMe interaction. n=3 (**C**) Binding of Deltarasin, Deltaflexin-1 and compounds **14, 17** and **19** to PDE6D determined in the fluorescence anisotropy assay; n=1. The correlation between IC_{50} values of compounds obtained by SPR analysis and fluorescence anisotropy assay is presented (right). (**D**) Schematic binding mode of compounds **5**, **9**, **13** and **15** to PDE6D based on computational docking results. (**E**) Comparison of the PDE6D binding mode (left) of the Deltasonamide (compound **8** in Martin-Gago et al. 2017, PDB code 5ML6) and **15** (Deltaflexin-1). (D) Residues involved in interactions similar to those of the depicted Deltasonamide (E) are shown in grey. Hydrogen bonds are depicted with dashed lines. Aromatic stacking interactions are shown with squiggled lines.

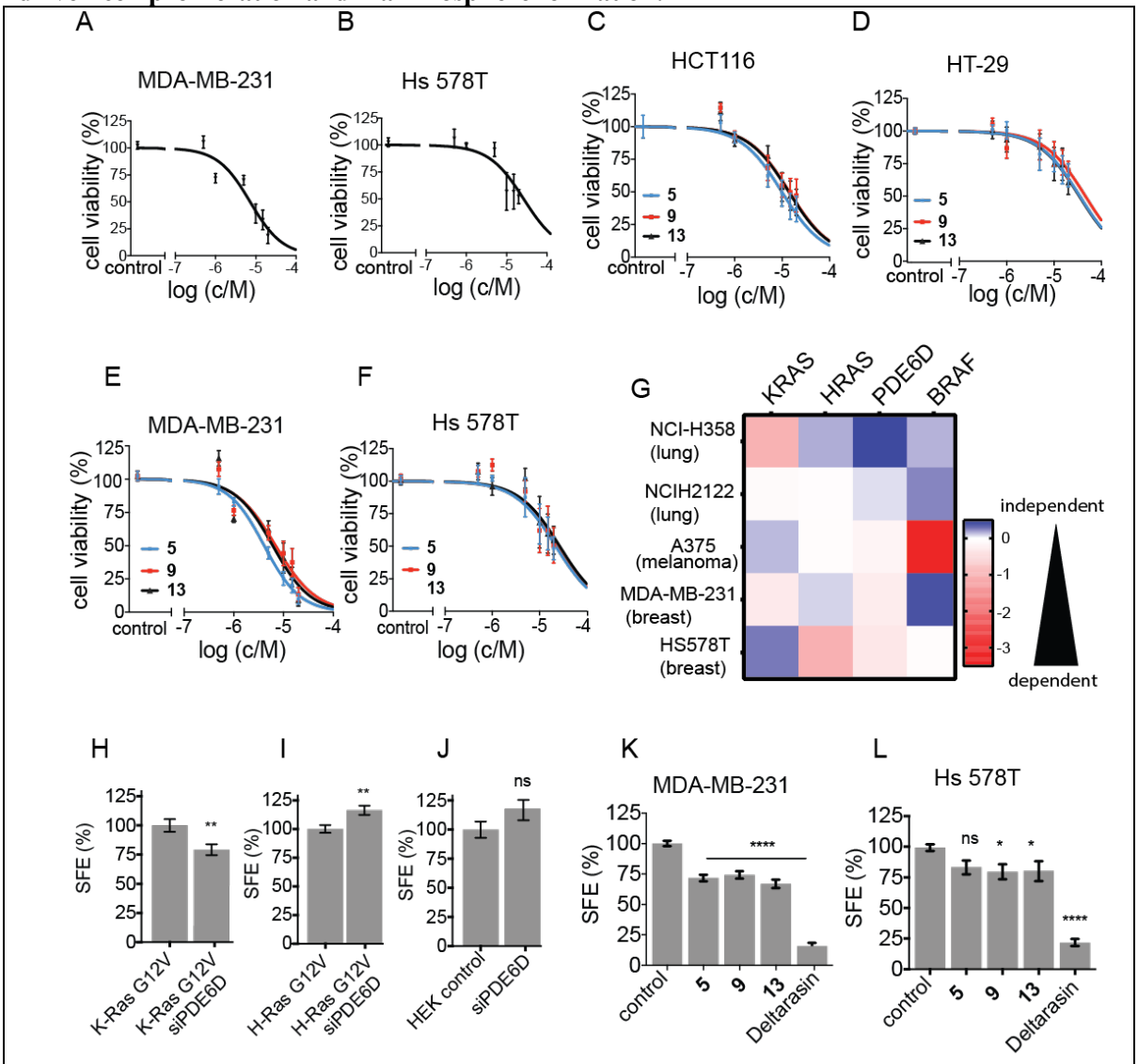
Supplemental Figure S2. Related to Figure 2 - Deltaflexin-1 suppresses K-Ras/ PDE6D interaction and selectively K-Ras membrane organization.



(**A**) K-Ras/ PDE6D interaction by FLIM-FRET. HEK cells were co-transfected with mGFP tagged K-RasG12V and mCherry tagged PDE6D and treated with 0.1% DMSO control or 5 µM of **5, 9, 13**, Deltarasin or 0.5 µM of FTI-277 for 24 h, n = 4. (**B**) PDE6D-Rheb interaction by FLIM-FRET. HEK

cells were co-transfected with mCitrine tagged Rheb and mCherry tagged PDE6D. Transfected cells were treated with 0.1% DMSO control or 5 μM of compounds **5, 9, 13**, Deltaflexin-1, **14** (Deltaflexin-1 precursor lacking the cell penetration moiety), Deltarasin or 0.5 μM FTI-2628 for 24 h, n = 3. The overall higher FRET of this latter reporter agreed with the higher soluble fraction of Rheb and a more efficient FRET-fluorophore pair. (**C**) Confocal imaging of MDCK cells expressing mGFP-K-RasG12V at 5 μM of indicated compounds treatments. Scale bar = 100 μm, n = 2. **(D)** Representative Western blot data showing the knock-down efficacy for PDE6D in siPDE6D-transfected cells, n ≥ 3. Cells were treated with 50 nM of siRNA for 48 h. (**E, F**) Ras membrane organization by nanoclustering-FRET in HEK cells co-expressing mGFP and mCherry tagged K-RasG12V (E) or H-RasG12V (F). Cells were co-transfected with siRNA PDE6D for 48 h or treated with 0.1% DMSO control or 5 μM of **5, 9 13**, Deltarasin or 0.5 μM FTI-277 for 24 h, n = 3. **(G)** K-Ras nanoclustering-FRET in HEK cells co-expressing mGFP or mCherry tagged K-RasG12V. The cells were treated with 2.5 μM Deltaflexin-1, **14, 19**, or Deltarasin. Freshly thawed compound aliquots were immediately diluted in ice cold medium (ice) and then added to cells or incubated for 1 h at room temperature (RT), or for 30 minutes at 55°C (55°C) before addition to cells, n = 3. The latter two conditions led to the premature, thermal deprotection of the compounds from their cell-penetration groups. For all FRET-data the numbers on the bars indicate the number of analysed cells and the bars represent mean values ± SEM. (**A,B,D,F,G**) Statistical significance levels are annotated as ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant..

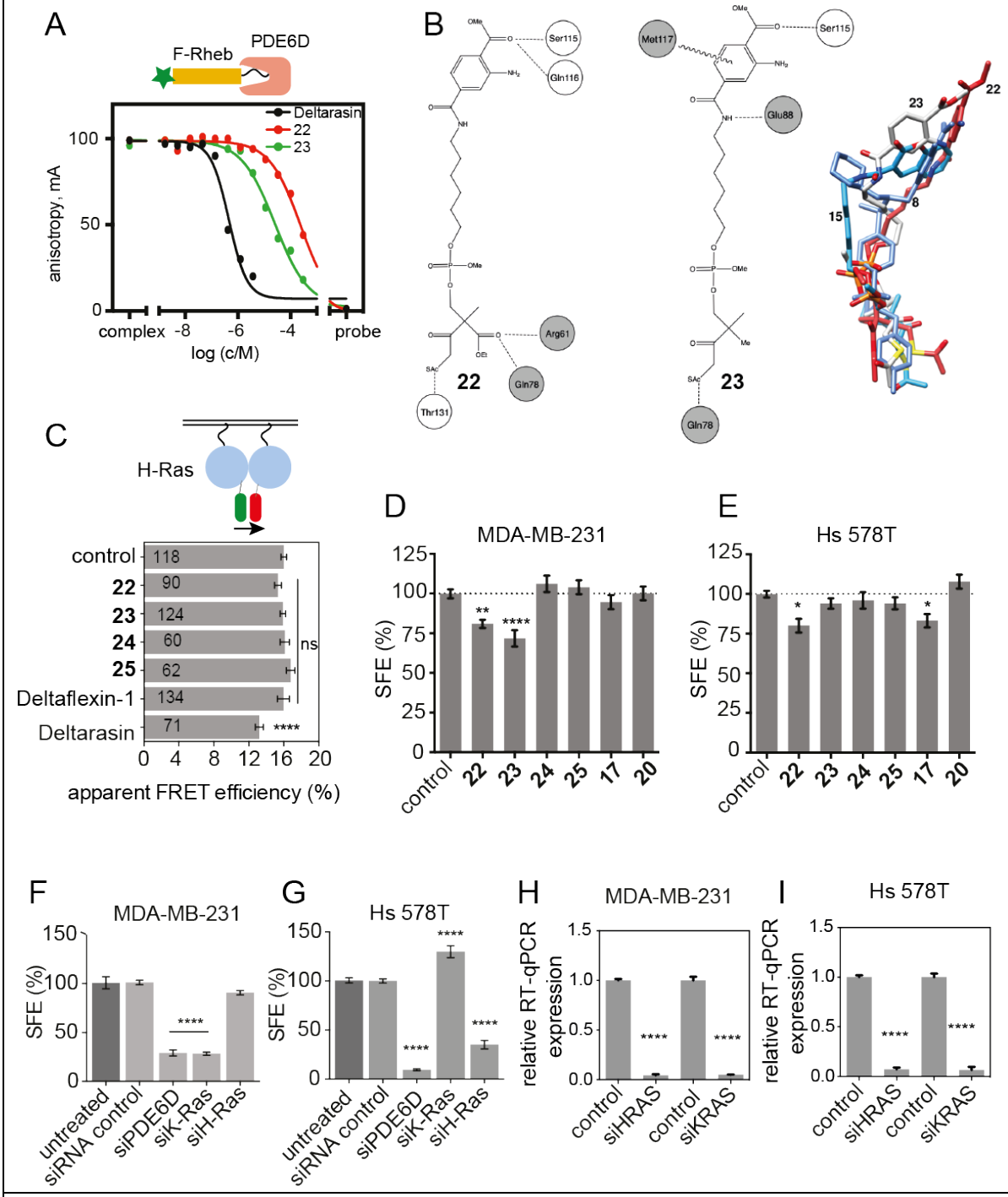
Supplemental Figure S3. Related to Figure 3 - Deltaflexin-1 selectively inhibits oncogenic K-Ras driven cell proliferation and mammosphere formation.



(**A, B**) Cell viability of MDA-MB-231 cells (A) and Hs578T cells (B) in response to Deltaflexin-1; n=3. (**C-F**) Cell viability of HCT116 (C), HT-29 (D), MDA-MB-231 (E) and Hs578T (F) cells in response to Deltaflexin derivatives. Cell viability curves (A-F) are expressed as % cell viability relative to 0.1% DMSO-treated control plotted against the log-transformed drug concentrations; n=3. (**G**) ATARis gene sensitivity profile of indicated cell lines and genes. Gene dependency/sensitivity data wer collected from the Project drive (ATARis) database at https://oncologynibr.shinyapps.io/drive/. The ATARis study is a large-scale RNAi screen in which viability effects of mRNA knockdown were assessed for 7,837 genes using an average of 20 shRNAs per gene in 398 cancer cell lines. The study outlines the classes of cancer dependency genes and their relationships to genetic, expression and lineage features. (**H,I,J**) Tumorosphere forming efficiency (SFE) after knockdown of PDE6D in HEK cells transiently transfected with K-RasG12V (H), H-

RasG12V (I) with or without 50 nM of scrambled siRNA or siPDE6D for 48 h (J). SFE is calculated as percent relative to scrambled siRNA control. Statistical comparison was done against the scrambled siRNA control. (**K,L**) SFE in MDA-MB-231 (K) and Hs 578T (L) cells in response to treatment with 5 μM Deltaflexin derivatives or Deltarasin for 72 h. All values are mean values ± SEM, n = 4. (**H-L**) Statistical significance levels are annotated as ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

Supplemental Figures S4. Related to Figure 4 - Partial scaffold hybridization creates second generation inhibitors.



(**A**) PDE6D binding of compounds **22** and **23** (Deltaflexin-2) as compared to Deltarasin determined in fluorescence anisotropy assay; n = 1. (**B**) Schematic binding mode of compounds **22** and **23** to PDE6D based on computational docking results. New contacts as compared to the Deltasonamide compound 8 (Figure S1E) are shown with white background. Superposition of **23**, **23**, Deltasonamide compound 8, and **15** is shown on the right in stick representation. (**C**) H-Ras membrane organization by nanoclustering-FRET in

HEK cells co-expressing mGFP or mCherry tagged H-RasG12V. After 24 h of transfection, cells were treated with 5 μM of indicated compounds, n = 2. (**D,E**) Sphere formation efficiency (SFE) of MDA-MB-231 cells (D), n ≥ 4, and Hs 578T cells (E) n ≥ 4, cultured in suspension culture followed by a 72 h incubation with 5 μM of indicated compounds. (**F,G**) SFE of MDA-MB-231 cells (F) and Hs 578T cells (G) either untreated or transfected with 50 nM of scrambled siRNA, siPDE6D, siKRAS or siHRAS. (**H,I**) RT-qPCR analysis of knockdown efficiency of siKRAS and siHRAS in MDA-MB-231 cells (H) and Hs578T cells (I); n = 1. The figures represent mean values ± SEM. Statistical significance levels are annotated as ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

Supplementary Data S1: Related to "Compound synthesis" in the Methods section. Chemical synthesis and analytical data

Table of Contents

SYNTHESES

Seven different coumarin-phosphate derivatives (**5**, **9**, **13**, **15**, **17**, **19** and **20**, Figure S4 and Scheme 1) and four terepthalic acid-phosphate derivatives (**22**, **23**, **24** and **25,** Figure S4), bearing a bioactivatable and thermolabile phosphate protecting groups (4-acetylthio-2-ethoxycarbonyl-3-oxo-2-methylbutyl, **26**[1] or 4-acetylthio-2,2-dimethyl-3-oxobutyl, **27**[1]), were prepared as outlined in Schemes 2-8. The coumarin or terepthalic acid and phosphate moieties are connected to each other through the *O*-hexyl oxime (**5**), *N*-arylsulfonamide-*O*-hexyl oxime (**9**), *O*-hexyl (**13**), hexylamide (**15, 22** and **23**), butylamide (**20**, **24** and **25**) and methyl cyclohexylmethylamide (**17**) linkers. The 2,2-disubstituted 4-acylthio-3-oxobutyl group is an esterase- and thermolabile phosphate protecting group that enhances cellular uptake of the drug candidates. The enzymatic and nonenzymatic deprotection of 4-acetylthio-2-ethoxycarbonyl-3-oxo-2-methylbutyl and 4-acetylthio-2,2-dimethyl-3-oxobutyl group takes place by intramolecular cyclization to give the negatively charged phosphodiester and a substituted tetrahydrothiophenone (deprotection for **15** given as an example in Scheme 1).[1] Additionally, the 4,4-disubstituted dihydrothiophen-3(2H)-one byproduct is not markedly alkylating, confirmed by glutathione adduct experiments in our earlier studies.[1] If the enzymatic reaction becomes retarded, the thermolytic removal takes a place.[2]

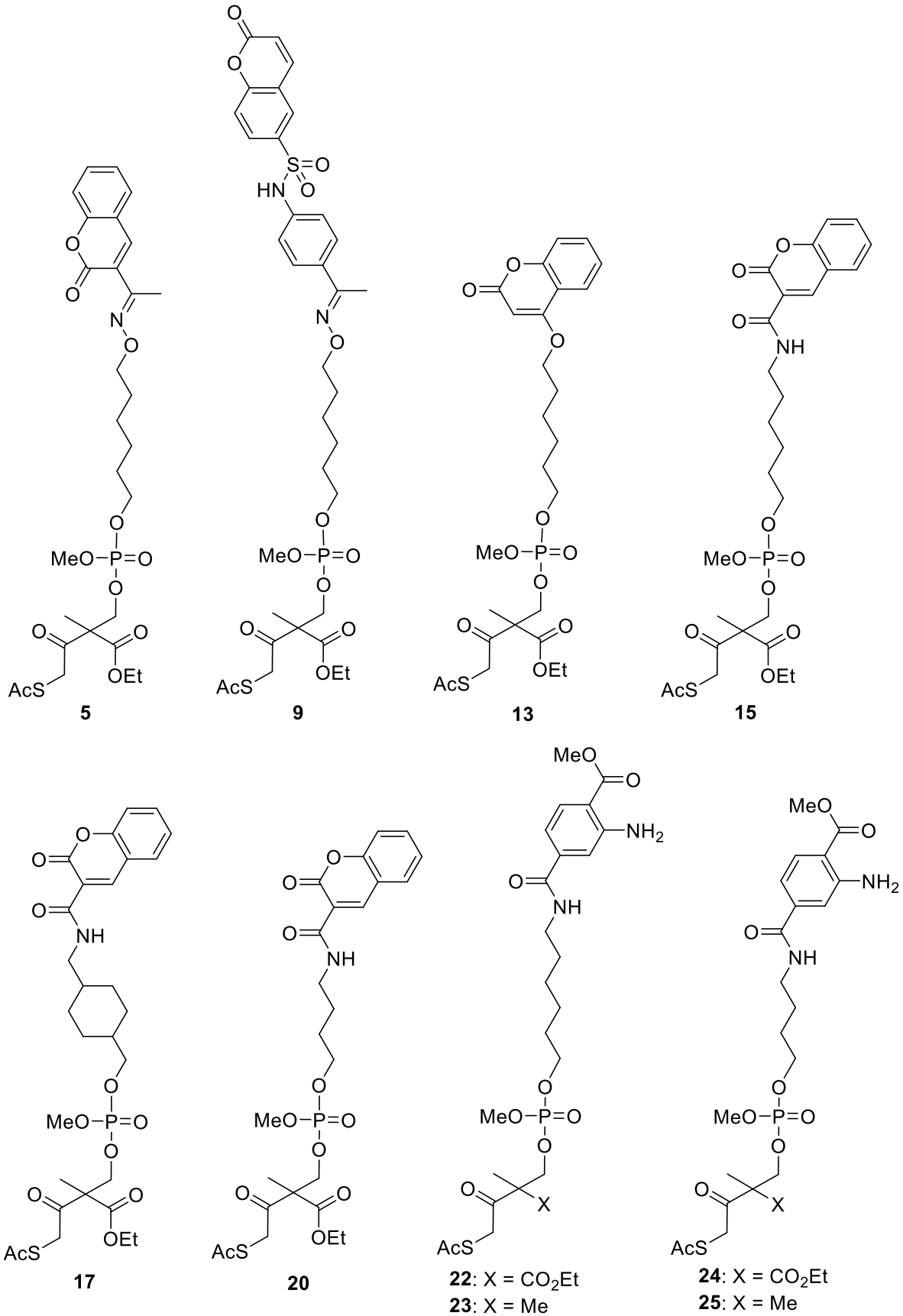
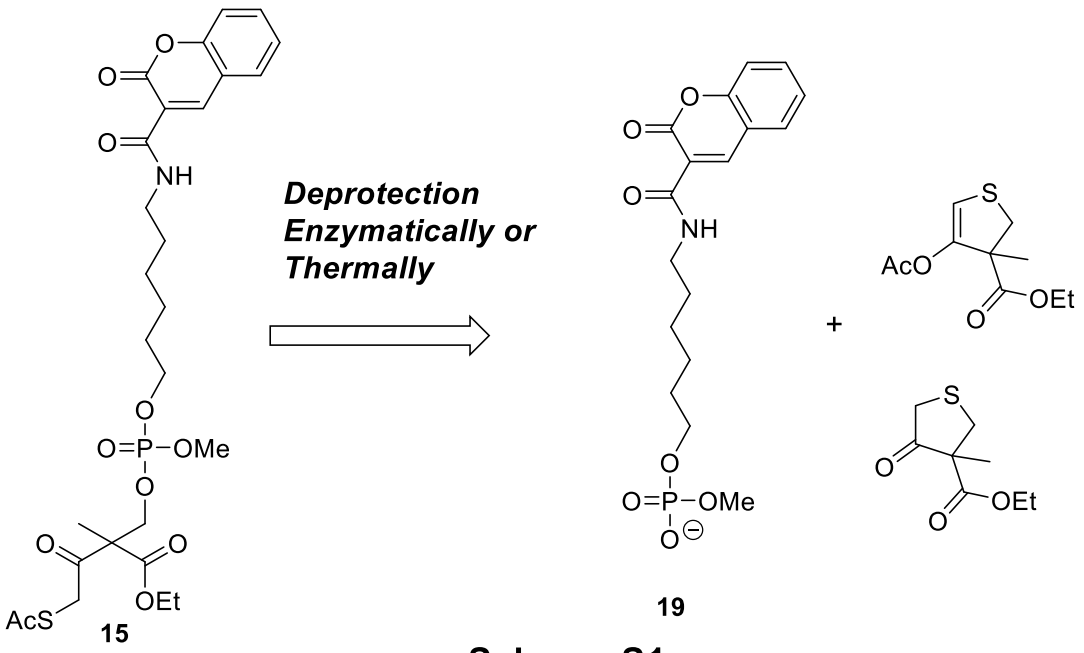
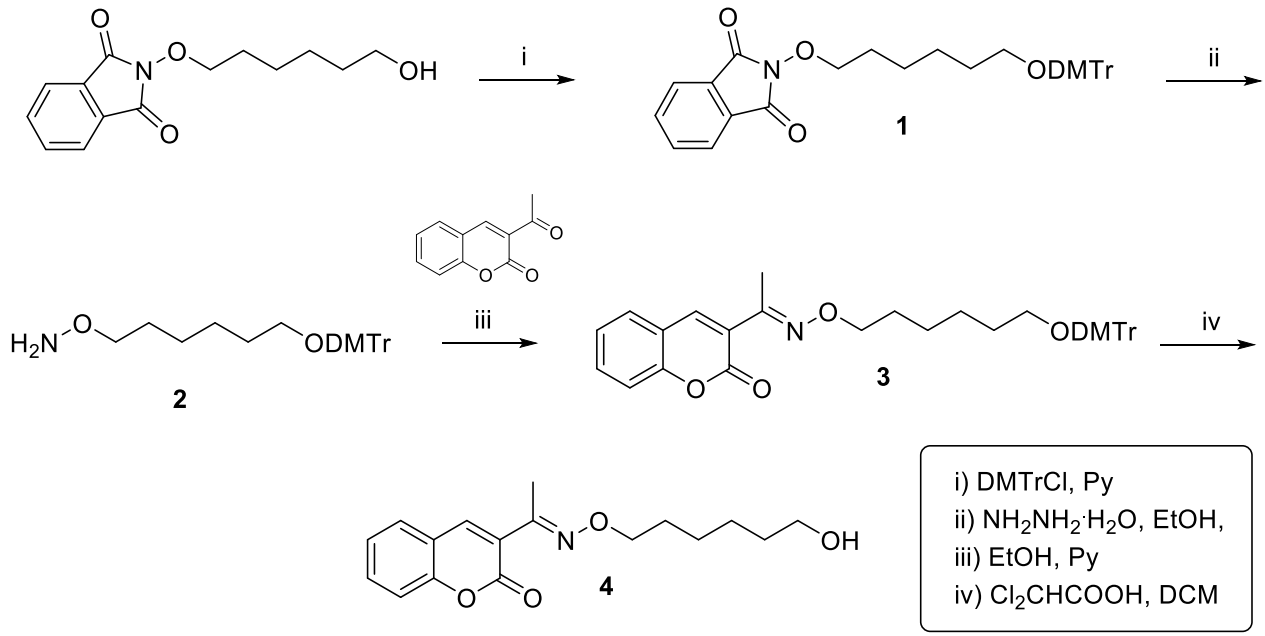


Figure S5. Structures of protected coumarin-phosphate and terepthalic acid-phosphate derivatives.



Scheme S1

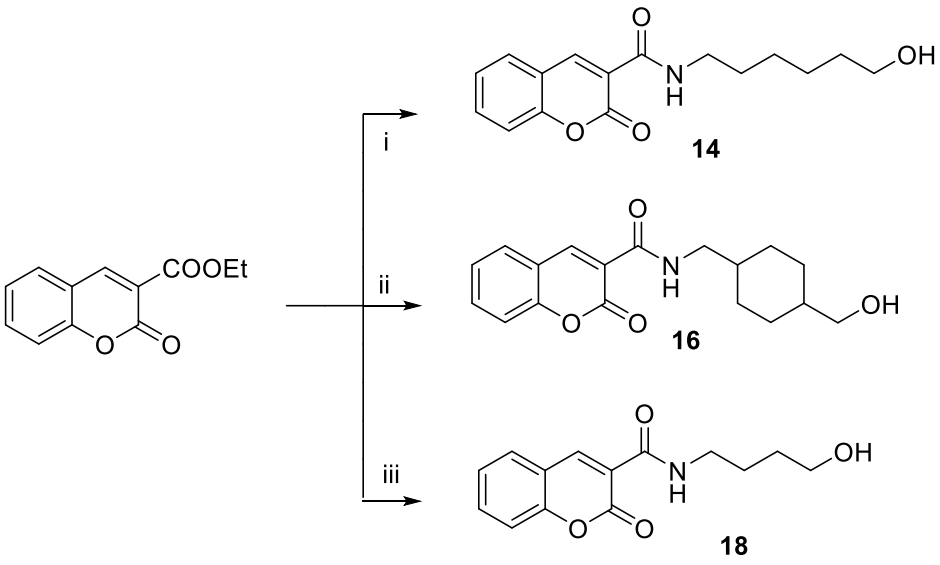
For the synthesis of 3-substituted coumarin phosphotriester derivatives **5** and **15** (see Scheme S6), compounds **4** and **14** were first prepared as depicted in Scheme S2 and S3, respectively. Accordingly, heating of 6-bromohexanol and *N*-hydroxyphthalimide in DMF in the presence of potassium carbonate[3], gave *N*-(6-hydroxyhexyloxy)phthalimide[4] which was further tritylated to produce **1** (Scheme S2). Deprotection of the phthaloyl group by hydrazine in ethanol and a subsequent conversion of the *O*-6-dimethoxytritylated hexylhydroxylamine[3] **2** with 3-acetyl-2H-chromen-2-one[5] in a mixture of ethanol and pyridine at 50 ºC gave *O*-alkyl oxime **3**. Finally, DMTr group was removed to give 3-(1-(((6-hydroxyhexyl)oxy)imino)ethyl)-2H-chromen-2-one (**4**).



Scheme S2

N-(6-hydroxyhexyl)-2-oxo-2*H*-chromene-3-carboxamide (**14**) was obtained from 6-aminohexanol and ethyl 2-oxo-2*H*-chromene-3-carboxylate (prepared by Knoevenagel condensation of 2′-hydroxybenzaldehyde with diethylmalonate[6]) in ethanol using piperidine as a base (Scheme S3).

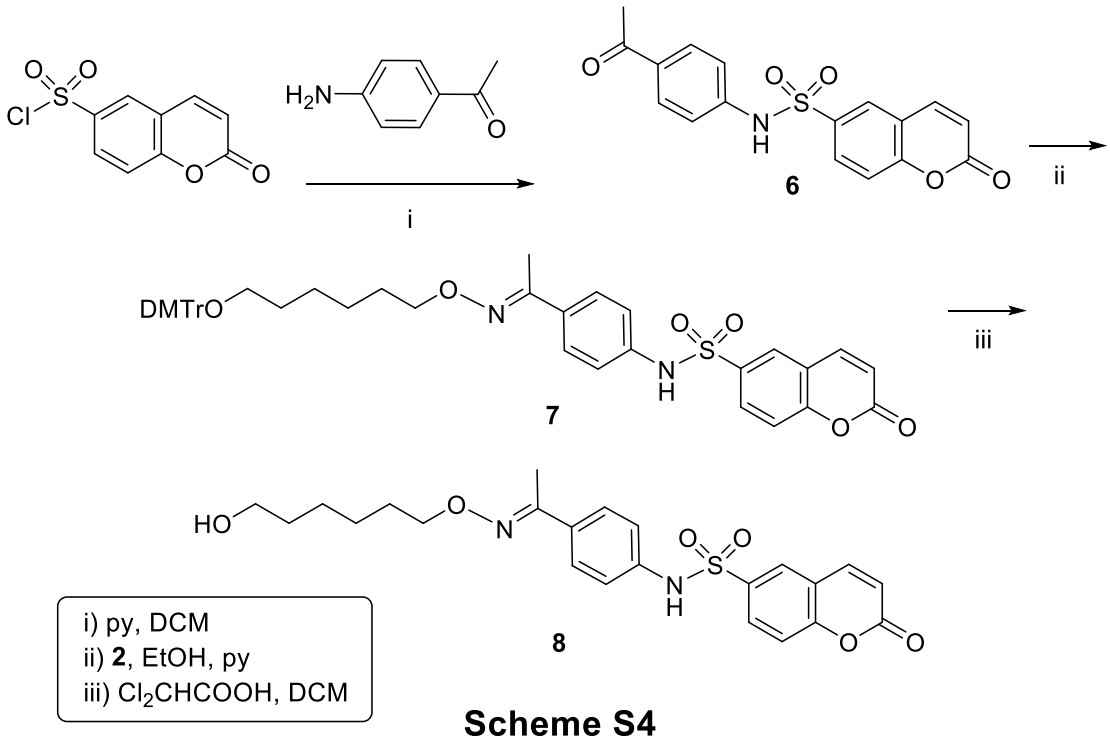
For the synthesis of phosphate protected 3-substituted coumarin derivatives **17** and **20** (see Scheme S6), bearing methyl cyclohexylmethylamide and butylamide linker, compounds **16** and **18** were first prepared. Accordingly, ethyl 2-oxo-2*H*-chromene-3-carboxylate[6] was refluxed with 4-aminobutanol or (4-(aminomethyl)cyclohexyl)methanol in ethanol to give *N*-(4-hydroxybutyl)-2-oxo-2*H*-chromene-3-carboxamide (**18**) and *N*-((4-(hydroxymethyl)cyclohexyl)methyl)-2-oxo-2*H*-chromene-3-carboxamide (**16**), respectively.



i) 6-aminohexanol, EtOH, piperidine, reflux
ii) (4-(aminomethyl)cyclohexyl)methanol, EtOH, reflux
iii) 4-aminobutanol, EtOH, reflux

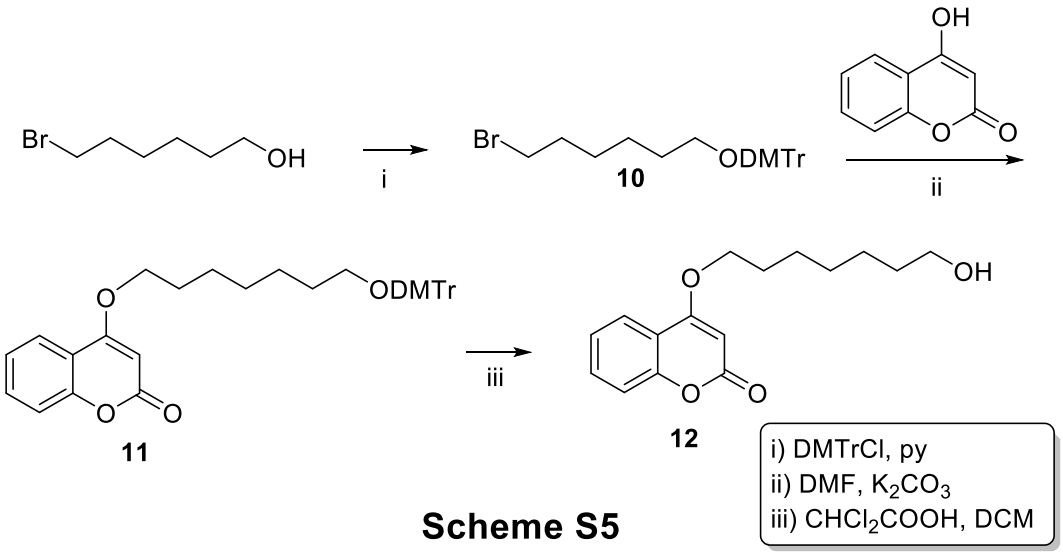
Scheme S3

To prepare 7-substituted coumarin phosphotriester derivative **9**, 2-oxo-2H-chromen-6-sulfonyl chloride[7] was first treated with 4-aminoacetophenone in a mixture of pyridine and DCM. After that, sulphonamide **6** was converted to *O*-alkyl oxime **7** using *O*-6-dimethoxytritylated hexylhydroxylamine **2** in pyridine.[8,9] Finally, DMTr group was removed to give **8** (Scheme S4).



Scheme S4

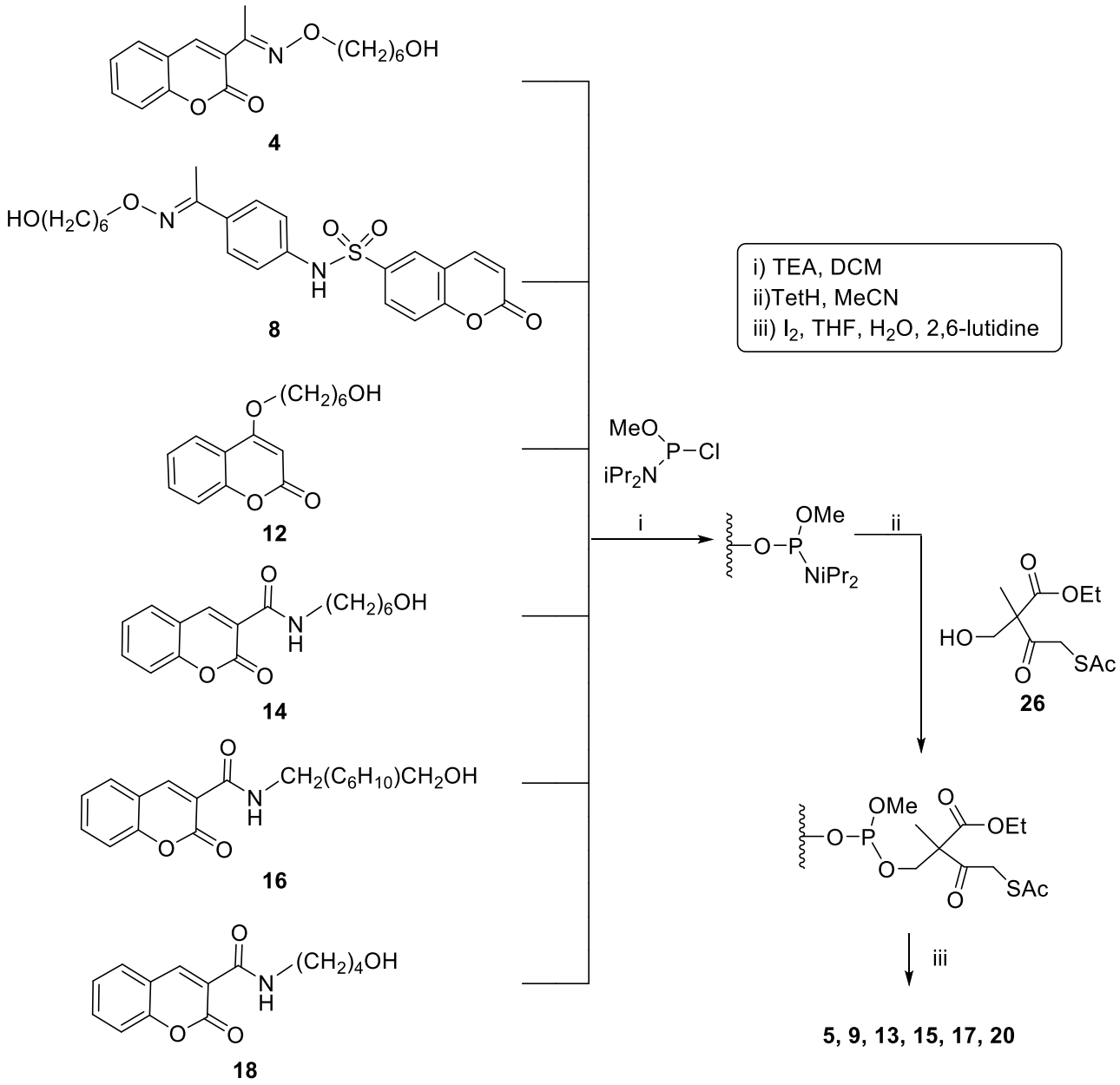
For the synthesis of 4-substituted coumarin phosphotriester derivative **13**, 6-bromohexanol was first dimethoxytritylated in pyridine to give **10** (Scheme S5). Alkylation with 4-hydroxycoumarin (**11**)[10], followed by DMTr-removal gave **12**.



Scheme S5

To prepare the coumarin-phosphate derivatives, compounds **4**, **8**, **12**, **14**, **16** and **18** were phosphitylated with 1-chloro-*N*,*N*-diisopropyl-1-methoxyphosphinamine,[1] followed by tetrazole promoted displacement

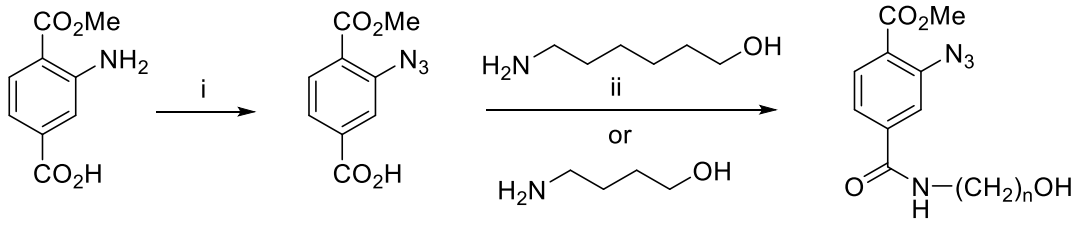
of the diisopropylamino group by 4-acylthio-2-hydroxymethyl-2-methyl-3-oxobutanoate (**26**) (Scheme S6). Oxidation of the resulting phosphite triester to phosphate triester with iodine in THF/H_2O/2,6-lutidine completed the synthesis to give **5**, **9**, **13**, **15**, **17** and **20**, respectively. The unprotected phosphodiester **19** was prepared analogously with that of the phosphotriester **15**, by hydrolysis of the phosphoramidite intermediate.



Scheme S6

Methyl 2-azido-4-((4-hydroxybutyl)carbamoyl)benzoate (**21a**) and methyl 2-azido-4-((6-hydroxyhexyl)carbamoyl)benzoate (**21b**) were prepared as described in Scheme S7. The amino group of

2-amino-terepthalic acid methyl ester was first converted to an azido group, and amide coupling with 4-aminobutanol or 6-aminohexanol using HATU and DIPEA in DMF afforded **21a** and **21b**, respectively.

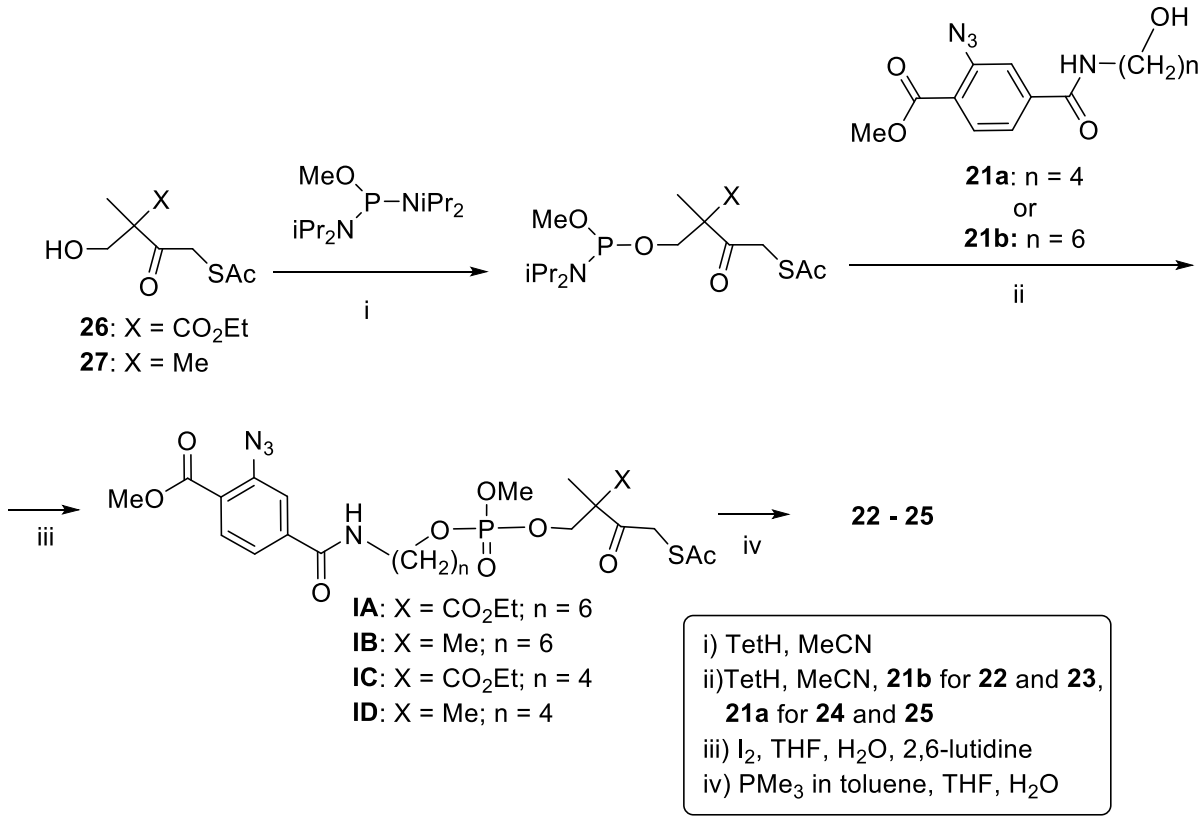


21a: n = 4
21b: n = 6

i) $NaNO_2$, NaN_3, Conc.HCl , H_2O
ii) HATU, DIPEA, DMF, 4-aminobutanol for **21a** and 6-aminohexanol for **21b**

Scheme S7

To prepare the terepthalic acid-phosphate derivatives **22**-**25**, the 4-acylthio-2-hydroxymethyl-2-methyl-3-oxobutanoate (**26**) and *S*-(4-hydroxy-3,3-dimethyl-2-oxobutyl)ethanethioate (**27**) were phosphitylated with *N*,*N*,*N*',*N*'-tetraisopropyl-1-methoxyphosphanediamine. The remaining diisopropylamino group was displaced by methyl 2-azido-4-((4-hydroxybutyl)carbamoyl)benzoate (**21a**) or methyl 2-azido-4-((6-hydroxyhexyl)carbamoyl)benzoate (**21b**) using tetrazole as an activator (Scheme S8).[1] ^{31}P NMR analyses revealed that the phosphite intermediate hydrolyzed partly during this latter step (The signals at δ 15.0 – -5.0 ppm were observed). In preparation of **24**, shorter reaction time (5 min) was used with replacement of diisopropylamino group compared to that of **22**, **23** and **25** (10-15 min) to increase the yield. The other side reaction (i.e. Staudinger reaction, in which the azido group was reduced by the phosphite intermediate) could be prevented by adding **21a** or **21b** together with an excess of tetrazole to the reaction solution. Oxidation of the resulting phosphite triester to phosphate triester with iodine in THF/H_2O/2,6-lutidine gave **IA**, **IB**, **IC** and **ID**. **IA-ID** were used in the next step without further purification. Staudinger reaction with trimethylphosphine completed the synthesis to give **22**-**25**.



