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Supporting Information

Ruthenium-Catalyzed Azide–Thioalkyne Cycloadditions in Aqueous Media: A Mild, Orthogonal, and Biocompatible Chemical Ligation

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Supporting Information

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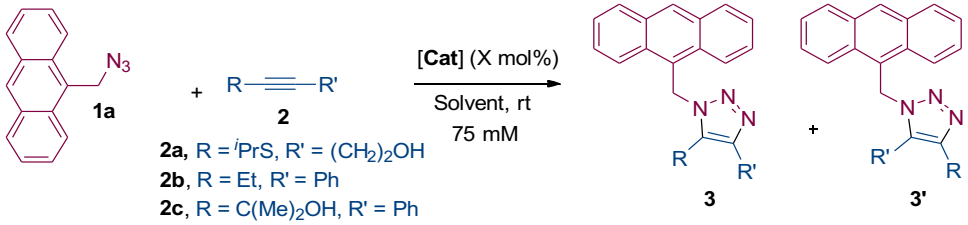
1. General procedures

Reactions were conducted in dry solvents under nitrogen atmosphere unless otherwise stated. Dry solvents were freshly distilled under argon from an appropriate drying agent before use. The abbreviation "rt" refers to reactions carried out approximately at 23 °C. Reaction mixtures were stirred using Teflon-coated magnetic stirring bars. Reaction temperatures were maintained using Thermo watch-controlled silicone oil baths. Thin-layer chromatography (TLC) was performed on silica gel plates and components were visualized by observation under UV light, and / or by treating the plates with *p*-Anisaldehyde followed by heating. Flash chromatography was carried out in silica gel unless otherwise stated. Dryings were performed with anhydrous Na_2SO_4 or $MgSO_4$. Concentration refers to the removal of volatile solvents via distillation using a Büchi rotary evaporator followed by residual solvent removal under high vacuum. NMR spectra were recorded in $CDCl_3$, CD_2Cl_2, CD_3OD or DMSO-d_6, at 300 MHz (Varian), 400 MHz (Varian) or 500 MHz (Bruker and Varian). Carbon types and structure assignments were determined from DEPT-NMR and two-dimensional experiments (HMQC and HMBC, COSY and NOESY). NMR spectra were analyzed using MestreNova© NMR data processing software (www.mestrelab.com). The following abbreviations are used to indicate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; p, pentuplet; hept, septuplet; dd, double doublet; ddd, doublet of doublet of doublets; td, triple doublet; dt, doublet of triplets; dq, doublet of quartet; dtd, doublet of triplet of doublets; m, multiplet; br, broad. Mass spectra were acquired using IT-MS Bruker AmaZon SL at CIQUS and also using electrospray ionization (ESI) and were recorded at the CACTUS facility of the University of Santiago de Compostela. UV and fluorescence spectra were acquired using Jasco V-670 spectrometer and Varian Cary Eclipse fluorescence spectrofluorometer as well as using Tecan 1000 plate reader. LC-MS analysis was carried out using Bruker Amazon IT/MS with C18 column.

9-(Azidomethyl)anthracene (**1a**),[1] (2-azidoethyl)benzene (**1c**),[2] 2-azidoethan-1-ol (**1d**),[3] 3-azido-7-hydroxy-2*H*-chromen-2-one (**1g**),[4] 3-azido-7-(diethylamino)-2*H*-chromen-2-one(**1h**),[4] 4-(phenylthio)but-3-yn-1-ol (**2d**),[5] hex-1-yn-1-yl(isopropyl)-sulfane(**2h**),[6] isopropyl(phenylethynyl)sulfane (**2e**),[7] ethynyl(phenyl)-sulfane(**2g**),[8] trimethyl((phenylthio)ethynyl)silane (**2f**),[8] ethyl *N*-(((benzyloxy)carbonyl)-*L*-tryptophyl)-*S*-(7-hydroxyhept-1-yn-1-yl)-*D*-cysteinate (**2j**),[9] (2*R*,3*R*,4*S*,5*R*,6*S*)- 2-(acetoxymethyl)-6-((7-hydroxyhept-1-yn-1-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**2k**),[9] are known compounds and were synthesized according to those previously reported procedures. Ruthenium complexes (**Ru1**)[10] and (**Ru2**)[11] were prepared according to literature procedures. Triazole (**3bi**) is a known compound and the spectra is in accordance to that previously reported in the literature.[6] Cp*Ru(cod)Cl, $[Cp^*RuCl]_4$, $[Ir(cod)Cl]_2$, $RuH_2(CO)(PPh_3)_3$ were purchased from Strem or Aldrich and used as received. All other reagents used were bought from Aldrich, Alfa Aesar, TCI, Strem or Acros and used without further purifications.

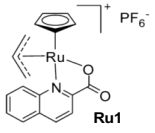
2. Details of the identification of optimal reaction conditions in water at rt. Comparison of the performances in water and in organic solvents

Table S1.

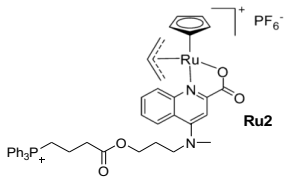


Media	entry	[Cat]	X mol%	2	1a : 2 ratio	Solvent	time (h)	Conv (%)[b]	3 : 3' ratio[b]	yield (%)[b,c]
In organic solvents	1[d]	$[Ir(cod)Cl]_2$	2.5	**2a**	1 : 1	CH_2Cl_2	15	99	**3aa** : **3aa'**, 1 : 0	78
	2[d]	Cp*Ru(cod)Cl	5	**2a**	1 : 1	CH_2Cl_2	24	99	**3aa** : **3aa'**, 18 : 1	74
	3[d]	Cp*Ru(cod)Cl	5	**2b**	1 : 1	Toluene	24	67	**3ab** : **3ab'**, 7 : 1	60
	4[d]	Cp*Ru(cod)Cl	5	**2c**	1 : 1	Toluene	24	99	**3ac** : **3ac'**, 1 : 0	85
in water	5	Cp*Ru(cod)Cl	5	**2a**	1 : 1	H_2O	24	65	**3aa** : **3aa'**, 19 : 1	58
	6	Cp*Ru(cod)Cl	5	**2b**	1 : 1	H_2O	24	60	**3ab** : **3ab'**, 5 : 1	54
	7	Cp*Ru(cod)Cl	5	**2c**	1 : 1	H_2O	24	10	-	<5
	8	$[Ir(cod)Cl]_2$	2.5	**2a**	1 : 1	H_2O	12	42	**3aa** : **3aa'**, 1 : 0	29
	9	Cp*Ru(cod)Cl	5	**2a**	2 : 1	H_2O	19	48	**3aa** : **3aa'**, 19 : 1	36
	10	Cp*Ru(cod)Cl	5	**2a**	1 : 2	H_2O	9	99	**3aa** : **3aa'**, 19 : 1	99
	11[e]	Cp*Ru(cod)Cl	5	**2a**	1 : 2	H_2O	19	78	**3aa** : **3aa'**, 19 : 1	70
	12	Cp*Ru(cod)Cl	5	**2b**	1 : 2	H_2O	9	99	**3ab** : **3ab'**, 5 : 1	95
	13	Cp*Ru(cod)Cl	5	**2c**	1 : 2	H_2O	24	10	**3ac** : **3ac'**, -	<5
	14	$[Ir(cod)Cl]_2$	2.5	**2a**	1 : 2	H_2O	24	36	**3aa** : **3aa'**, 1 : 0	20
	15	$Cp^*Ru(PPh_3)_2Cl$	5	**2a**	1 : 2	H_2O	24	47	**3aa** : **3aa'**, 23 : 1	17
	16	$RuH_2(CO)(PPh_3)_3$	5	**2a**	1 : 2	H_2O	24	0	-	0
	17	**Ru1**	5	**2c**	1 : 2	H_2O	24	60	**3aa** : **3aa'**, 1 : 0	10
	18	**Ru2**	5	**2a**	1 : 2	H_2O	24	0	-	0
	19	$[Cp^*RuCl]_4$	1.25	**2a**	1 : 2	H_2O	24	99	**3aa** : **3aa'**, 14 : 1	99
	20	$[Cp^*RuCl]_4$	1.25	**2b**	1 : 2	H_2O	24	99	**3ab** : **3ab'**, 5 : 1	84
	21[f]	Cp*Ru(cod)Cl	5	**2a** + **2b**	1 : 4	H_2O	4	99	**3aa**:**3aa'**[g], 19: 1	98[g]
in organic solvents	22[d]	Cp*Ru(cod)Cl	5	**2a**	1 : 2	CH_2Cl_2	2	99	**3aa** : **3aa'**, 17 : 1	99
	23[h]	Cp*Ru(cod)Cl	5	**2a**	1 : 2	CH_2Cl_2	2	44	**3aa** : **3aa'**, 18 : 1	37
	24[h]	Cp*Ru(cod)Cl	5	**2a**	1 : 2	CH_2Cl_2	24	99	**3aa** : **3aa'**, 17: 1	65