Scheme S8

EXPERIMENTAL SECTION

General. The preparation of 2-((6-hydroxyhexyl)oxy)isoindoline-1,3-dione,[4] 3-acetyl-2*H*-chromen-2-one,[5] 2-oxo-2*H*-chromene-6-sulfonyl chloride,[7] 2-oxo-2*H*-chromene-3-carboxylate,[6] ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate[1] and *S*-(4-Hydroxy-3,3-dimethyl-2-oxobutyl)ethanethioate,[1] have been described previously. The synthesis of methyl 2-azido-4-((4-hydroxybutyl)carbamoyl)benzoate (**21a**), methyl 2-azido-4-((6-hydroxyhexyl)carbamoyl)benzoate (**21b**) and the large scale synthesis of 4-((6-(((4-(acetylthio)-2,2-dimethyl-3-oxobutoxy)(methoxy)phosphoryl)oxy)hexyl)carbamoyl)-2-aminobenzoate (**23)** were outsourced to Piramal Enterprises Ltd. *N*,*N*,*N*',*N*'-tetraisopropyl-1-methoxyphosphanediamine, 1-chloro-*N*,*N*-diisopropyl-1-methoxyphosphinamine, 0.45 M 1*H*-tetrazole solution in CH_3CN, 1 M trimethylphosphine solution in toluene, 3-amino-4-(methoxycarbonyl)benzoic acid and 2,6-lutidine were commercial products of Sigma-Aldrich. 4-Aminobutanol and 6-aminohexanol were commercial products of TCI. (4-(Aminomethyl)cyclohexyl)methanol was commercial products of Carbosynth. Solvents were purchased from Sigma Aldrich, VWR and Thermo Fisher Scientific. Pyridine,

DMF, MeCN, DCM, EtOAc and 0.45 M 1*H*-tetrazole solution in CH_3CN were dried over molecular sieves (3 or 4 Å). TEA was dried by refluxing over CaH_2 and distilled before use. Reagents were dried or tested for dryness before use when appropriate. The NMR spectra were recorded with a Bruker Avance 400, 500 or 600 MHz spectrometer. 2D NMR spectra were used for peak assignment. The mass spectra were recorded with a Bruker Daltonics microTOF-Q instrument. RP- HPLC purification of the products was performed with a Merck Hitachi instrument using Phenomenex Oligo-RP C18 (250 × 10 mm, 5 μm) semipreparative column for the compounds **5**, **9**, **13** and **15** and Thermo Scientific ODS Hypersil (250 × 10 mm, 5 μm) semipreparative column for the compounds **17**, **20**, **22-25** (flow-rate 3.0 mL min^{-1} and wavelength 260 nm).

2-((6-(Bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)oxy)isoindoline-1,3-dione (1). 2-((6-hydroxyhexyl)oxy)isoindoline-1,3-dione[3,4] (1.00 g, 3.79 mmol) was dissolved in dry pyridine (20 ml). 4,4-Dimethoxytritrylchloride (1.54 g, 4.55 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was evaporated to dryness under reduced pressure and the residue was extracted between DCM and 5% aqueous $NaHCO_3$. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel chromatography by eluting with hexane that contained 20% EtOAc to yield **1** (1.95g, 90%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83-7.78 (m, 4H, NH), 7.41-7.07 (m, 9H, DMTr), 6.86 (d, 4H, J = 9.2 Hz, DMTr), 4.11 (t, 2H, J = 6.4 Hz, CH_2ON), 3.70 (s, 6H, OCH_3), 2.99 (t, 2H, J = 6.0 Hz, OCH_2), 1.64 and 1.56 (m, 4H, 2 × CH_2), 1.40-1.32 (m, 4H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.7 (2 × C=O), 158.5 (DMTr), 145.8, 136.6 (DMTr), 135.1 (NH), 130.1, 129.4, 129.0, 128.2 and 126.9 (DMTr), 123.6 (NH), 113.5, 113.2 (DMTr), 85.9 (quaternary C), 78.1 (CH_2ON), 63.2 (OCH_2), 55.4 (OCH_3), 29.9, 28.1, 26.0, 25.5 and 21.1 (CH_2); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{35}H_{35}NNaO_6^+$ 588.2357; found 588.2386.

***O*-(6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)hydroxylamine (2)**. Compound **1** (1.90 g, 3.35 mmol) was dissolved in EtOH (25 ml) and hydrazine monohydrate (0.34 g, 6.72 mmol) was added. The

solution was stirred at room temperature for 4 h. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel chromatography eluting with a mixture of DCM, MeOH and TEA (98:1:1, *v/v/v*). Compound **2** was obtained as a white oil in 59% yield (0.86 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49-7.20 (m, 9H, DMTr), 6.83 (d, 4H, J = 7.6 Hz, DMTr), 3.79 (s, 6H, 2 × OCH_3), 3.65 (t, 2H, J = 7.0 Hz, CH_2ON), 3.08 (t, 2H, J = 7.0 Hz, OCH_2), 1.67, 1.59, 1.42 and 1.31 (m, 8H, 4 x CH_2); ^{13}C NMR (126 MHz, $CDCl_3$): δ =158.4, 145.5, 136.8, 130.1, 128.2, 128.2, 127.7, 126.6, 113.5 (DMTr), 85.7 (quaternary C), 76.1 (CON), 63.4 (CH_2ON), 60.4 (OCH_2), 55.2 (OCH_3), 30.1, 29.8, 28.4, 26.3, 26.0 (CH_2); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{27}H_{33}NNaO_4^+$ 458.2302; found 458.2319.

3-(1-(((6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)oxy)imino)ethyl)-2H-chromen-2-one (3). 3-Acetyl-2*H*-chromen-2-one (3-acetylcoumarin)[5] (0.31 g, 1.65 mmol) was dissolved in a mixture of EtOH (10 ml) and pyridine (1 ml). Compound **2** (0.72 g, 1.65 mmol) was added and the mixture was stirred at 50 °C for 12 h and evaporated to dryness. The residue was purified by silica gel chromatography eluting with a mixture of hexane, EtOAc and TEA (89:10:1, *v/v/v*) to yield **3** as a yellow oil (0.73 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.54 (s, 1H, H5 of coumarin), 7.51 and 7.47 (m, 4H, coumarin); 7.38-7.19 (9H, DMTr); 6.91 (d, 4H, J = 7.0 Hz, DMTr); 4.21 (t, 2H, J = 6.8 Hz, $NOCH_2$); 3.80 (s, 6H, 2 × OCH_3); 3.10 (t, 2H, J = 6.8 Hz, OCH_2); 2.25 (s, 3H, CH_3); 1.76 and 1.68 (m, 4H, 2 × CH_2); 1.50-1.40 (m, 4H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.0, 158.3, 158.0, 154.0, 157.0, 145.5, 141.0, 136.7, 132.0, 130.0, 129.2, 128.5, 128.2, 127.8, 127.7, 127.0, 126.6, 125.4, 124.6 (coumarin and DMTr), 85.7(quaternary C), 74.5 ($NOCH_2$), 63.4 (OCH_2), 55.2 (OCH_3), 30.1, 29.2, 26.2, 25.9, (CH_2), 14.36 (CH_3); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{38}H_{39}NNaO_6^+$ 628.2670; found 628.2678

3-(1-(((6-hydroxyhexyl)oxy)imino)ethyl)-2*H*-chromen-2-one (4). Compound **3** (0.85 g, 2.80 mmol) was dissolved in a mixture of dichloroacetic acid (0.8 ml) and DCM (24.2 ml) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated aq. $NaHCO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was

purified by silica gel chromatography eluting with a mixture of hexane and EtOAc (60:40, *v/v*). The product **4** was obtained as a yellow solid (0.22 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84 (s, 1H, H5 of coumarin), 7.49 and 7.27 (m, 4H, coumarin), 4.14 (t, 2H, *J* = 6.8 Hz, OCH_2), 3.59 (t, 2H, *J* = 6.8 Hz, $NOCH_2$), 2.20 (s, 3H, CH_3), 1.68 and 1.53 (m, 4H, 2 × CH_2), 1.45-1.37 (m, 4H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.8 and 153.9 (CN and CO), 153.2, 141.0, 132.0, 128.5, 125.3, 124.6, 118.9, 116.4 (coumarin), 74.41 ($NOCH_2$), 62.69 (OCH_2), 36.6, 32.6, 29.1, 25.8 and 25.57 (CH_2), 14.3 (CH_3); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{17}H_{21}NNaO_4^+$ 326.1363, found 326.1359.

Ethyl 4-(acetylthio)-2-(((methoxy((6-(((1-(2-oxo-2H-chromen-3-yl) ethylidene) amino) oxy) hexyl)oxy)phosphoryl)oxy)methyl)-2-methyl-3-oxobutanoate (5). Compound **4** (0.25 g, 0.82 mmol), dried over P_2O_5 overnight, was dissolved in dry DCM (2.90 ml) under nitrogen. TEA (0.57 ml, 4.12 mmol) and methyl-*N*,*N*-diisopropylchlorophosphoramidite (0.18 ml, 0.91 mmol) were added and the mixture was stirred at room temperature for 40 minutes. The reaction mixture was filtrated through a short silica gel column by eluting with EtOAc that contained 0.5% TEA. The product fractions were combined, evaporated to dryness under reduced pressure, and the residue was co-evaporated three times with dry CH_3CN. The residue was dissolved in dry CH_3CN (1.1 ml), and ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate[1] (0.23 g, 0.91 mmol) in dry CH_3CN (1.1 ml) and 0.45 M 1*H*-tetrazole solution in CH_3CN (2.93 ml, 1.32 mmol) were added under nitrogen. The mixture was stirred at room temperature for 3 h. The phosphite ester was oxidized with I_2 (0.30 g) in a mixture of THF (6 ml), H_2O (3 ml) and 2,6-lutidine (1.5 ml) by stirring at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (LichroCHART 250 × 10 Hypersil ODS 5µm, flow rate 3 ml/min) by isocratic elution with a mixture of CH_3CN and H_2O (70:30, *v/v*) to yield **5** as a yellow oil (200 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.87 (s, 1H, H5 of coumarin), 7.54-7.50 (m, 2H, coumarin), 7.32-7.25 (m, 2H, coumarin), 4.42-4.33 (m, 2H, $POCH_2$), 4.25 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 4.16 (t, 2H, *J* = 6.8 Hz, OCH_2), 4.05-4.00 (m, 2H, OCH_2), 4.01-3.88 (m, 2H SCH_2), 3.75 and 3.72 (d, *J* = 11.2 Hz, 3H, $POCH_3$), 2.34 (s, 3H,

AcS), 2.20 (s, 3H, N=CCH$_3$), 1.75-1.65 (m, 4H, CH$_2$CH$_2$), 1.55 (s, 3H, CCH$_3$), 1.45-1.38 (m, 4H, CH$_2$CH$_2$), 1.27 (t, 3H, J = 7.2 Hz, OCH$_2$C*H*$_3$); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 198.6 (CO), 193.6 (SCO), 169.5 (OCO), 159.7, 154.0 (CN and CO of coumarin), 153.2, 141.0, 132.3, 128.5, 125.3, 124.6, 119.0 and 116.5 (coumarin), 74.3 (NOCH$_2$), 69.0, 68.2, and 68.1 (OCH$_2$), 62.3 (O*C*H$_2$CH$_3$), 60.2 and 60.1 (quaternary C), 54.4 and 54.4 (OCH$_3$), 36.7 (CH$_2$S), 30.2, 30.1 and 30.1 (OCH$_2$*C*H$_2$ and *C*H$_3$CO), 29.0, 25.5 and 25.2 (CH$_2$), 17. 6 (CH$_3$), 14.3 (NCCH$_3$), 13.9 (*C*H$_3$CH$_2$O); ^{31}P NMR (162 MHz, CDCl$_3$): δ = -0.34 ppm; HRMS (ESI-TOF) *m/z*: [M+H]$^+$ calcd for C$_{28}$H$_{39}$NO$_{11}$PS$^+$ 628.1976, found 628.1984.

N-(4-acetylphenyl)-2-oxo-2H-chromene-6-sulfonamide (6). 2-Oxo-2*H*-chromene-6-sulfonyl chloride[7] (1.00 g, 4.08 mmol) was dissolved in a mixture of dry DCM (20 ml) and pyridine (1 ml). 4-Aminoacetophenone (0.55g, 4.08 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was poured to ice and acidified with HCl. The precipitate was filtrated and crystallized from ethanol to yield **6** as pale yellow needle crystals (0.86, 61%). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 10.99 (s, 1H, NH), 8.30 (d, 1H, J = 2.4 Hz, H5 of coumarin), 8.17 (d, 1H, J = 9.6, H4 of coumarin), 7.97 (dd, 1H, J = 8.8 and 2.4 Hz, H7 of coumarin), 7.83 (d, 2H, J = 8.8 Hz, Ar), 7.56 (d, 1H, J = 8.8 Hz, H8 of coumarin), 7.20 (d, J = 8.8 Hz, 2H, Ar), 6.60 (d, 1H, J = 10.0 Hz, H3 of coumarin), 2.45 (s, 3H, COCH$_3$); ^{13}C NMR (101 MHz, CDCl$_3$): δ = 196.90 (CO), 159.5 (C2 of coumarin), 156.5 (C9 of coumarin), 143.8 (C4 of coumarin), 142.4, 135.7, 132.6 and 130.3 (Ar and coumarin), 130.0 (C7 of coumarin), 128.3 (C5 of coumarin), 119.5, 118.6 and 118.4 (Ar and C8 and C3 of coumarin), 26.9 (CH$_3$); HRMS (ESI-TOF) *m/z*: [M+Na]$^+$ calcd for C$_{17}$H$_{13}$NNaO$_5$S$^+$ 366.0407; found 366.0409.

***N*-(4-(1-(((6-hydroxyhexyl)oxy)imino)ethyl)phenyl)-2-oxo-2H-chromene-6-sulfonamide (8)**. Compound **6** (0.50 g, 1.45 mmol) was dissolved in a mixture of EtOH (15 ml) and pyridine (1 ml). *O*-(6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)hydroxylamine **2** (0.64g, 1.47 mmol) was added and the reaction mixture was stirred at 50 °C for 12 h. Volatiles were removed under reduced pressure. The crude product was purified by a silica gel chromatography using gradient elution from 5% to 15% EtOAc in

hexane that contained 1% TEA. Compound **7** was obtained as a white oil in 50% yield (0.56 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.48 (s, NH), 7.87 (s, 1H, H5 of coumarin), 7.62 (m, 1H), 7.52-7.43 (m, 4H), 7.36-17.17 (m, 11H), 6.84 (m, 6H, DMTr and H3 of coumarin), 4.18 (t, 2H, *J* = 6.4 Hz, CH_2ON), 3.76 (s, 6H, OCH_3), 3.08 (t, 2H, *J* = 6.4 Hz, OCH_2), 2.23 (s, 3H, CH_3), 1.75 and 1.67 (m, 4H, 2 × CH_2), 1.49-1.39 (m, 4H CH_2CH_2). To remove the 4,4′-dimethoxy trityl protecting group, **7** (0.95 g, 1.25 mmol) was dissolved in a mixture dichloroacetic acid (0.8 ml) and DCM (24.2 ml) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated aq. $NaHCO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by a silica gel chromatography eluting with a mixture of hexane and EtOAc (60:40, *v/v*). The product **(8)** was obtained as a yellow oil (0.50 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ =7.98 (d, 1H, *J* = 2.4 Hz, H5 of coumarin), 7.90 (dd, 1H, *J* = 8.8 and 2.4 Hz, H7 of coumarin), 7.82 (br. s, 1H, NH), 7.68 (d, 1H, *J* = 9.6 Hz, H4 of coumarin), 7.52 (d, 2H, *J* = 8.8 Hz, Ar), 7.33 (d, 1H, *J* = 8.8 Hz, H8 of coumarin), 7.10 (d, *J* = 8.8 Hz, 2H, Ar), 6.49 (d, 1H, *J* = 9.6 Hz, H3 of coumarin), 4.13 (t, 2H, *J* = 6.4 Hz, OCH_2), 3.65 (t, 2H, *J* = 6.8 Hz, OCH_2), 2.14 (s, 3H, $COCH_3$), 1.83 (br. s, 1H, OH), 1.69 and 1.58 (m, 4H, 2 × CH_2), 1.43-1.38 (m, 4H CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.5, 156.4 and 153.2 (C2, C9 of coumarin and C=N), 142.6 (C4 of coumarin), 136.7, 135.4 and 134.1 (Ar and coumarin), 130.1 (C7 of coumarin), 127.7 (C5 of coumarin),127.1, 121.1, 118.9 (Ar and coumarin), 118.3 and 118.0 (C8 and C3 of coumarin), 74.18 (CON), 62.87 (COH), 32.6, 29.2, 25.8, 25.6 (CH_2), 12.5 (CH_3); HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{23}H_{27}N_2O_6S^+$ 459.1584; found 459.1582.

Ethyl (E)-4-(acetylthio)-2-(((methoxy((6-(((1-(4-((2-oxo-2H-chromene)-6-sulfonamido) phenyl)ethylidene)amino)oxy)hexyl)oxy)phosphoryl)oxy)methyl)-2-methyl-3-oxobutanoate (9). Compound **8** (0.27 g, 0.59 mmol), dried over P_2O_5 overnight, was dissolved in dry DCM (2.1 ml) under nitrogen. TEA (0.41 ml, 2.94 mmol) and methyl-*N*,*N*-diisopropylchlorophosphoramidite (0.13 ml, 0.65 mmol) were added and the mixture was stirred at room temperature for 40 minutes. The reaction mixture was filtrated through a short silica gel column by eluting with EtOAc that contained 0.5% TEA. The product fractions were combined, evaporated to dryness under reduced pressure, and the residue was co-

evaporated three times with dry CH_3CN. The residue was dissolved in dry CH_3CN (0.77 ml), and ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate[1] (0.16 g, 0.65 mmol) in dry CH_3CN (0.77 ml) and 0.45 M 1*H*-tetrazole solution in CH_3CN (2.09 ml, 0.94 mmol) were added under nitrogen. The mixture was stirred at room temperature for 3 h. The phosphite triester was mixed with I_2 (0.30 g) in a mixture of THF (6 ml), H_2O (3 ml) and 2,6-lutidine (1.5 ml) and the mixture was stirred at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (LichroCHART 250 × 10 Hypersil ODS 5µm, flow rate 3 ml/min) using an isocratic elution with a mixture of CH_3CN and H_2O (70:30, v/v) to yield **9** as a yellow oil (104 mg, 22%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (br s, 1H, NH), 7.93 (d, 1H, *J* = 2.4 Hz, coumarin), 7.93-7.88 (dd, 1H, *J* = 8.8 and 6.4 Hz, coumarin), 7.70 (d, 1H, *J* = 9.6 Hz, coumarin), 7.50 (d, 1H, *J* = 8.4 Hz, Ar), 7.33 (d, 1H, *J* = 8.8 Hz, coumarin), 7.13 (d, 2H, *J* = 8.8 Hz, Ar), 6.49 (d, 1H, *J* = 9.6 Hz, coumarin), 4.44-4.37 (m, 2H, $POCH_2$), 4.25 (q, 2H, *J* = 7.2 Hz, OCH_2CH_3), 4.12 (t, 2H, *J* = 6.4 Hz, OCH_2), 4.07-4.02 (m, 2H, OCH_2), 4.03-3.90 (m, 2H SCH_2), 3.77 and 3.74 (d, *J* = 11.6 Hz, 3H, $POCH_3$), 2.35 (s, 3H, AcS), 2.13 (s, 3H, N=CCH_3), 1.70-1.66 (m, 4H, CH_2CH_2), 1.57 (s, 3H, CCH_3), 1.43-1.38 (m, 4H, CH_2CH_2), 1.28 (t, 3H, *J* = 7.2 Hz, OCH_2CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 198.7 (CO), 193.8 (SCO), 169.5 (OCO), 159.4 and 156.4 (CN and CO of coumarin), 153.2, 142.6, 137.0, 135.6, 133.8, 130.2, 127.7, 127.0, 121.1, 118.8, 118.2 and 117.9 (coumarin and Ar), 74.0 ($NOCH_2$); 69.2, 68.3, and 68.3 (OCH_2), 62.4 (OCH_2CH_3), 60.2 and 60.1 (quaternary C), 54. 6 and 54.5 (OCH_3), 36.7 (CH_2S), 30.2, 30.1, 29.0, 25. 6, 25.2 (CH_3CO and CH_2), 17.6 (CH_3), 14.0 (CH_3CH_2O), 12.4 ($NCCH_3$); ^{31}P NMR (162 MHz, $CDCl_3$): δ = -0.50 ppm. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{34}H_{44}N_2O_{13}PS_2^+$ 783.2017; found 783.2023.

4,4'-(((6-bromohexyl)oxy)(phenyl)methylene)bis(methoxybenzene) (10). 6-Bromohexanol (1.00 g, 5.52 mmol) was dissolved in dry pyridine (15 ml) and 4,4-dimethoxytritrylchloride (2.25 g, 6.62 mmol) was added. The reaction mixture was stirred at room temperature overnight and evaporated to dryness under reduced pressure. The residue was dissolved in DCM and washed with aq 5% $NaHCO_3$. The

organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography by eluting with a mixture of hexane, EtOAc and TEA (85:15:1, *v/v/v*) to yield **10** as a yellow oil (2.10 g, 79%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.49-7.18 (9H, DMTr), 6.87 (d, 4H, *J* = 9.0 Hz, DMTr), 3.82 (s, 6H, OCH_3), 3.56 (t, 2H, *J* = 7.2 Hz, CH_2Br), 3.11 (t, 2H, *J* = 6.6 Hz, OCH_2), 1.79 and 1.67 (m, 4H, 2 × CH_2), 1.46-1.42 (m, 4H CH_2CH_2); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 158.4, 145.5, 136.7, 130.06, 128.2, 127.8, 126.6, 113.0 (MMTr), 85.7 (quaternary C), 63.2 (OCH_2), 55.2 (OCH_3), 45.1 (CH_2Br), 32.6, 30.0, 26.8, 25.7 (CH_2); HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{27}H_{32}BrNaO_3^+$ 505.1349; found 505.1331.

4-((6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)oxy)-2H-chromen-2-one (11). Compound **10** (3.60 g, 7.45 mmol) was dissolved in dry DMF, and 4-hydroxy-2*H*-chromen-2-one (1.24 g, 7.45 mmol) and K_2CO_3 (2.11 g, 15.29 mmol) were added. The reaction mixture was stirred overnight at 80 °C under argon, filtered and evaporated to dryness. The residue was dissolved in EtOAc and washed with 0.1 N NaOH and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product was purified by silica gel chromatography eluting with a mixture of EtOAc, hexane and TEA (25:74:1, *v/v/v*), to obtain **11** as a yellow oil (1.30 g, 32%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.81 (dd, 1H, *J* = 7.8 and 1.8 Hz, H5 of coumarin), 7.55 (m, 1H, H7 of coumarin), 7.47-7.16 (m, 11H, DMTr and coumarin), 6.84 (d, 4H, *J* = 6.6 Hz, DMTr), 5.68 (s, 1H, H3 of coumarin), 4.12 (t, 2H, *J* = 6.0 Hz, OCH_2), 3.80 (s, 6H, OCH_3), 3.10 (t, 2H, *J* = 6.6 Hz, OCH_2), 1.91 and 1.69 (m, 4H, 2 × CH_2), 1.51-1.50 (m, 4H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ =165.7 and 163.0 (CO), 158.4 (DMTr), 153.4, 145.4, 136.7, 132.3, 130.0, 128.2, 127.7, 126.6, 123.8, 123.0 116.8, 115,8, 113.0 (DMTr and coumarin), 90.4 (C3 of coumarin); 85.7 (quaternary C), 69.4, 63.2 (OCH_2), 55.2 (OCH_3), 29.9, 28.46, 26.02, 21.03 (CH_2); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{36}H_{36}NaO_6^+$ 587.2404; found 587.2399.

4-((6-hydroxyhexyl)oxy)-2H-chromen-2-one (12). Compound **11** (1.30 g, 2.40 mmol) was dissolved in a mixture dichloroacetic acid (0.9 ml) and DCM (29.1 ml) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated aq. $NaHCO_3$ (100 ml). The

organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel chromatography, eluting with a mixture of hexane and EtOAc (60:40, *v/v*). The product **12** was obtained as a white solid (0.40 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, 1H, *J* = 8.0 Hz, H5 of coumarin), 7.63 (t, 1H, *J* = 8.4 Hz, H7 of coumarin), 7.37-7.31 (m, 2H, coumarin), 5.84 (s, 1H, H3 of coumarin), 4.36 (t, 2H, *J* = 5.2 Hz, OH), 4.17 (t, 2H, *J* = 6.4 Hz, OCH_2), 3.40 (m, 2H, C*H*$_2$OH), 1.80 (m, 2H, CH_2), 1.46-1.40 (m, 4H, CH_2CH_2), 1.36 (m, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.4 (C4 of coumarin), 162.1 (CO of coumarin), 133.1, 124.6, 123.2, 116.9 and 115.7 (coumarin), 90.9 (H3 of coumarin), 69.9 (OCH_2), 61.1 (CH_2OH), 32.9, 28.5, 25.8 and 25.64 (CH_2); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{15}H_{18}NaO_4^+$ 285.1097; found 285.1096.

Ethyl 4-(acetylthio)-2-(((methoxy((6-((2-oxo-2H-chromen-4-yl) oxy) hexyl) oxy) phosphoryl) oxy) methyl)-2-methyl-3-oxobutanoate (13). Compound **12** (0.25 g, 0.95 mmol), dried over P_2O_5 overnight, was dissolved in dry DCM (3.36 ml) under nitrogen. TEA (0.66 ml, 4.76 mmol) and methyl-*N*,*N*-diisopropylchlorophosphoramidite (0.20 ml, 1.05 mmol) were added and the mixture was stirred at room temperature for 40 minutes. The reaction mixture was filtrated through a short silica gel column by eluting with EtOAc that contained 0.5% TEA. The product fractions were combined and evaporated to dryness under reduced pressure and the residue was co-evaporated three times with dry CH_3CN. The residue was dissolved in dry CH_3CN (1.24 ml), and ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate[1] (0.26 g, 1.05 mmol) in dry CH_3CN (1.24 ml) and 0.45 M 1*H*-tetrazole solution in CH_3CN (3.39 ml, 1.52 mmol) were added under nitrogen. The mixture was stirred at room temperature for 3 h. The phosphite triester was mixed with I_2 (0.30 g) in THF (6 ml), H_2O (3 ml) and 2,6-lutidine (1.5 ml) and the mixture was stirred at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (LichroCHART 250 × 10 Hypersil ODS 5µm, flow rate 3 ml/min) by using an isocratic elution with a mixture of CH_3CN and H_2O (50:50, *v/v*) to yield **13** as a yellow oil (0.11 g, 19%). ^{1}H NMR (500 MHz, CD_3CN): δ = 7.88 (m, H5 of coumarin), 7.63 (m, H7 of coumarin), 7.36-7.33 (m, H4 and H6 of coumarin), 5.72 (s, 1H, H3 of

coumarin), 4.38-4.34 (m, 2H, $POCH_2$), 4.22 (q, 2H, J = 7.2 Hz, OCH_2CH_3), 4.20 (t, 2H, J = 6.3 Hz, OCH_2), 4.06-4.03 (m, 2H, OCH_2), 4.06-3.96 (m, 2H SCH_2), 3.72, and 3.70 (d, J = 11.2. Hz, 3H, $POCH_3$), 2.37 (s, 3H, AcS), 1.95-1.87 (m, 2H, CH_2), 1.75-1.70 (m, 2H, CH_2), 1.62-1.54 (m, 2H, CH_2), 1.52 (s, 3H, CCH_3), 1.55-1.45 (m, 2H, CH_2), 1.27 (t, 3H, J =7.2 Hz, OCH_2CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 198.6 (CO), 193.6 (SCO), 169.5 (OCO), 165.7 (C4 of coumarin), 162.9 (CO of coumarin), 153.4, 132.3, 123.9, 123.0, 116.8, 115.8, (coumarin), 90.4 (C3 of coumarin), 69.2, 69.1, 68.0 and 67.9 (OCH_2), 62.3 (OCH_2CH_3), 60.2 (quaternary C), 54.4 (OCH_3), 36.69 (CH_2S), 30.1, 30.1, 28.4, 25.6 and 25.1 (CH_2 and CH_3CO), 17.57 (CH_3), 14.0 (CH_3CH_2O); ^{31}P NMR (202 MHz, $CDCl_3$): δ = -0.52 ppm; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{26}H_{36}O_{11}PS_2^+$ 587.1710; found 587.1739.

***N*-(6-hydroxyhexyl)-2-oxo-2*H*-chromene-3-carboxamide (14)**. Ethyl 2-oxo-2*H*-chromene-3-carboxylate[6] (0.88g, 4.07 mmol) was dissolved in EtOH (20 ml), and four drops of piperidine and 6-aminohexanol were added. The mixture was refluxed overnight. The mixture was concentrated and purified by silica gel chromatography eluting with a mixture of hexane and EtOAc (60:40, *v/v*) to give **14** as a white solid (0.46 g, 39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.88 (s, 1H, H4 of coumarin), 8.81 (br s, 1H, NH), 7.78-7.63 (m, 2H, coumarin), 7.42-7.35 (m, 2H, coumarin), 3.63 (t, 2H, J = 6.6 Hz, OCH_2), 3.44 (m, 2H, CH_2-NH), 2.02 (br s, 1H, OH), 1.64 and 1.55 (m, 4H, 2 x CH_2), 1.42-1.38 (m, 4H CH_2CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 161.4 (CO of coumarin and CO of amide), 154.4, 133.9, 129.8, 125.3, 118.7, 118.5 and 116.6 (coumarin), 62.6 (OCH_2), 39.7 ($NHCH_2$), 32.6, 29.3, 26.6 and 25.3 (CH_2). HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{16}H_{19}NNaO_4^+$ 312.1206; found 312.1204.

Ethyl 4-(acetylthio)-2-(((methoxy((6-(2-oxo-2H-chromene-3-carboxamido) hexyl) oxy) phosphoryl)oxy)methyl)-2-methyl-3-oxobutanoate (15). Compound **14** (0.25 g, 0.86 mmol), dried over P_2O_5 overnight, was dissolved in dry DCM (3.05 ml) under nitrogen. TEA (0.60 ml, 4.32 mmol) and methyl-*N*,*N*-diisopropylchlorophosphoramidite (0.185 ml, 0.95 mmol) were added and the mixture was stirred at room temperature for 40 minutes. The reaction mixture was filtrated through a short silica gel column by eluting with a mixture of 0.5% TEA in EtOAc. The product fractions were combined and

evaporated to dryness under reduced pressure and the residue was co-evaporated three times with dry CH_3CN. The residue was dissolved in dry CH_3CN (1.12 ml) and mixed with ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate[1] (0.24 g, 0.95 mmol) in dry CH_3CN (1.12 ml). 1*H*-tetrazole solution (0.45 M) in CH_3CN (3.07 ml, 1.38 mmol) was added under nitrogen and the mixture was stirred at room temperature for 3 h. The phosphite ester was oxidized with I_2 (0.30 g) in a mixture of THF (6 ml), H_2O (3 ml) and 2,6-lutidine (1.5 ml) by stirring at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (LichroCHART 250 × 10 Hypersil ODS 5µm, flow rate 3 ml/min) by using an isocratic elution with a mixture of CH_3CN and H_2O (50:50, *v/v*) to yield **15** as a yellow oil (200 mg, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.86 (s, 1H, H4 of coumarin), 8.76 (br s, 1H, NH), 7.67-7.57 (m, 2H, coumarin), 7.37-7.32 (m, 2H, coumarin), 4.38-4.31 (m, 2H, $POCH_2$), 4.19 (q, 2H, J = 7.2 Hz, OCH_2CH_3), 4.00 (m, 2H, OCH_2), 3.99-3.87 (m, 2H SCH_2), 3.75 and 3.70 (d, J = 11.2 Hz, 3H, $POCH_3$), 3.44-3.39 (m, 2H, NCH_2), 2.32 (s, 3H, AcS), 1.67-1.57 (m, 4H, CH_2CH_2), 1.52 (s, 3H, CCH_3), 1.41-1.36 (m, 4H, CH_2CH_2), 1.25 (t, 3H, J = 7.2 Hz, OCH_2CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 198.6 (CO), 193.6 (SCO), 169.5 (OCO), 161.4 and 161.4 (CO of coumarin and CO of amide), 154.4, 148.1, 133.9, 129.7, 125.2, 111.6, 118.5 and 116.5 (coumarin), 69.0, 68.1, and 68.0 ($POCH_2$ and OCH_2), 62.2 (OCH_2CH_3), 60.2 and 60.1 (quaternary C), 54.4 and 54.3 (OCH_3), 39.68 (NCH_2), 36.66 (CH_2S), 30.1, 30.0, 29.3, 26.5 and 25.1 (CH_2 and CH_3CO), 17.53 (CH_3), 13.92 (CH_3CH_2O). ^{31}P NMR (162 MHz, $CDCl_3$): δ = -0.34 ppm.; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{27}H_{36}NO_{11}PSNa^+$ 636.1639; found 636.1685.

***N*-((4-(Hydroxymethyl)cyclohexyl)methyl)-2-oxo-2H-chromene-3-carboxamide (16).** Ethyl 2-oxo-2*H*-chromene-3-carboxylate[6] (0.60 g, 2.75 mmol) was dissolved in EtOH (10 ml), and (4-(aminomethyl)cyclohexyl)methanol (0.39 g, 2.75 mmol) was added. The mixture was refluxed overnight. The solvent was evaporated and the residue was purified by silica gel chromatography eluting with a mixture of hexane and EtOAc (80:20, *v/v*) to give **16** as a white solid (0.80 g, 83%). ^{1}H NMR (500 MHz,

$(CD_3)_2SO$): δ = 8.84 (s, 1H, H4 of coumarin), 8.67 (t, 1H, J = 6.0 Hz, NH), 7.97 (m, 1H, coumarin), 7.74 (m, 1H, coumarin), 7.49 (d, J = 8.5 Hz, 1H, coumarin), 7.42 (m, 2H, coumarin), 4.34 (t, 1H, J = 5.5 Hz, OH), 3.21-3.18 (m, 4H, OCH_2 and NCH_2), (m, 4H, CH_2CH_2), 1.48-1.45 (m, 1H, CH), 1.31-1.28 (m, 1H, CH), 0.99-0.84 (m, 4H CH_2CH_2); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 161.5 and 161.0 (CO of coumarin and CO of amide), 154.3, 147.7, 134.5, 130.7, 125.6, 118.7, 119.6, 119.0 and 116.6 (coumarin), 67.0 (OCH_2), 45.7 ($NHCH_2$), 40.8 and 38.2 (CH), 30.4 and 29.3 (CH_2); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{18}H_{21}NO_4Na^+$ 338.1363; found 338.1382.

Ethyl 4-(acetylthio)-2-(((methoxy((4-((2-oxo-2H-chromene-3-carboxamido)methyl)cyclohexyl)methoxy)phosphoryl)oxy)methyl)-2-methyl-3-oxobutanoate (17). Compound **16** (0.15 g, 0.48 mmol) was dissolved in DCM (1.70 ml) under nitrogen and TEA (0.33 ml, 2.37 mmol) was added. To increase the solubility of **16**, the reaction solution was heated 5 min at 37 °C. Methyl-*N*,*N*-diisopropylchlorophosphoramidite (0.102 ml, 0.52 mmol) were added and the mixture was stirred at room temperature for 40 minutes. The reaction mixture was filtrated through a short silica gel column by eluting with a mixture of 0.5% TEA in EtOAc. The mixture was evaporated to dryness under reduced pressure and the residue was co-evaporated three times from dry CH_3CN. The residue was dissolved in dry CH_3CN (2.70 ml), and the reaction solution was hetead 5 min at 37 °C. Ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate (**26**)[1] (0.13 g, 0.52 mmol) in dry 0.45 M 1*H*-tetrazole solution in CH_3CN (2.00 ml, 0.90 mmol) was added under nitrogen. The mixture was stirred at room temperature for 3 h. The phosphite ester was oxidized with I_2 (0.30 g) in a mixture of THF (6 ml), H_2O (3 ml) and 2,6-lutidine (1.5 ml) by stirring at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified twice by RP-HPLC (Thermo Scientific 250 × 10 Hypersil ODS 5µm, flow rate 3 ml/min) by eluting with a mixture of CH_3CN and H_2O (40:60, *v/v*) to yield **17** as a white solid (57 mg, 19%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.91 (2 x s, 1H, H4 of coumarin); 8.86 (br s, 1H, NH); 7.73-7.67 (m, 2H, coumarin), 7.44-7.39 (m, 2H, coumarin), 4.47-4.27 (m, 2H, $POCH_2C_q$), 4.27 (q, 2H, J = 7.0 Hz, OCH_2CH_3), 4.07-3.92

(m, 2H SCH_2), 3.88-3.84 (m, 2H, OCH_2), 3.77 (2 x d, 3H, *J* = 11.5 Hz, $POCH_3$), 3.37-3.35 (m, 2H, NCH_2), 2.39 (s, 3H, AcS), 1.92-1.84 (m, 4H, CH_2CH_2), 1.70—1.59 (m, 2H, 2 × CH), 1.58 (s, 3H, CCH_3); 1.32 (t, 3H, *J* = 7.0 Hz, $OCH_2C\mathit{H}_3$), 1.15-0.99 (m, 4H, CH_2CH_2); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.6 (CO), 193.6 (SCO), 169.5 (OCO), 161.6 and 161.5 (CO of coumarin and CO of amide); 154.4, 148.3, 134.0, 129.8, 125.3, 118.7, 118.6 and 116.6 (coumarin), 72.9 and 72.8 ($POCH_2$), 69.1 ($PO\mathit{C}H_2C_q$), 62.3 ($O\mathit{C}H_2CH_3$), 60.2 (quaternary C), 54.5 and 54.4 (OCH_3), 45.9 (NCH_2), 38.3, 38.2, 37.8 (CH), 36.7 (CH_2S), 30.1, 29.9 and 28.5 (CH_2CH_2 and $\mathit{C}H_3CO$), 17.6 (CH_3); 14.0 ($\mathit{C}H_3CH_2O$); ^{31}P NMR (202 MHz, $CDCl_3$): δ = -0.37 ppm. HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{29}H_{38}NO_{11}PSNa^+$ 662.1795; found 662.1767.

***N*-(4-hydroxybutyl)-2-oxo-2*H*-chromene-3-carboxamide (18)**. Ethyl 2-oxo-2*H*-chromene-3-carboxylate[6] (1.09 g, 5.00 mmol) was dissolved in EtOH (20 ml) and 4-amino-1-butanol (0.45g, 5 mmol) was added. The mixture was refluxed overnight. The solvent was evaporated and the residue was purified by silica gel chromatography eluting first with a mixture of hexane and EtOAc (35:65, *v/v*) and then with a mixture of hexane and EtOAc (20:80, *v/v*) to give **18** as white solid (0.91 g, 69%). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.91 (s, 1H, H4 of coumarin), 8.89 (br s, 1H, NH), 7.72-7.66 (m, 2H, coumarin), 7.42-7.37 (m, 2H, coumarin), 3.73 (t, 2H, *J* = 5.5 Hz, OCH_2), 3.53 (m, 2H, CH_2-NH); 2.02 (br s, 1H, OH), 1.76 and 1.69 (m, 4H, 2 x CH_2); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 161.6 and 161.5 (CO of coumarin and CO of amide), 154.4, 148.3, 134.0, 129.8, 125.3, 118.7, 118.5 and 116.6 (coumarin), 62.4 (OCH_2), 39.6 ($NHCH_2$), 29.9 and 26.0 (CH_2); HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{14}H_{15}NNaO_4^+$ 284.0893; found 284.0886.

Methyl (6-(2-oxo-2H-chromene-3-carboxamido)hexyl) phosphate (19). Compound **14** (26.3 mg, 0.09 mmol), dried over P_2O_5 overnight, was dissolved in dry DCM (0.5 ml) under nitrogen. TEA (63 µl, 0.45 mmol) and methyl-*N*,*N*-diisopropylchlorophosphoramidite (19 µl, 0.1 mmol) were added and the mixture was stirred at room temperature for 40 minutes. The reaction mixture was filtrated through a short silica gel column by eluting with a mixture of 0.5% TEA in EtOAc. The product fractions were combined and

evaporated to dryness under reduced pressure. The residue was co-evaporated three times with dry CH_3CN. A solution of 0.45 M 1*H*-tetrazole in CH_3CN (3.02 ml, 1.36 mmol) and water (200 µl, 5.55 mmol) were added, and the mixture was stirred at room temperature for 3 h. The phosphite triester was oxidized with I_2 (0.15 g) in a mixture of THF (3 ml), H_2O (1.5 ml) and 2,6-lutidine (0.75 ml) by stirring at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed twice with 5% aq $NaHSO_3$ (4.5 ml). The crude product was purified by RP-HPLC (SunFire 250 × 10 Prep C18 5µm, flow rate 3 ml/min) using a gradient elution (25 mM TEAA and MeCN (from 14% to 63% MeCN). The product was desalted on the same column by eluting with a mixture of H_2O and MeCN. Finally, the product was passed through a Na^+- form Dowex 50WX8 (100-200 mesh) cation exchange column to give compound **19** (3.4 mg, 9.2 %) as a white solid. ^{1}H NMR (500 MHz, D_2O): δ = 9.09 (br s, 1H, NH), 8.87 (s, 1H, H4 of coumarin), 7.87 (m, 1H, coumarin), 7.76 (m, 1H, coumarin), 7.48-7.44 (m, 2H, coumarin), 3.89 (q, 2H, J = 6.4 Hz, OCH_2), 3.60 (d, 3H, J = 10.8 Hz, $POCH_3$), 3.46 (m, 2H, NCH_2), 3.33 (MeOH), 1.64-1.72 (m, 4H, 2 × CH_2), 1.42-1.53 (m, 4H, CH_2CH_2); ^{13}C NMR (125 MHz, D_2O): δ = 162.3 and 161.1 (CO of coumarin and CO of amide), 154.5 (coumarin), 147.8 (C4 of coumarin), 134.0, 129.9, 125.1, 118.6, 118.3 and 116.1 (coumarin), 65.3 and 65.2 (OCH_2), 51.7 and 51.7 (OCH_3), 39.5 and 39.4 (NCH_2), 30.3, 30.3, 28.9, 30.3, 26.4, 25.2 (4 × CH_2); ^{31}P NMR (202 MHz, D_2O): δ = 1.73 ppm; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{17}H_{22}NNaO_7P^+$ 406.1026; found 406.1018.

Ethyl 4-(acetylthio)-2-(((methoxy((4-(2-oxo-2*H*-chromene-3-carboxamido) butoxy) phosphoryl)oxy)methyl)-2-methyl-3-oxobutanoate (20). Compound **18** (0.30 g, 1.15 mmol) was dissolved in DCM (3.40 ml) under nitrogen and TEA (0.80 ml, 5.74 mmol) and methyl-*N*,*N*-diisopropylchlorophosphoramidite (0.245 ml, 1.26 mmol) were added. The mixture was stirred at room temperature for 40 minutes and then filtrated through a short silica gel (3 cm) column by eluting with a mixture of 0.5% TEA in EtOAc. The solvent was evaporated under reduced pressure and the residue was co-evaporated three times from dry CH_3CN. The residue was dissolved in CH_3CN (2.04 ml), and ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate (**26**)[1] (0.31 g, 1.26 mmol) in dry CH_3CN (2.04

ml) and 0.45 M 1*H*-tetrazole solution in CH_3CN (4.08 ml, 1.84 mmol) were added under nitrogen. The mixture was stirred at room temperature for 3 h. The phosphite ester was oxidized with I_2 (0.30 g) in a mixture of THF (6 ml), H_2O (3 ml) and 2,6-lutidine (1.5 ml) by stirring at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic layer was dried with Na_2SO_4, filtrated and evaporated to dryness. The crude product was purified twice by RP-HPLC (Thermo Scientific 250 × 10 Hypersil ODS 5μm, flow rate 3 ml/min) by using first elution with a mixture of CH_3CN and H_2O (40:60, v/v) and then elution with a mixture of CH_3CN and H_2O (50:50, v/v) to yield **20** as yellow oil (81 mg, 12%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.91 (s, 1H, H4 of coumarin), 8.86 (br s, 1H, NH), 7.72-7.66 (m, 2H, coumarin), 7.42-7.38 (m, 2H, coumarin), 4.47-4.37 (m, 2H, $POCH_2$), 4.26 (q, 2H, *J* = 7.00 Hz, OCH_2CH_3), 4.17-4.08 (m, 2H, OCH_2), 4.04-3.93 (m, 2H SCH_2), 3.78 and 3.76 (d, 3H, *J* = 11.0 Hz, $POCH_3$), 3.53-3.49 (m, 2H, NCH_2), 2.37 (s, 3H, AcS), 1.57 (s, 3H, CCH_3), 1.83-1.73 (m, 4H, CH_2CH_2), 1.29 (t, 3H, *J* = 7.00 Hz, OCH_2CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.6 (CO), 193.7 (SCO), 169.5 (OCO), 161.6 and 161.46 (CO of coumarin and CO of amide), 154.41, 148.33, 134.04, 129.81, 125.30, 118.65, 118.44 and 116.6 (coumarin), 69.1, 69.1, 69.0, 69.1, 67.7, and 67.6 ($POCH_2$ and OCH_2), 62.3 (OCH_2CH_3), 60.2 and 60.1 (quaternary C), 54.5 and 54.4 (OCH_3), 39.2 (NCH_2), 36.7 (CH_2S), 30.1, 27.7, 27.6 and 25.6 (CH_2 and CH_3CO), 17.6 (CH_3), 13.9 (CH_3CH_2O); ^{31}P NMR (202 MHz, $CDCl_3$): δ = -0.47 ppm; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{25}H_{33}NO_{11}PS^+$ 586.1506; found 586.1511.

Methyl 2-azido-4-((4-hydroxybutyl)carbamoyl)benzoate (21a). 3-Amino-4-(methoxycarbonyl)benzoic acid (5.0 g, 25.6 mmol) was dissolved in a mixture of H_2O (35.7 ml) and concentrated HCl (43 ml) at 0 °C and a solution of sodium nitrite (2.00 g, 29.4 mmol) was added. the mixture was stirred for 20 min, and sodium azide (5.13 g, 78.9 mmol) was added dropwise upon 1 h. The mixture was allowed to warm up and stirred overnight at room temperature. The reaction mixture was diluted in water and extracted with ethyl acetate. The organic phase was separated, dried over Na_2SO_4, filtrated and evaporated to dryness to obtain 5.0 g (88%) of the crude azide. To a solution of the crude azide (3-azido-4-(methoxycarbonyl)benzoic acid) (5.00 g, 22.6 mmol) and HATU (12.9 g, 33.6 mmol) in DMF (50 ml),

DIPEA (11.8 ml, 67.8 mmol) was added. After 5 min, a solution of 4-aminobuthanol (3.02 g, 33.9 mmol) in DMF (10 ml) was added and the reaction solution was stirred for 1 h at 60 °C. The mixture was evaporated to dryness and the residue was purified by silica gel chromatography eluting with a mixture of DCM and MeOH (98:1, *v/v*). Compound **21a** was obtained as a white solid in 48% yield (3.2 g). ^{1}H NMR (600 MHz, CD_3CN): δ = 7.83 (d, 1H, J = 8.1 Hz, Ar), 7.68 (d, 1H, J = 1.5 Hz, Ar), 7.62 – 7.59 (m, 1H , Ar), 7.39 (br s, NH), 3.88 (s, 3H, OCH_3), 3.56 (m, 2H, CH_2OH), 3.39 (m, 2H, NCH_2), 2.76 (m, 1H, OH), 1.69 – 1.63 (m, 2H, CH_2), 1.60 – 1.55 (m, 2H, CH_2); ^{13}C NMR (151 MHz, CD_3CN): δ = 165.2 (OCO), 165.1 (NCO), 139.8, 139.2, 131.4, 125.0, 123.0 and 119.2 (Ar), 61.3 (CH_2OH), 52.1 (OCH_3), 39.6 (NCH_2), 29.9 and 25.8 (2 × CH_2); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{13}H_{16}N_4O_4Na^+$ 315,1064; found 315,1063.

Methyl 2-azido-4-((6-hydroxyhexyl)carbamoyl)benzoate (21b). To a solution of 3-azido-4-(methoxycarbonyl)benzoic acid (5.00 g, 22.6 mmol) and HATU (12.9 g, 33.6 mmol) in DMF (50 ml), DIPEA (11.0 ml, 67.8 mmol) was added. After 5 min, a solution of 6-aminohexanol (3.97 g, 33.9 mmol) in DMF (10 ml) was added and the reaction mixture was stirred for 1 h at 60 °C. The mixture was evaporated to dryness and the residue was purified by silica gel chromatography by eluting with a mixture of DCM and MeOH (98:1, *v/v*). Compound **21b** was obtained as a white solid in 51% yield (3.0 g). ^{1}H NMR (600 MHz, CD_3CN): δ = 7.86 (d, 1H, J = 7.9 Hz, Ar), 7.70 (s, 1H, Ar), 7.61 (m, 1H , Ar), 7.23 (br s, NH), 3.90 (s, 3H, OCH_3), 3.51 (m, 2H, CH_2OH), 3.38 (m, 2H, NCH_2), 2.51 (m, 1H, OH), 1.66 – 1.58 (m, 2H, CH_2), 1.54 – 1.48 (m, 2H, CH_2), 1.43 – 1.37 (m, 4H, 2 x CH_2); ^{13}C NMR (151 MHz, CD_3CN): δ = 165.3 (OCO), 165.1 (NCO), 139.8, 139.3, 131.3, 125.1, 123.1, and 119.2 (Ar), 61.5 (CH_2OH), 52.1 (OCH_3), 39.6 (NCH_2), 32.5, 29.1, 26.5, 25.3 (4 x CH_2); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{15}H_{20}N_4O_4Na^+$ 343,1377; found 343,1374.

Methyl 4-((6-(((4-(acetylthio)-2-(ethoxycarbonyl)-2-methyl-3-oxobutoxy)(methoxy)phosphoryl)oxy)hexyl)carbamoyl)-2-aminobenzoate (22). Ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate (**26**) (0.17 g, 0.67 mmol) was dissolved in CH_3CN (3.0 ml)

under nitrogen. *N*,*N*,*N*',*N*'-tetraisopropyl-1-methoxyphosphanediamine (0.23 ml, 0.79 mmol) and 0.45 M 1*H*-tetrazole solution in CH_3CN (1.49 ml, 0.67 mmol) were added and the mixture was stirred at room temperature for 30 minutes. The course of the phosphitylation was followed by ^{31}P NMR spectroscopy (126 MHz, CD_3CN, the product: δ = 149.4 and 149.2 ppm). Methyl 2-azido-4-((6-hydroxyhexyl)carbamoyl)benzoate (**21b**) (0.30 g, 0.93 mmol) together with 0.45 M solution of 1*H*-tetrazole in CH_3CN (4.15 ml, 1.86 mmol) was then added to the reaction mixture under nitrogen (^{31}P NMR: δ = 139.9 ppm for the phosphite trimester). The mixture was stirred for 15 min and the phosphite ester was oxidized with I_2 (0.20 g, 0.79 mmol) in a mixture of THF (3.0 ml), H_2O (1.5 ml) and 2,6-lutidine (0.75 ml) by stirring overnight at room temperature. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. To reduce N_3 to NH_2 group, the crude product (**IA**) was dissolved in a mixture of THF (6.0 ml) and H_2O (1.2 ml). 1 M trimethylphosphine solution (0.76 ml) in toluene was added and the mixture was stirred for 15 min. The residue was dissolved in DCM and washed with H_2O and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (Phenomenex 250 × 10, Synenergi 4µm Fusion-RP 80Å, flow rate 3 ml/min) by using an isocratic elution with 46% CH_3CN in H_2O and then a gradient elution from 46% to 80% CH_3CN in H_2O over 5 min. The product fractions were combined and lyophilized to yield **22** as a viscous oil (46 mg, 11%). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.89 (d, 1H, *J* = 8.0 Hz, Ar), 7.17 (d, 1H, J = 1.5 Hz, Ar), 6.94 (m, 1H, Ar), 6.43 (br m, NH_2), 4.48-4.37 (m, 2H, $POCH_2C_q$), 4.27 (q, 2H, *J* = 7.5 Hz, OCH_2CH_3), 4.07 (m, 2H,$POCH_2$), 4.04-3.93 (m, 2H, and SCH_2), 3.90 (s, 3H, OCH_3), 3.77 (2 x d, 3H, *J* = 11.0 Hz, $POCH_3$), 3.45 (m, 2H, NCH_2), 2.37 (2 x s, 3H, AcS), 1.71 (quintet, *J* = 6.5 Hz, 2H, CH_2), 1.64 (quintet, *J* = 7.0 Hz, 2H, CH_2), 1.58 (s, 3H, CH_3), 1.47-1.43 (m, 4H, CH_2CH_2), 1.30 (t, 3H, *J* = 7.5 Hz, OCH_2CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.8 (CO), 193.7 (SCO), 169.6 and 169.5 (*C*OOEt), 168.0 (*C*OOMe), 167.0 (NCO), 150.5, 139.8, 131.7, 115.6, 113.7 and 112.5 (Ar), 69.1 and 69.0 ($POCH_2$), 67.9 and 67.9 ($POCH_2$), 62.4 (OCH_2CH_3), 60.2 and 60.1 (quaternary C), 54.5 and 54.4 ($POCH_3$), 51.7 (OCH_3), 39.7 (NCH_2), 36.7 (CH_2S), 30.1 (CH_3CO), 30.0, 29.9 and 29.3

(CH_2), 26.1 (CH_2), 24.7 (CH_2), 17.6 (CH_3), 14.0 (OCH_2CH_3). ^{31}P NMR (202 MHz, CD_3CN): δ = -0.38 ppm; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{26}H_{39}N_2O_{11}PSNa^+$ 641.1904; found 641.1897.

Methyl 4-((6-(((4-(acetylthio)-2,2-dimethyl-3-oxobutoxy)(methoxy)phosphoryl)oxy)hexyl)carbamoyl)-2-aminobenzoate (**23**). *S*-(4-hydroxy-3,3-dimethyl-2-oxobutyl)ethanethioate (**27**) (0.13 g, 0.70 mmol) was dissolved in CH_3CN (3.0 ml) under nitrogen. *N*,*N*,*N*',*N*'-tetraisopropyl-1-methoxyphosphanediamine (0.22 ml, 0.78 mmol) and 0.45 M 1*H*-tetrazole solution in CH_3CN (1.56 ml, 0.70 mmol) were added and the mixture was stirred at room temperature for 30 minutes. The course of the phosphitylation was followed by ^{31}P NMR spectroscopy (126 MHz, CD_3CN: δ = 148.5 ppm). Methyl 2-azido-4-((6-hydroxyhexyl)carbamoyl)benzoate (**21b**) (0.30 g, 0.94 mmol) was added together with a 0.45 M 1*H*-tetrazole solution in CH_3CN (4.16 ml, 1.87 mmol) under nitrogen (^{31}P NMR: δ = 139.5 ppm for the phosphite trimester). The mixture was stirred for 15 min, and the resulted phosphite ester was oxidized with I_2 (0.20 g, 0.78 mmol) in a mixture of THF (3.0 ml), H_2O (1.5 ml) and 2,6-lutidine (0.75 ml) by stirring at room temperature overnight. The solvent was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. To reduce N_3 to NH_2 group, the crude product **(IB)** was dissolved in a mixture of THF (4.0 ml) and H_2O (0.8 ml). 1 M trimethylphosphine solution (0.46 ml) in toluene was added and the mixture was stirred for 15 min. The residue was dissolved in DCM and washed with H_2O and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (Phenomenex 250 × 10, Synenergi 4µm Fusion-RP 80Å, flow rate 3 ml/min) by using an isocratic elution with 46% CH_3CN in H_2O and then a gradient elution from 46% to 80% CH_3CN over 5 min. Compound **23** was obtained as a viscous oil (22 mg, 6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.85 (d, 1H, *J* = 8.0 Hz, Ar), 7.17 (d, 1H, *J* = 1.5 Hz, Ar), 7.04 (br s, NH), 6.93 (dd, 1H, *J* = 8.0, and 1.5 Hz, Ar), 6.17 (br s, NH_2), 4.07 (d, 2H, *J* = 5.0 *Hz*, $POCH_2C_q$), 4.04-4.00 (m, 2H, $POCH_2$ and SCH_2), 3.86 (s, 3H, OCH_3), 3.71 (d, 3H, *J* = 11.0 Hz, $POCH_3$), 3.44 (m, 2H, NCH_2), 2.35 (s, 3H, AcS), 1.68 (quintet, *J* = 7.0 Hz, 2H, CH_2), 1.59 (quintet, *J* = 7.0 Hz, 2H, CH_2), 1.45-1.40 (m, 4H, CH_2CH_2), 1.24 (s, 6H, 2 x CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 205.6 (CO), 194.4

(SCO), 167.9 (*C*OOMe), 166.4 (NCO), 151.0, 140.3, 131.2, 115.4, 113.4 and 111.6 (Ar), 72.3 and 72.2 ($POCH_2$), 67.7 and 67.6 ($POCH_2$), 54.0 and 53.9 ($POCH_3$), 51.3 (OCH_3), 48.5 (quaternary C), 39.2 (NCH_2), 36.1 (CH_2S), 29.8 and 29.7 (CH_2), 29.4 (CH_3CO), 29.0 (CH_2), 26.0 (CH_2), 24.7 (CH_2), 20.7 (CH_3); ^{31}P NMR (202 MHz, CD_3Cl): δ = -0.43 ppm. HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{24}H_{37}N_2O_9PSNa^+$ 583.1850; found 583.1825. The synthesis of **23** was repeated by the company of Piramal Enterprises Ltd with a similar procedure, but with slightly different reagent ratios. *S*-(4-Hydroxy-3,3-dimethyl-2-oxobutyl)ethanethioate (**27**) (1.0 g, 5.26 mmol) was dissolved in CH_3CN (10.0 ml) under nitrogen. *N*,*N*,*N*',*N*'-tetraisopropyl-1-methoxyphosphanediamine (1.52 g, 1.66 ml, 5.79 mmol) and 1*H*-tetrazole (0.37 g, 5.26 mmol) were added and the mixture was stirred at room temperature for 30 minutes. Methyl 2-azido-4-((6-hydroxyhexyl)carbamoyl)benzoate (**21b**) (0.84 g, 2.63 mmol) was added together with a 0.45 M 1*H*-tetrazole solution in CH_3CN (11.7 ml, 5.26 mmol) under nitrogen. The mixture was stirred for 15 min., and the resulted phosphite ester was oxidized with I_2 (3.0 g, 6.3 mmol) in a mixture of THF (11.4 ml), H_2O (5.6 ml) and 2,6-lutidine (6.5 ml) by stirring at room temperature for 1 h. LCMS analysis showed 33% product conversion. The solvent was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was passed through a silica column eluting with a 7:3 (*v/v*) mixture of hexane and ethylacetate and used in a next step without further purification. To reduce N_3 to NH_2 group, the crude product **IB** (1.0 g, 1.70 mmol) was dissolved in a mixture of THF (10.0 ml) and H_2O (6.0 ml). Trimethylphosphine (0.16 g, 2.04 mmol) (1 M solution in toluene) was added and the mixture was stirred for 15 min. The residue was dissolved in DCM and washed with H_2O and brine. The organic phase was dried with Na_2SO_4 and the solvent evaporated to dryness. The crude product was purified by RP-HPLC by using an isocratic elution with 46% CH_3CN in H_2O. Compound **23** was obtained as a viscous oil (0.4 g, 42 %). The synthesis was repeated to yield compound **23** 2.7 g. ^1H NMR (500 MHz, CD_3CN): δ = 7.85 (d, 1H, *J* = 8.2 Hz, Ar), 7.17 (d, 1H, *J* = 1.6 Hz, Ar), 7.06 (br s, NH), 6.93 (dd, 1H, *J* = 8.2, and 1.6 Hz, Ar), 6.17 (br s, NH_2), 4.07 (d, 2H, *J* = 4.7 Hz, $POCH_2C_q$), 4.04-4.00 (m, 4H, $POCH_2$ and SCH_2), 3.86 (s, 3H, OCH_3), 3.71 (d, 3H, *J* = 11.1 Hz, $POCH_3$), 3.34 (m, 2H, NCH_2), 2.35 (s, 3H, AcS), 1.68 (quintet, *J* = 6.8 Hz, 2H, CH_2), 1.59 (quintet, *J* = 7.0 Hz, 2H, CH_2), 1.44-1.39 (m, 4H,

CH_2CH_2), 1.24 (s, 6H, 2 x CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 205.6 (COC_q), 194.4 (SCO), 167.9 ($COOMe$), 166.4 (NCO), 151.0, 140.3, 131.2, 115.4, 113.4 and 111.6 (Ar), 72.4 and 72.3 ($POCH_2C_q$), 67.7 and 67.6 ($POCH_2$), 54.0 and 53.9 ($POCH_3$), 51.3 (OCH_3), 48.5 (quaternary C), 39.3 (NCH_2), 36.1 (CH_2S), 29.9 and 29.8 (CH_2), 29.4 (CH_3CO), 29.0 (CH_2), 26.0 (CH_2), 24.8 (CH_2), 20.7 (CH_3); ^{31}P NMR (202 MHz, CD_3Cl): δ = -0.43 ppm; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{24}H_{38}N_2O_9PS^+$ 561.2030; found 561.2032.

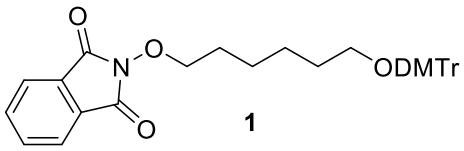
Methyl 4-((4-(((4-(acetylthio)-2-(ethoxycarbonyl)-2-methyl-3-oxobutoxy)(methoxy)phosphoryl)oxy)butyl)carbamoyl)-2-aminobenzoate (24). Ethyl 4-(acetylthio)-2-(hydroxymethyl)-2-methyl-3-oxobutanoate (**26**) (0.18 g, 0.73 mmol) was dissolved in CH_3CN (3.0 ml) under nitrogen. *N*,*N*,*N*',*N*'-tetraisopropyl-1-methoxyphosphanediamine (0.24 ml, 0.84 mmol) and 0.45 M 1*H*-tetrazole solution in CH_3CN (1.61 ml, 0.73 mmol) were added and the mixture was stirred at room temperature for 30 minutes. The course of the phosphitylation was followed by ^{31}P NMR spectroscopy (126 MHz, CD_3CN: δ = 149.4 and 149.2 ppm for the product). Methyl 2-azido-4-((4-hydroxybutyl)carbamoyl)benzoate (**21a**) (0.28 g, 0.96 mmol) was added in 0.45 M 1*H*-tetrazole solution in CH_3CN (4.27 ml, 1.92 mmol) under nitrogen (^{31}P NMR: δ = 139.9 ppm for the phosphite trimester). The mixture was stirred for 5 min, and the resulted phosphite ester was oxidized with I_2 (0.21 g, 0.84 mmol) in a mixture of THF (3.0 ml), H_2O (1.5 ml) and 2,6-lutidine (0.75 ml) by stirring at room temperature overnight. The mixture was evaporated to dryness and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. To reduce N_3 to NH_2 group, the crude product (**IC)** was dissolved in a mixture of THF (6.0 ml) and H_2O (1.2 ml). 1 M trimethylphosphine solution (0.70 ml) in toluene was added and the mixture was stirred for 15 min. The residue was dissolved in DCM and washed with H_2O and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (Thermo Scientific 250 × 10 Hypersil ODS 5µm, flow rate 3 ml/min) by using an isocratic elution with 35% CH_3CN in H_2O to yield **24** as a viscous oil (60 mg, 18%). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.91 (d, 1H, *J* = 8.4 Hz, Ar), 7.20 (d, 1H, *J* = 1.2 Hz, Ar), 7.00 (d, 1H, *J* = 8.4 Hz, Ar), 6.60 (br s, NH), 4.50 – 4.37 (m,

2H, $POCH_2C_q$), 4.27 (quartet, 2H, J = 7.2 Hz, OCH_2CH_3), 4.13 (m, 2H, $POCH_2$), 4.00 (m, 2H, SCH_2), 3.91 (s, 3H, OCH_3), 3.78 (dd, 3H, J = 11.2 and 1.0 Hz, $POCH_3$), 3.50 (m, 2H, NCH_2), 2.36 (s, 3H, AcS), 1.83 – 1.73 (m, 4H, CH_2CH_2), 1.58 (d, 3H, J = 2.4 Hz, CH_3C_q), 1.31 (t, 3H, J = 7.1 Hz, OCH_2CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 198.9 (CO), 193.9 (SCO), 169.6 (*C*OOEt), 168.0 (*C*OOMe), 166.9 (NCO), 150.0, 139.6, 131.7, 115.9, 114.3 and 112.9 (Ar), 69.2 and 69.1 ($POCH_2C_q$), 68.0 and 67.9 ($POCH_2$), 62.4 (OCH_2CH_3), 60.2 and 60.1 (quaternary C), 54.6 and 54.5 ($POCH_3$), 51.8 (OCH_3), 39.5 (NCH_2), 36.8 and 36.7 (CH_2S), 30.1 (CH_3COS), 27.6 and 25.5 (CH_2CH_2), 17.6 (CH_3C_q), 14.0 (CH_3CH_2O). ^{31}P NMR (202 MHz, $CDCl_3$): δ = -0.46 and -0.50 ppm; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{24}H_{35}N_2O_{11}PSNa^+$ 613.1591; found 613.1579.

Methyl 4-((4-(((4-(acetylthio)-2,2-dimethyl-3-oxobutoxy)(methoxy)phosphoryl)oxy)butyl)carbamoyl)-2-aminobenzoate (25). *S*-(4-hydroxy-3,3-dimethyl-2-oxobutyl)ethanethioate (**27**) (0.136 g, 0.71 mmol) was dissolved in CH_3CN (3.0 ml) under nitrogen. *N*,*N*,*N*',*N*'-tetraisopropyl-1-methoxyphosphanediamine (0.24 ml, 0.83 mmol) and 0.45 M 1*H*-tetrazole solution in CH_3CN (1.59 ml, 0.72 mmol) were added and the mixture was stirred at room temperature for 30 minutes. The course of the phosphitylation was followed by ^{31}P NMR spectroscopy (126 MHz, CD_3CN: δ = 149.2 ppm for the product). Methyl 2-azido-4-((4-hydroxybutyl)carbamoyl)benzoate (**21a**) (0.29 g, 0.94 mmol) was added together with a 0.45 M 1*H*-tetrazole solution in CH_3CN (4.17 ml, 1.88 mmol) under nitrogen (^{31}P NMR: δ = 139.9 ppm for the phosphite trimester). After 10 min stirring, the phosphite ester was oxidized with I_2 (0.21 g, 0.83 mmol) in a mixture of THF (3.0 ml), H_2O (1.5 ml) and 2,6-lutidine (0.75 ml) by stirring at room temperature overnight. The solvent was evaporated and the residue was dissolved in DCM and washed with 5% aq $NaHSO_3$ (100 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. To reduce N_3 to NH_2 group, the crude product (**ID**) was dissolved in a mixture of THF (6.0 ml) and H_2O (1.2 ml). 1 M trimethylphosphine solution (0.70 ml) in toluene was added and the mixture was stirred for 15 min. The residue was dissolved in DCM and washed with H_2O and brine. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by RP-HPLC (Thermo Scientific

250 × 10 Hypersil ODS 5µm, flow rate 3 ml/min) by using an isocratic elution with 34% CH_3CN in H_2O to yield **25** as a viscous oil (23 mg, 6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.90 (d, 1H, J = 8.5 Hz, Ar), 7.17 (d, 1H, J = 1.5 Hz, Ar), 6.98 (dd, 1H, J = 8.5, and 1.5 Hz, Ar), 6.63 (br s, NH), 5.92 (br s, NH_2), 4.14-4.07 (m, 4H, $POCH_2C_q$, $POCH_2$), 3.99 (s, 2H, SCH_2), 3.90 (s, 3H, OCH_3), 3.78 (d, 3H, J = 11.0 Hz, $POCH_3$), 3.55-3.47 (m, 2H, NCH_2), 2.35 (s, 3H, AcS), 1.87-1.73 (m, 4H, CH_2CH_2), 1.30 and 1.29 (2 × s, 6H, 2 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 205.7 (CO), 194.5 (SCO), 168.0 (*C*OOMe), 167.0 (NCO), 150.5, 139.6, 131.7, 115.7, 113.8 and 112.5 (Ar), 72.9 and 72.8 ($POCH_2$), 67.8 and 67.7 ($POCH_2$), 54.5 and 54.4 ($POCH_3$), 51.7 (OCH_3), 48.8 and 48.7 (quaternary C), 39.5 (NCH_2), 36.5 (CH_2S), 30.2 (CH_3CO), 27.7, 27.6 and 25.5 (CH_2CH_2), 21.5 and 21.5 (CH_3); ^{31}P NMR (202 MHz, $CDCl_3$): δ = -0.35 ppm; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{22}H_{33}N_2O_9PSNa^+$ 555.1537; found 555.1520.

Copies of the ^{13}C spectra of compounds 1-25.



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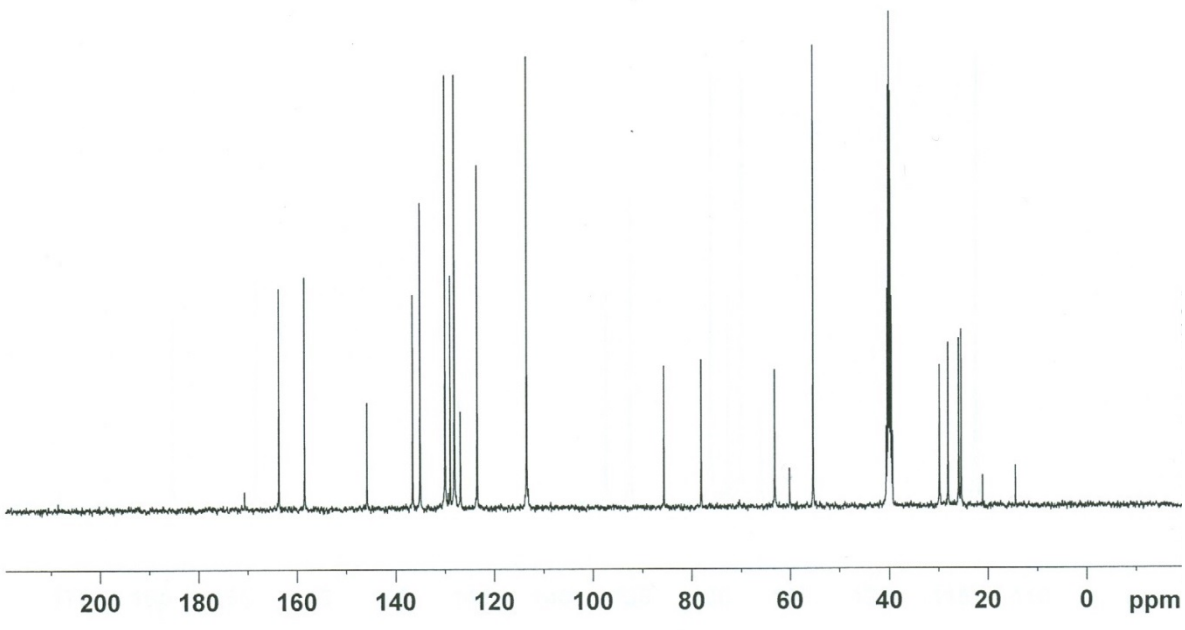


Figure S6. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **1**.

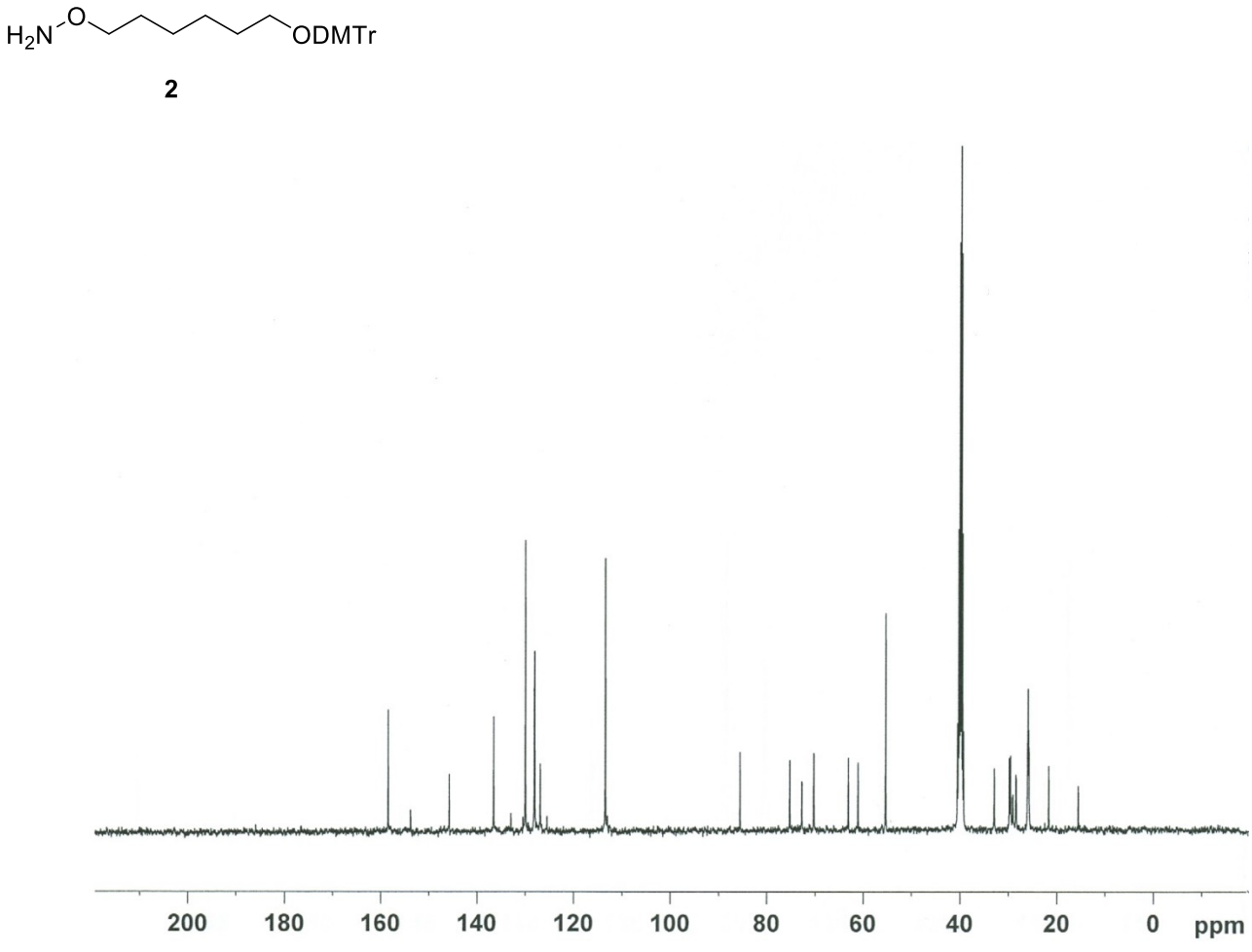


Figure S7. 126 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **2**.

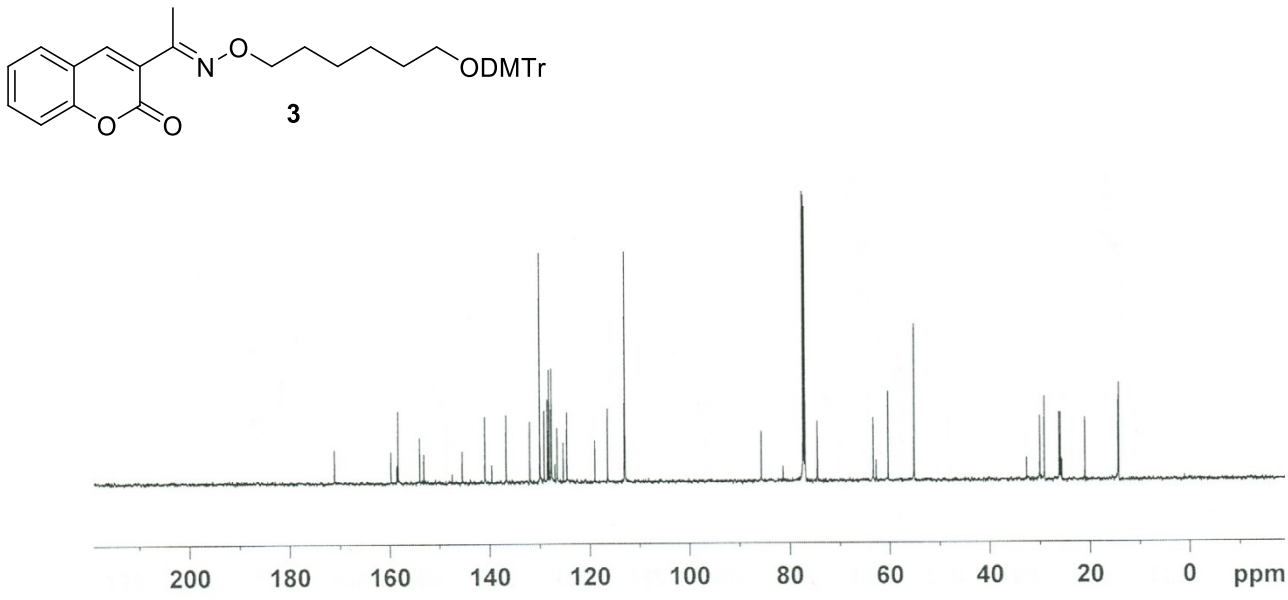


Figure S8. 151 MHz ($CDCl_3$) ^1H NMR spectra of compound **3**.

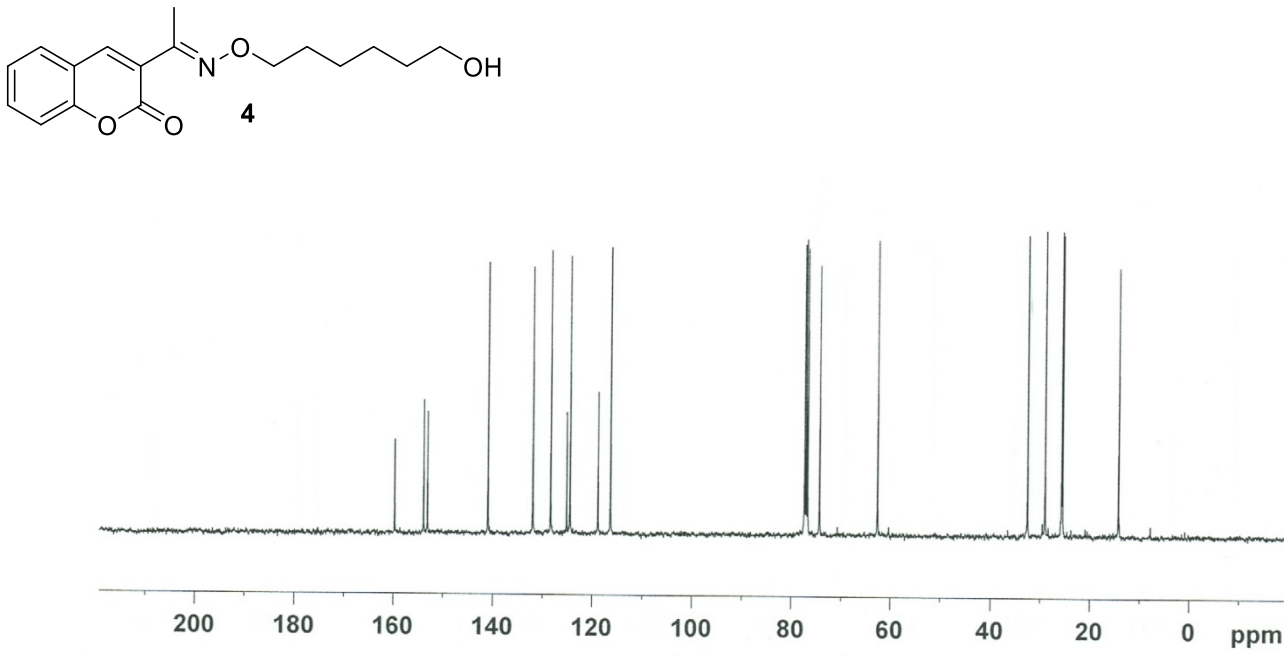
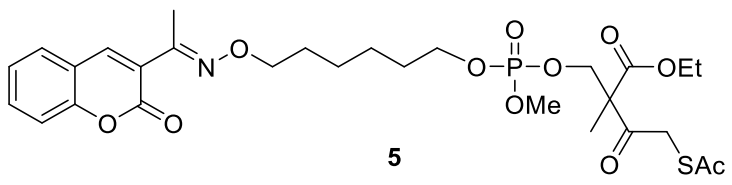


Figure S9. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **4**.



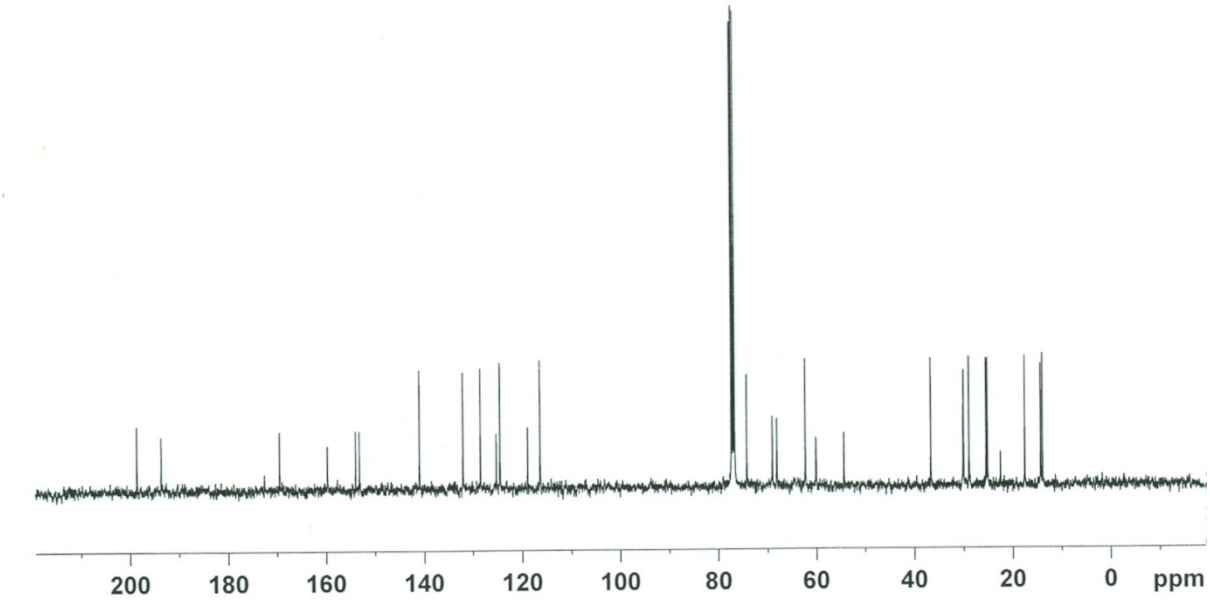
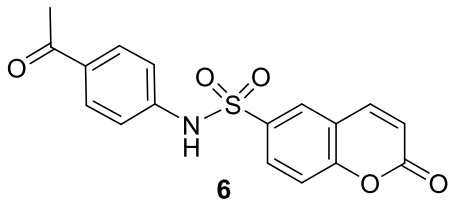


Figure S10. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **5**.