[a] Reaction conditions: Unless otherwise noted, **2** (1 - 2 equiv), water and **1a** (1 equiv, 75 mM) were sequentially added under air to a vial containing the catalyst [**Cat**] (x mol%) (that had been kept under N_2). Then, the vial was closed and the resulting mixture was stirred at rt, for the indicated time. [b] Determined by ^{1}H-NMR of the crude mixture using, 1,3,5-$(MeO)_3C_6H_4$ as internal standard. [c] Combined yield of **3** / **3'**. [d] Carried out under inert atmosphere (N_2) in anhydrous solvent. [e] Result when the [Ru] catalyst **is handled under air** (instead of N2), before the addition of reagents and water under air. [f] Carried out with both **2a** (2 equiv) and **2b** (2 equiv). [g] **3ab** or **3ab'** were not even observed in the ^{1}H-NMR spectra of the crude mixture. **3** : **3'** ratios and yield correspond to **3aa** / **3aa'** mixtures. [h] Reaction carried out under air.



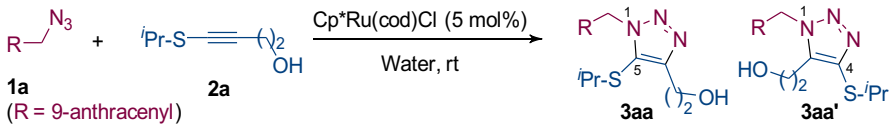
Ru1



Ru2

3. Monitoring of the RuAtAC reaction *versus* time and comparison of the reactivity of thioalkynes and regular alkynes.

Table S2. Monitoring of the RuAtAC between the azide **1a** and the thioalkyne **2a** with time [a]



Entry	Time (h)	Conversion (%)[b]	**3aa : 3aa'**[b]	Yield (%)[b,c]
1	0.5	78	22 : 1	70
2	1.5	85	19 : 1	81
3	3	90	19 : 1	85
4	9	99	19: 1	97

[a] Reaction conditions: **2** (1 - 2 equiv), water and **1a** (1 equiv, 75 mM) were sequentially added under air to a vial containing the catalyst (5 mol%), that had been kept under N_2. The vial was closed and the resulting mixture was stirred at rt, for the indicated time, followed by addition of CH_2Cl_2. The two phases were immediately shaked and separated. The organic phase was filtered through florisil (washing with EtOAc) and the solvent was evaporated. [b] Determined by ^{1}H-NMR of the crude mixture using, 1,3,5-$(MeO)_3C_6H_4$ as internal standard. [c] Combined yield of both isomers (**3aa** : **3aa'**).

The same analysis using alkyne **2b** revealed that the RuAAC in water with this regular internal alkyne is feasible but somewhat slower (10% less conversion after 30 min). Moreover, the following comparison of the RuAAC between the azide **1c** and alkynes **2e** and **2b** highlights more clearly the differences in their reactivity. Thus, as can be seen in the Figure S1, the cycloaddition between **1c** and **2e** provides a 65% yield of **3ce** after 3.5h, whereas the analog reaction with **2b** provides less than 20% yield of **3cb** after the same period of time.

<u>Comparison of the RuAtAC cycloadditions between azide **1c** and alkynes **2e** and **2b**</u>

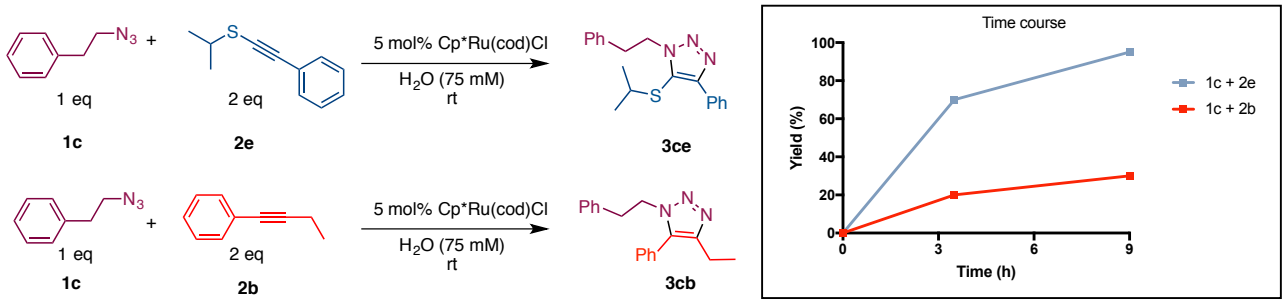
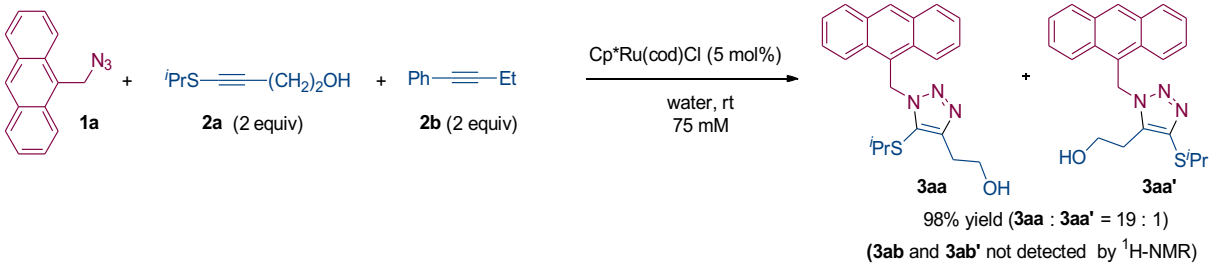


Figure. S1 Comparison of the RuAAC of **1c** with **2e** or **2b**. (yields by ^{1}H-NMR with internal standard)

According to this higher reactivity of thioalkynes, when the azide **1a** was reacted with a 1 : 1 mixture of **2a** and **2b** (2 equiv each), the triazoles **3aa** / **3aa'**, arising from the cycloaddition with the thioalkyne, were exclusively observed, in 98% yield (Table 1, entry 12, main manuscript and Scheme S1).



Scheme S1. Cross-competition experiment between **2a** and **2b**

4. NMR analysis of the interaction between alkynes and "Cp*RuCl" species in CD_2Cl_2

Analysis of the interaction between thialkyne **2a** and Cp*Ru(cod)Cl: Cp*Ru(cod)Cl (8.0 mg, 0.021 mmol, 1 eq) and 4-(isopropylthio)but-3-yn-1-ol (**2a**, 6.1 mg, 0.042 mmol, 2 eq) were successively added to a Schlenk tube with CD_2Cl_2 (0.600 mL) under N_2. The brown mixture was stirred at rt for 30 min. Then, the mixture was transferred into a nitrogen purged NMR tube and NMR analysis was taken. Release of the cod ligand from the metal center is clearly observed (Figure S2).