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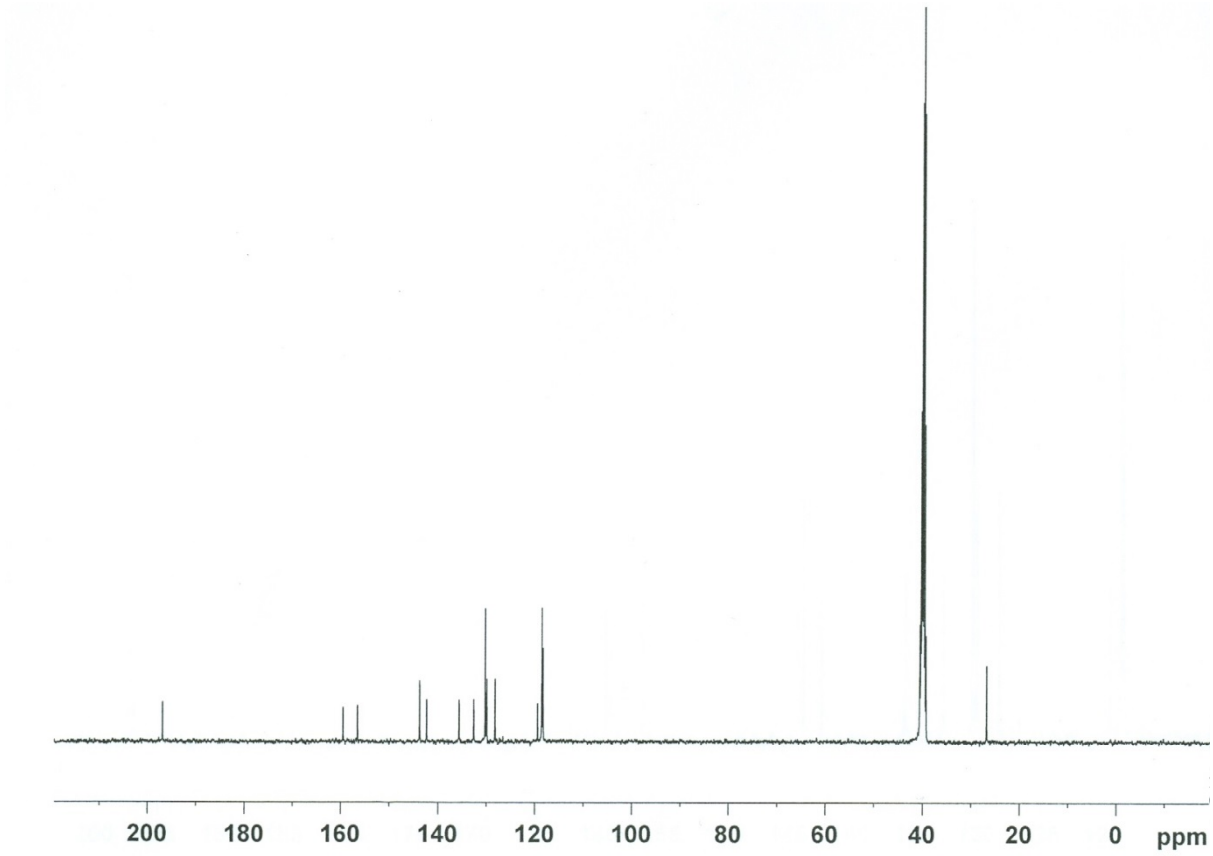
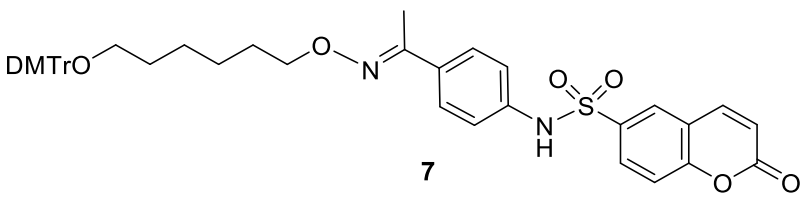


Figure S11. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **6**.



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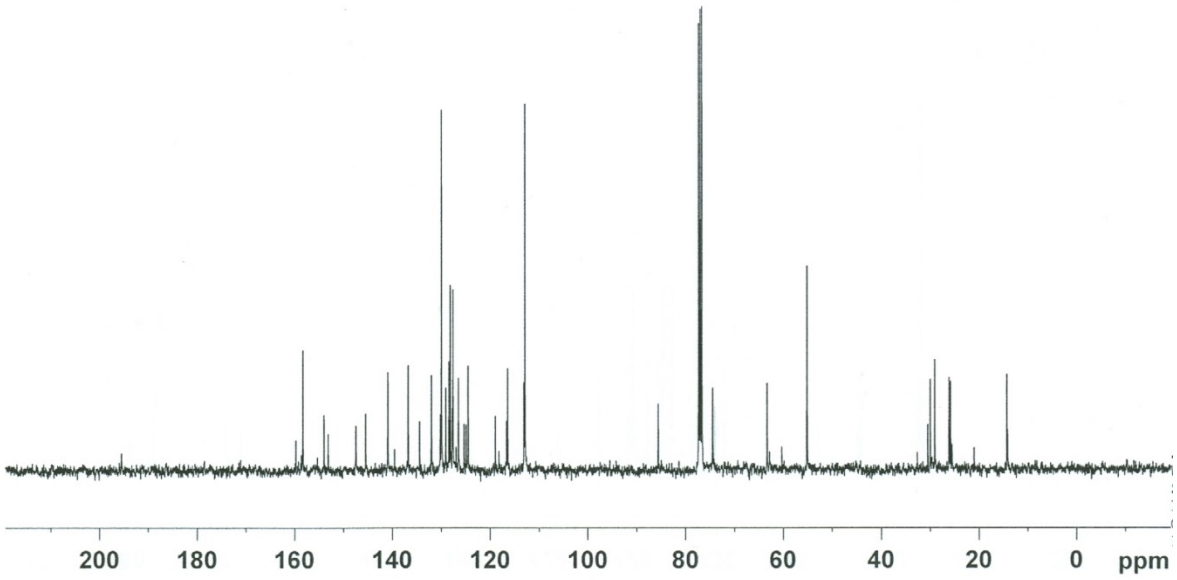
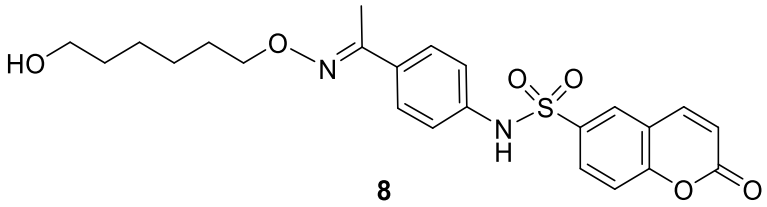


Figure S12. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **7**.



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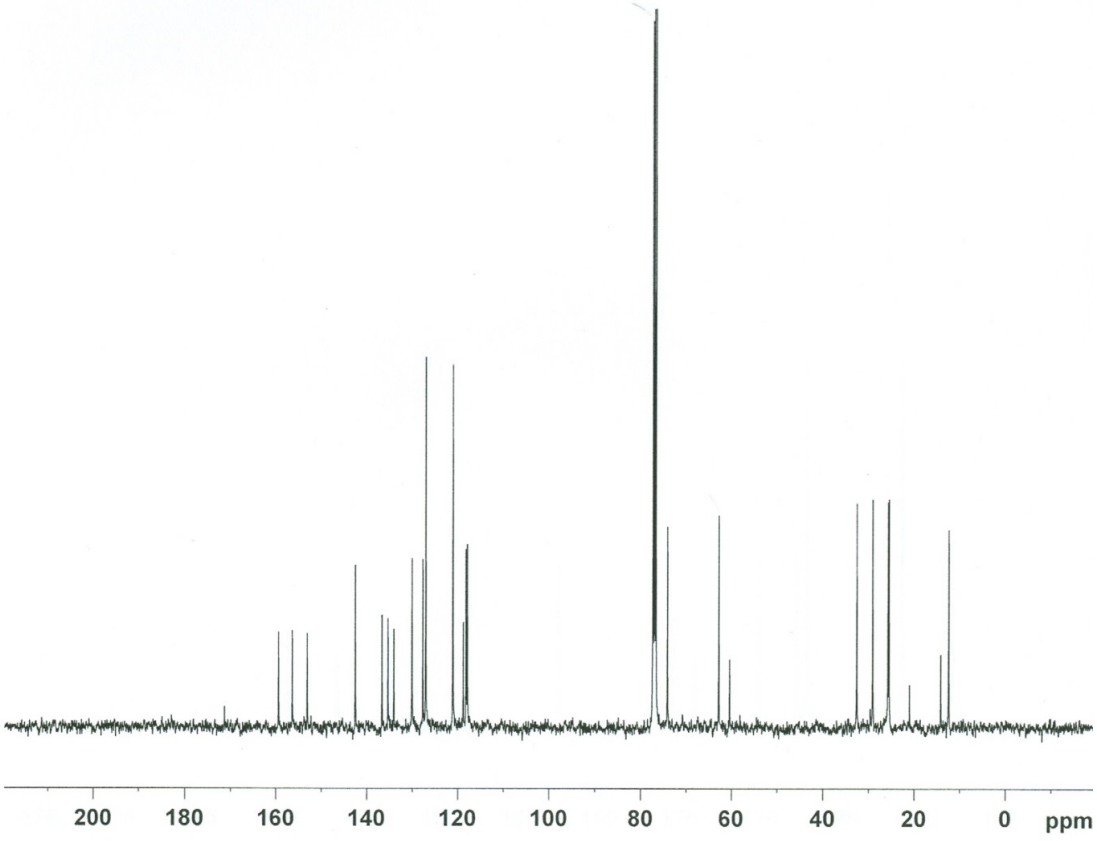
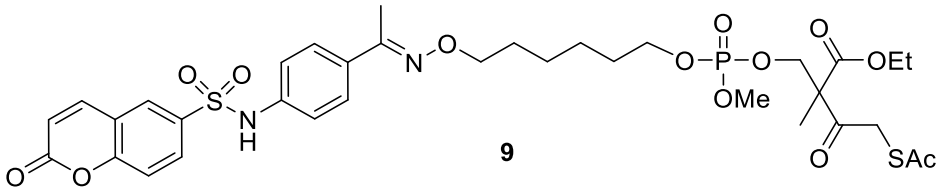


Figure S13. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **8**.



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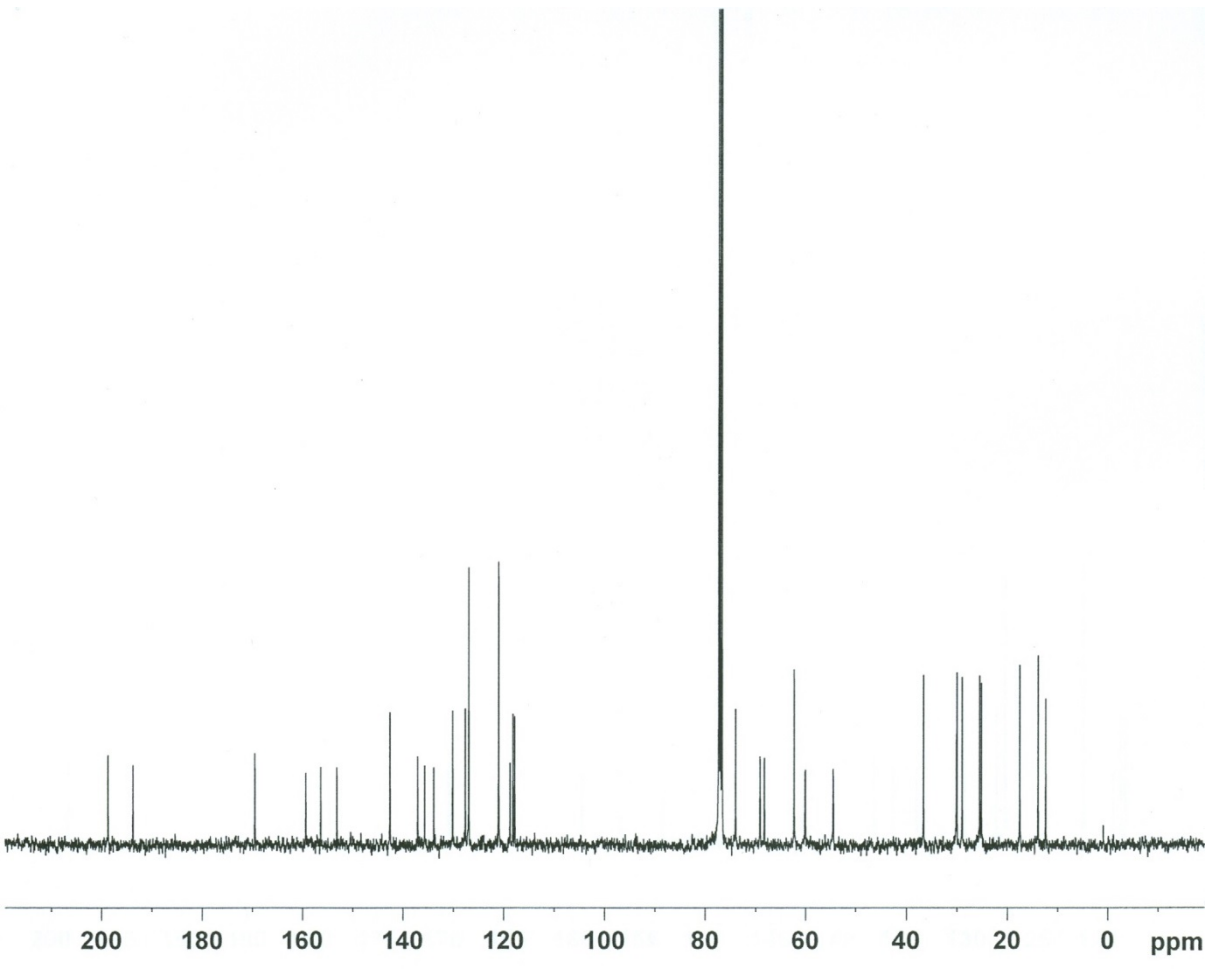
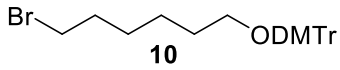


Figure S14. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **9**.



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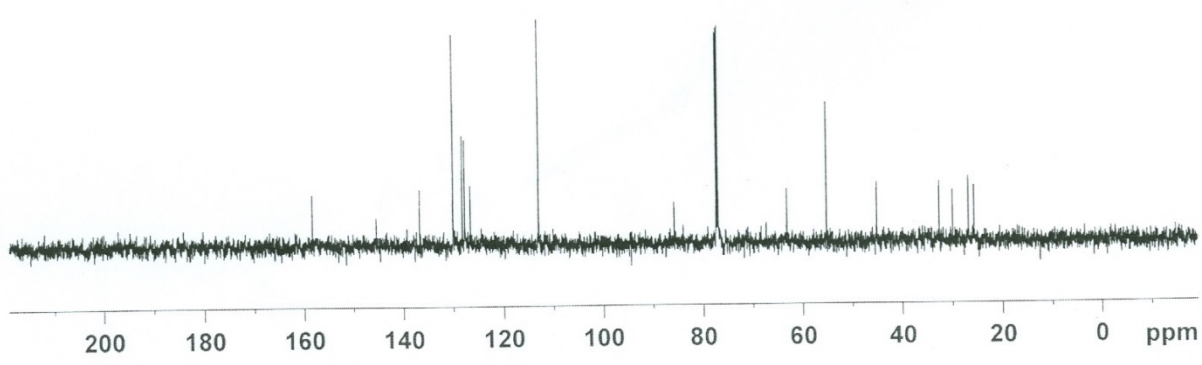
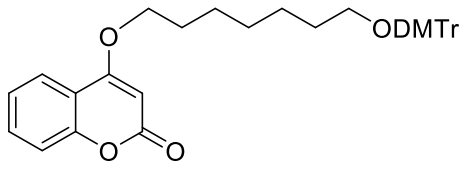


Figure S15. 151 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **10**.



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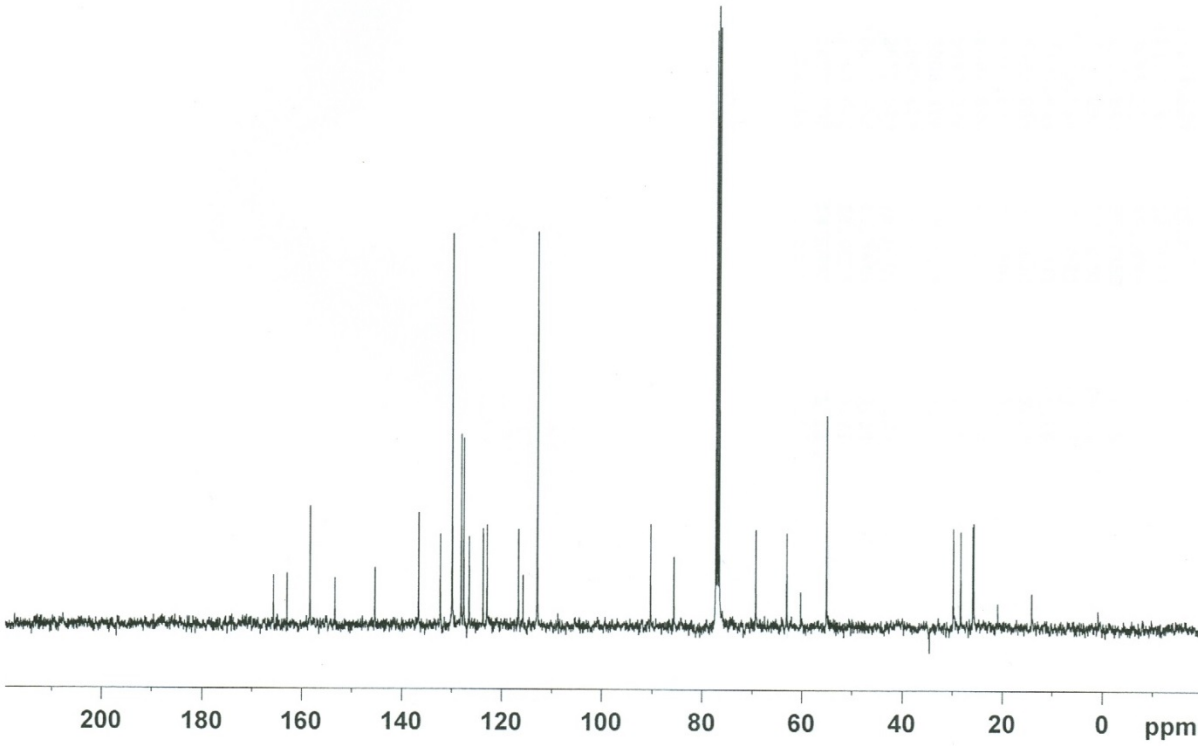
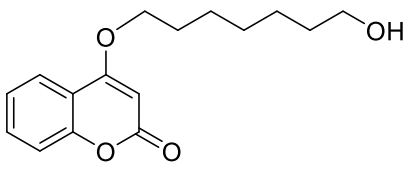


Figure S16. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **11**.



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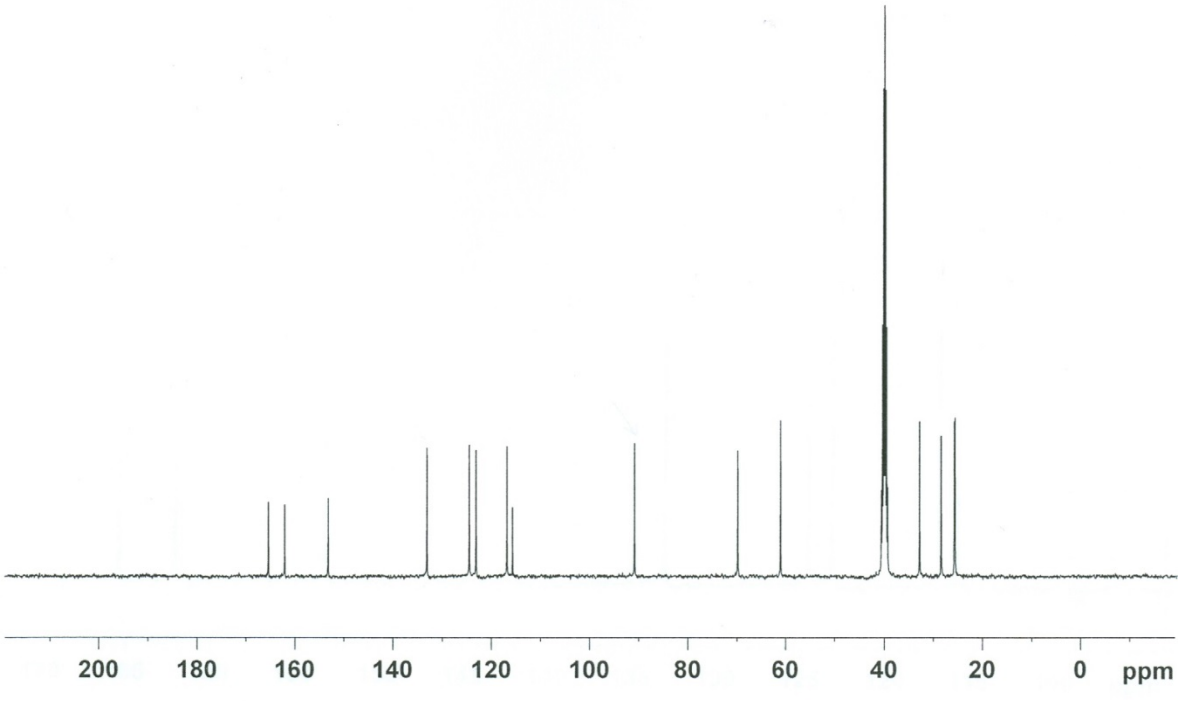
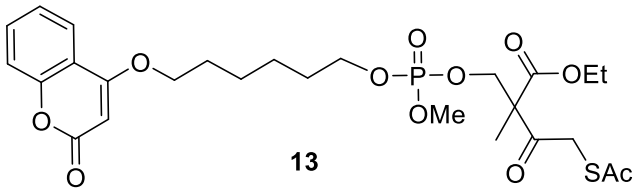


Figure S17. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **12**.



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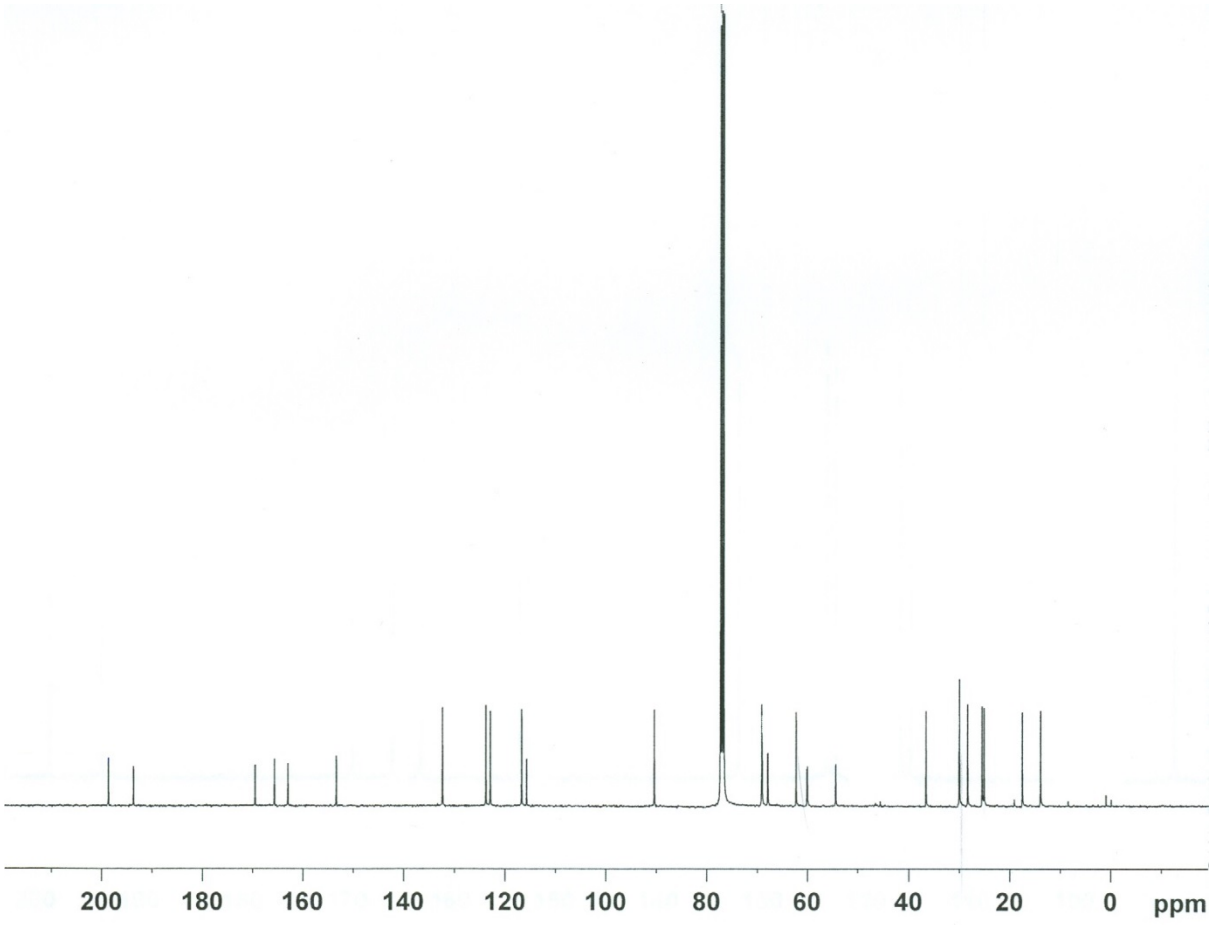
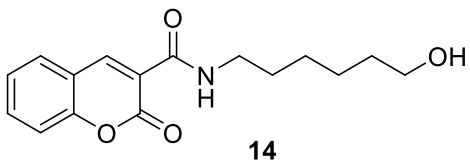


Figure S18. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **13**.



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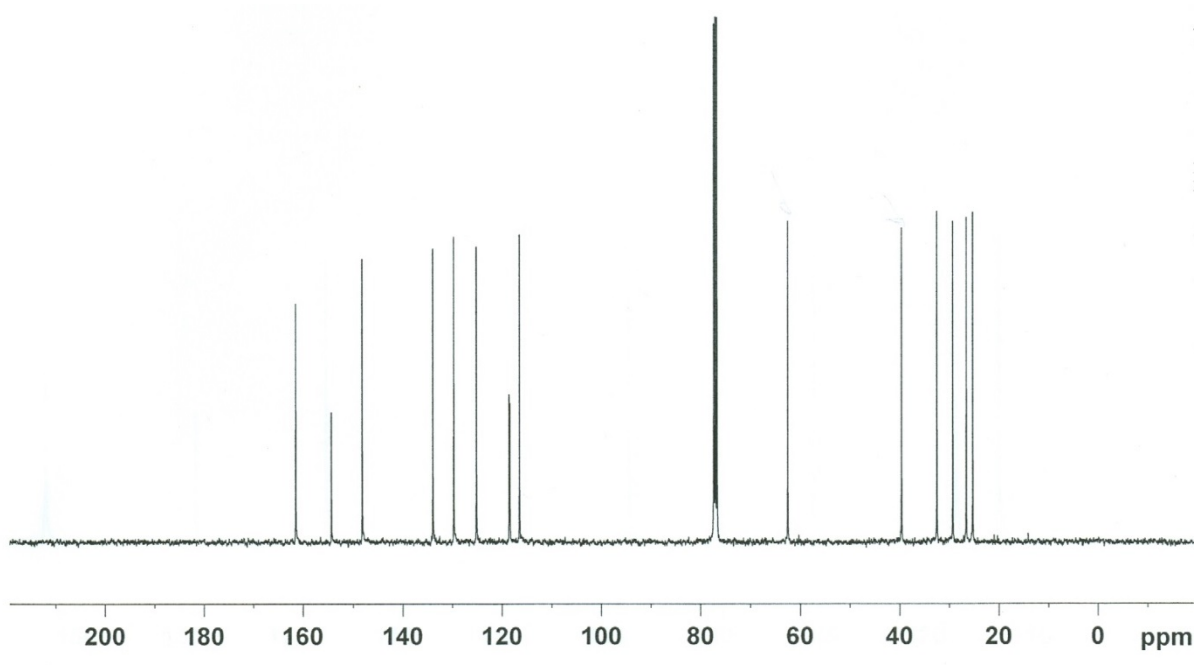
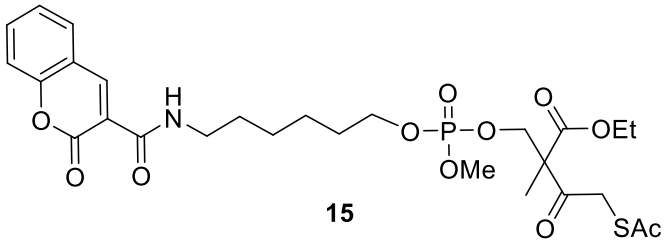


Figure S19. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **14**.



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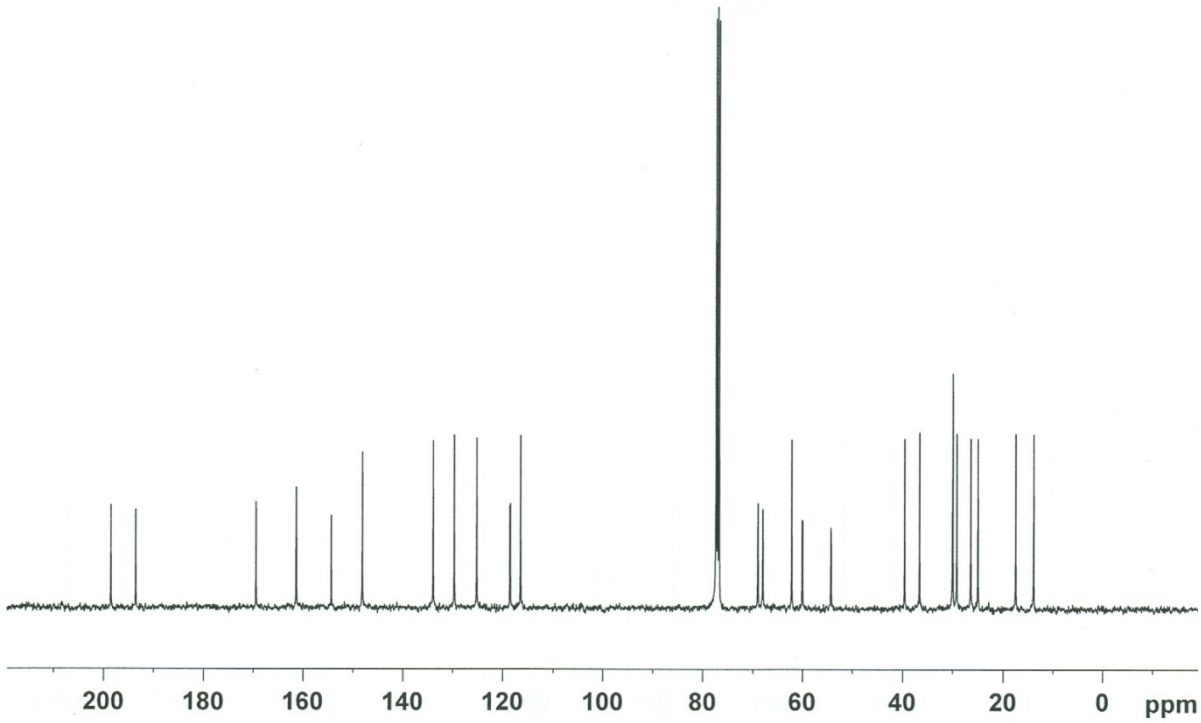
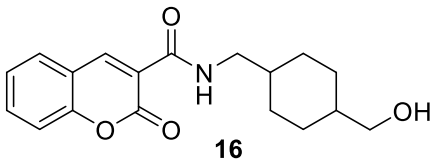


Figure S20. 101 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **15**.



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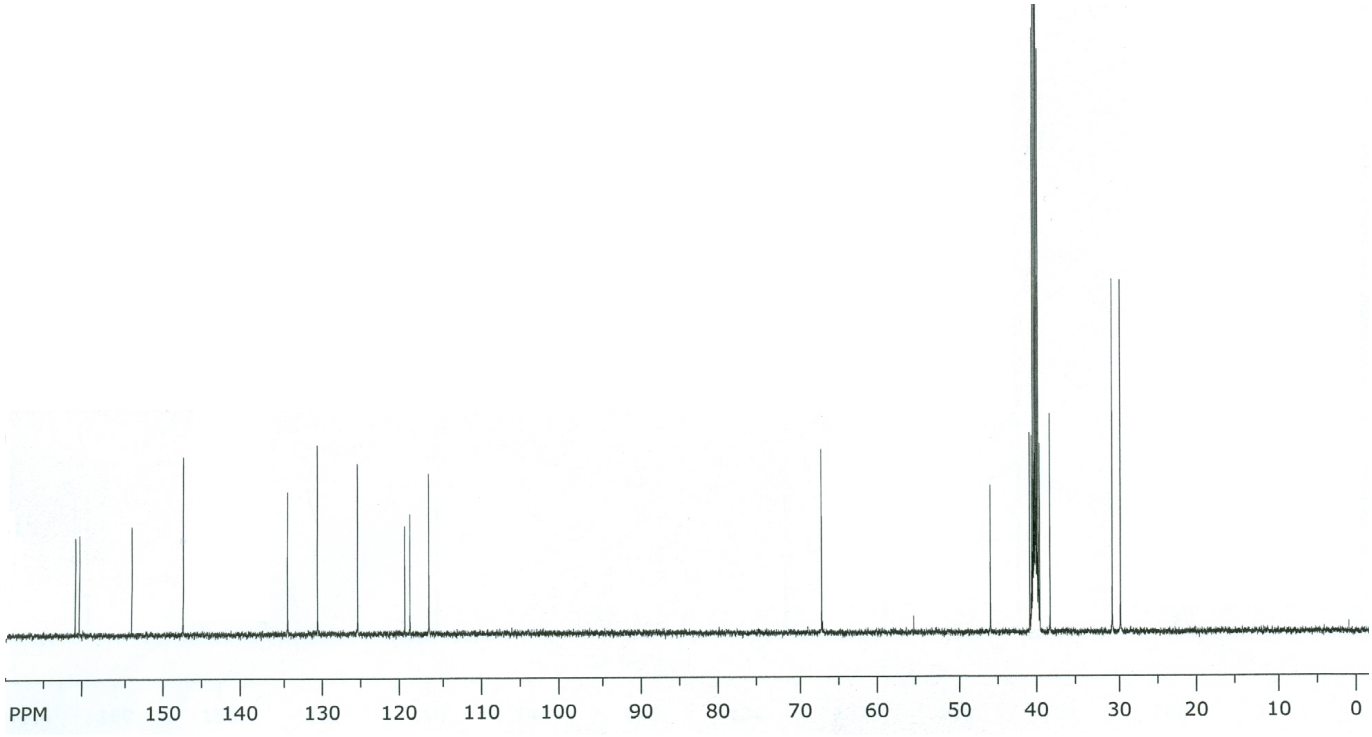
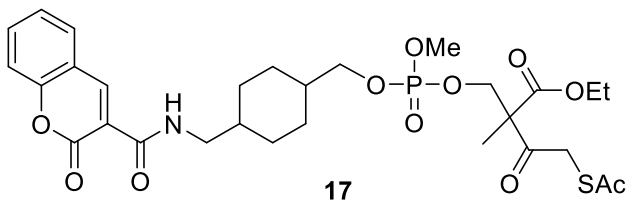


Figure S21. 126 MHz ($(CD_3)_2SO$) ^{13}C NMR spectra of compound **16**.



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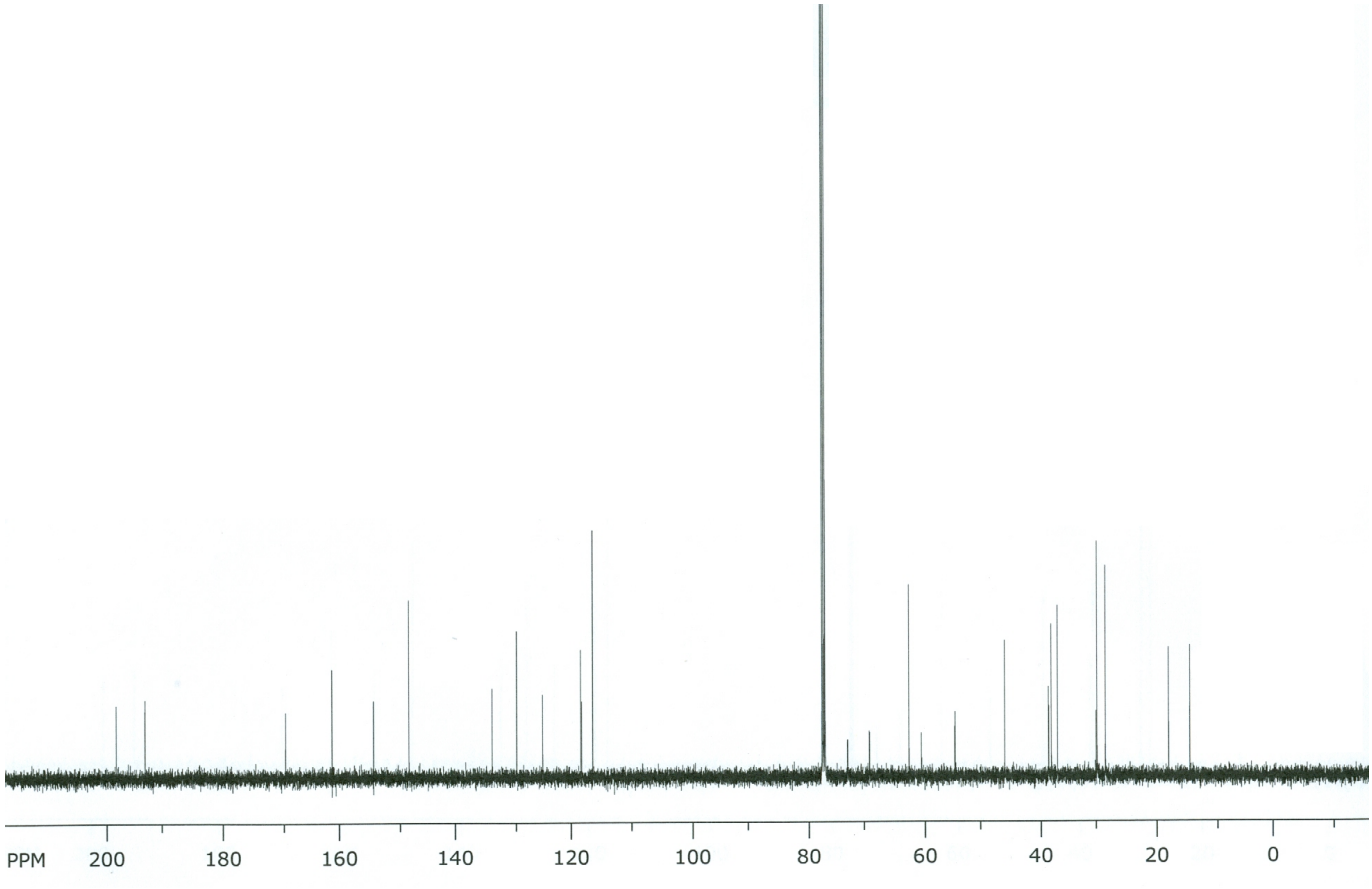
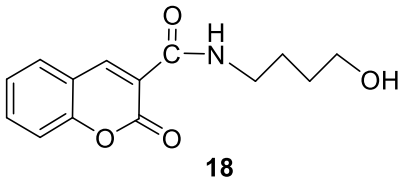


Figure S22. 126 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **17**.



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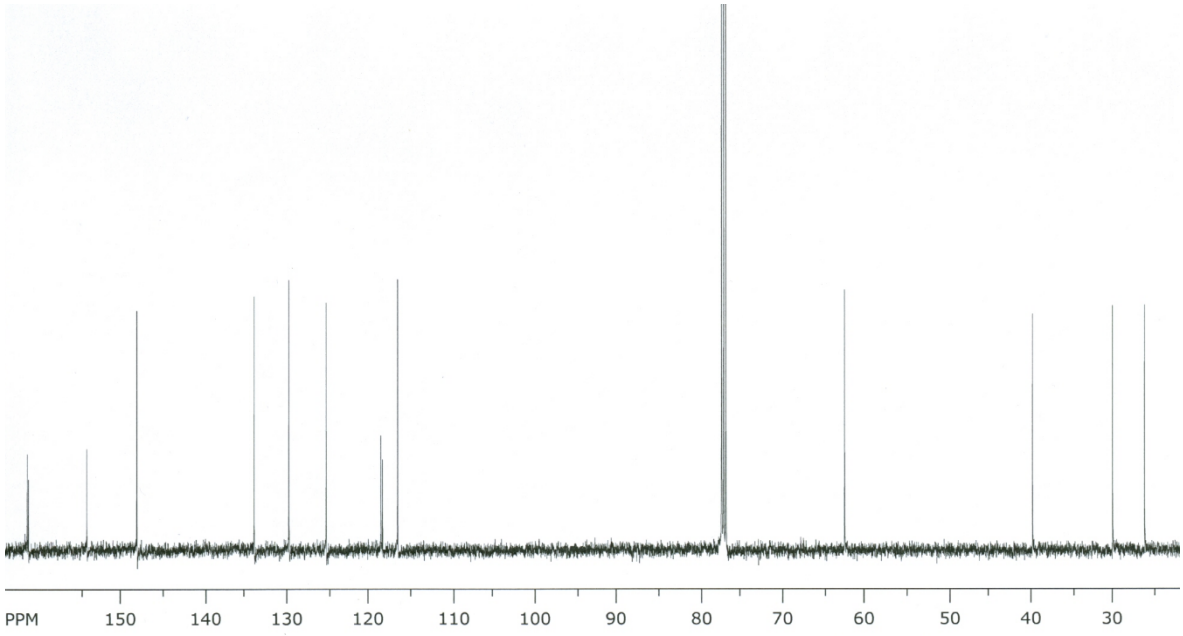
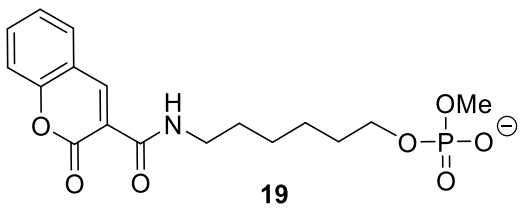


Figure S23. 126 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **18**.



19

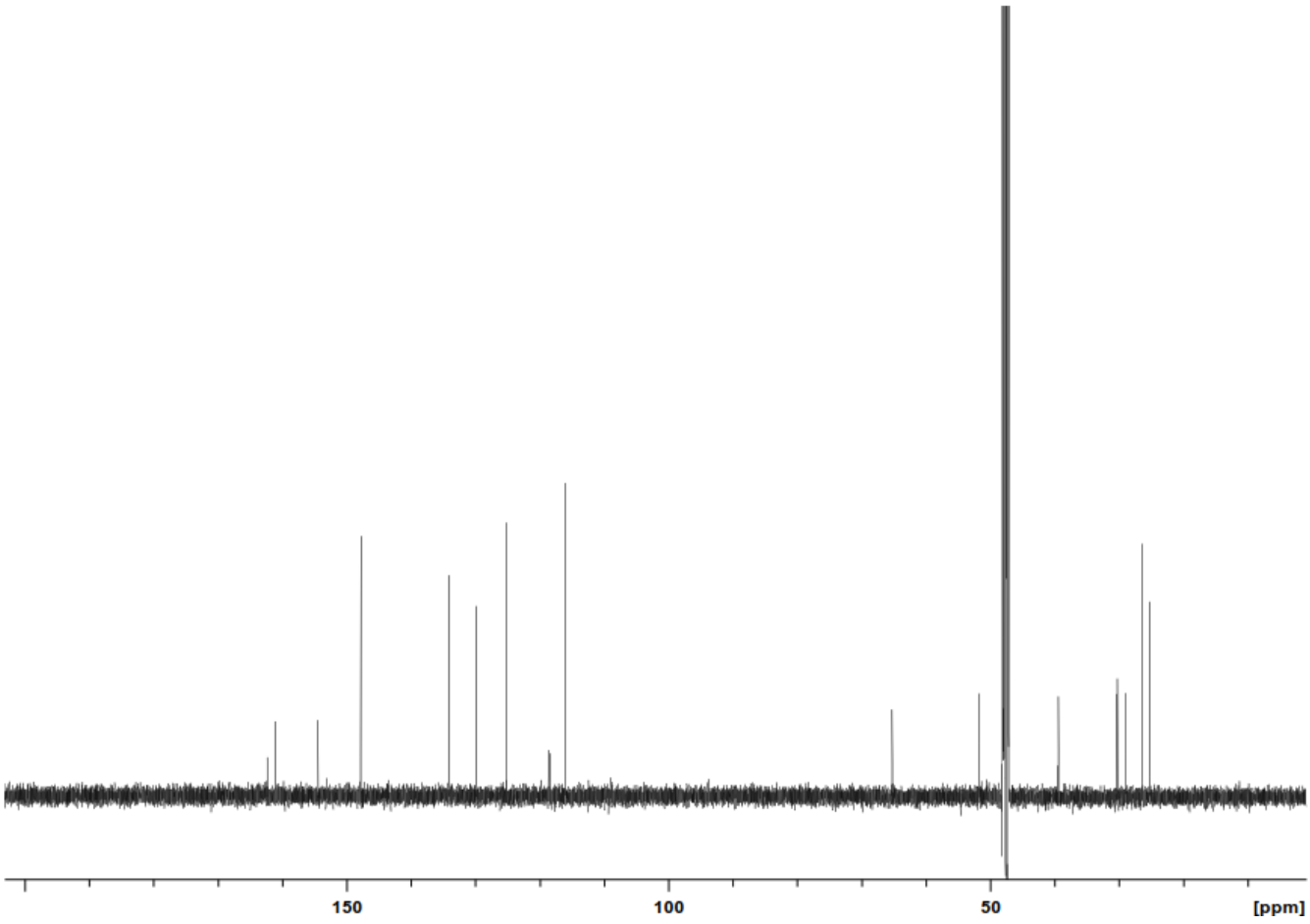


Figure S24. 125 MHz (D_2O) ^{13}C NMR spectra of compound **19**.

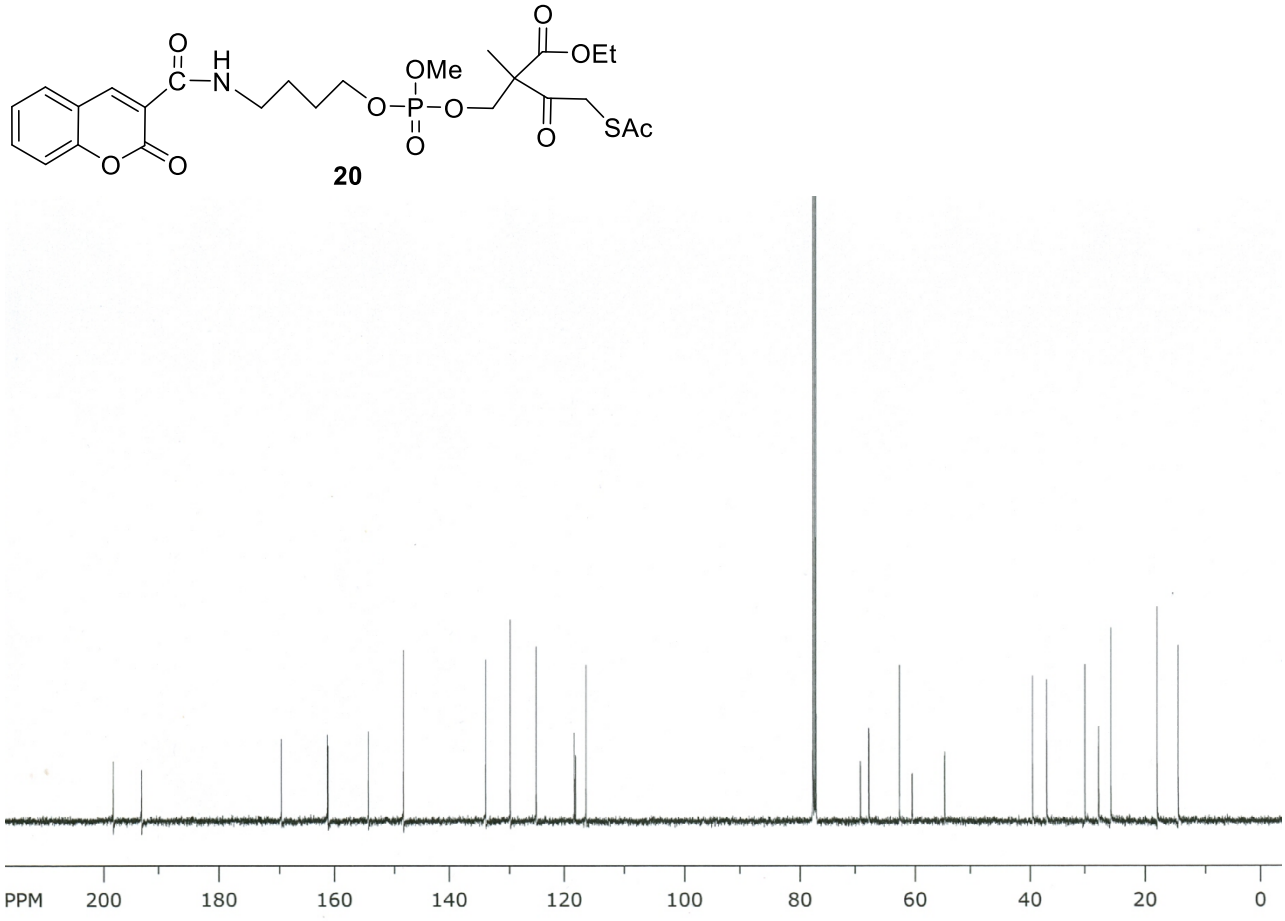


Figure S25. 126 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **20**.

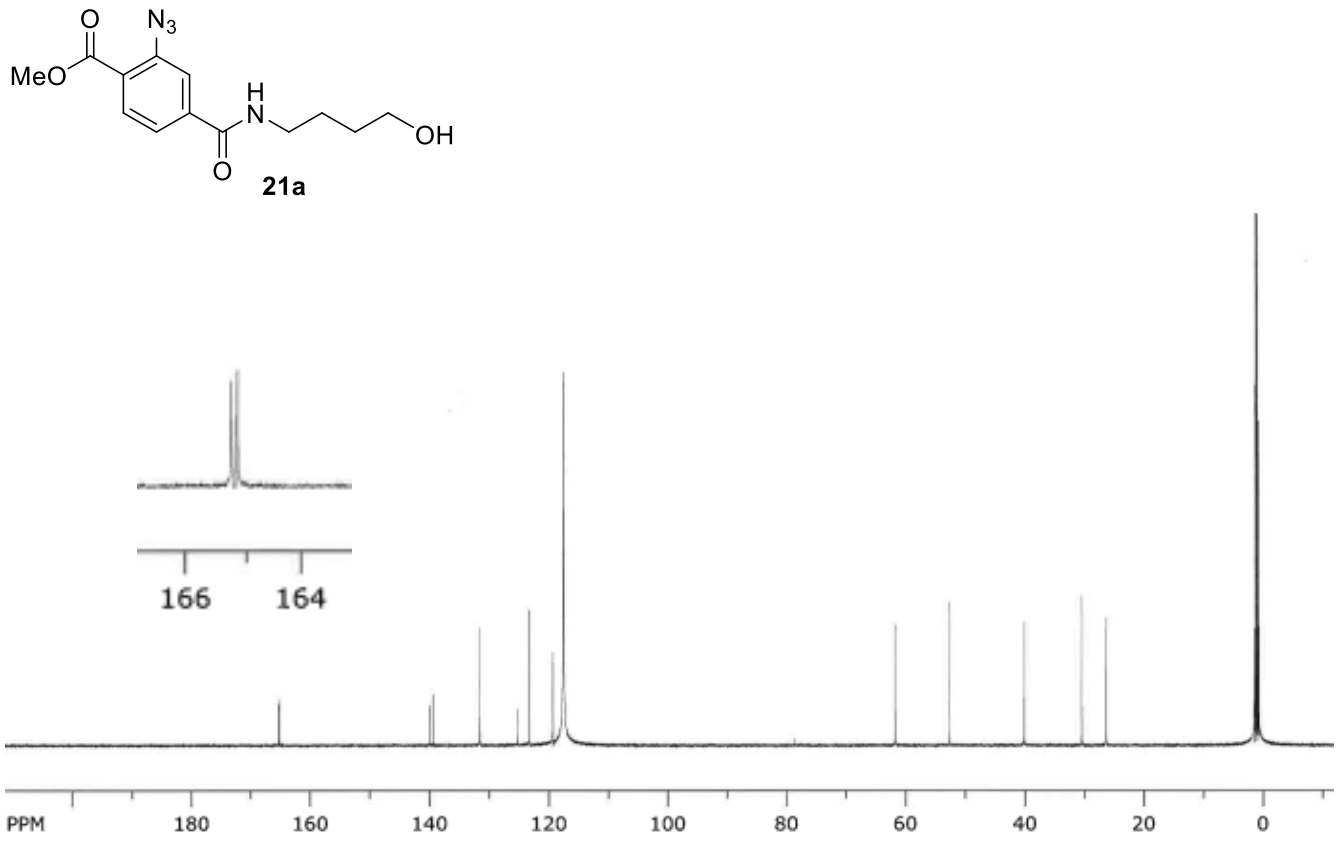
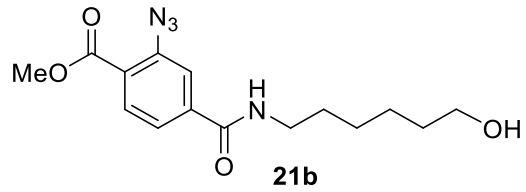


Figure S26. 151 MHz (CD_3CN) ^{13}C NMR spectra of compound **21a**.



21b

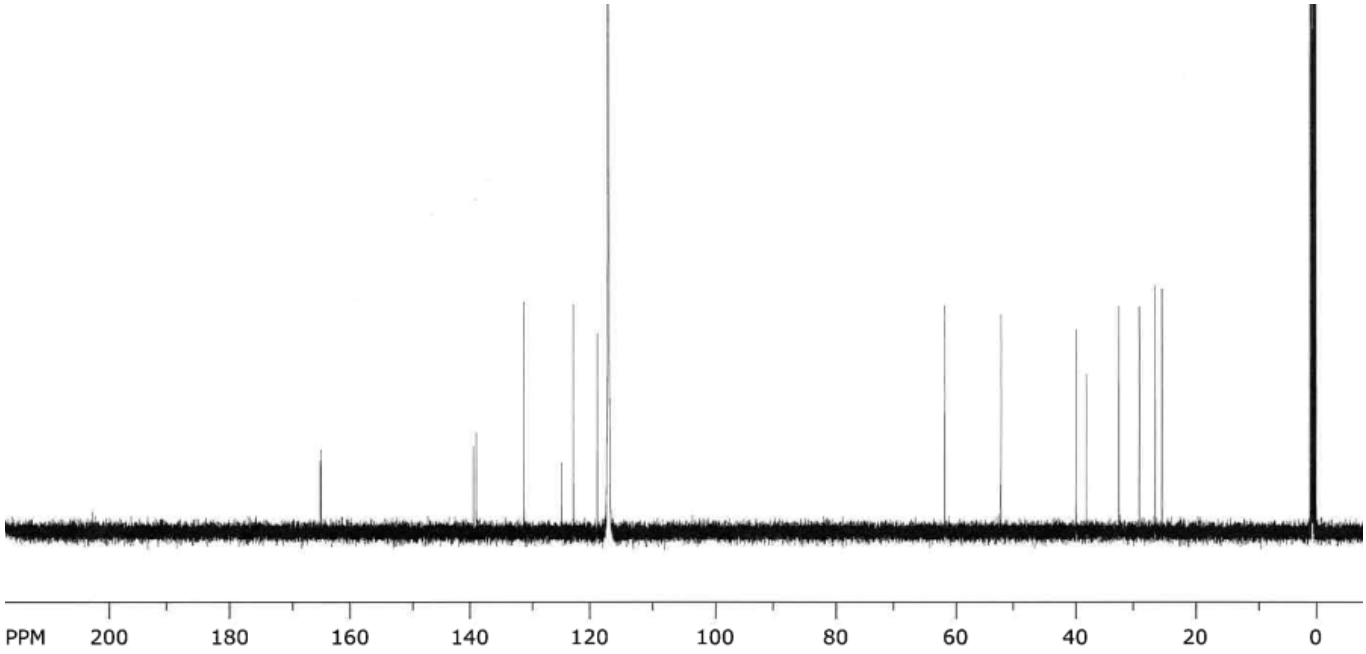
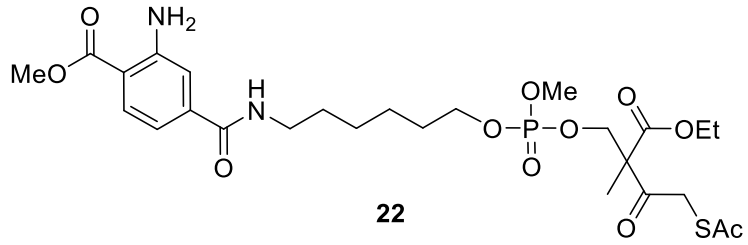


Figure S27. 126 MHz (CD_3CN) ^{13}C NMR spectra of compound **21b**.



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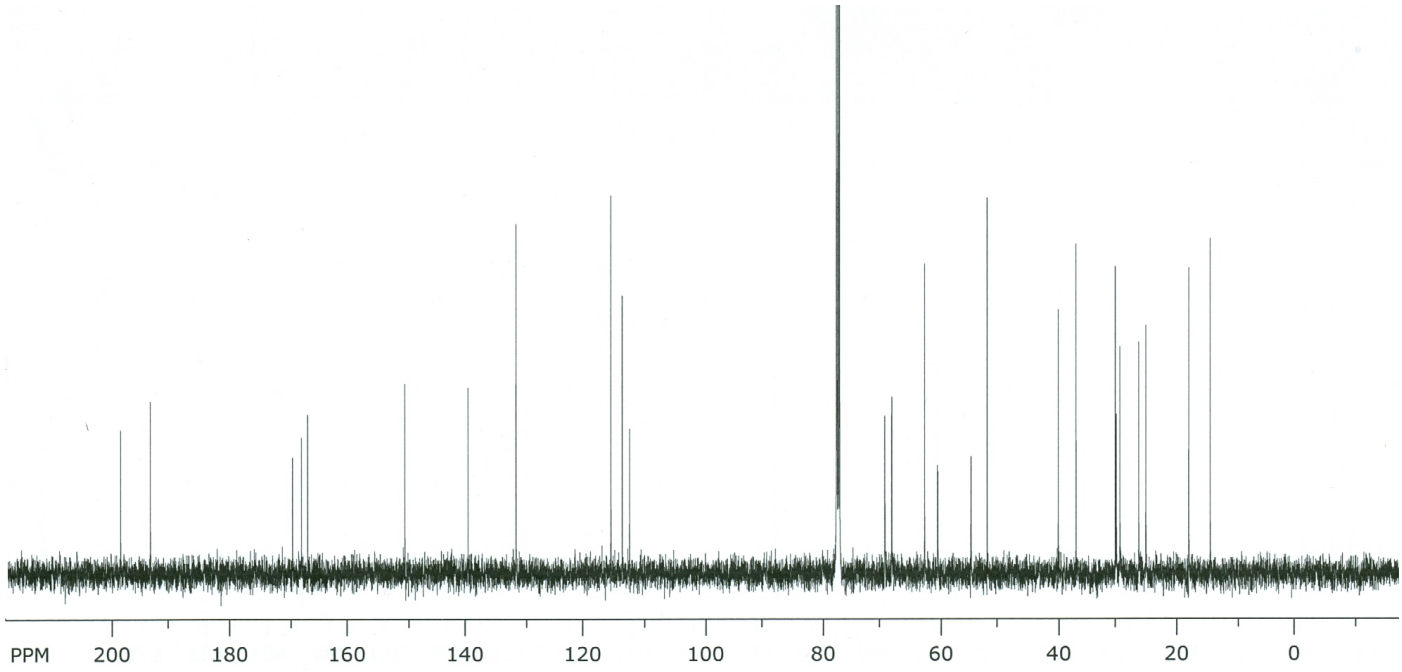


Figure S28. 126 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **22**.

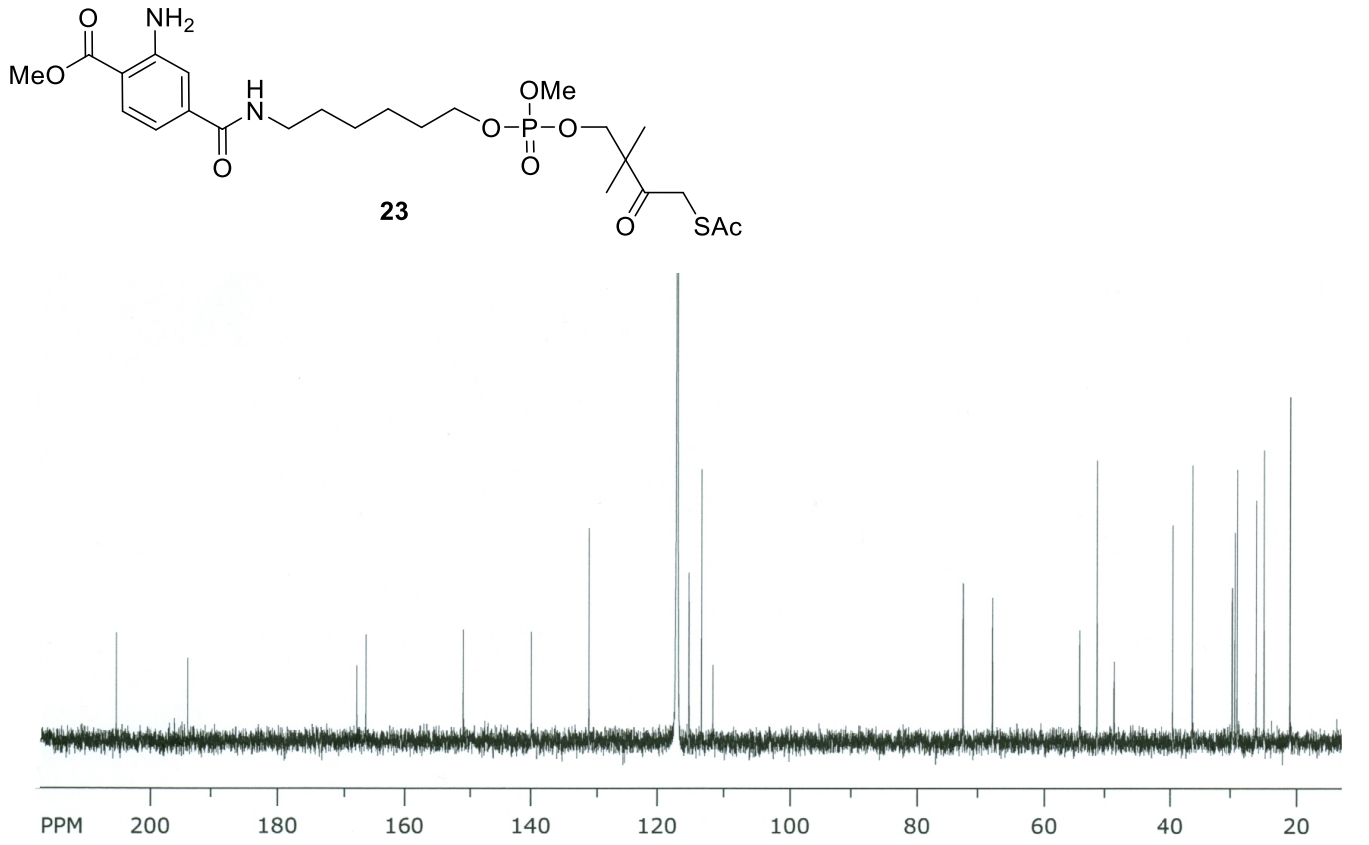


Figure S29. 126 MHz (CD_3CN) ^{13}C NMR spectra of compound **23**.

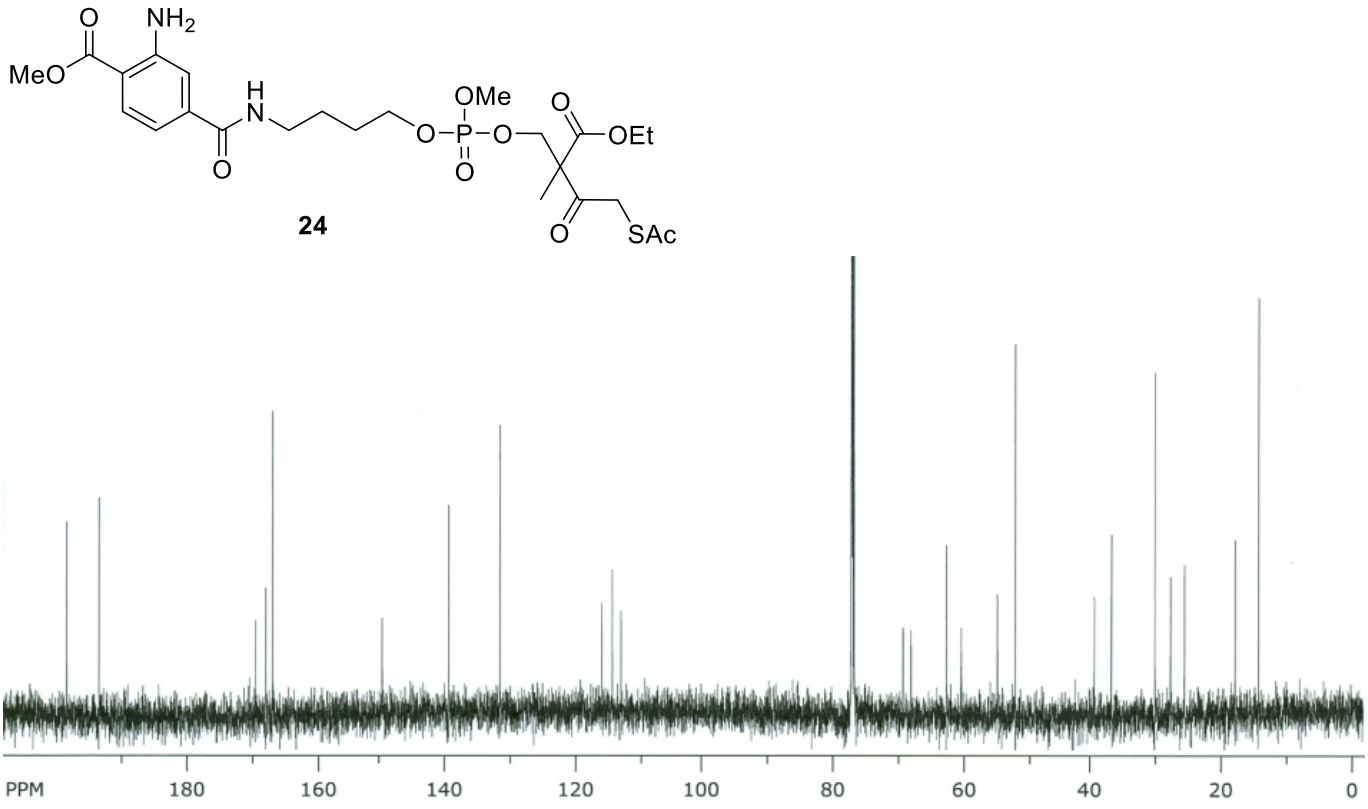


Figure S30. 126 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **24**.

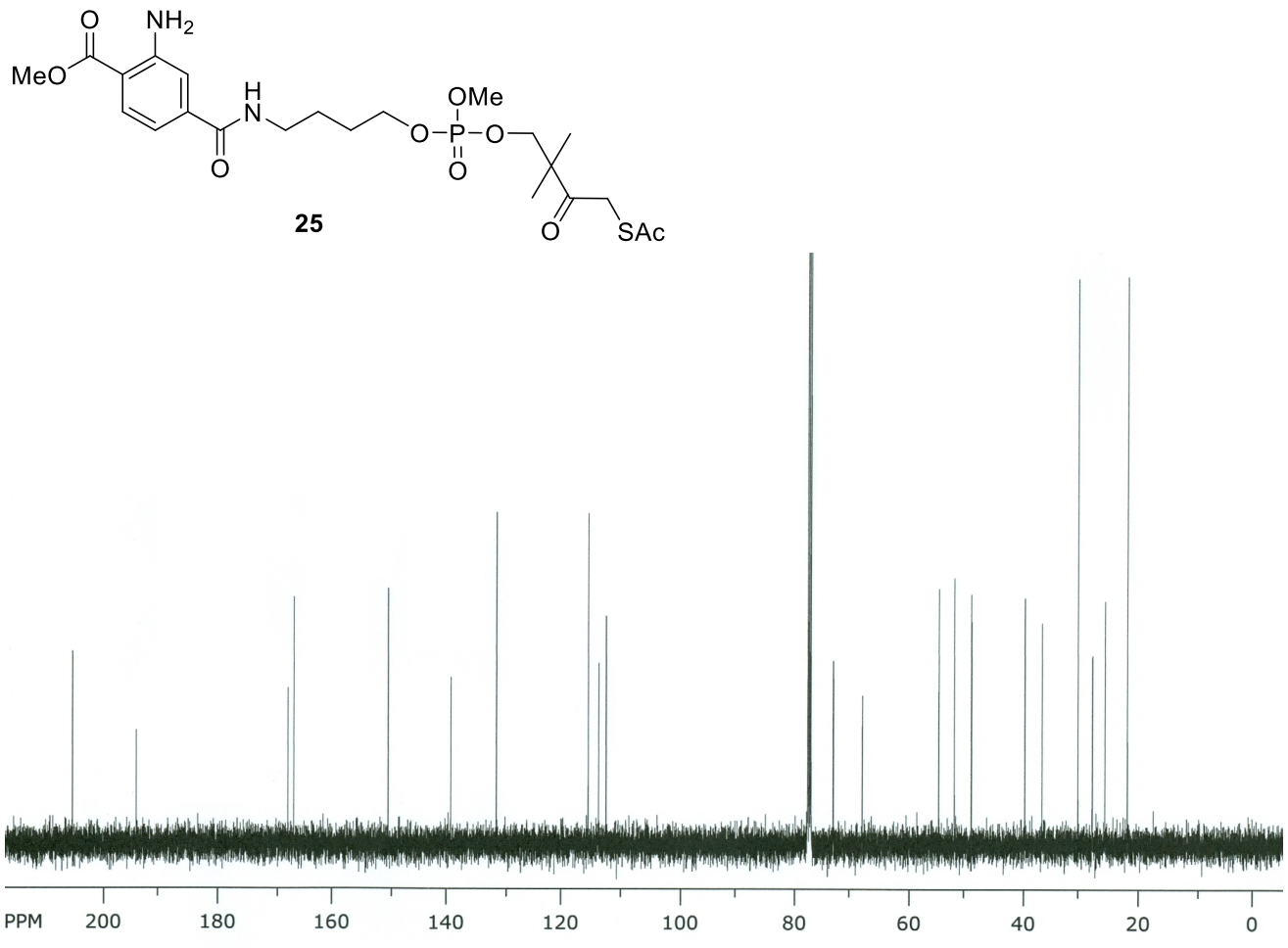
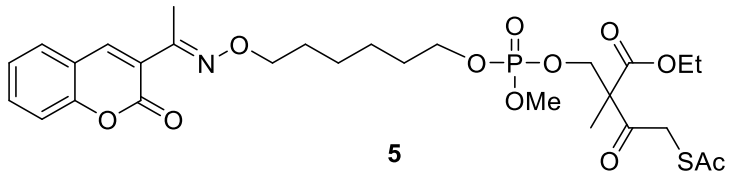


Figure S31. 126 MHz ($CDCl_3$) ^{13}C NMR spectra of compound **25**.

Copies of the ^{31}P NMR spectra of compounds 5, 9, 13, 15, 17, 19, 20 and 22-25.



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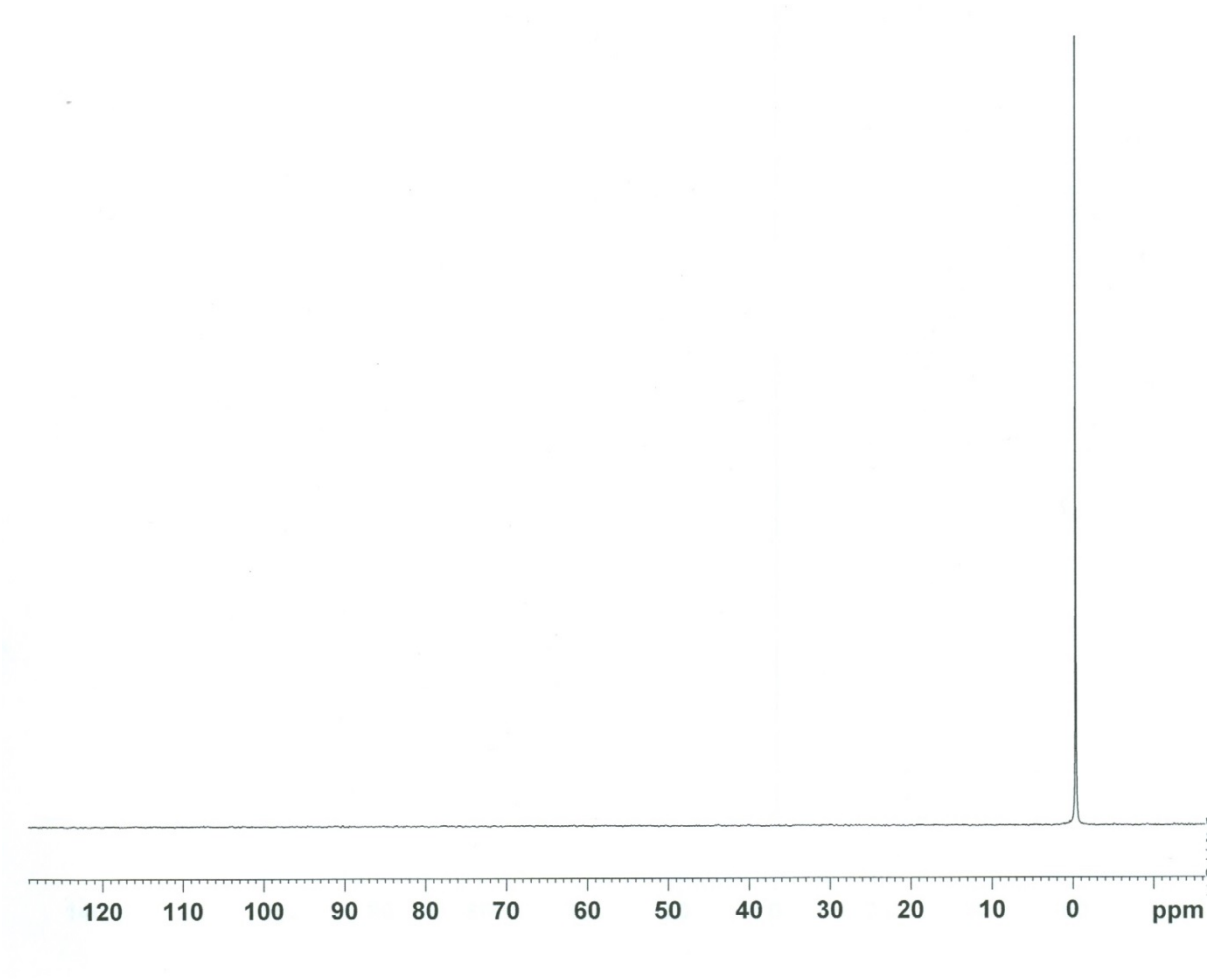


Figure S32. 162 MHz ($CDCl_3$) ^{31}P NMR spectra of compound **5**.

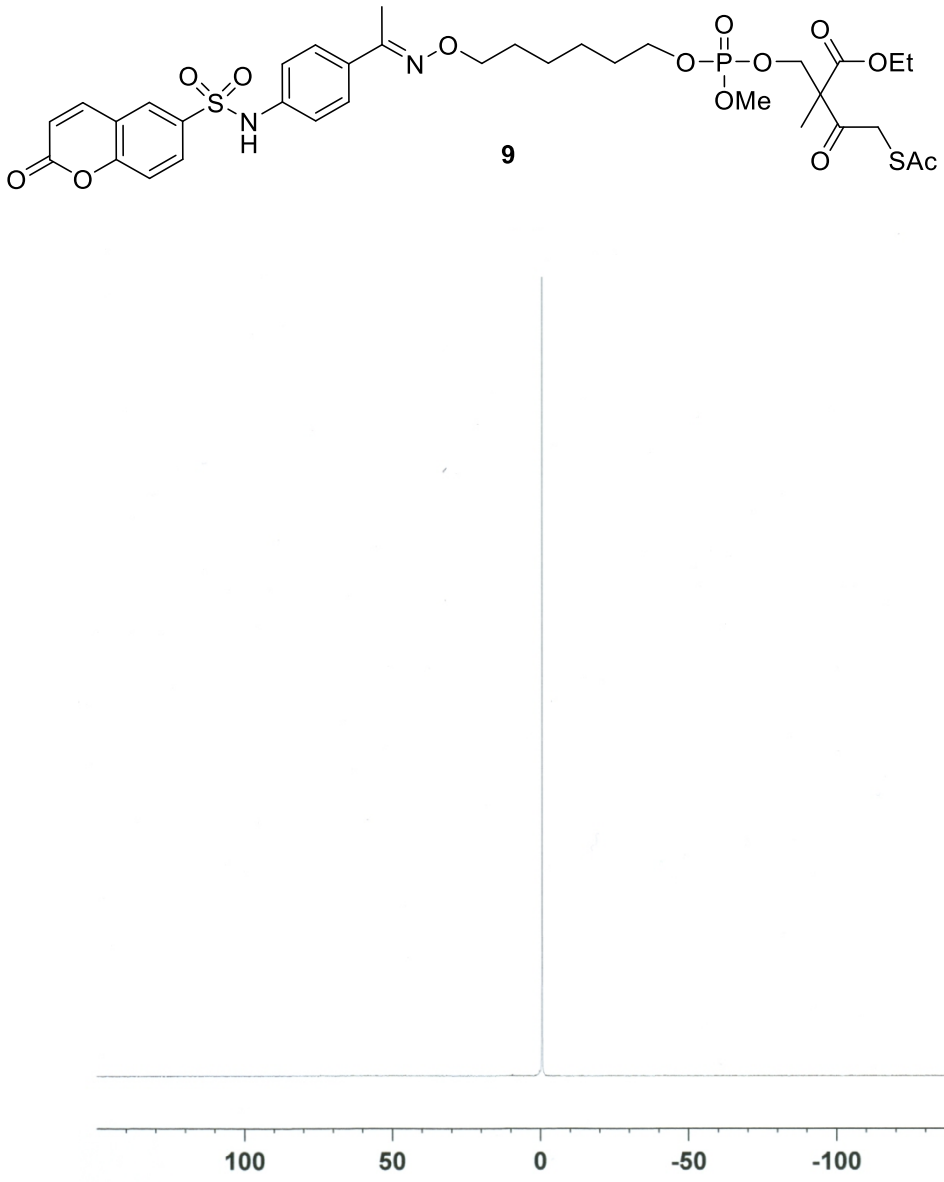


Figure S33. 162 MHz ($CDCl_3$) ^{31}P NMR spectra of compound **9**.

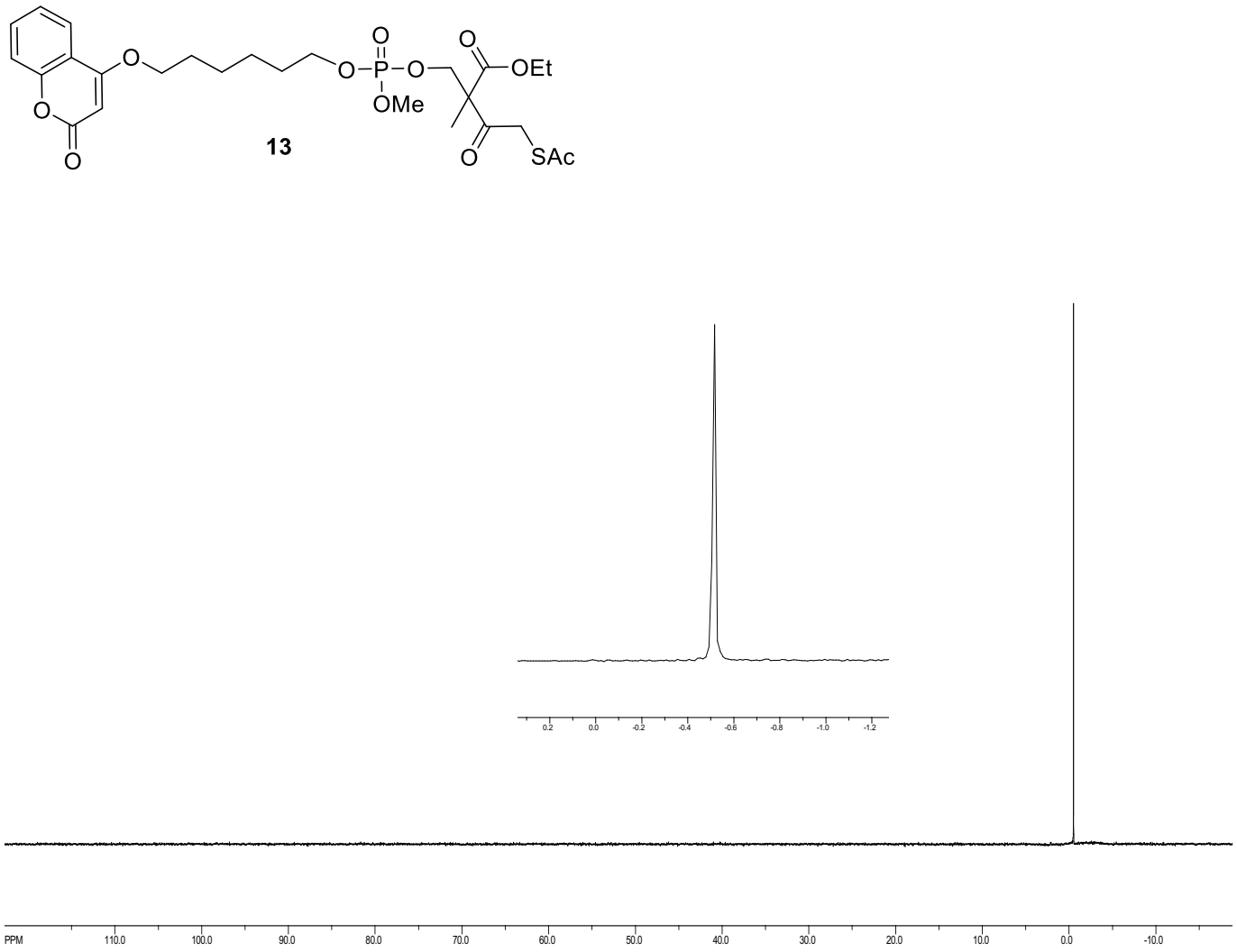
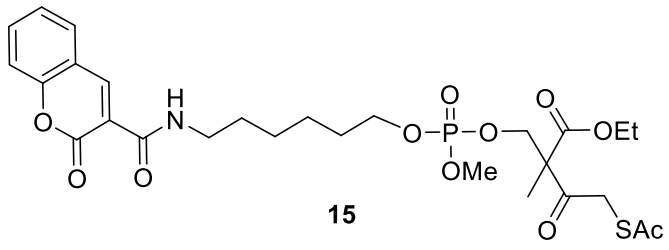


Figure S34. 202 MHz (CD_3CN) ^{31}P NMR spectra of compound **13**.