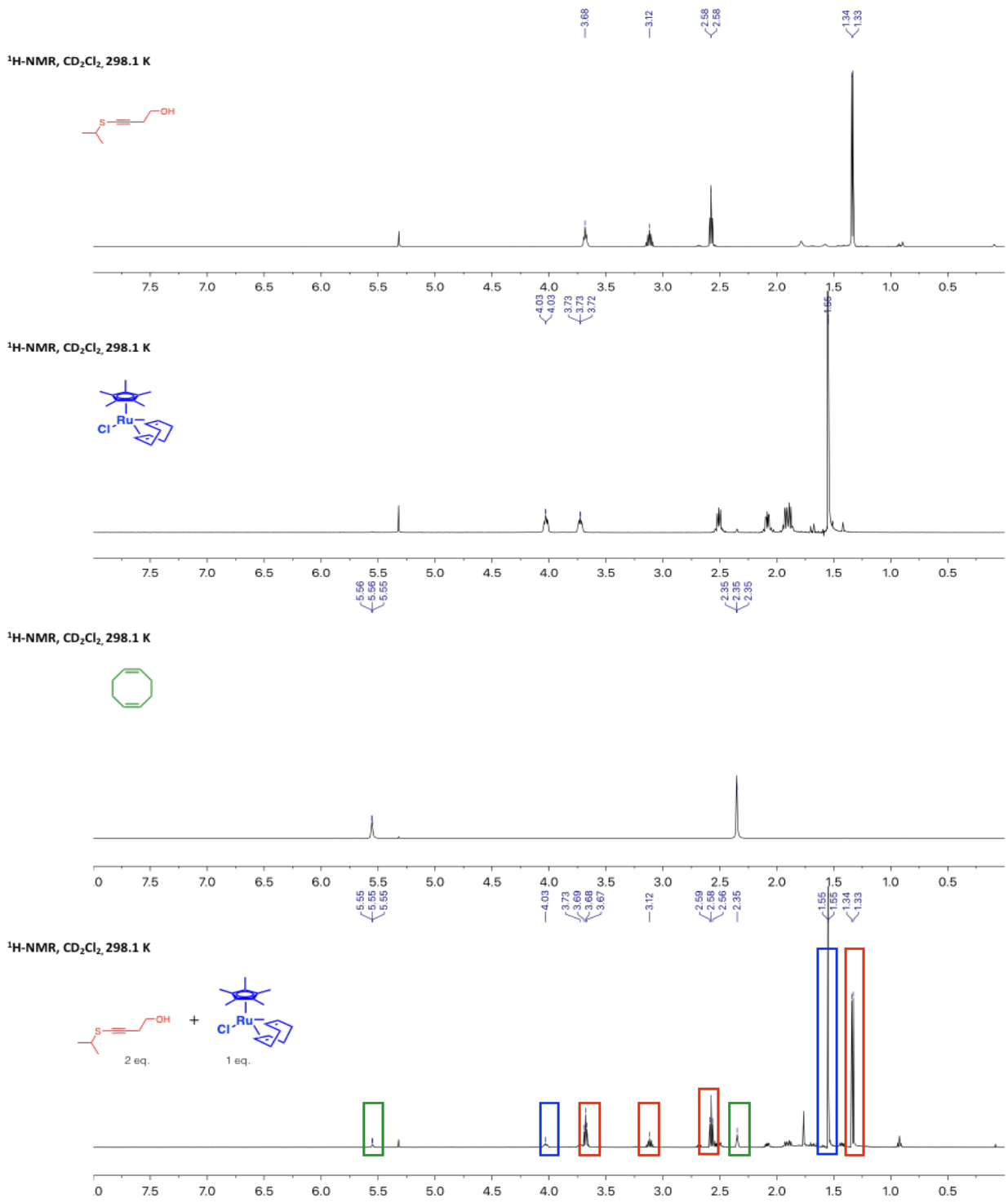


Figure S2. ^{1}H spectra for the interaction between **2a** and **Cp*Ru(cod)Cl**.

Analysis of the interaction between internal alkyne **2b** and and Cp*Ru(cod)Cl: Cp*Ru(cod)Cl (8.0 mg, 0.021 mmol, 1 eq) and 1-phenyl-1-butyne (**2b**, 5.5 mg, 0.042 mmol, 2 eq) were successively added to a Schlenk tube with CD_2Cl_2 (0.600 mL) under N_2. The brown mixture was stirred at rt for 30 min. Then, the mixture was transferred into a nitrogen purged NMR tube and NMR analysis was taken. No significant changes were observed (Figure S3).

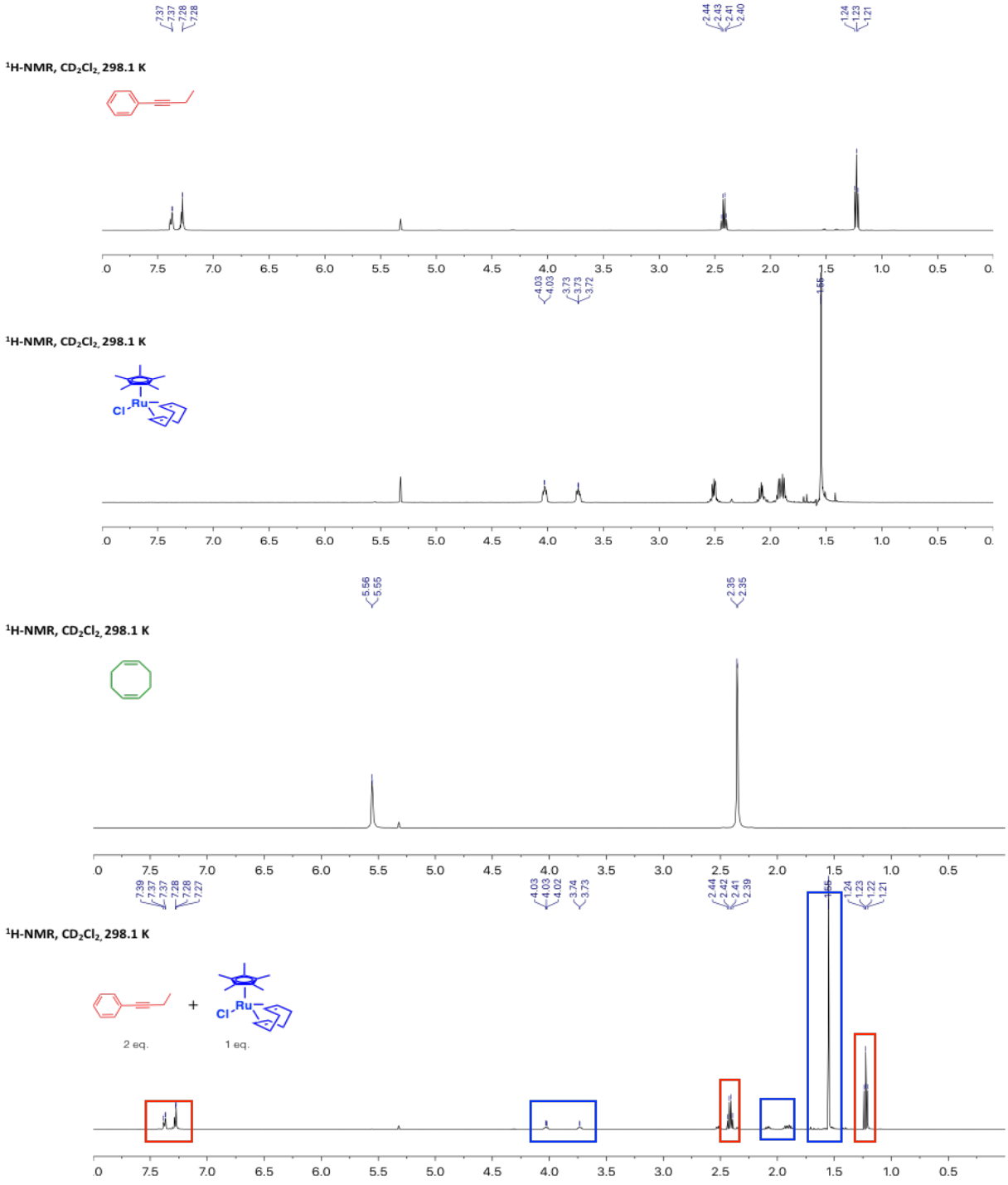


Figure S3 ^{1}H spectra for the interaction between **2b** and **Cp*Ru(cod)Cl**.

Analysis of the interaction between thioalkyne **2a** and and [Cp*RuCl]$_4$: (Isopropylthio)but-3-yn-1-ol (**2a**, 12.7 mg, 0.088 mmol, 4 eq) and [Cp*RuCl]$_4$ (23.9 mg, 0.022 mmol, 1 eq) were added to a dried Schlenk tube under nitrogen containing CD_2Cl_2 (0.560 mL). The dark brown solution turned to a cherry red colour after 30 seconds. The stirring was continued for 20 min and the crude was transferred into a N_2-purged NMR tube and NMR was recorded showing the formation of a new Ru complex (blue color boxes) that was identified as Cp*Ru(**2a**)Cl, in accordance with the precedents reported by Fürstner and coworkers (see reference 25 of the main manuscript). The most salient features of this new complex are the two ^{13}C signals at 158 and 133 ppm, that correspond to the alkynic carbons of the thioalkyne, which behaves as a 4e- donor ligands. Moreover, the ^{1}H-NMR spectra also shows a new signal for the Cp* and all the hydrogens of the thioalkyne.

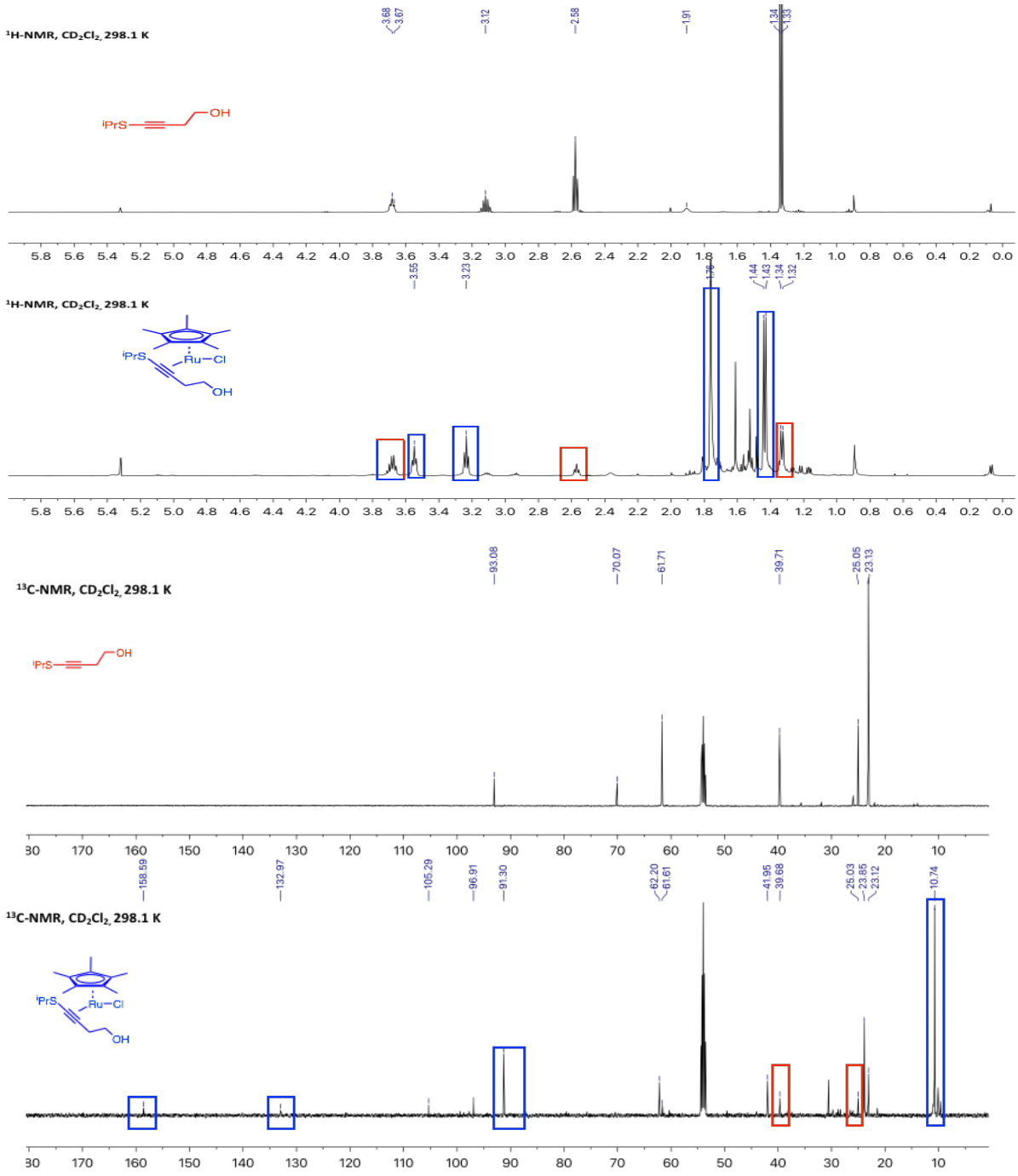


Figure S4 ^{1}H and ^{13}C NMR spectra for the interaction between **2a** and **[Cp*RuCl]$_4$**.

Analysis of the catalytic competence of [Cp*Ru(**2a**)Cl].

The prepared complex [Cp*Ru(**2a**)Cl] in $CDCl_2$ was transferred to a Schlenk tube and the solvent was removed under vacuum. Water, thioalkyne **2a** and the azide **1a** were added, and the mixture was stirred for 20h and analyzed by ^{1}H-NMR, showing the formation of a 81% yield of **3aa**/**3aa'** (19:1 ratio).

Analysis of the interaction between alkyne **2b** and and $[Cp^*RuCl]_4$: 1-Phenyl-1-butyne (**2b**, 11.5 mg, 0.088 mmol, 4 eq) CD_2Cl_2 (0.560 mL) and $[Cp^*RuCl]_4$ (23.9 mg, 0.022 mmol, 1 eq) were added to a dried Schlenk tube under N_2 containing CD_2Cl_2 (0.560 mL). The dark brown solution did not turn to a cherry red colour. The stirring was continued for 3h, the crude was transferred into a N_2-purged NMR tube, and NMR was recorded. Any new Ru complex was detected (Figure S4).

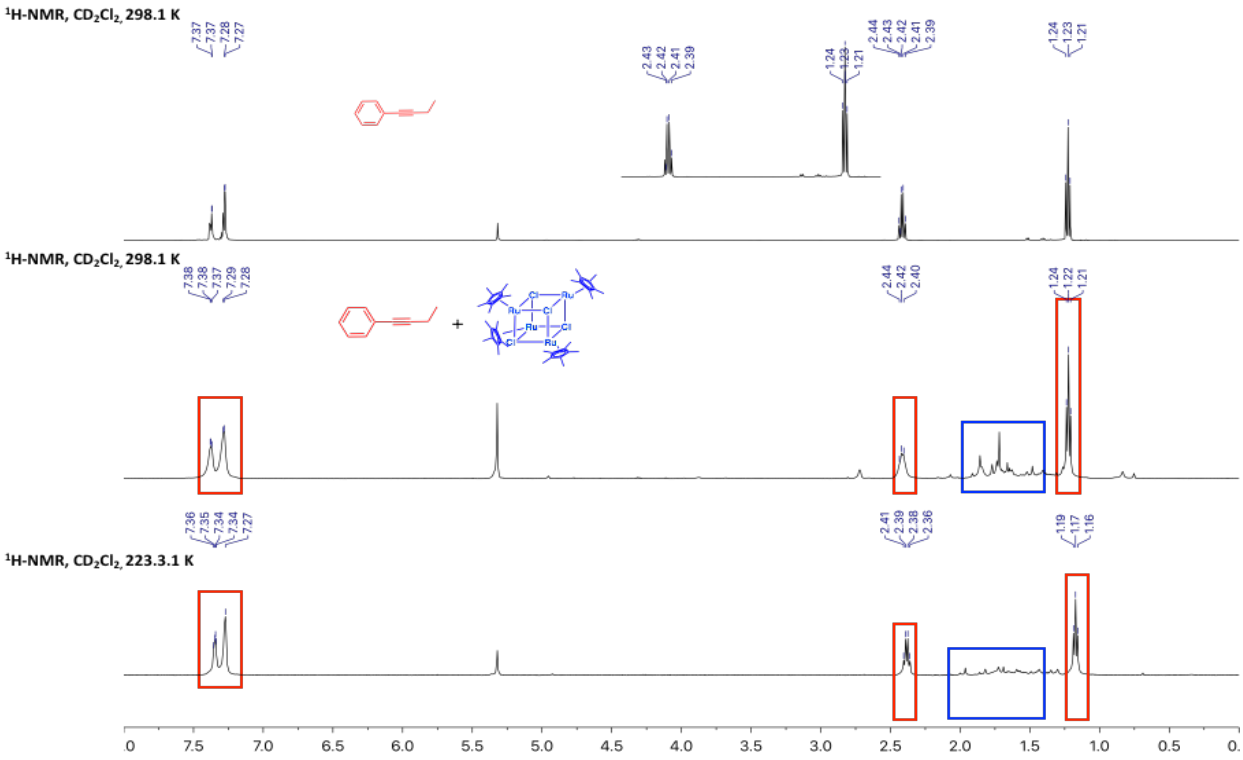
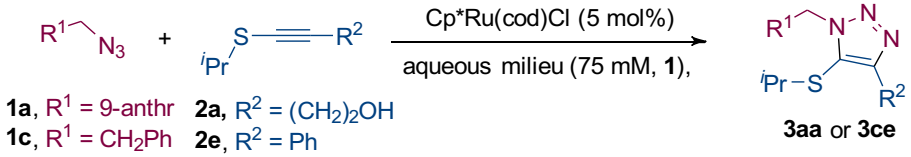


Figure S5 ^{1}H NMR spectra for the interaction between **2b** and **$[Cp^*RuCl]_4$**.