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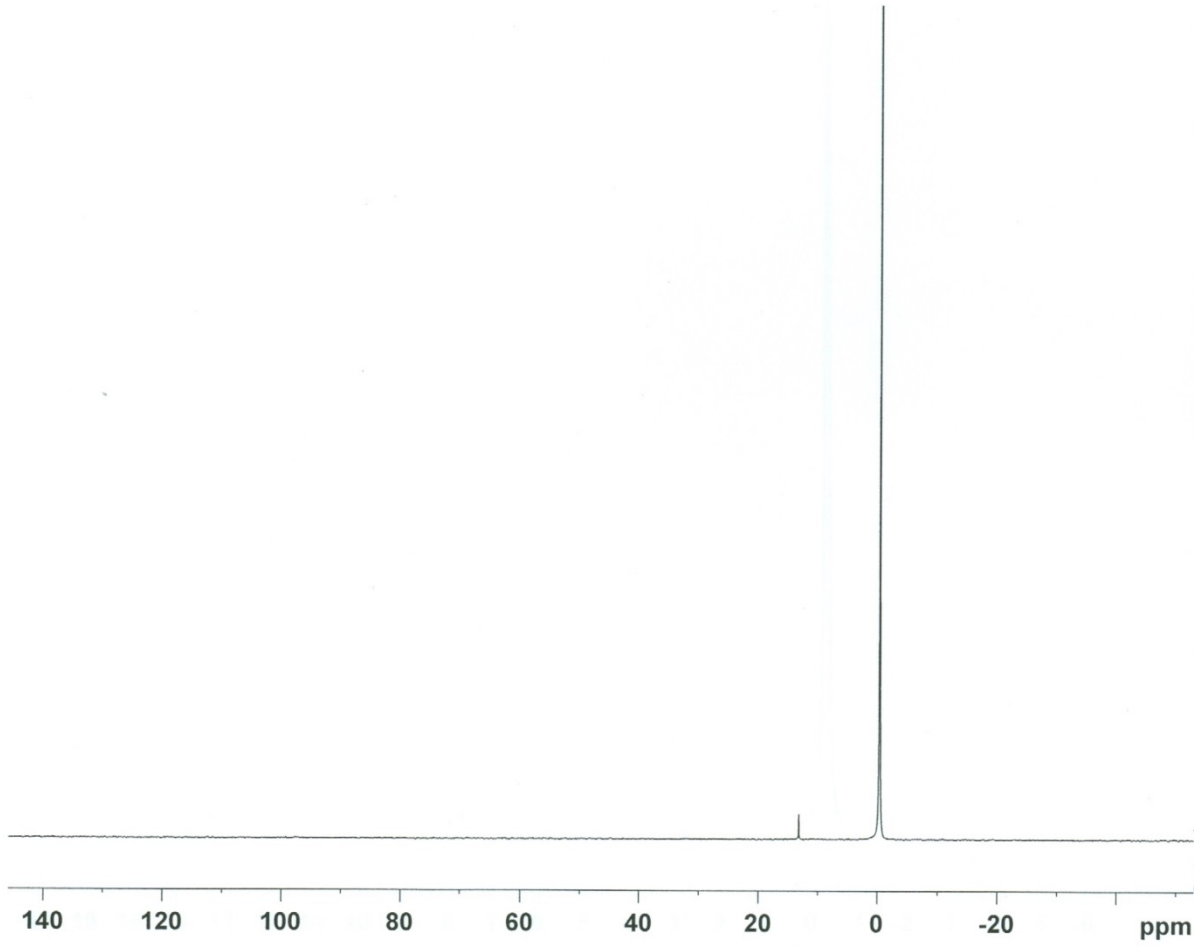
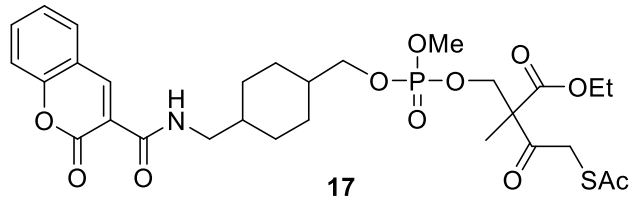


Figure S35. 162 MHz ($CDCl_3$) ^{31}P NMR spectra of compound 1**5**.



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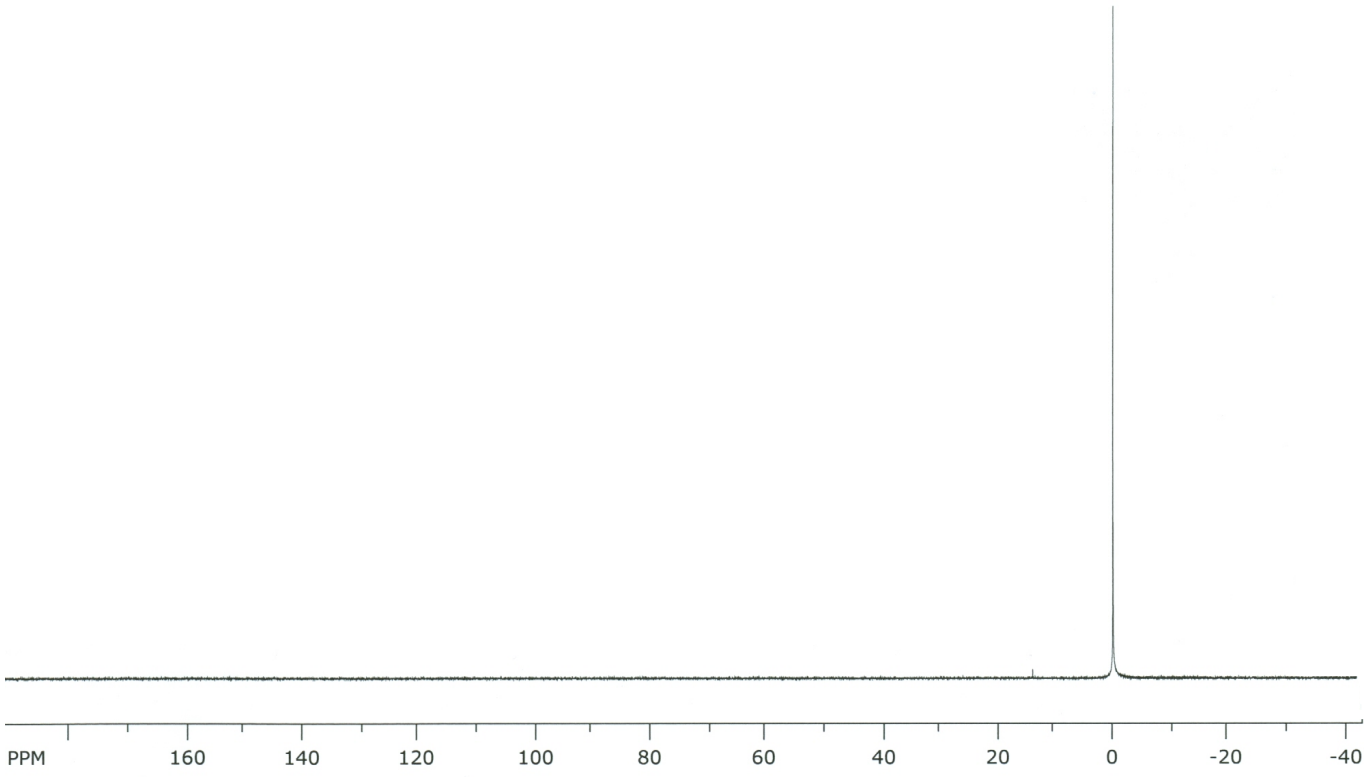
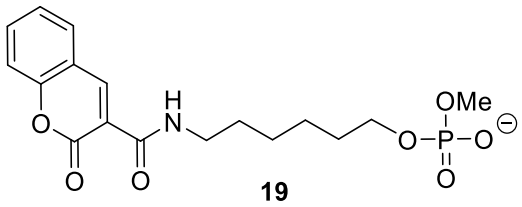


Figure S36. 202 MHz (CD_3CN) ^{31}P NMR spectra of compound **17**.



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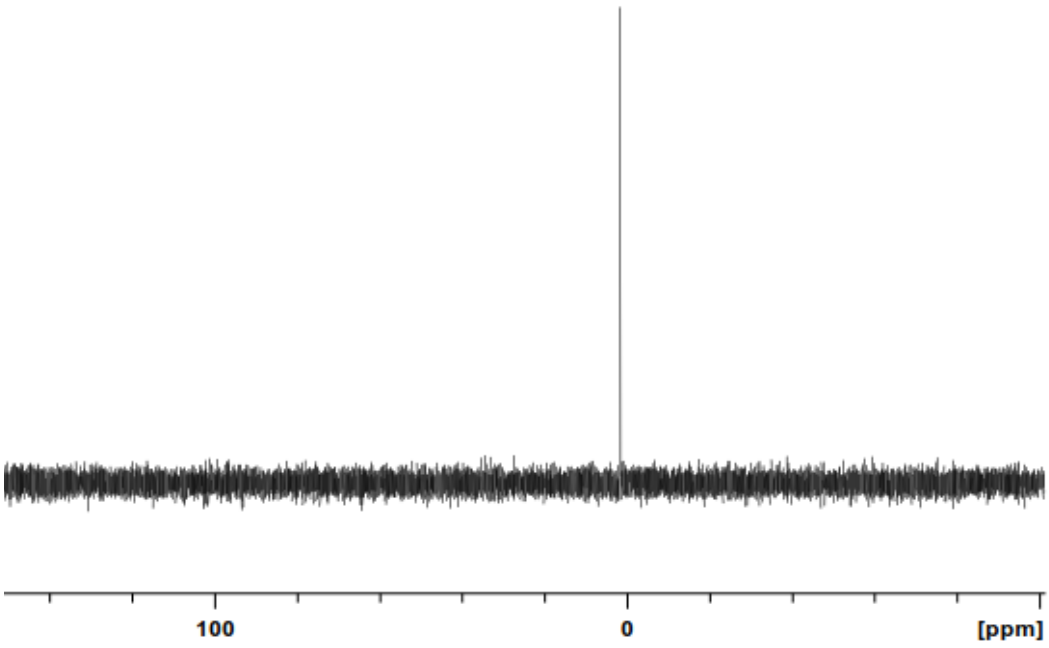
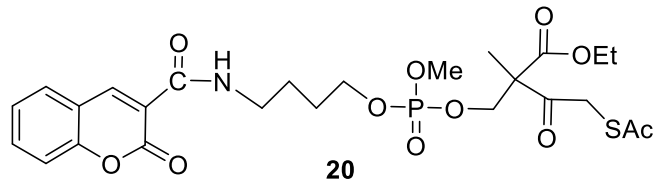


Figure S37. 202 MHz (D_2O) ^{31}P NMR spectra of compound **19**.



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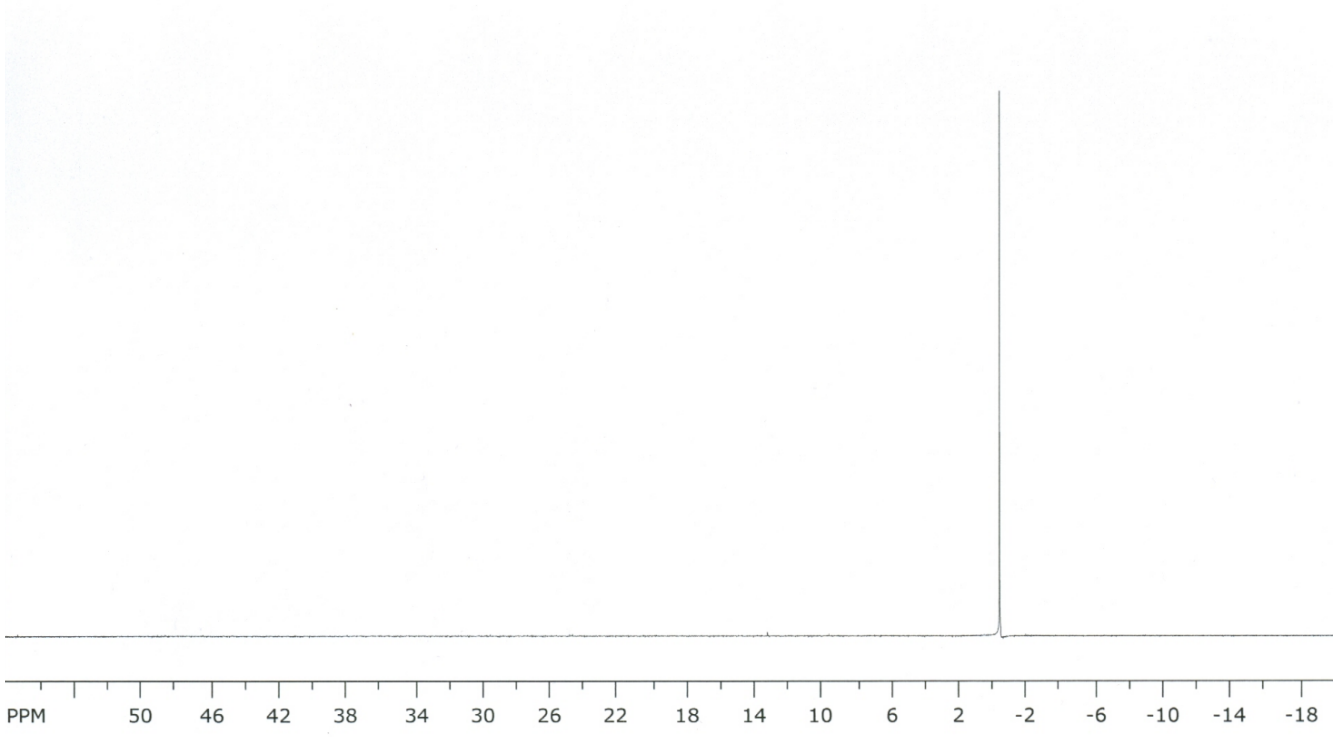
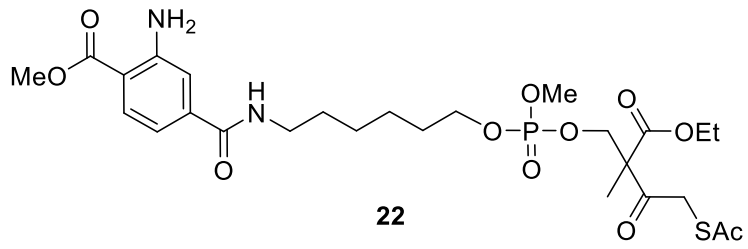


Figure S38. 202 MHz ($CDCl_3$) ^{31}P NMR spectra of compound **20.**



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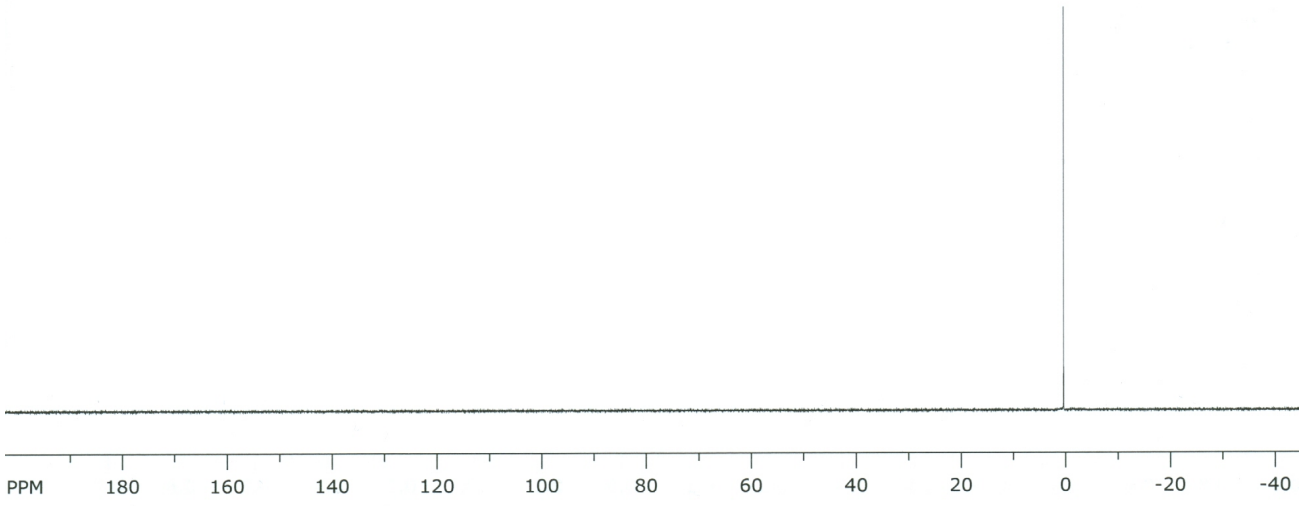
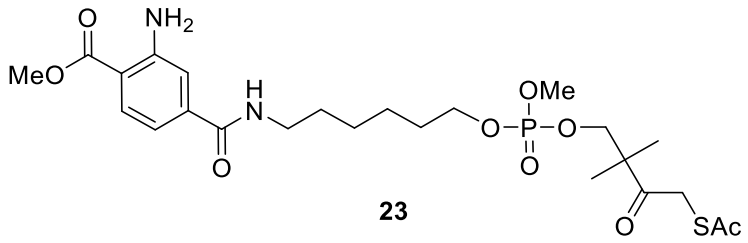


Figure S39. 202 MHz ($CDCl_3$) ^{31}P NMR spectra of compound **22**.



23

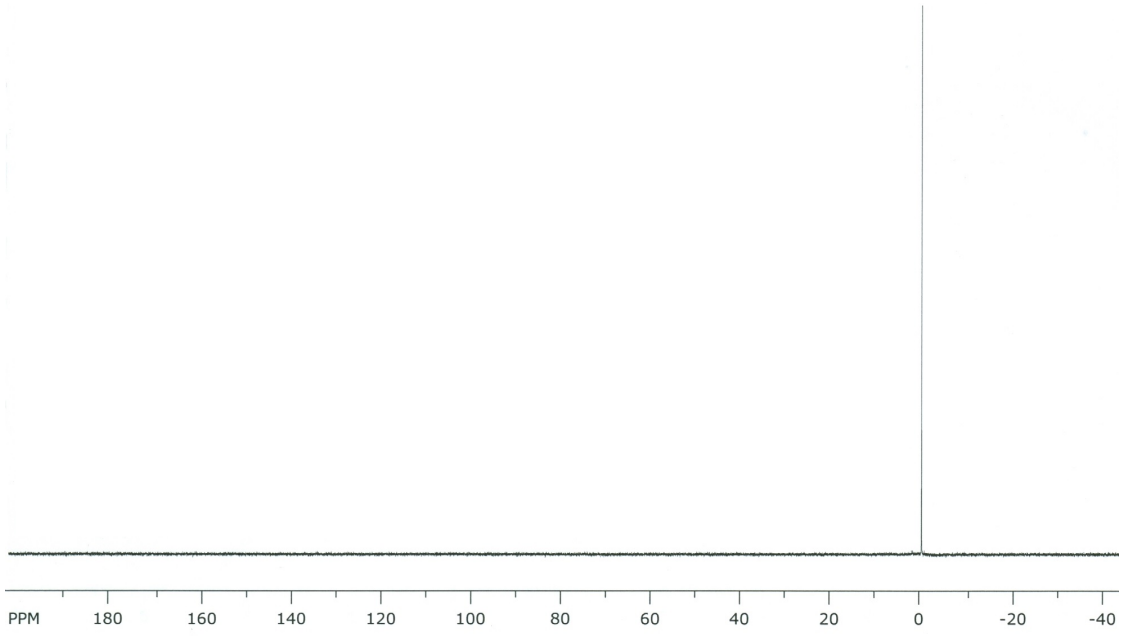
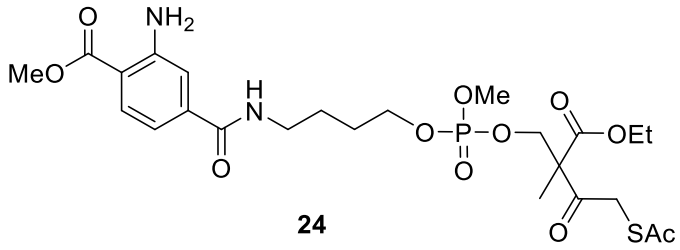


Figure S40. 202 MHz (CD_3CN) ^{31}P NMR spectra of compound **23**.



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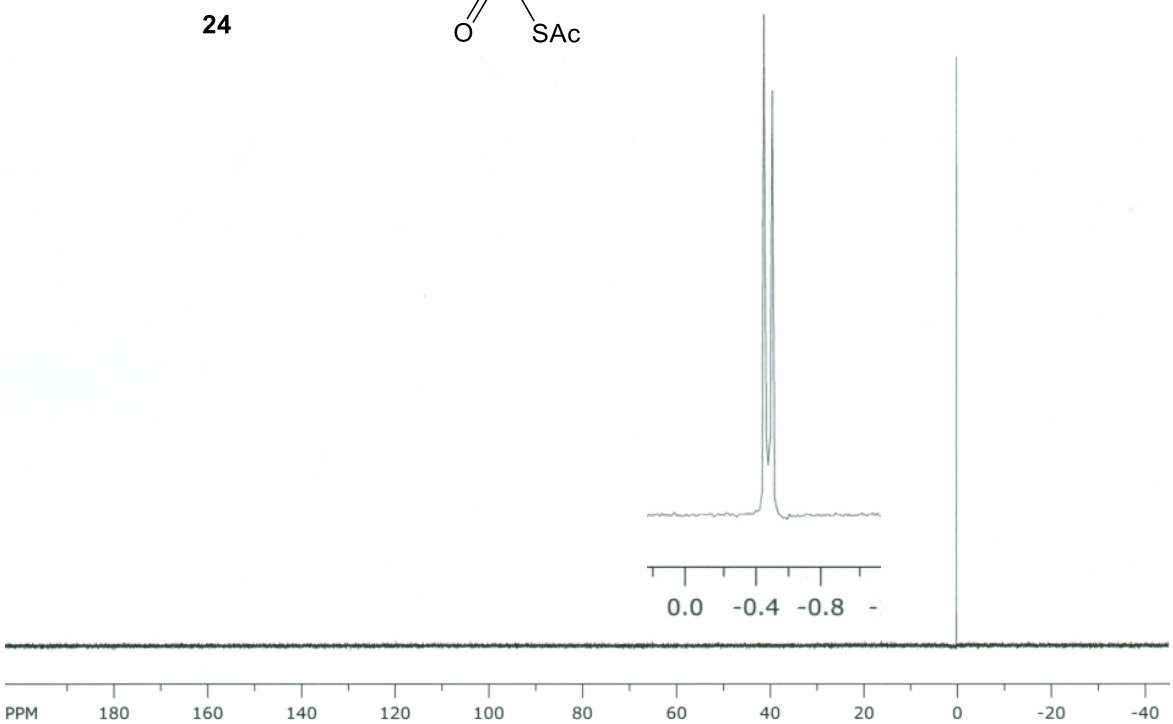
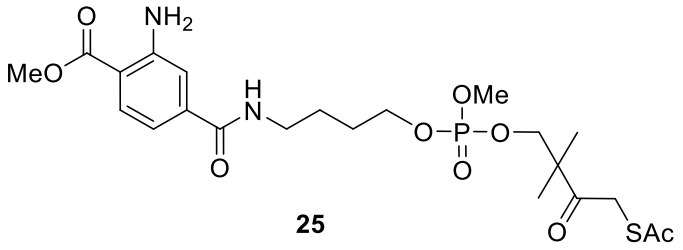


Figure S41. 202 MHz ($CDCl_3$) ^{31}P NMR spectra of compound **24**.



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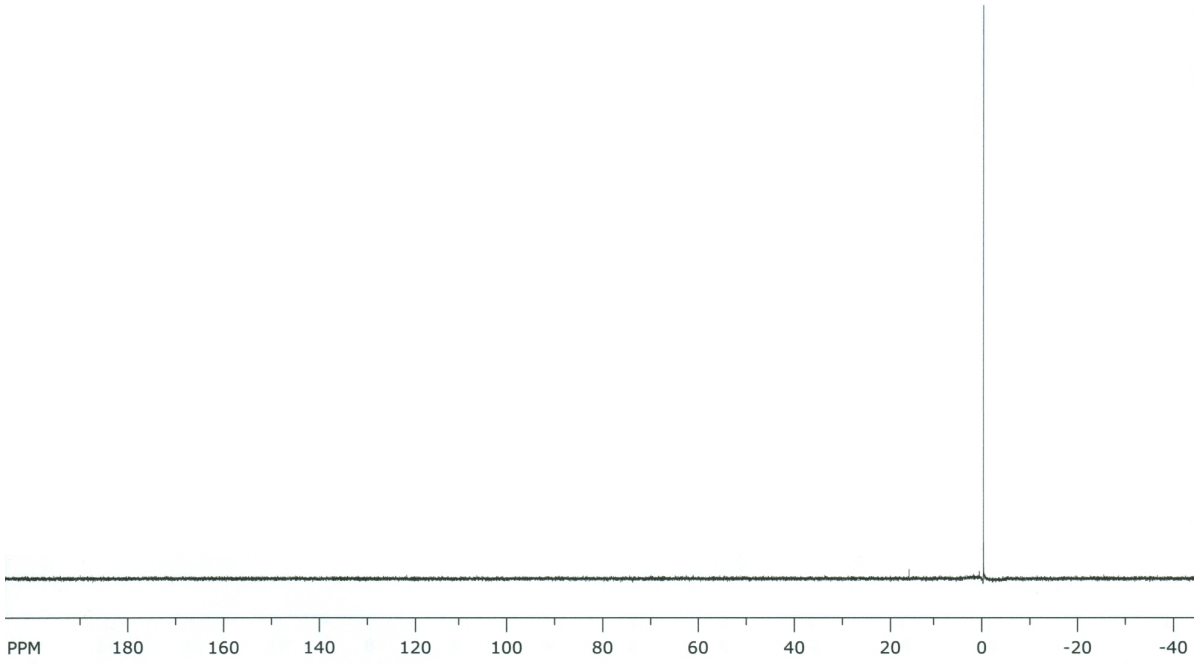
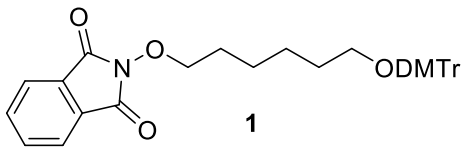


Figure S42. 202 MHz ($CDCl_3$) ^{31}P NMR spectra of compound **25**.

Copies of the ^{1}H spectra of compounds 1-25.



1

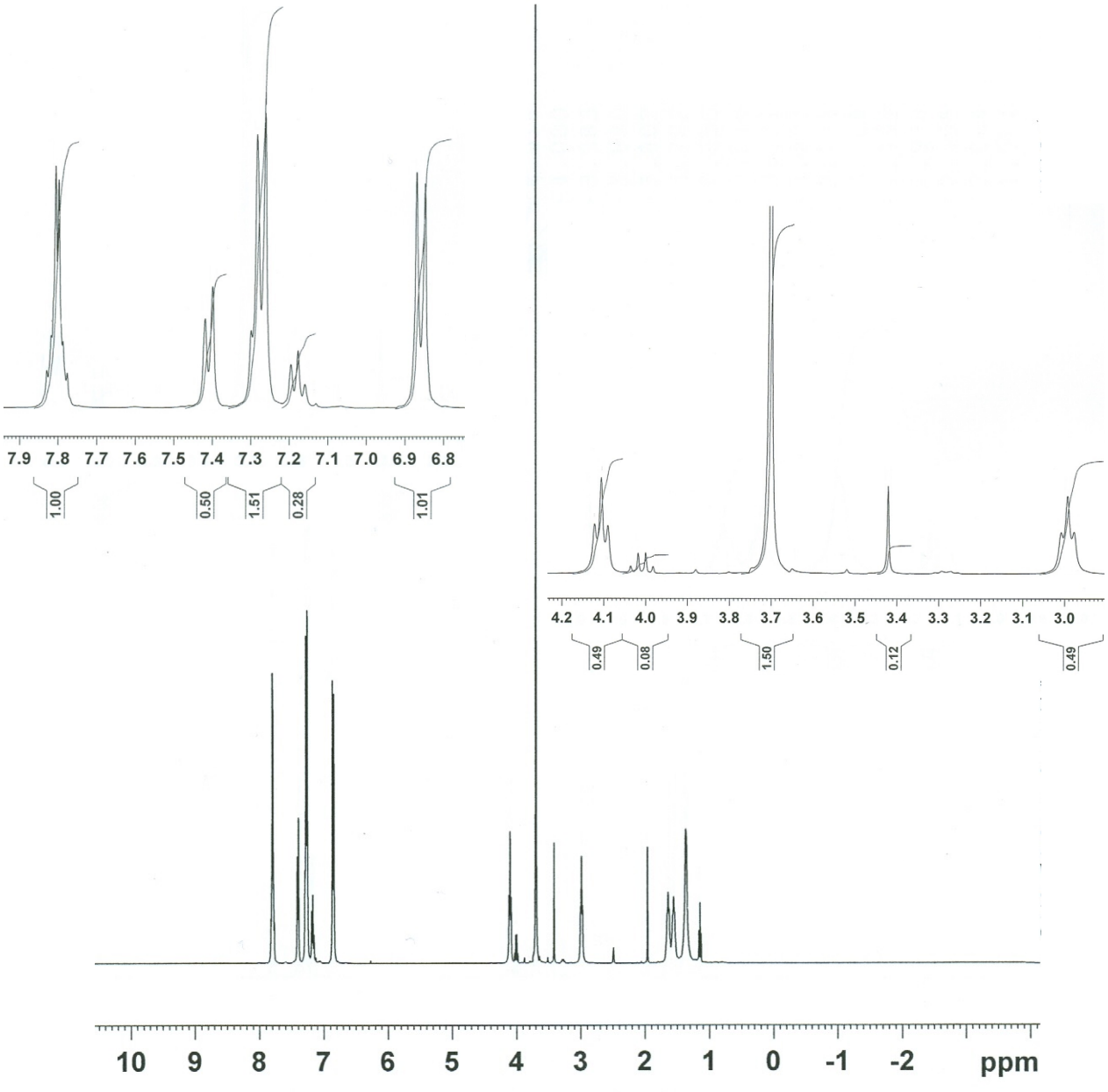
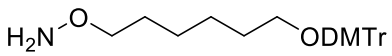


Figure S43. 400 MHz ($CDCl_3$) ^{1}H NMR spectra of compound **1**.



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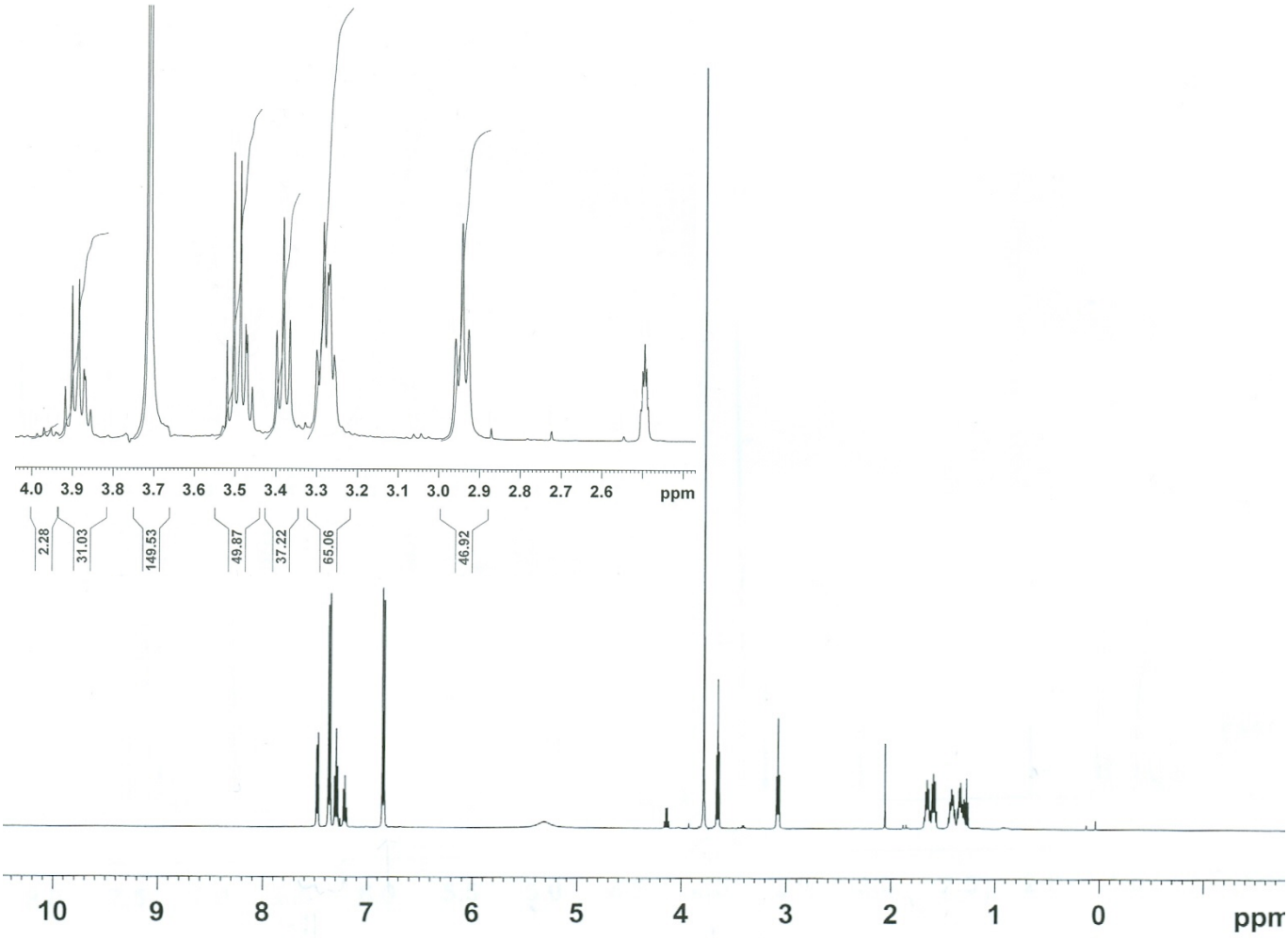
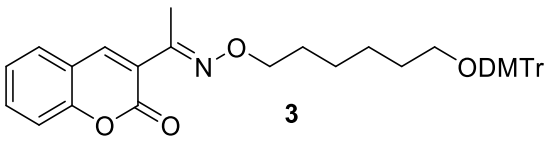


Figure S44. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **2**.



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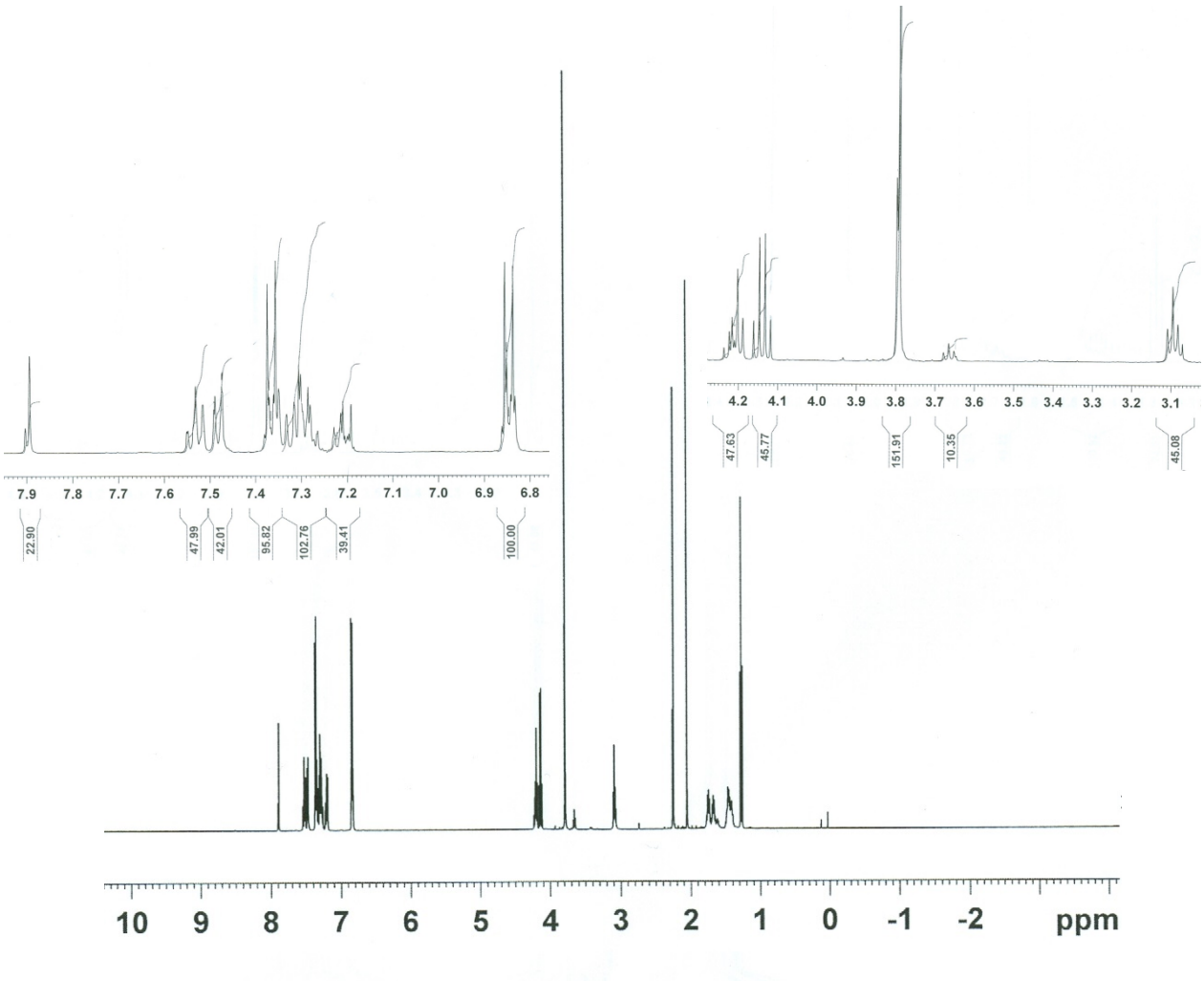
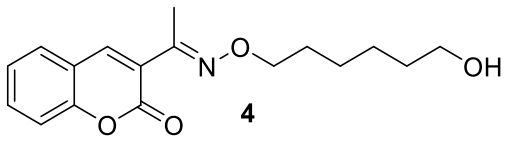


Figure S45. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **3**.



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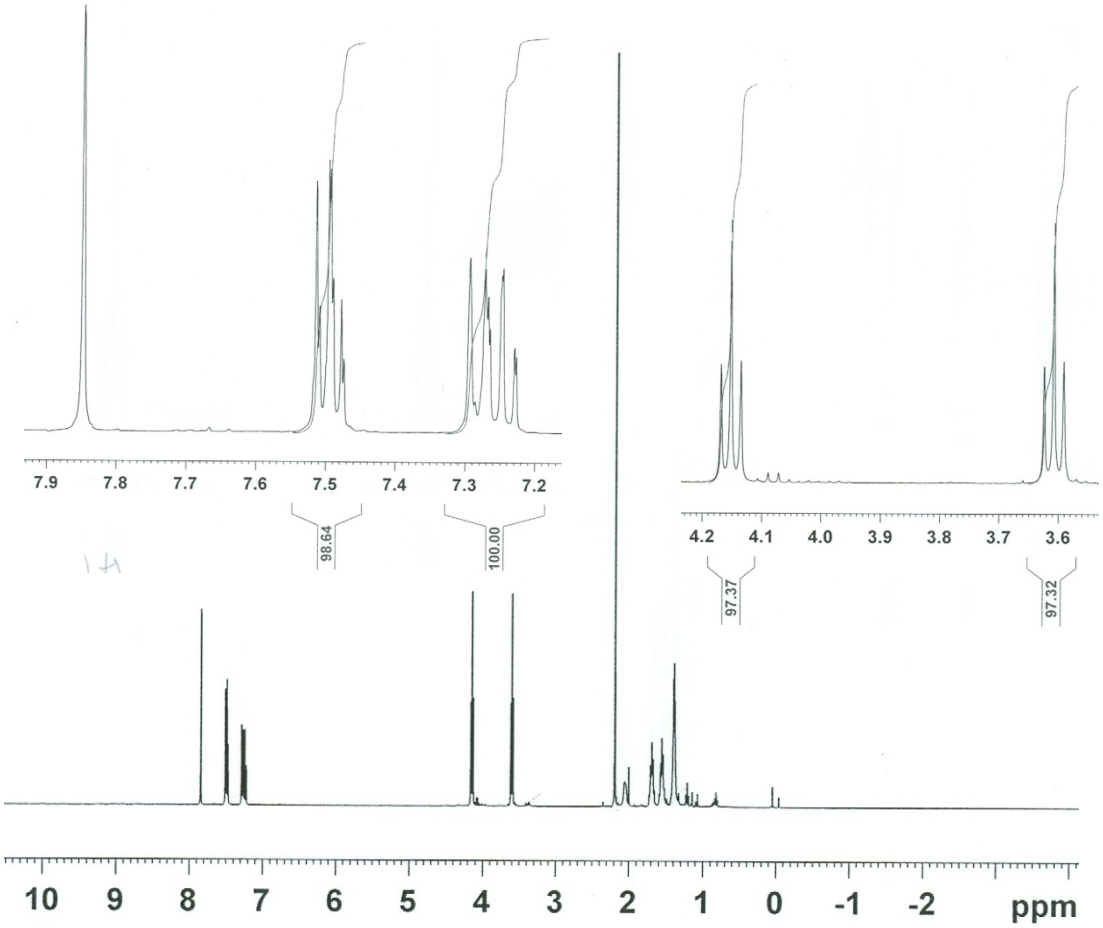


Figure S46. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **4**.