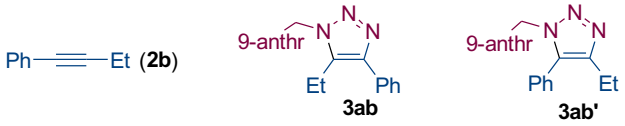
5. Details on the orthogonality and compatibility with complex aqueous mixtures

Table S3. Analysis of the biocompatibility of the method with azides **1a** and **1c** and alkynes **2a**, **2b** and **2e**.[a]



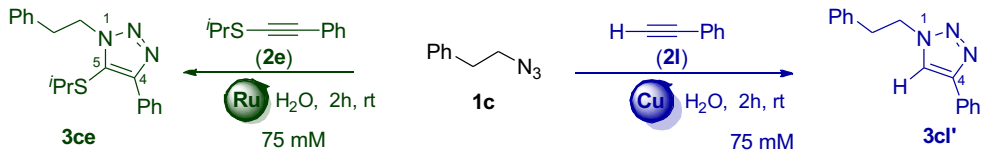
entry	1	2	milieu[b]	Conv (%)	3 : 3', ratio [c]	yield (%)[c,d]
1	**1a**	**2a**	H_2O / none	99	**3aa** : **3aa'** , 19 : 1	76 [e]
2	**1c**	**2e**	H_2O / none	99	**3ce** : **3ce'** , 19: 1	88 [e]
3	**1a**	**2a**	H_2O / Glutathione	80	**3aa** : **3aa'** , 19 : 1	60
4	**1c**	**2e**	H_2O / Glutathione	99	**3ce** : **3ce'** , 14 : 1	75
5	**1c**	**2e**	H_2O / Hist + Fmoc-ala	99	**3ce** : **3ce'** , 1 : 0	50
6	**1a**	**2a**	H_2O / Hist + Fmoc-ala	93	**3aa** : **3aa'** , 18 : 1	82
7	**1a**	**2a**	H_2O / Peptide (500μM) [f]	99	**3aa** : **3aa'** , 23 : 1	98
8	**1a**	**2a**	PBS / none	99	**3aa** : **3aa'** , 18 : 1	97
9	**1c**	**2e**	PBS / none	99	**3ce** : **3ce'** , 12 : 1	81
10	**1a**	**2a**	Cell Lysates	99	**3aa** : **3aa'** , 16 : 1	91
11	**1c**	**2e**	Cell Lysates	99	**3ce** : **3ce'** , 10 : 1	90
12	**1a**	**2a**	Cell Cultured media (DMEM)	99	**3aa** : **3aa'** , 15 : 1	84
13	**1a**	**2a**	Fetal Bovine serum (FBS)	88	**3aa** : **3aa'** , 17 : 1	77
14	**1a**	**2b**	PBS / none	94	**3ab** : **3ab'** , 5 : 1	90
15	**1a**	**2b**	Cell Lysates	85	**3ab** : **3ab'** , 5 : 1	68

[a] Reaction conditions: **2** (2 equiv) was added to a suspension of Cp*Ru(cod)Cl (5 mol%), **1** (1 equiv, 75 mM) and the additive, in the selected milieu, and the resulting heterogeneous mixture was stirred for 24 h. [b] The additives in entries 3-6 are in 20 fold excess (each one) with respect to the Ru catalyst. [c] Determined by ^{1}H-NMR of the crude mixture using, 1,3,5-$(MeO)_3C_6H_4$ as internal standard, unless otherwise noted. [d] Combined yield of **3** / **3'** unless otherwise noted. [e] Isolated yield of pure **3**. [f]Peptide (500 μM) = CYILSVQAEE-QKLISEEDLL-RKRREQLKHK-LEQLRNSSA



6. Comparison between the RuAtAC and the CuAAC in water

• **Table S4**. Comparison of the **RuAtAC** between **1c** (75 mM) and **2e** and the **CuAAC** between **1c** (75 mM) and **2l**

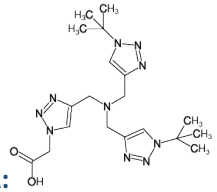


entry	**3ce** yield	**RuAtAC** conditions
1	**3ce**: 60%,	**[Ru]** (5%),
2	**3ce**: 75%,	**[Ru]** (5%), 75mM GSH (1 eq)

See below for reaction procedures.
[Ru] = Cp*Ru(cod)Cl ; GSH = glutathione

entry	**CuAAC** Conditions	**3cl'**, yield
3	**[Cu]** (5%), NaAsc (40%),	**3cl'**, 22%
4	**[Cu]** (5%), NaAsc (20%), **L** (10%),	**3cl'**, 75%
5	**[Cu]** (5%), NaAsc (20%), **L** (10%), GSH (1 eq)	**3cl'**, 0%,[a]

See below for reaction procedures.
[Cu] = $CuSO_4$; **L** = **BTTAA** ; GSH = glutathione, [a] Reaction time: 24 h

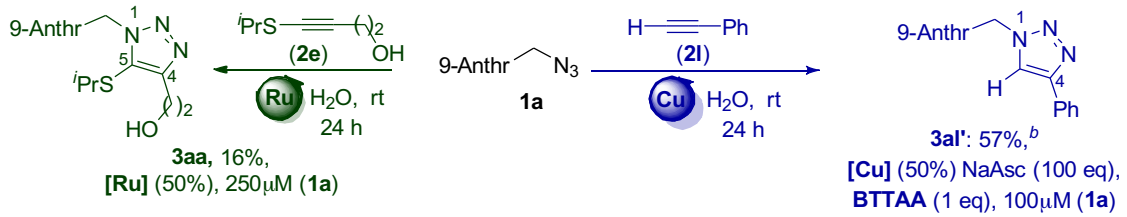


NOTE : The RuAtAC with **2e** was significantly faster than the Cu-counterpart using phenylacetylene (**2l**), $CuSO_4$ and sodium ascorbate (60% *vs* 22% yield, after 2h, entries 1 vs 3), although the CuAAC becomes faster using ligands such as BTTAA (75% yield after 2h, entry 4). As expected, the CuAAC failed with internal alkynes, including thioalkynes like **2a** and, importantly, it is essentially inhibited in the presence of thiols like glutathione (0% yield after 24 h, entry 5). In contrast, the RuAtAC works effectively even in the presence of a 20 fold excess of glutathione (75% yield, entry 2).

Procedure for the RuAtAC: Thioalkyne **2e** (2 equiv) was added to a suspension of Cp*Ru(cod)Cl (5 mol%) in H_2O. Then, azide **1c** (1 equiv, 75 mM) and the additive (none or glutathione) were added and the resulting heterogeneous mixture was stirred at rt for the indicated period of time. CH_2Cl_2 (2ml) was added, the two phases were immediately shaked and separated. The organic phase was filtered through florisil (washing with EtOAc) and the solvent was evaporated to give a crude residue that was analyzed by ^{1}H-NMR using, 1,3,5-$(MeO)_3C_6H_4$ as internal standard.

Procedure for the CuAAC: Phenylacetylene (**2l,** 2 equiv) was added to a solution of $CuSO_4$ (5 mol%), sodium ascorbate (40 mol%) in H_2O. Then, azide **1c** (1 equiv, 75 mM) and the additive (none, BTTAA or gluthatione) were added and the resulting mixture was stirred at rt for 2h. CH_2Cl_2 (2ml) was added, the two phases were immediately shaked and separated. The organic phase was filtered through florisil (washing with EtOAc) and the solvent was evaporated to give a crude residue that was analyzed by ^{1}H-NMR using, 1,3,5-$(MeO)_3C_6H_4$ as internal standard.

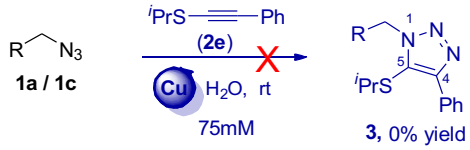
• Comparison between the **RuAtAC** (between **1a** and **2a**) and the **CuAAC** (between **1a** and **2l**) at 250 μM and 100 μM, respectively



Procedure for the RuAtAC: Thioalkyne **2a** (2 eq) was dissolved in H_2O (250 μM) followed by addition of Cp*Ru(cod)Cl (50 mol%) and **1a** (250 μM, 1 eq). The reaction mixture was stirred at rt for 24 h and analyzed by quantitative HPLC showing a 16% yield of **3aa** (see pages S12-S15 for quantification details).

Procedure for the CuAAC: **2l** (2 eq) was dissolved in H_2O (100 μM) followed by $CuSO_4$ (50 mol%), ligand BTTAA (1 eq) and **1a** (100μM, 1 eq). Finally sodium ascorbate (10 mM) was added and the reaction mixture was stirred at rt for 24 h and analyzed by quantitative HPLC (see pages S13-S15 for quantification details) showing a 57% yield of **3al'**.

• Reactivity of internal thioalkynes under CuAAC conditions. Recovery of starting materials was observed.



Quantification of RuAtAC and CuAAC yields under mM dilute conditions

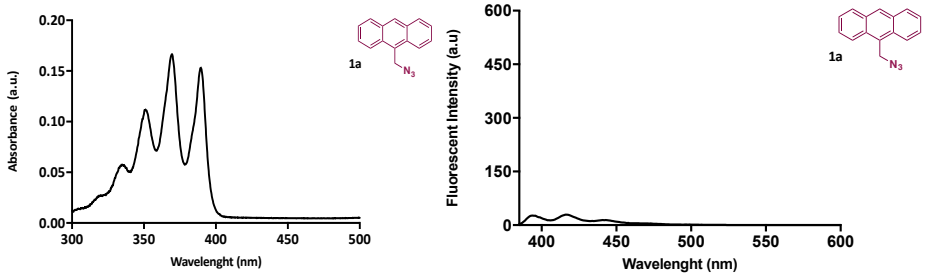


Figure S6. 1a UV spectra, 20 μM in $CHCl_3$ (left) and **1a** emission spectra, 10 μM, $CHCl_3$, λ_{exc} 370nm.

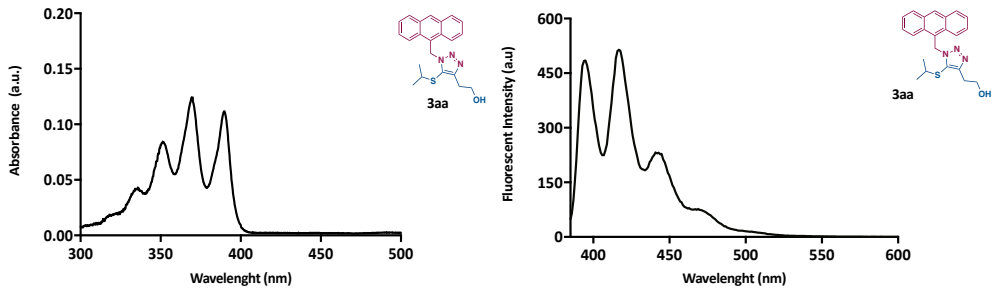
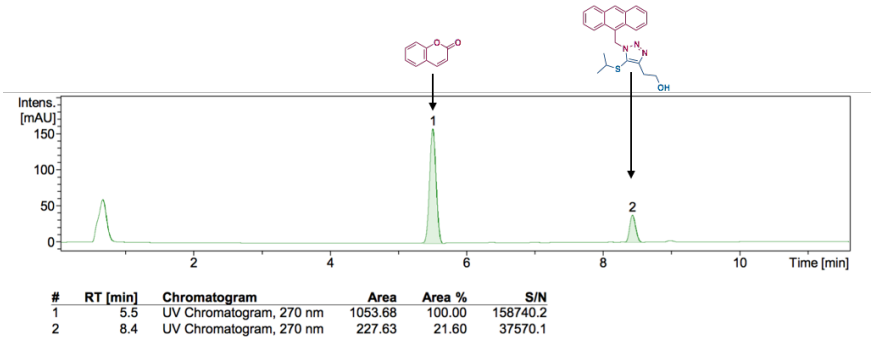


Figure S7. 3aa UV spectra, 20 μM in $CHCl_3$ (left) and **3aa** emission spectra, 10 μM, $CHCl_3$, λ_{exc}370 nm.



#	RT [min]	Chromatogram	Area	Area %	S/N
1	5.5	UV Chromatogram, 270 nm	1053.68	100.00	158740.2
2	8.4	UV Chromatogram, 270 nm	227.63	21.60	37570.1

Figure S8. HPLC chromatogram of **3aa** at 270 nm (2H-chromen-2-one was used as internal standard).

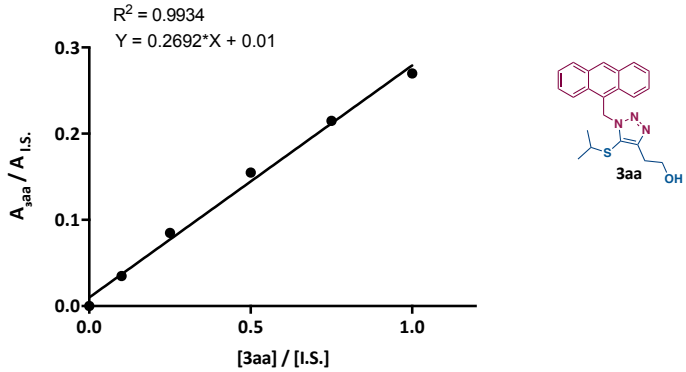
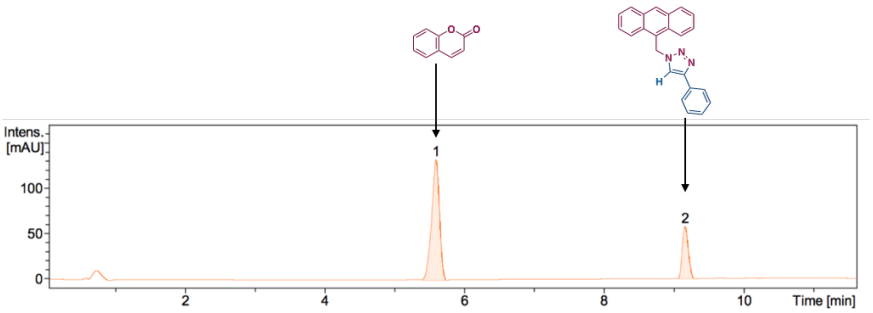


Figure S9. Calibration curve of **3aa**, average of three runs.



#	RT [min]	Chromatogram	Area	Area %	S/N
1	5.6	UV Chromatogram, 270 nm	1033.78	100.00	132920.8
2	9.2	UV Chromatogram, 270 nm	344.20	33.30	57833.5

Figure S10. HPLC chromatogram of **3al'** (60 μM) at 270 nm (2H-chromen-2-one used as internal standard).

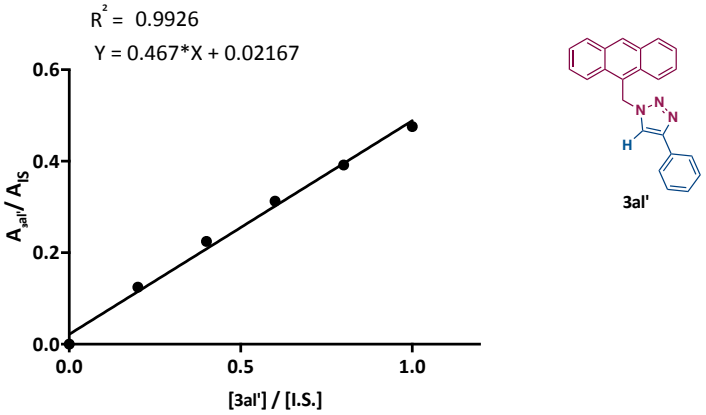
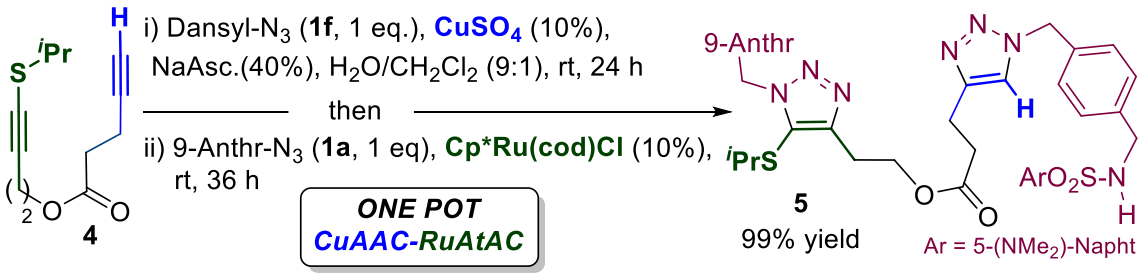


Figure S11. Calibration curve of **3al'**, average of three runs.