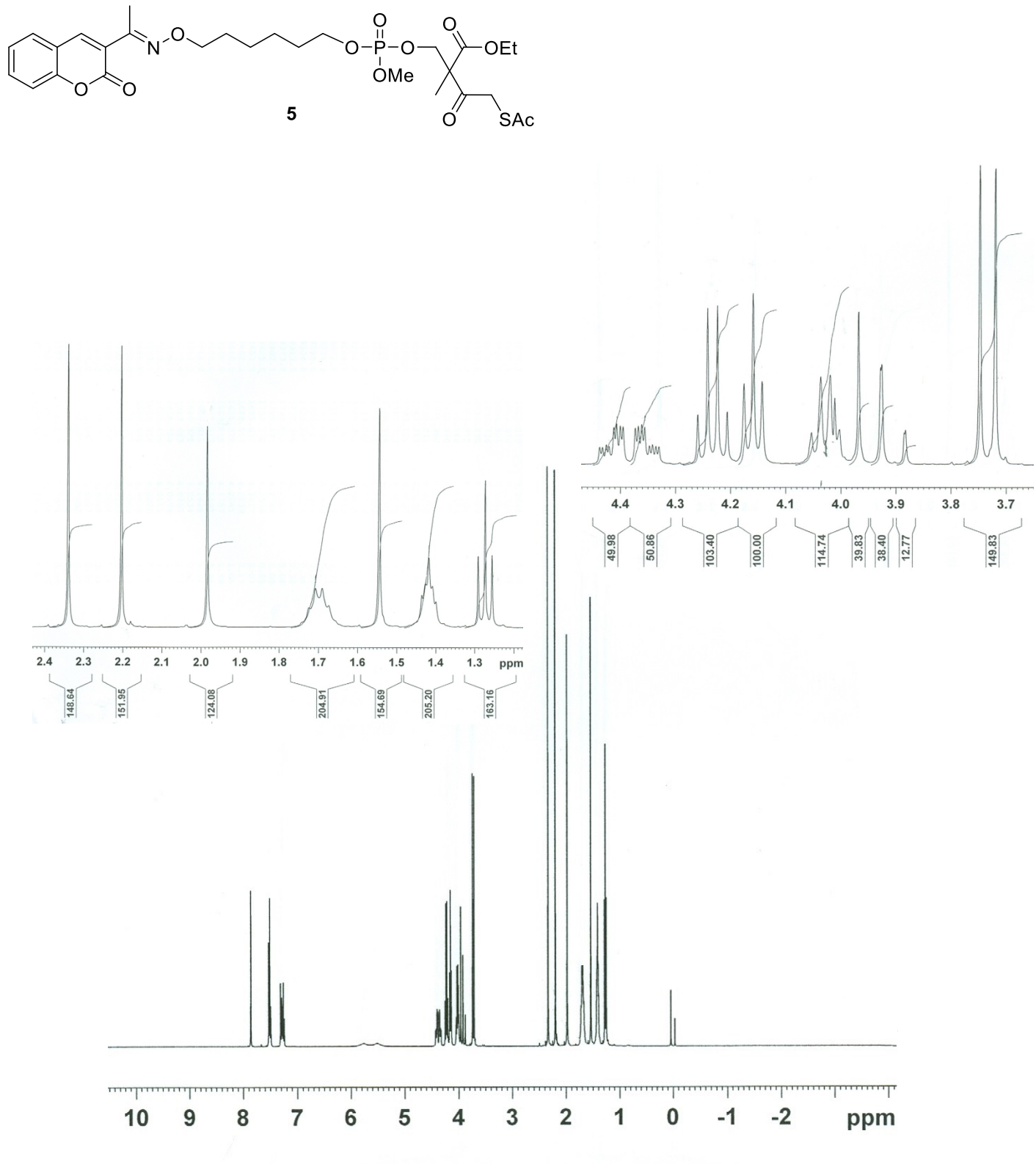
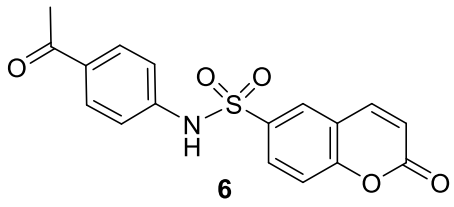


Figure S47. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **5**.



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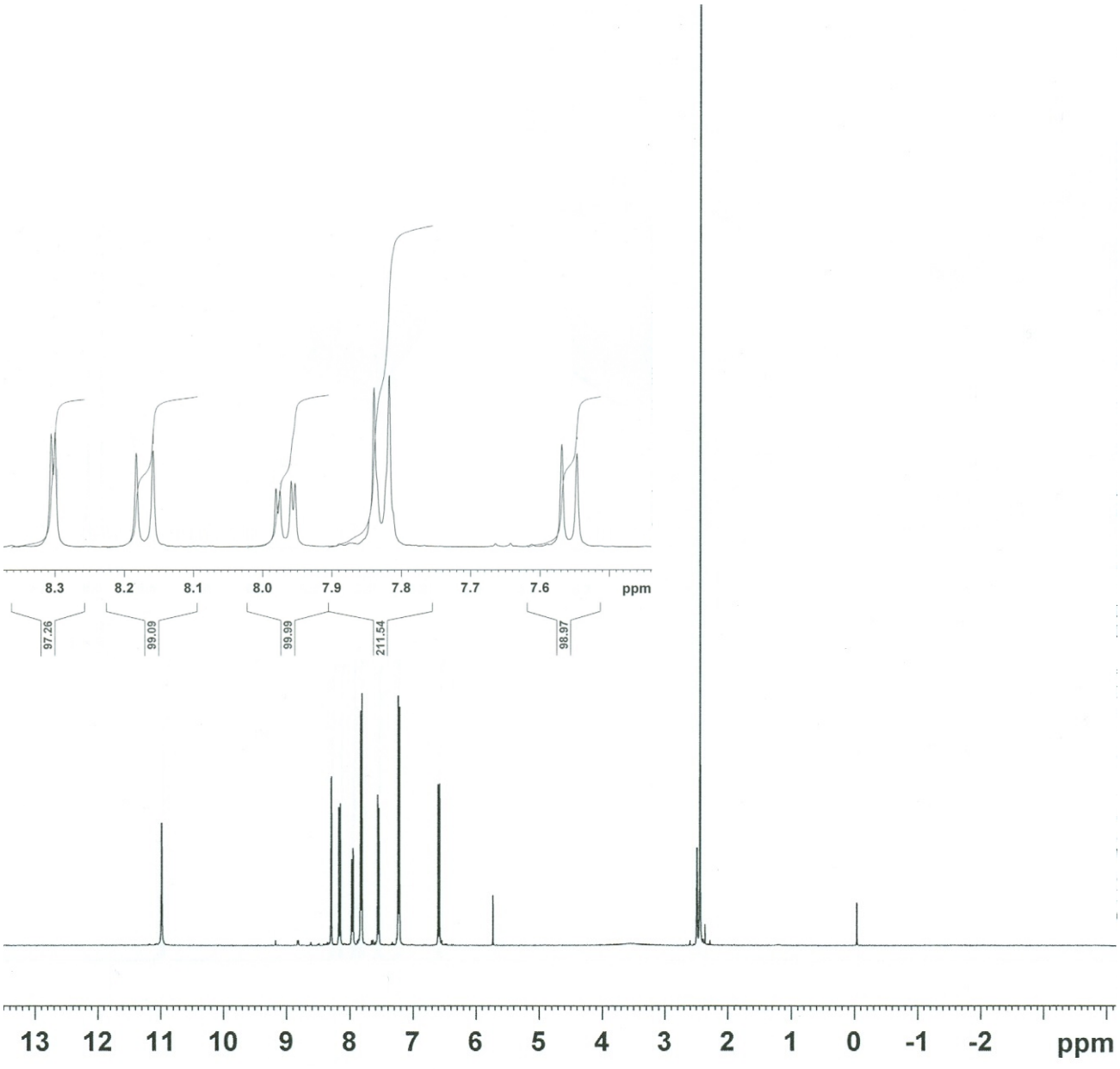


Figure S48. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **6**.

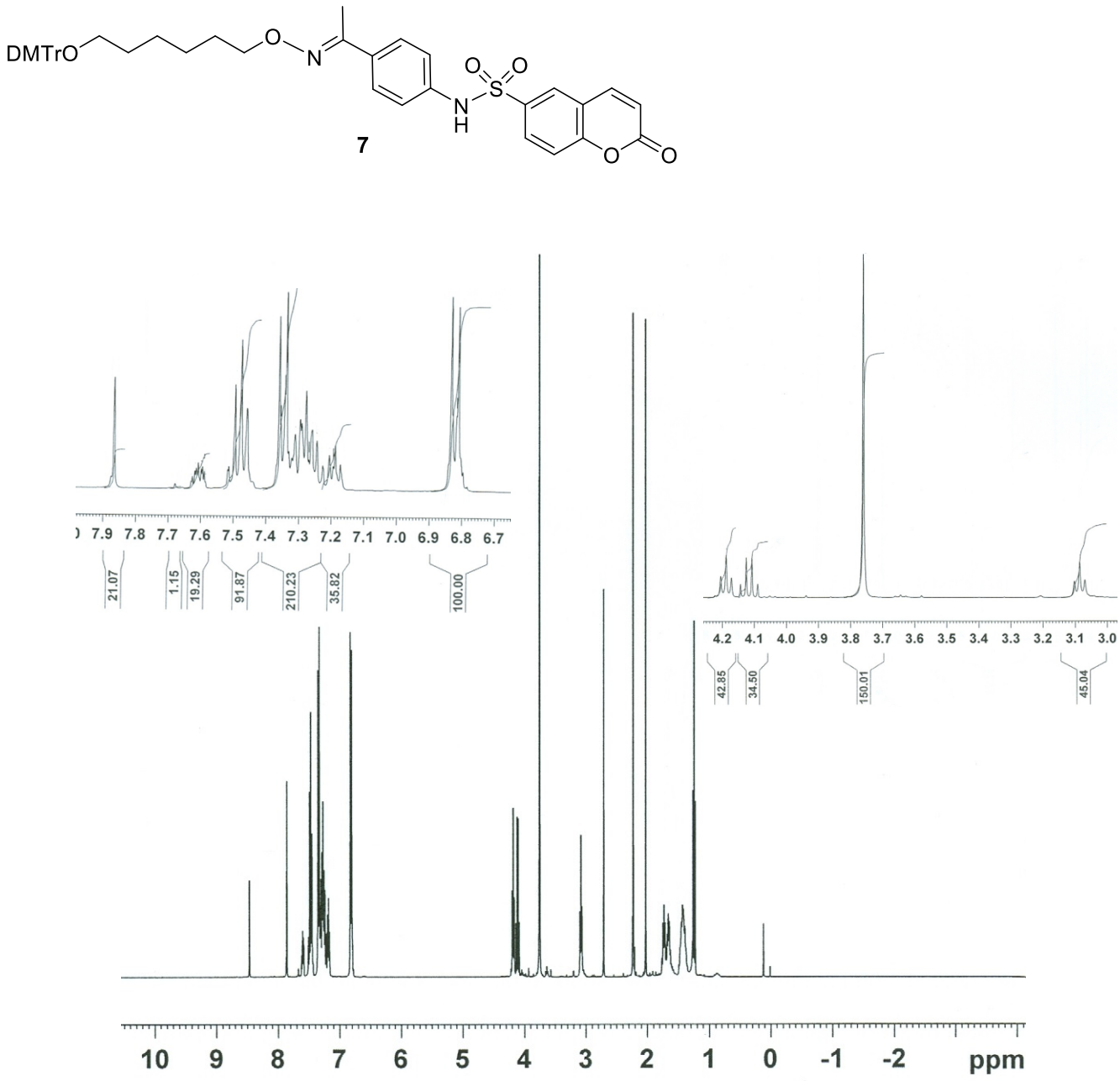
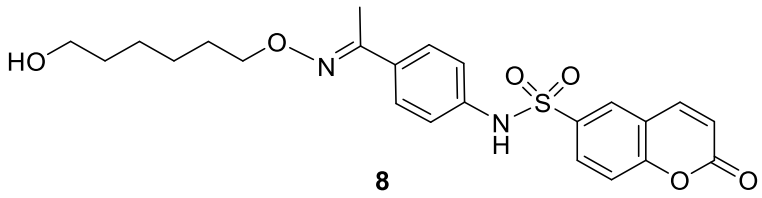


Figure S49. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **7**.



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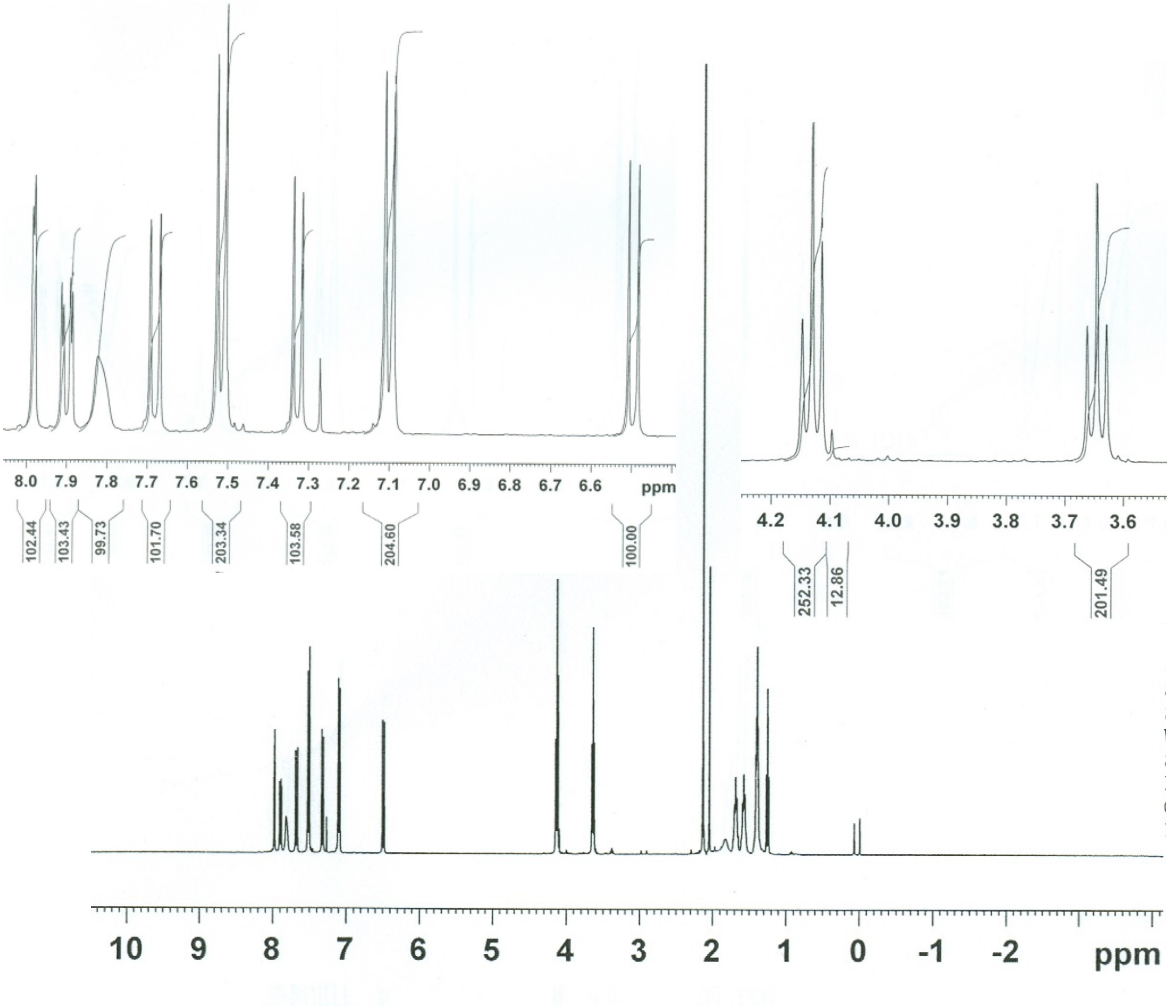


Figure S50. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **8**.

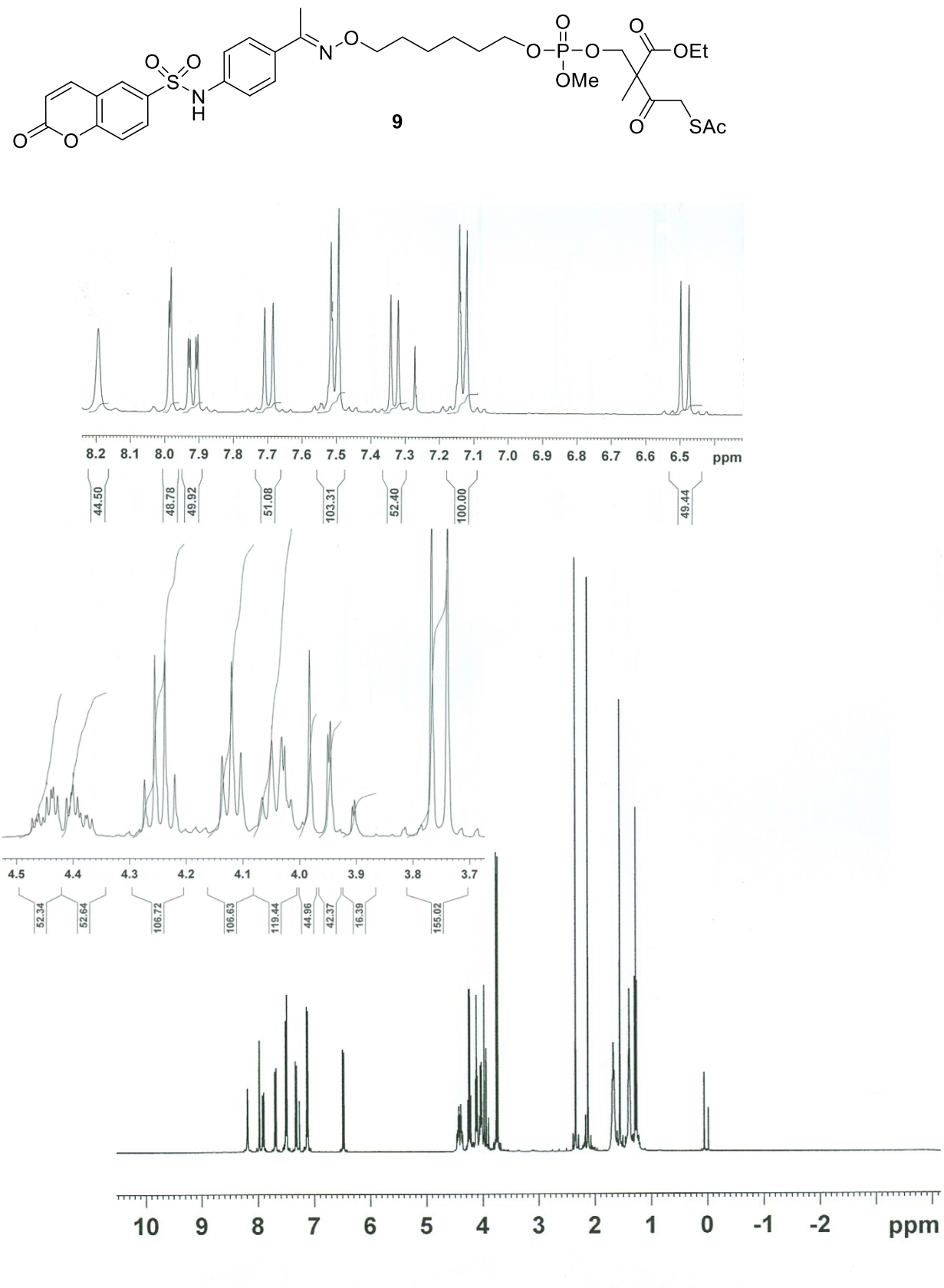
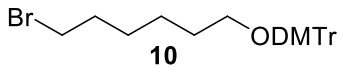


Figure S51. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **9**.



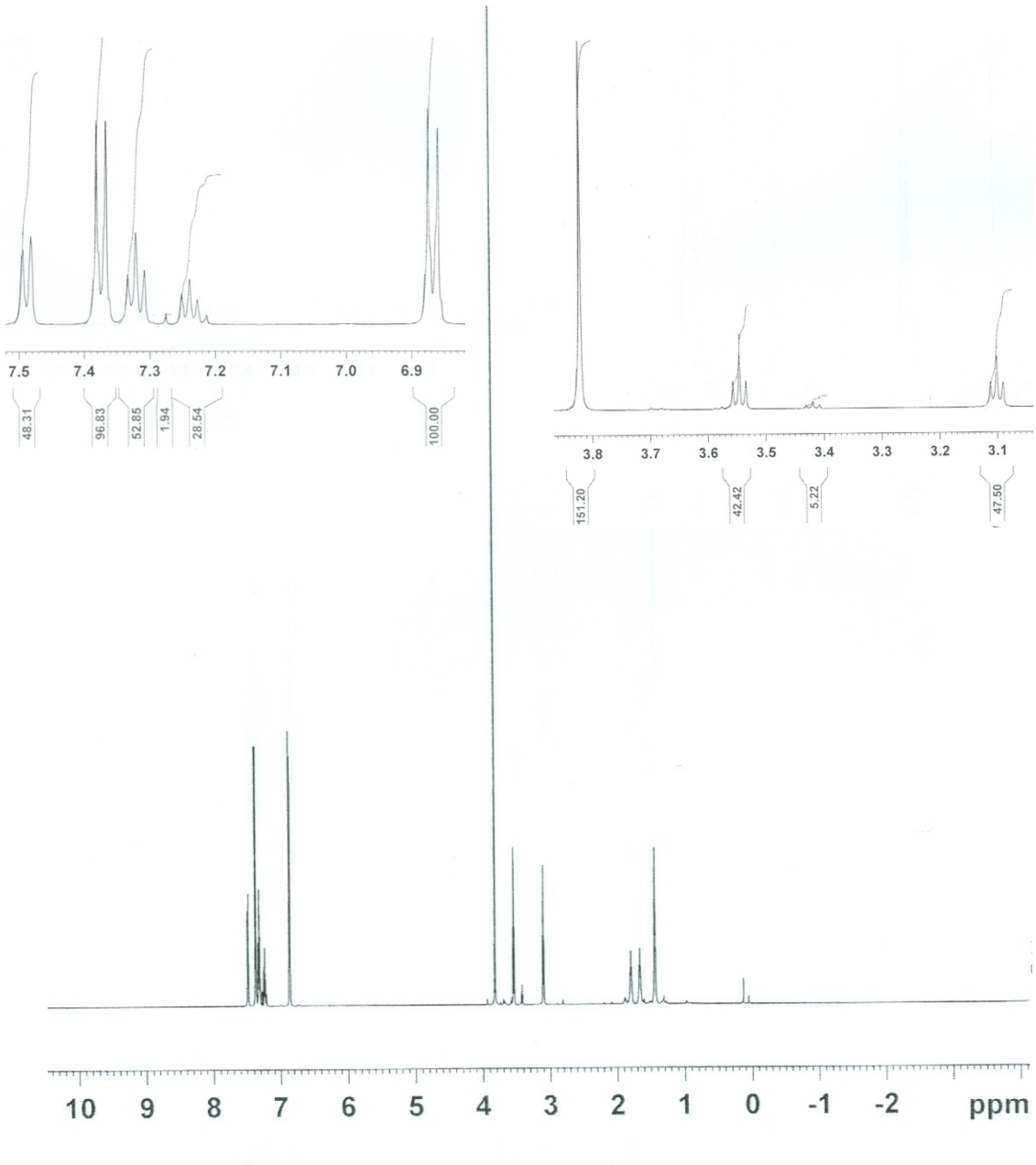
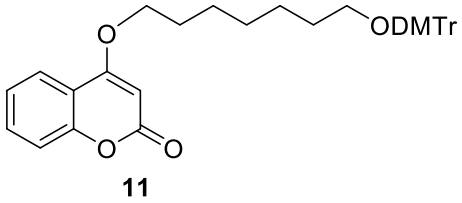


Figure S52. 600 MHz ($CDCl_3$) ^1H NMR spectra of compound **10**.



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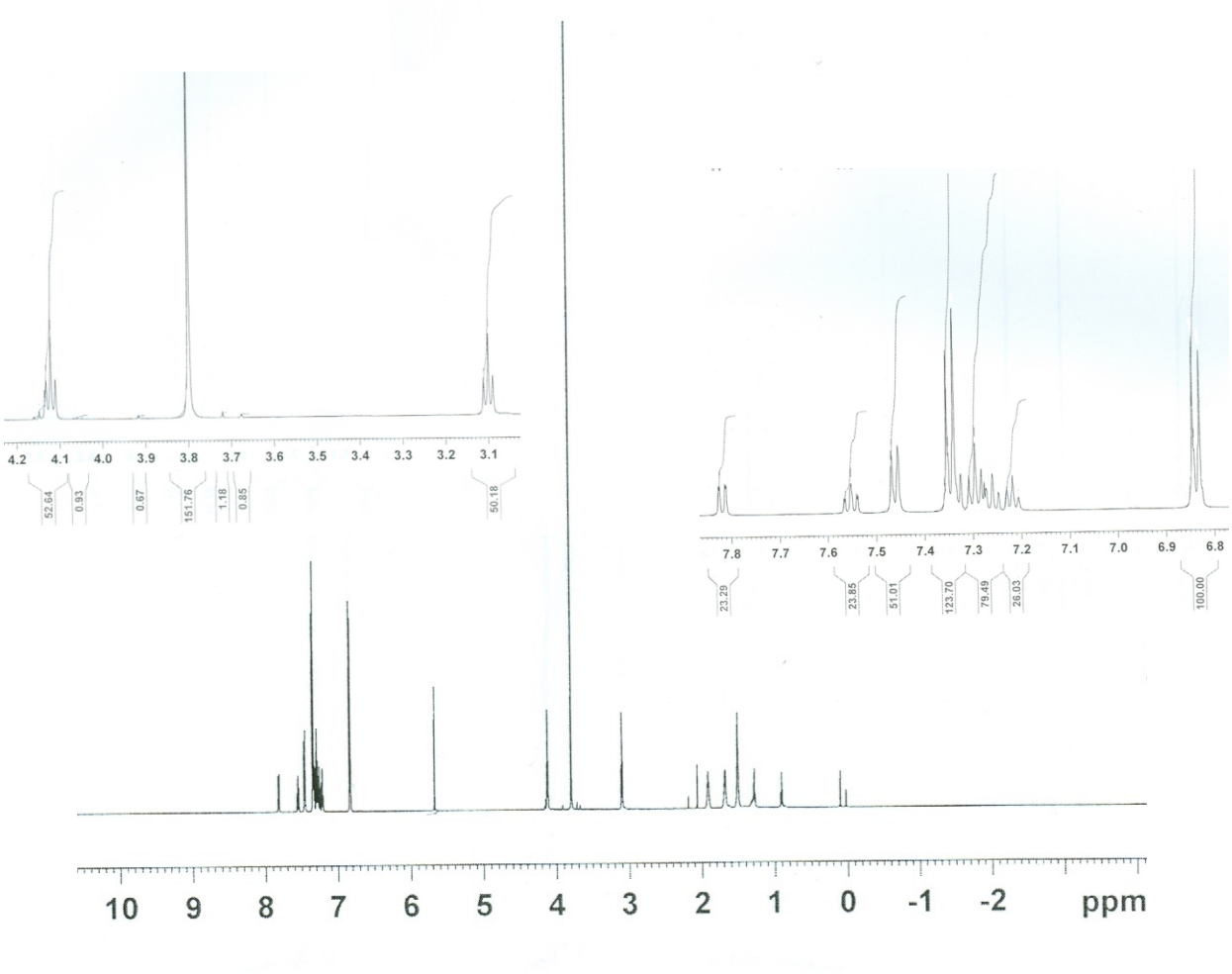
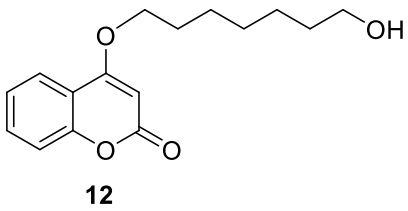


Figure S53. 600 MHz ($CDCl_3$) ^1H NMR spectra of compound **11**.



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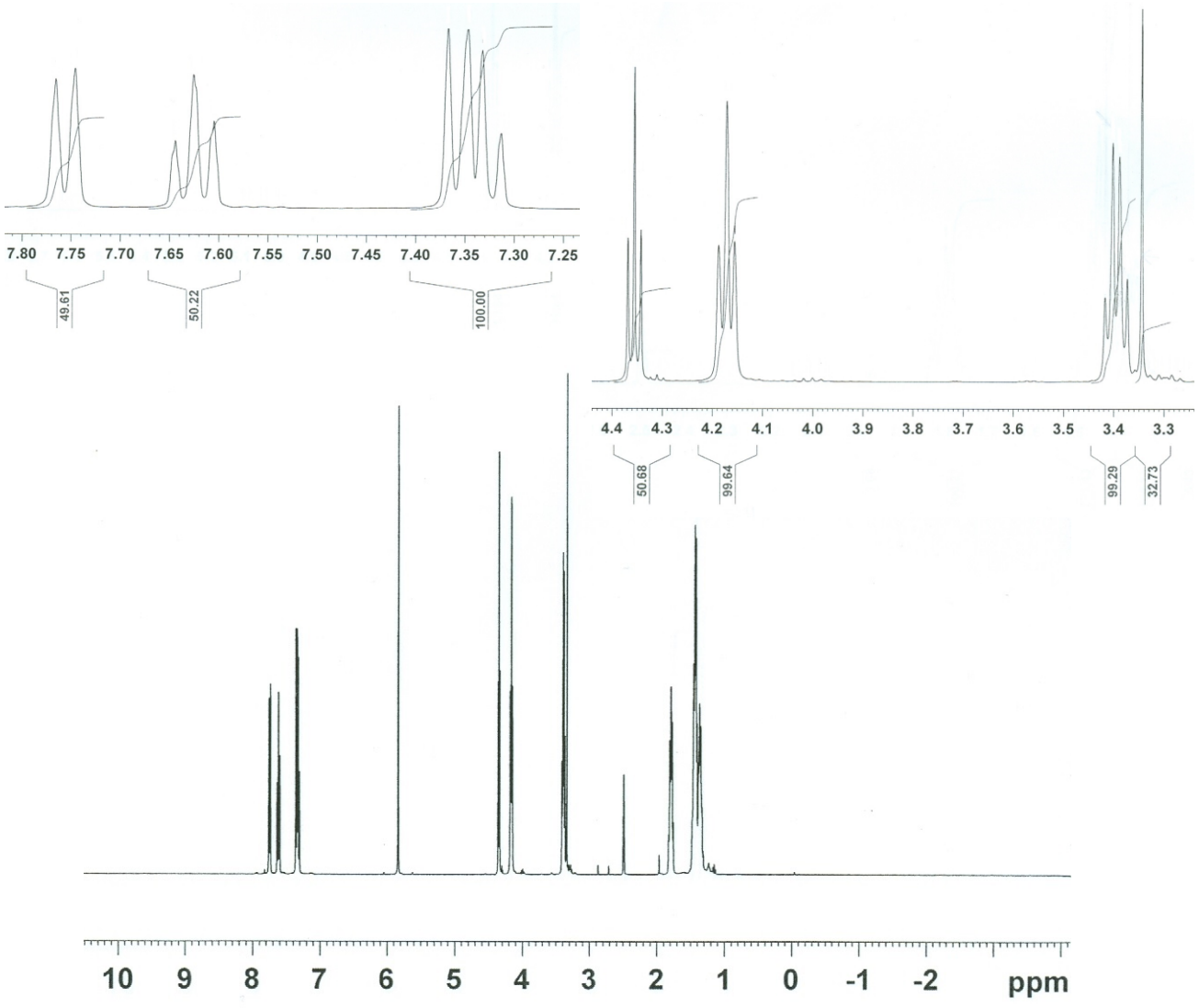


Figure S54. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **12**.

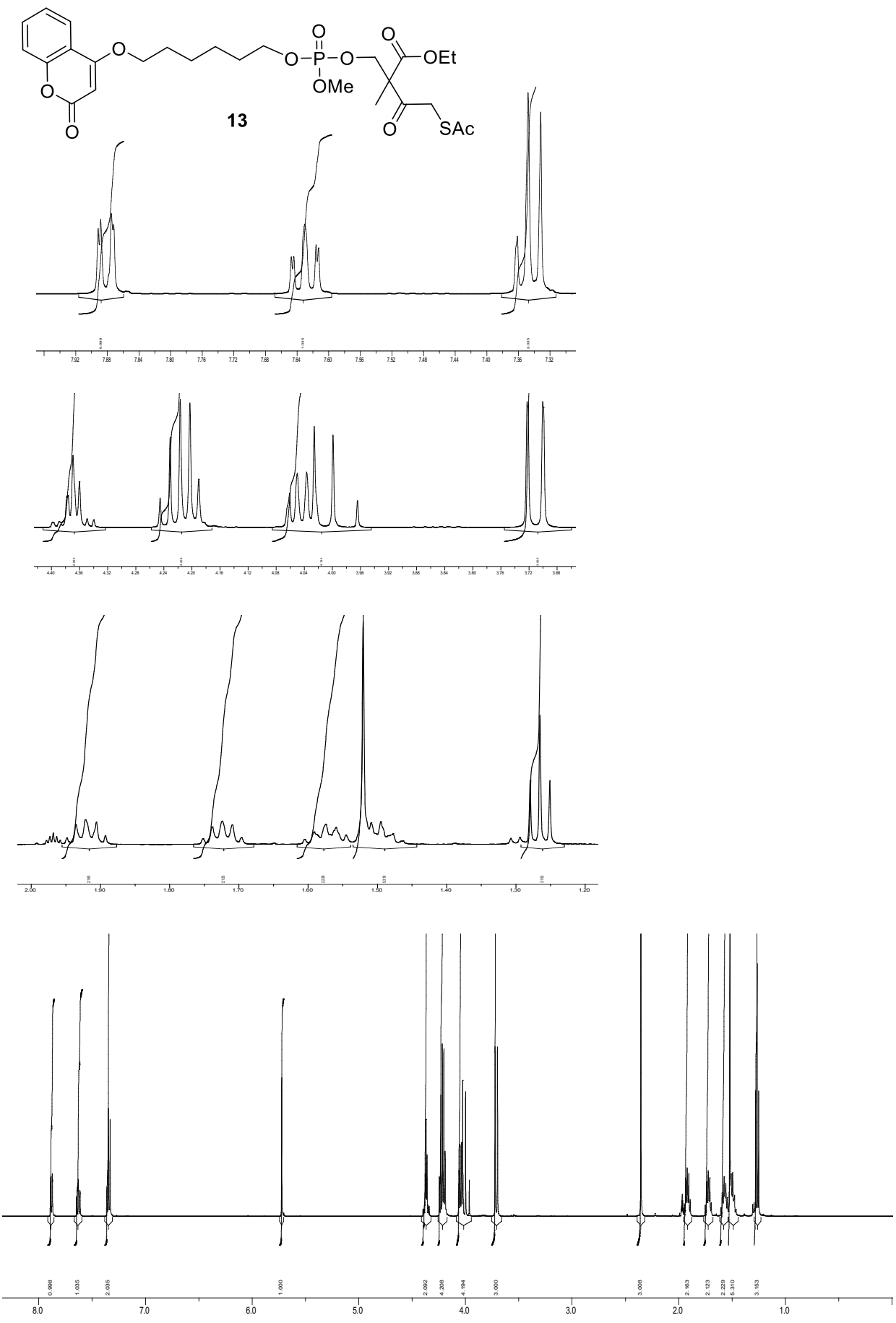


Figure S55. 500 MHz (CD_3CN) ^1H NMR spectra of compound **13**.

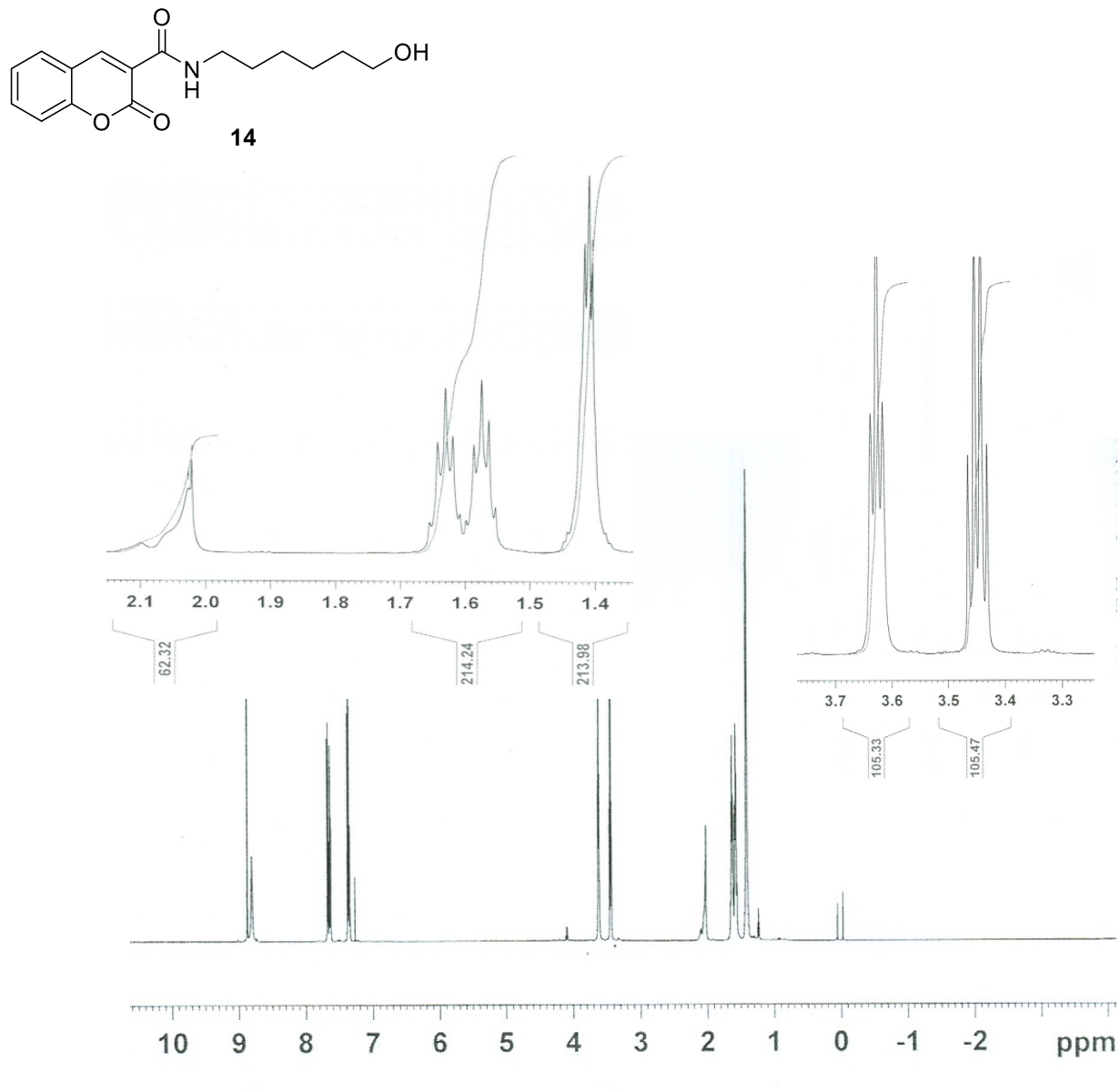
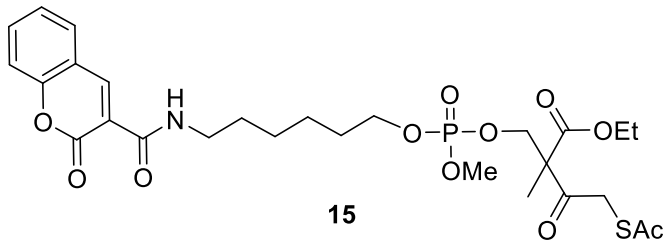


Figure S56. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **14**.



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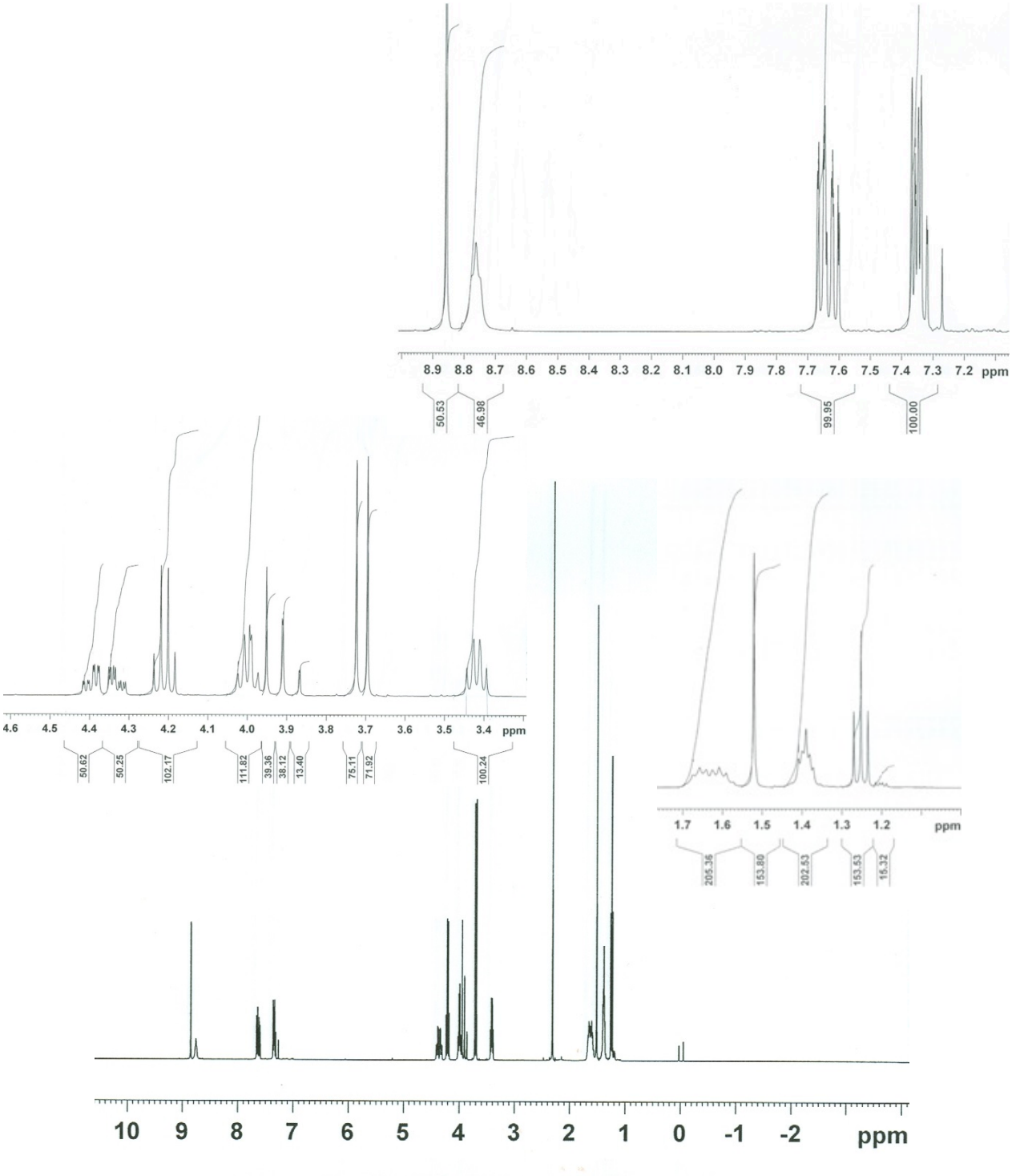
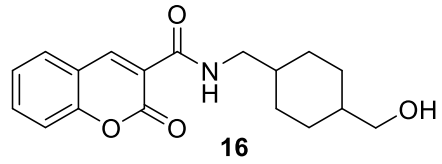


Figure S57. 400 MHz ($CDCl_3$) ^1H NMR spectra of compound **15**.