7. Mutual orthogonality of the RuAtAC and the CuAAC



General Procedure for the One-Pot CuAAC / RuAtAC process. Diyne **4** (24.0 mg, 0.107 mmol, 1 eq) was added to a 5 ml vial containing a H_2O/CH_2Cl_2 mixture (9:1, 1.4 mL, 75mM), followed by addition of $CuSO_4 \cdot 5 H_2O$ (2.7 mg, 0.01 mmol, 0.1 eq), sodium ascorbate (6.4 mg, 0.03 mmol, 0.3 eq) and dansyl azide **1f** (42.4 mg, 0.107, 1 eq). The reaction mixture was stirred at rt for 24 h. Upon full conversion of **1f** (as monitored by TLC), Cp*Ru(cod)Cl (4.1 mg, 0.01 mmol, 0.1 eq) was added followed by **2a** (25 mg, 0.107 mmol, 1 eq) . The reaction mixture was stirred at rt for 36 h, CH_2Cl_2 (5 mL) was added, and the reaction mixture was filtered through celite, eluted with EtOAc (5 x 1 mL) and concentrated. Purification by column chromatography yielded 2-(1-(anthracen-9-ylmethyl)-5-(isopropylthio)-1*H*-1,2,3-triazol-4-yl)ethyl3-(1-(4-(((5-(dimethylamino) naphtha-lene) -1-sulfonamido)methyl)benzyl)-1*H*-1,2,3-triazol-4-yl)propanoate (**5**) in 99% yield. Pale yellow solid. $\mathbf{R_f}$ = 0.32 (Hexanes:EtOAc 2:8).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 – 8.47 (m, 2H), 8.42 (d, *J* = 8.3 Hz, 2H), 8.26 (d, *J* = 8.6 Hz, 1H), 8.21 (d, *J* = 7.3 Hz, 1H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.56 – 7.47 (m, 2H), 7.45 (t, *J* = 7.9 Hz, 5H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.00 (s, 1H), 6.96 (d, *J* = 7.9 Hz, 2H), 6.86 (d, *J* = 8.1 Hz, 2H), 6.48 (s, 2H), 5.51 (t, *J* = 6.2 Hz, 1H), 5.16 (s, 2H), 4.29 (t, *J* = 6.7 Hz, 2H), 3.98 (d, *J* = 6.1 Hz, 2H), 2.98 (t, *J* = 6.7 Hz, 2H), 2.92 – 2.85 (m, 8H), 2.68 – 2.60 (m, 1H), 2.58 (t, *J* = 7.0 Hz, 2H), 1.06 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.53 (C), 151.97 (C), 148.90 (C), 146.74 (C), 136.99 (C), 134.82 (C), 134.36 (C), 131.47 (CH), 131.12 (CH), 130.54 (CH), 129.91 (C), 129.79 (CH), 129.70 (C), 129.62 (CH), 129.29 (CH), 128.54 (CH), 128.47 (CH), 128.16 (CH), 127.01 (CH), 126.76 (C), 125.27 (CH), 124.92 (C), 124.14 (CH), 123.28 (CH), 121.30 (CH), 118.91 (CH), 115.32 (CH), 63.13 (CH_2), 53.43 (CH_2), 46.90 (CH_2), 45.55 (CH_3), 45.46 (CH_2), 41.03 (CH), 33.72 (CH_2), 25.14 (CH_2), 23.01 (CH_3), 21.08 (CH_2). **LRMS** (*m/z, ESI*): 875.31 $(M+Na)^+$, 853.33 $(M+H)^+$, 701.20. **HRMS-ESI** Calculated for $C_{47}H_{48}N_8NaO_4S_2$: 875.3132, found 875.3127.

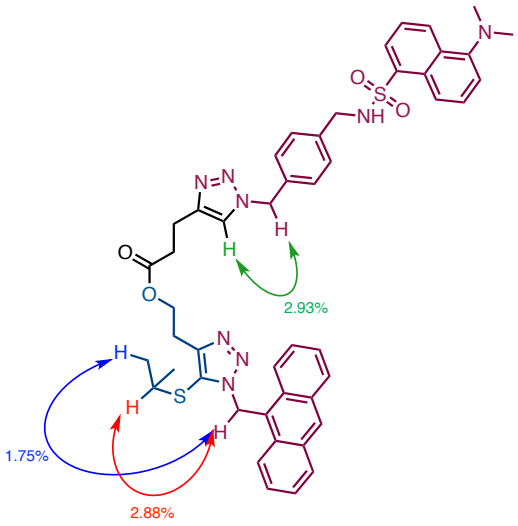
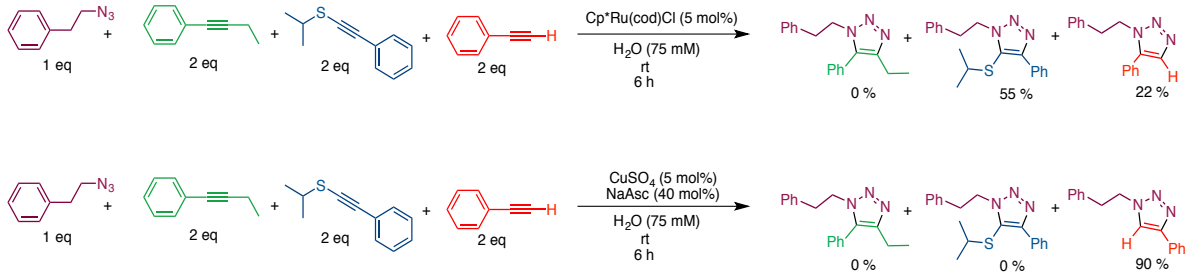


Figure S12. Representative nOe's observed for compound **5**.

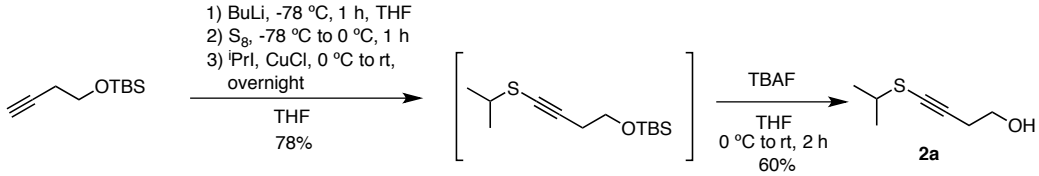
Additional experiment to highlight the mutual orthogonality of the CuAAC and RuAAC in water.



Scheme S2. Competitive experiment between RuAtAC and CuAAC.

8. Synthesis and characterization data of new thioalkynes and organic azides

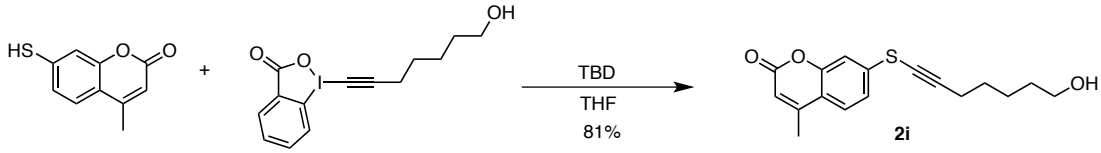
Synthesis of 4-(isopropylthio)but-3-yn-1-ol (**2a**)[7]



^{n}BuLi (2.5M in THF, 4.8 mL, 11.93 mmol, 1.1 eq) was slowly added to a THF solution (54 mL) of (but-3-yn-1-yloxy)(*tert*-butyl)dimethylsilane[12] (2.0 g, 10.85 mmol, 1 eq), at -78 ˚C, under nitrogen. The colourless mixture was stirred at the same temperature for 1 h, then sulphur powder (0.35 g, 1.36 mmol, 0.125 eq) was added portionless and the solution became immediately red. The reaction mixture was stirred for 30 min at -78 ˚C and for 30 min at 0 ˚C until complete consumption of sulphur (the solution turns brown or dark red 1 h after sulphur addition). At 0 ˚C, 2-iodopropane (1.083 mL, 10.849 mmol, 1 eq) was added via syringe followed by CuCl (0.054 g, 0.542 mmol, 0.05 eq) and the reaction mixture was left under stirring at rt, under nitrogen, overnight. Completion of the reaction was determined by TLC (Hexanes:Et_2O 95:5). NH_4Cl (sat) (70 mL) was then added, the aqueous phase was extracted with Et_2O(3 x 50 mL), washed with brine, dried and concentrated in vacuum. The crude residue was used for the next step without further