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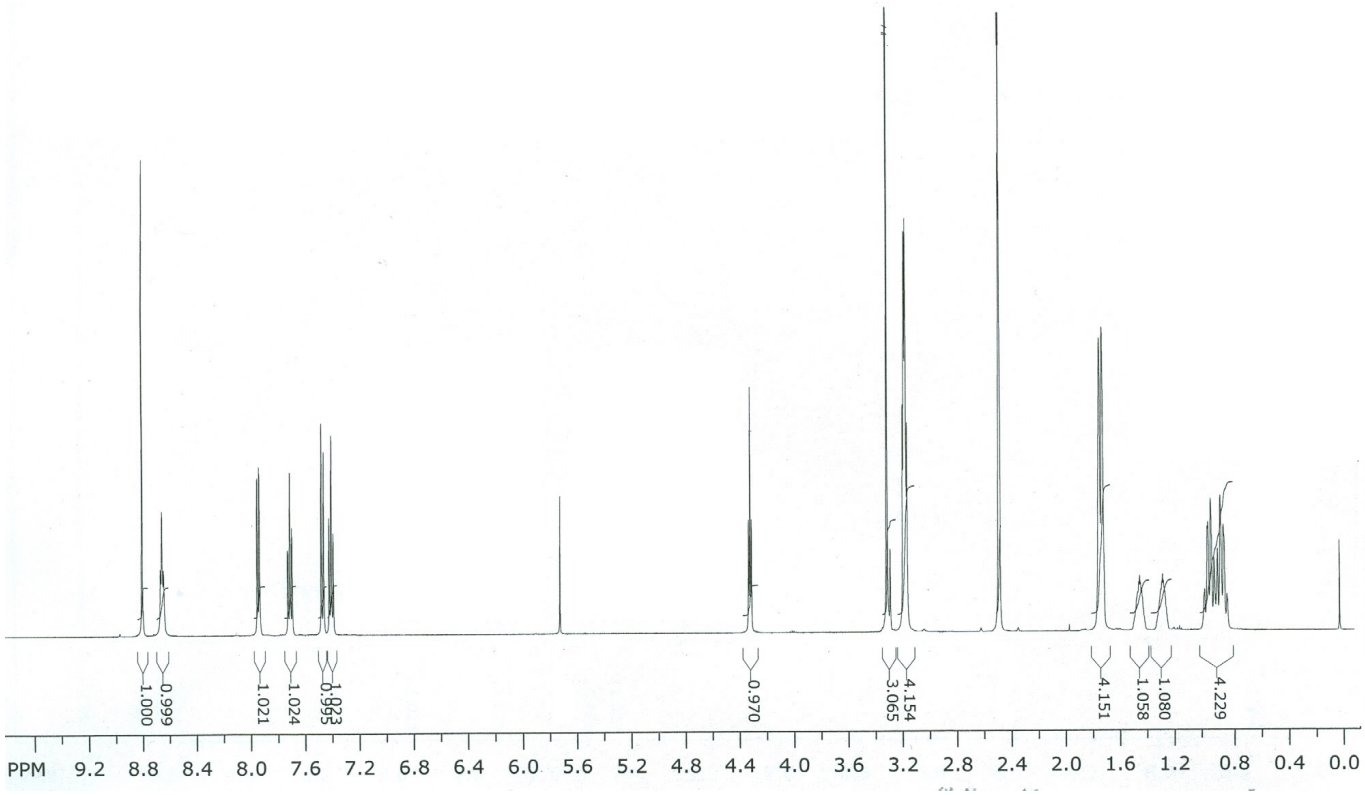


Figure S58. 500 MHz ($(CD_3)_2SO$) ^1H NMR spectra of compound **16**.

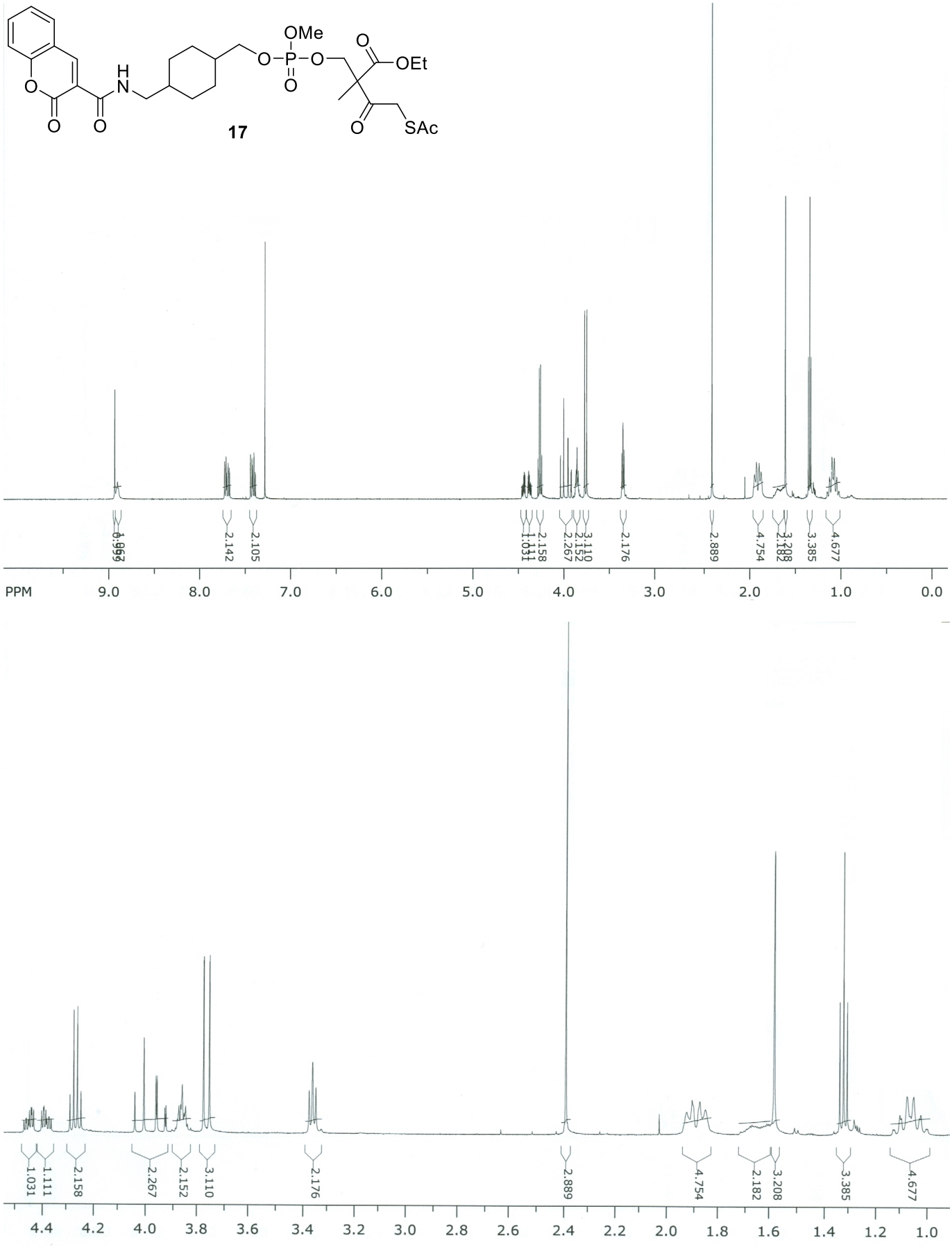


Figure S59. 500 MHz ($CDCl_3$) ^1H NMR spectra of compound **17**.

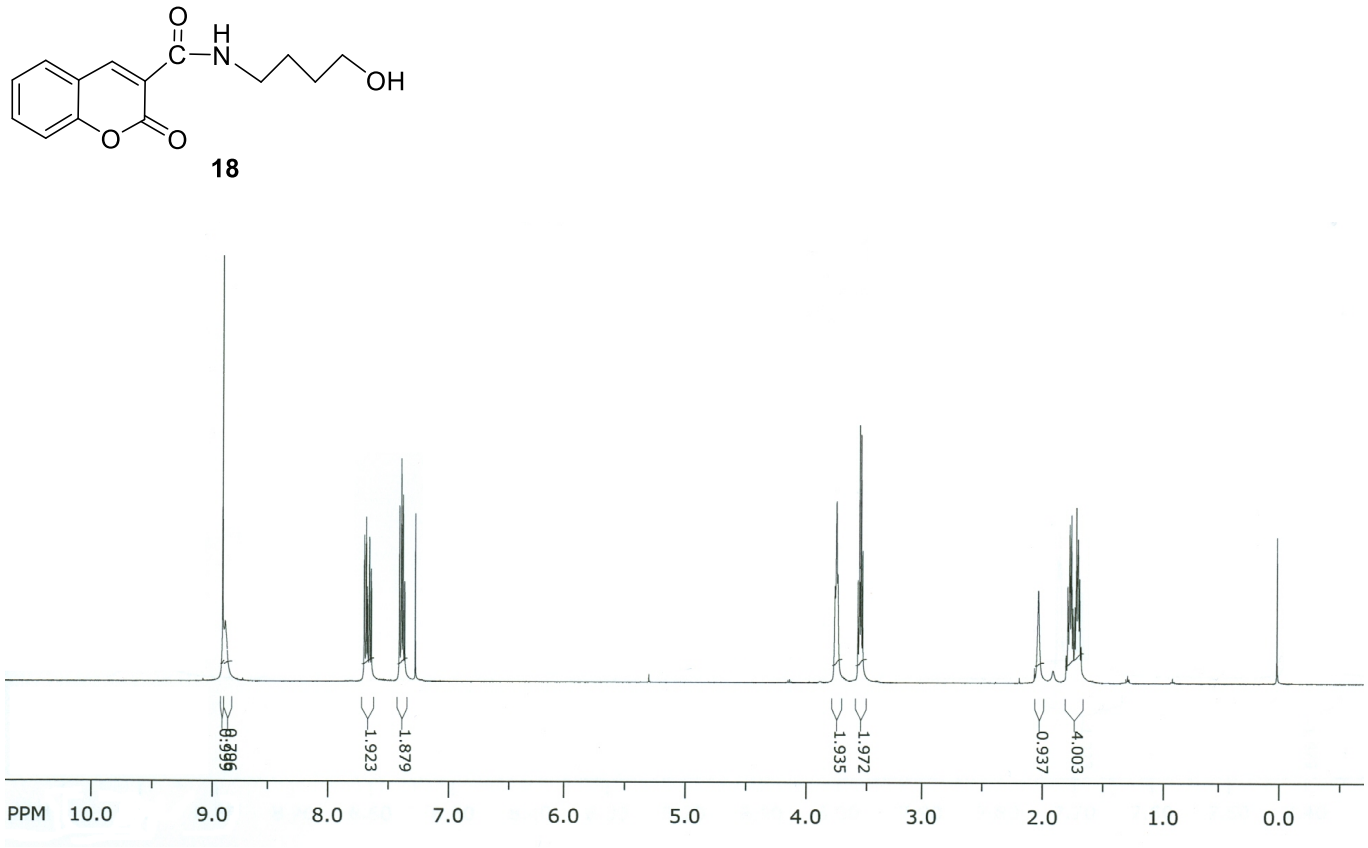
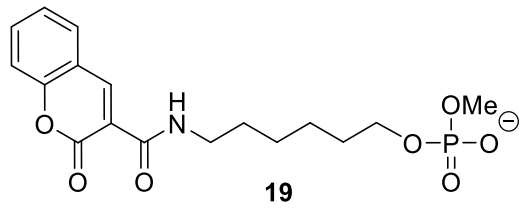
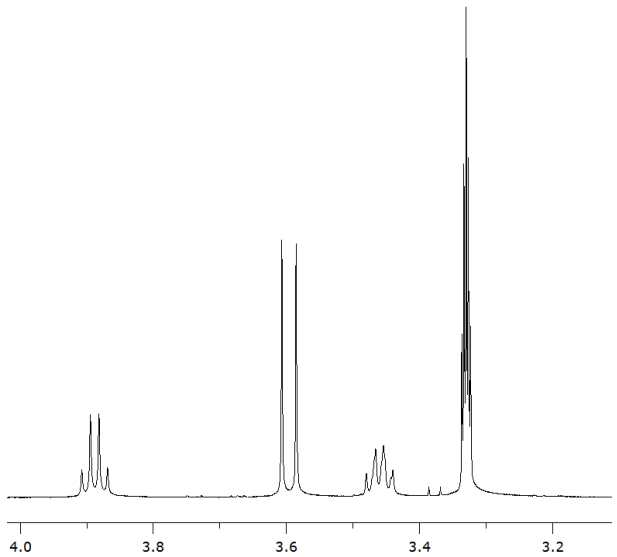


Figure S60. 500 MHz ($CDCl_3$) ^1H NMR spectra of compound **18**.





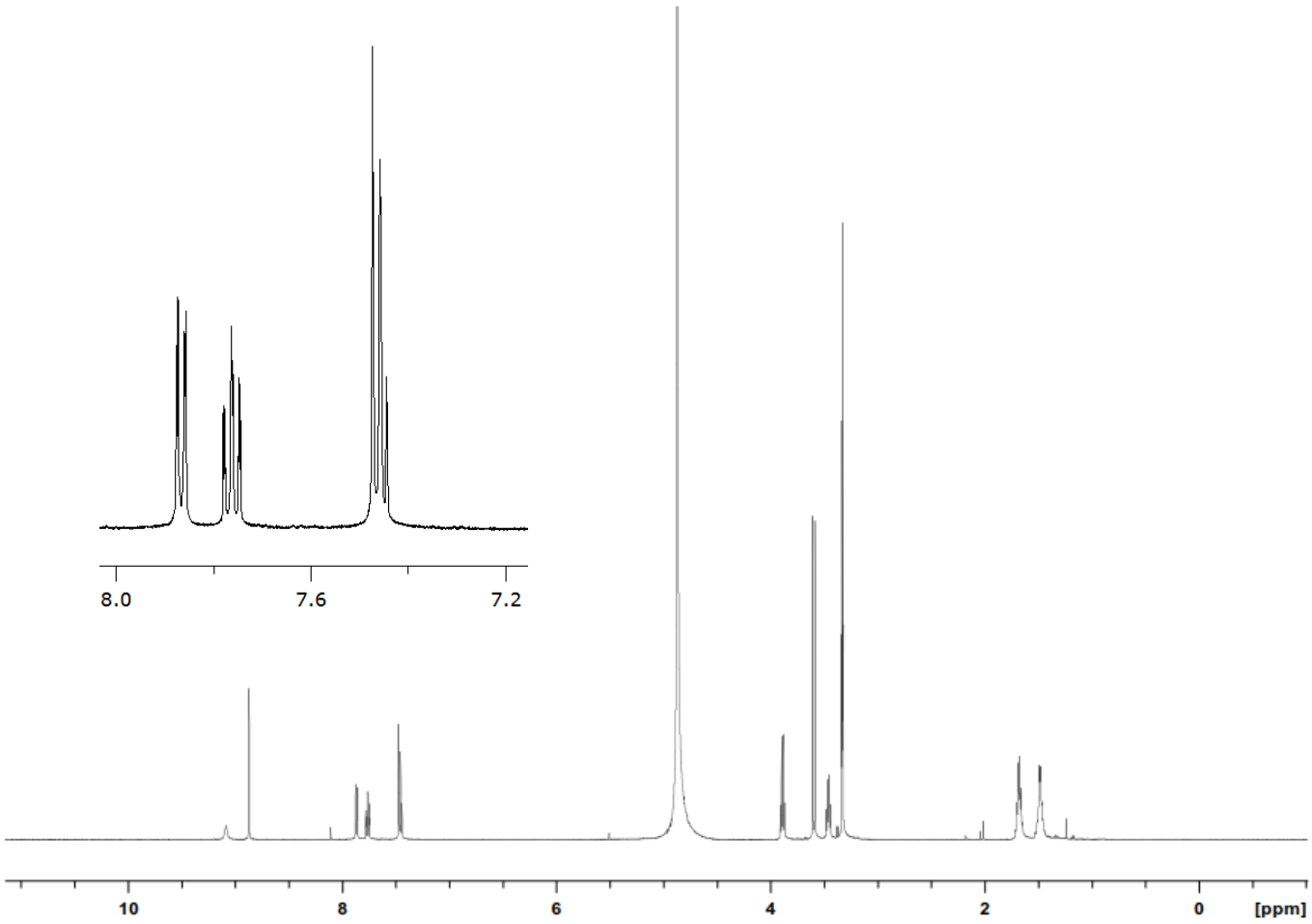
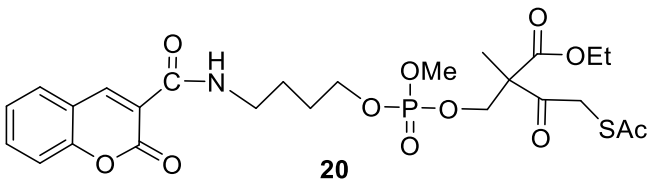


Figure S61. 500 MHz (D_2O) ^1H NMR spectra of compound **19**.



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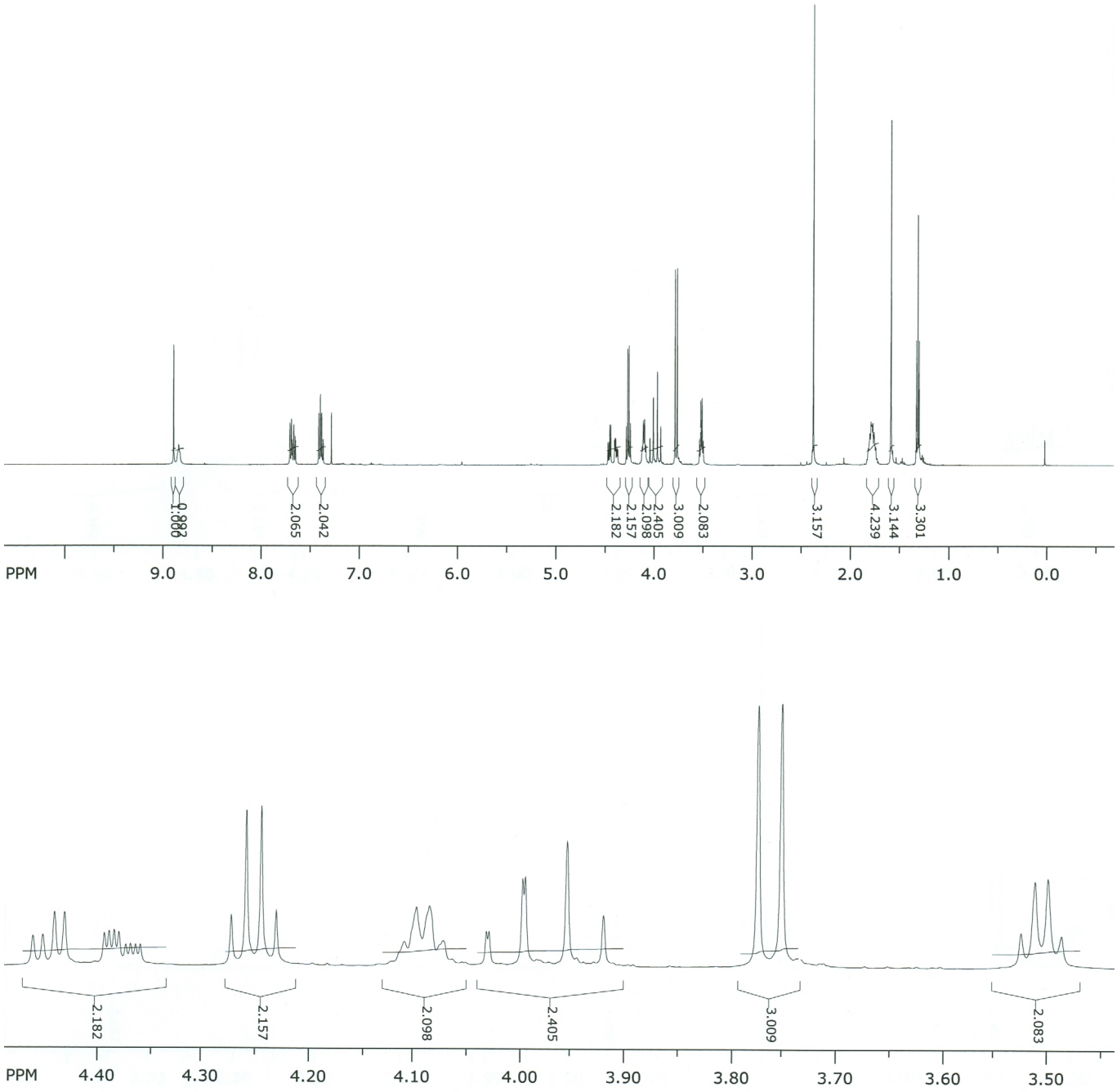
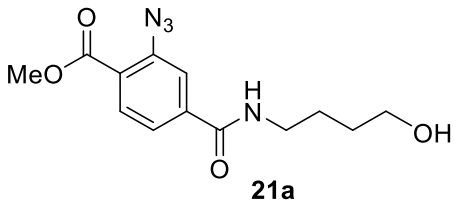


Figure S62. 500 MHz ($CDCl_3$) ^1H NMR spectra of compound **20**.



21a

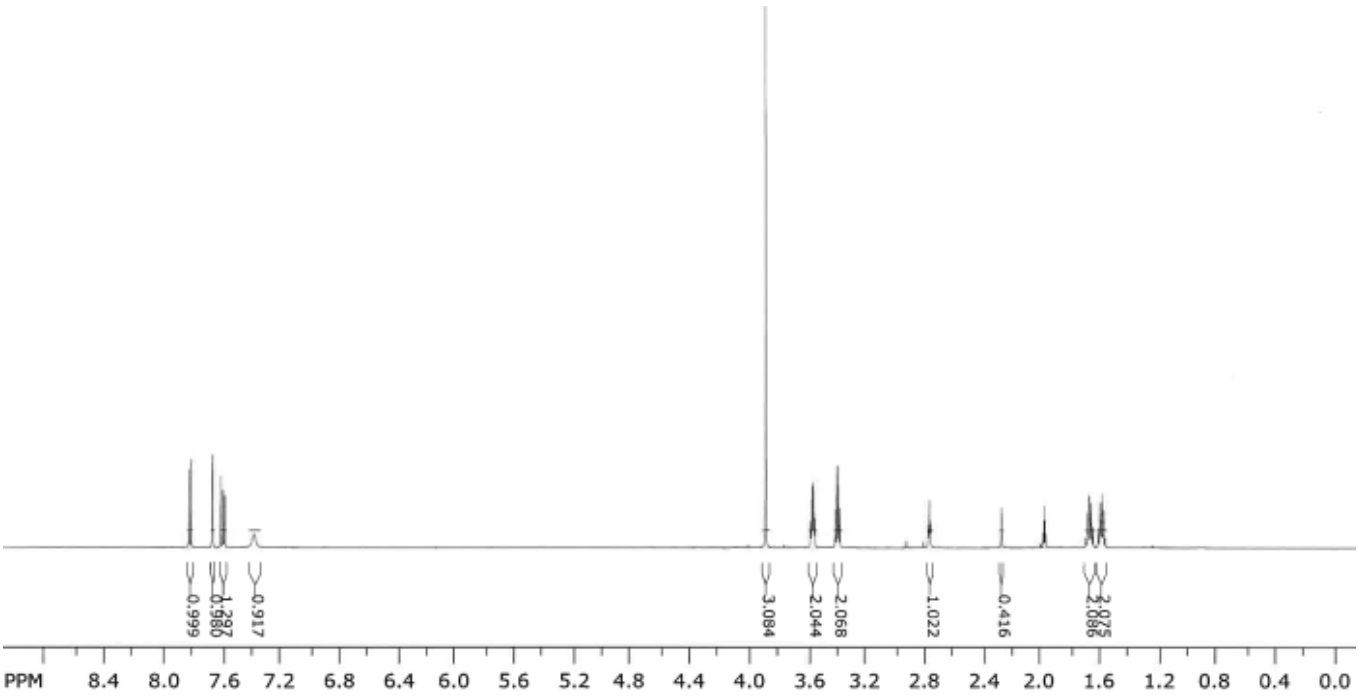
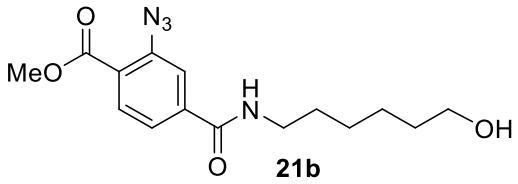


Figure S63. 600 MHz (CD_3CN) ^1H NMR spectra of compound **21a**.



21b

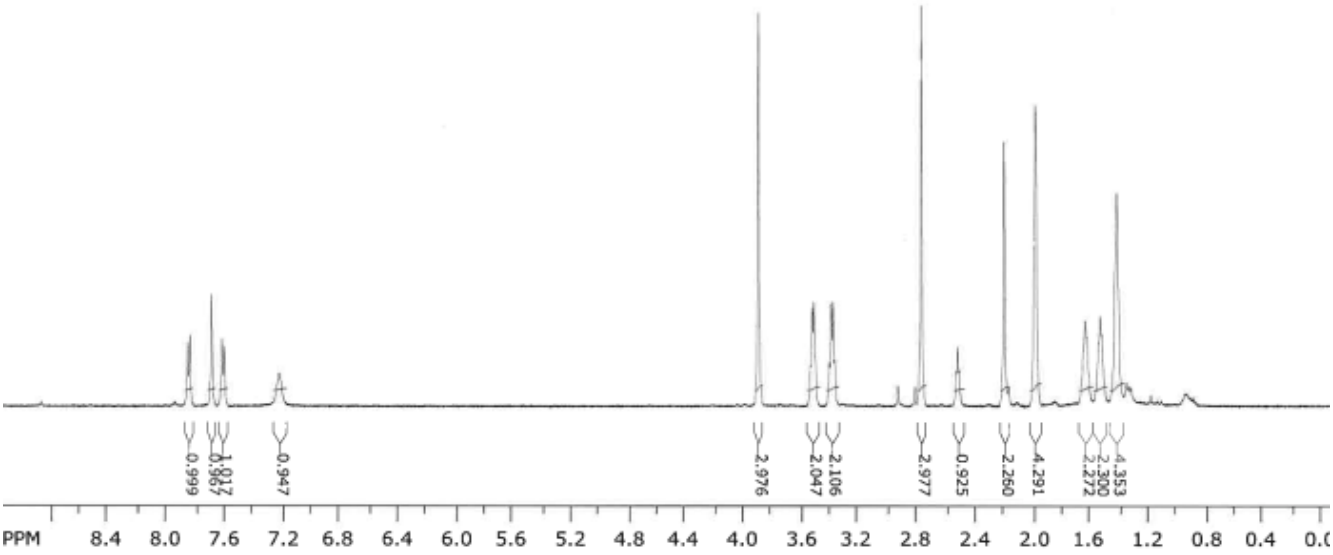


Figure S64. 500 MHz (CD_3CN) ^1H NMR spectra of compound **21b**.

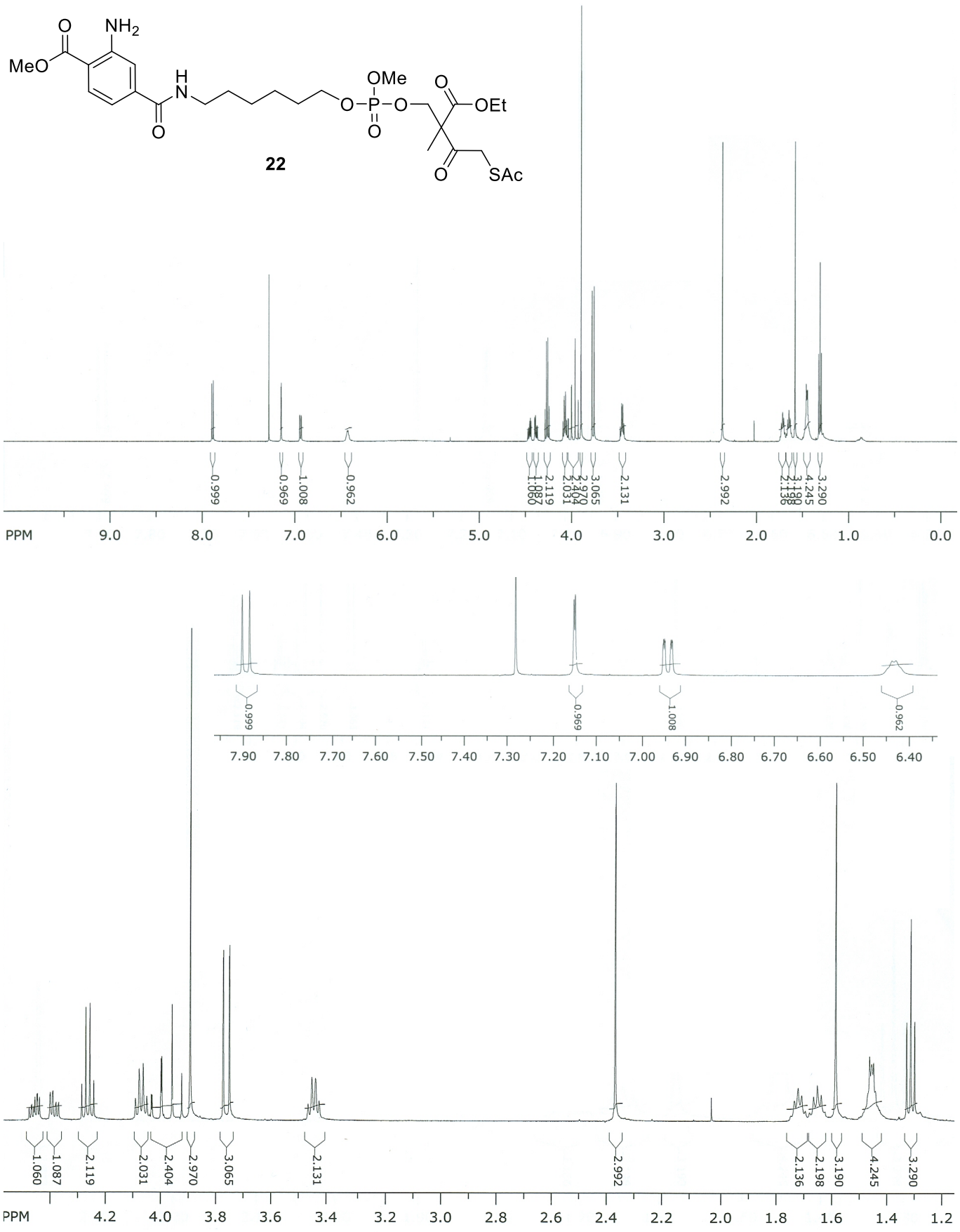


Figure S65. 500 MHz ($CDCl_3$) ^1H NMR spectra of compound **22**.

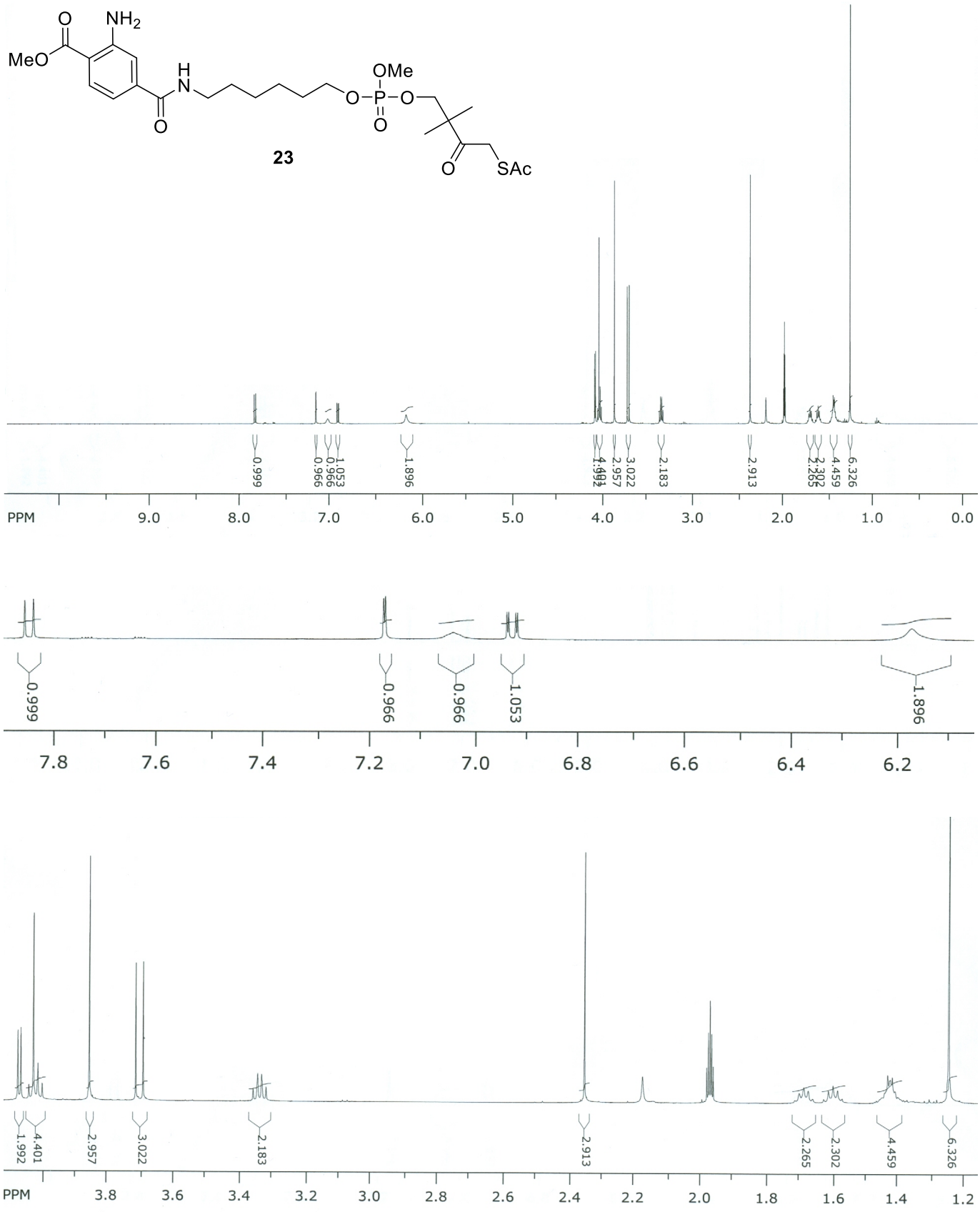
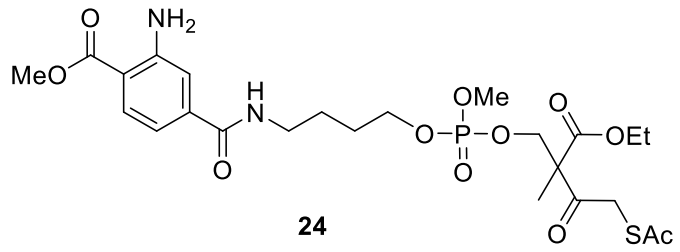


Figure S66. 500 MHz (CD_3CN) ^1H NMR spectra of compound **23**.



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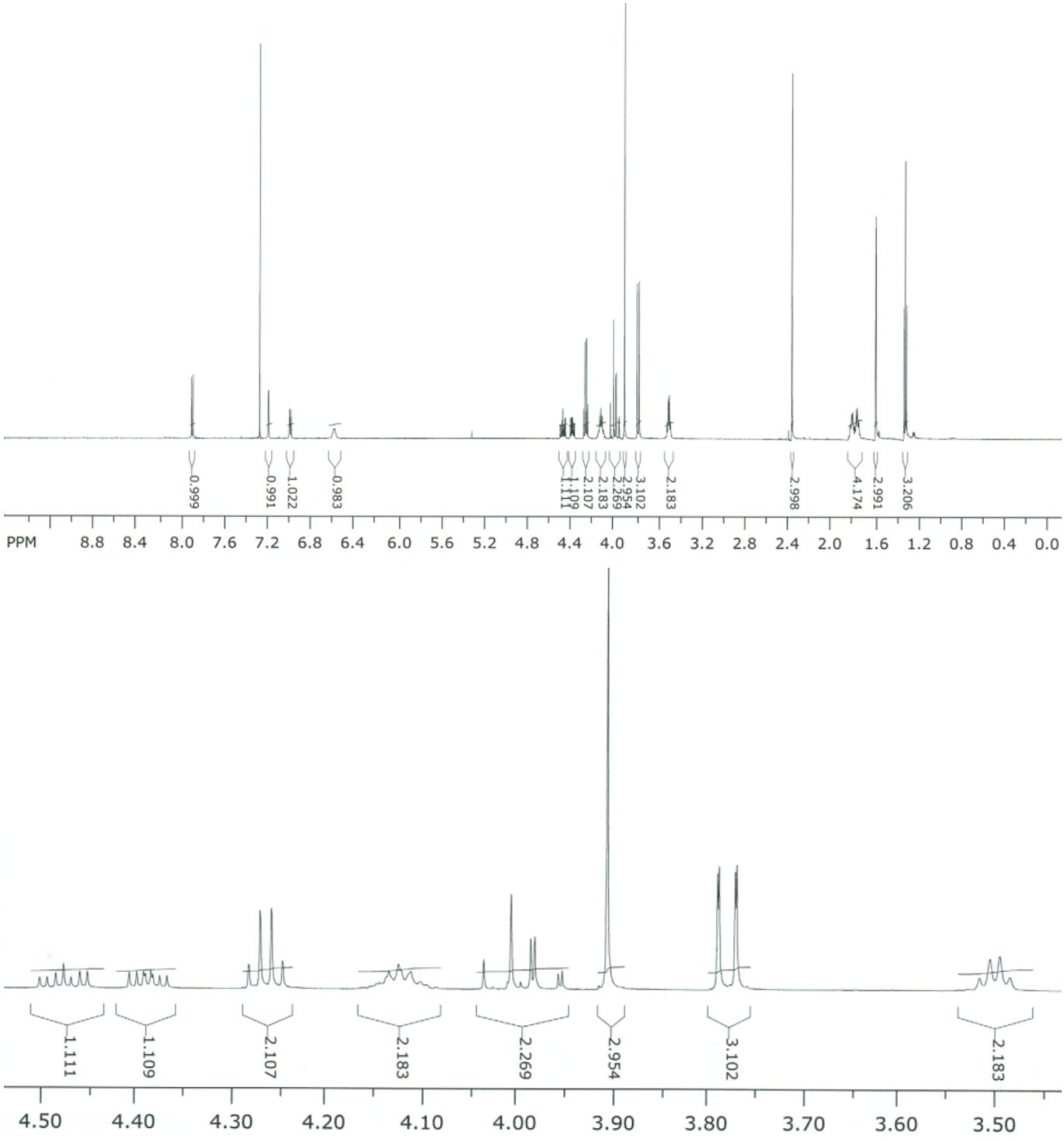

Figure S67. 500 MHz ($CDCl_3$) ^1H NMR spectra of compound **24**.

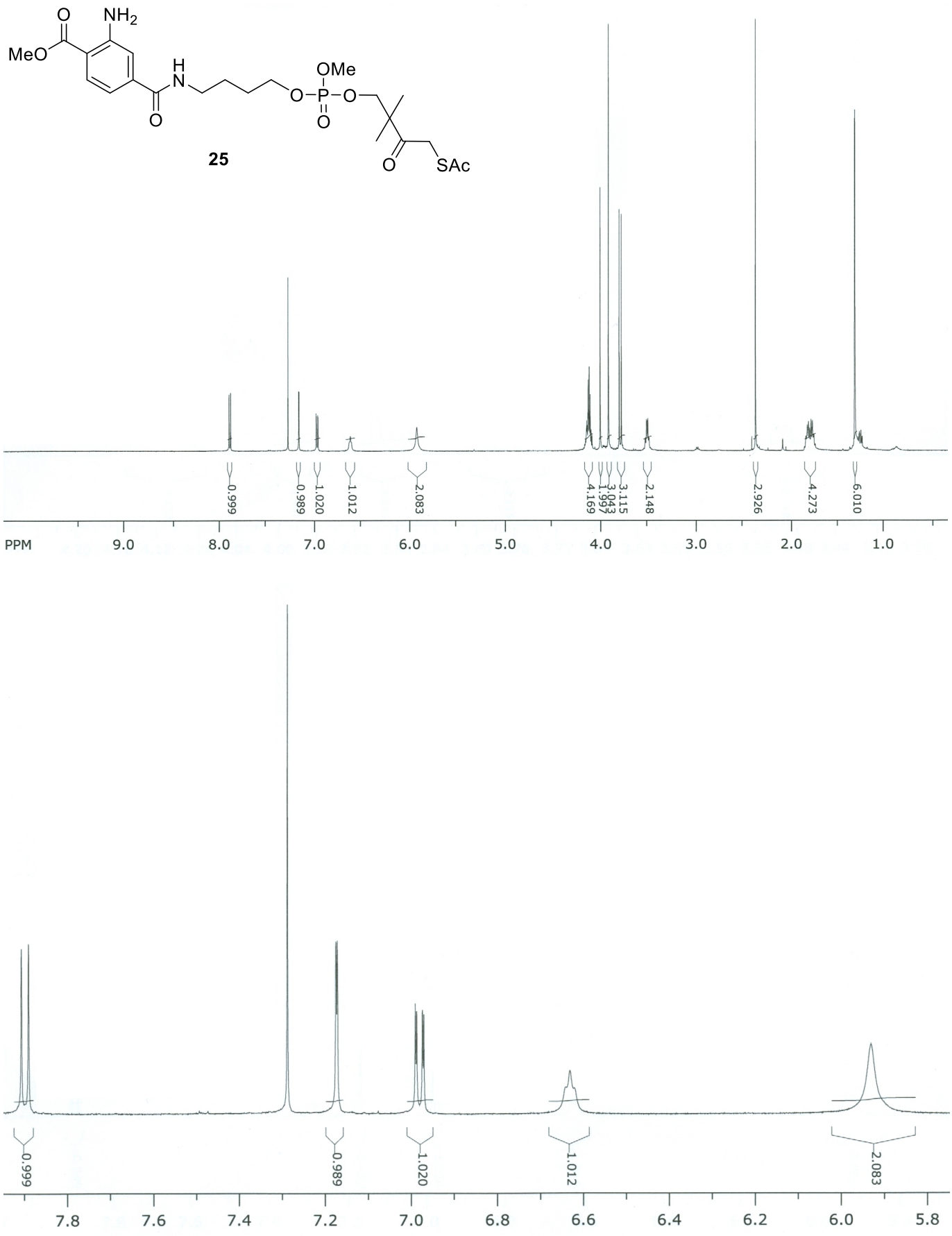

Figure S68. 500 MHz ($CDCl_3$) ^1H NMR spectra of compound **25**.

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