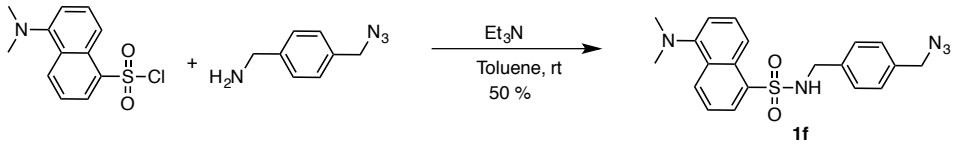
purifications. TBAF (1M in THF, 3.034 g, 11.61 mmol, 1.5 eq) was added dropwise to a solution of tert-butyl((4-(isopropylthio)but-3-yn-1-yl)oxy)dimethylsilane (2.000 g, 7.737 mmol, 1 eq) in THF (39 mL) at 0 ˚C. The reaction mixture was stirred for 2 h at rt, the solvent was concentrated and the crude was adsorbed onto silica gel and purified by flash column chromatography (Hexanes:Et_2O from 65:35 to 55:45) to afford the product 4-(isopropylthio)but-3-yn-1-ol (**2a)**, as a yellow oil (0.67 g, 4.64 mmol, 60% yield). $\mathbf{R_f}$ = 0.23 (Hexanes:Et_2O 6:4). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.72 (t, *J* = 6.2 Hz, 2H), 3.13 (hept, *J* = 6.7 Hz, 1H), 2.61 (t, *J* = 6.2 Hz, 2H), 2.25 (s, br, OH, 1H), 1.35 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 92.33 (C), 70.03 (C), 61.23 (CH_2), 39.17 (CH), 24.59 (CH_2), 22.87 (CH_3).

7-((7-hydroxyhept-1-yn-1-yl)thio)-4-methyl-2*H*-chromen-2-one (2i)[13]



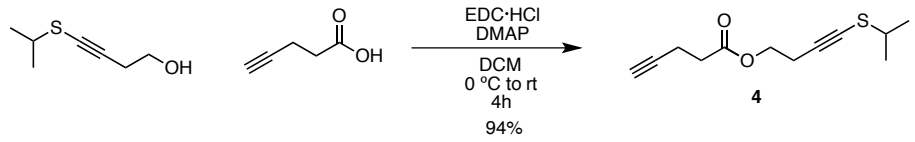
7-Mercapto-4-methyl-2*H*-chromen-2-one (0.150 g, 0.780 mmol, 1 eq) and triazabicyclodecene (TBD, 0.108 g, 0.780 mmol, 1 eq) were dissolved in THF (10 mL). After stirring for 5 min at rt, the EBX reagent (0.307 g, 0.858 mmol, 1.1 eq) was added as a solid in one portion. The resulting reaction mixture was stirred at rt for 5 min. Upon completion, the mixture was concentrated in vacuum and the residue was purified by flash column chromatography using Hexanes:EtOAc (from 4:6 to 3:7) to afford the product 7-((7-hydroxyhept-1-yn-1-yl)thio)-4-methyl-2*H*-chromen-2-one (**2i**) as white solid (0.190 g, 0.628 mmol, 81% yield). $\mathbf{R_f}$ = 0.42 (Hexanes:EtOAc 3:7) **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.0 Hz, 1H), 7.19 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.22 (s, 1H), 3.68 (t, *J* = 6.3 Hz, 2H), 2.51 (t, *J* = 6.8 Hz, 2H), 2.40 (s, 3H), 1.86 (s, 1H), 1.71 – 1.60 (m, 4H), 1.60 – 1.50 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 160.63 (C), 154.04 (C), 152.28 (C), 140.08 (C), 124.92 (CH), 121.08 (CH), 118.02 (C), 114.32 (CH), 113.33 (CH), 102.60 (C), 63.03 (C), 62.74 (CH_2), 32.32 (CH_2), 28.41 (CH_2), 25.29 (CH_2), 20.42 (CH_2), 18.74 (CH_3). **LRMS** (*m/z, ESI*): 325.09 $(M+Na)^+$, 303.10 $(M+H)^+$, 285.09, 231.05. **HRMS-ESI** Calculated for $C_{17}H_{19}O_3S$: 303.1049, found 303.1047.

***N*-(4-(Azidomethyl)benzyl)-5-(dimethylamino)naphthalene-1-sulfonamide (1f)**



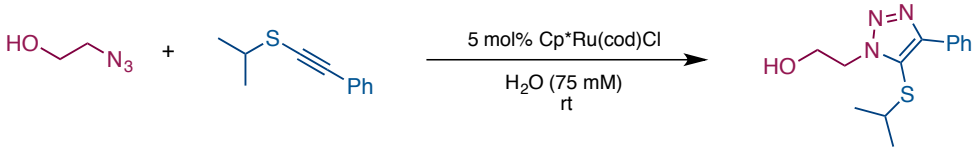
A solution of Et_3N (1.029 mL, 7.399 mmol, 2.4 eq) and (4-(azidomethyl)phenyl)methanamine[14] (0.500 g, 3.083 mmol, 1 eq) in toluene (5 mL) was added at once to a stirring suspension of dansyl chloride (0.997 g, 3.699 mmol, 1.2 eq) in toluene (9 mL). The resulting yellow mixture was stirred at rt for 15 h. Next, the reaction mixture was concentrated in vacuum and the crude oil was adsorbed onto silica and purified by flash column chromatography using Hexanes:EtOAc (from 8:2 to 7:3) to obtain the product *N*-(4-(azidomethyl)benzyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**1f**) as a solid (0.615 g, 1.55 mmol, 50% yield). $\mathbf{R_f}$ = 0.60 (Hexanes:EtOAc 6:4). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 8.5, 1H), 8.33 – 8.22 (m, 2H), 7.60 – 7.46 (m, 2H), 7.19 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.15 – 7.05 (m, 4H), 5.00 (t, *J* = 6.2 Hz, 1H), 4.24 (s, 2H), 4.08 (d, *J* = 6.2 Hz, 2H), 2.90 (s, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 152.17 (C), 136.53 (C), 135.06 (C), 134.62 (C), 130.75 (CH), 130.01 (CH), 129.99 (C), 129.71 (C), 128.64 (CH), 128.44 (CH), 128.37 (CH), 123.31 (CH), 118.72 (CH), 115.35 (CH), 54.43 (CH_2), 47.09 (CH_2), 45.55 (CH_3). **LRMS** (*m/z, ESI*): 418.13 $(M+Na)^+$, 396,15 $(M+H)^+$, 172.07, 157.08, 133.08. **HRMS** Calculated for $C_{20}H_{22}N_5O_2S$: 396.1489, found 396.1489.

4-(Isopropylthio)but-3-yn-1-yl pent-4-ynoate (4)



EDC (0.299 g, 1.56 mmol, 1.5 eq) and DMAP (0.318 g, 0.26 mmol, 0.25 eq) were added at 0°C, under nitrogen, to a solution of pent-4-ynoic acid (0.118 g, 1.144 mmol, 1.1 eq) in CH_2Cl_2 (10 mL). The white suspension was stirred for 15 min at 0 ˚C and 4-(isopropylthio)but-3-yn-1-ol (0.150 g, 1.04 mmol, 1 eq) was added at the same temperature. The reaction mixture was stirred at rt until complete consumption of the alcohol as monitored by TLC (4 h). HCl (20 mL, 0.5 M) was added and the organic phase was extracted with CH_2Cl_2 (3 x 10 mL). The organic phase was washed with $NaHCO_3$ (sat.) and brine. The combined organic phases were dried, filtered and concentrated in vacuum onto silica. Purification by flash column chromatography using Hexane : EtOAc (8:2) afforded the product 4-(isopropylthio)but-3-yn-1-yl pent-4-ynoate (**4**), as a yellow oil (0.219 g, 0.975 mmol, 94% yield). $\mathbf{R_f}$ = 0.70 (Hexane:EtOAc 7:3). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.21 (t, *J* = 6.9 Hz, 2H), 3.12 (hept, *J* = 6.8 Hz, 1H), 2.68 (t, *J* = 6.9 Hz, 2H), 2.65 – 2.44 (m, 4H), 1.98 (t, *J* = 2.5 Hz, 1H), 1.35 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.27 (C), 91.08 (C), 82.25 (C), 69.73 (C), 69.11 (C), 62.55 (CH_2), 38.91 (CH), 33.14 (CH_2), 22.71(CH), 20.53 (CH_2), 14.24 (CH_2). **LRMS** (*m/z, ESI*): 247.07 $(M+Na)^+$, 225.09 $(M+H)^+$, 162.09, 117.04. **HRMS-ESI** Calculated for $C_{12}H_{16}NaO_2S$: 247.0763, found 247.0761.

9. General procedure for the RuAtAC in water and characterization data of new triazoles (Exemplified for the preparation of **3de** from azide **1d** and thioalkyne **2e**)



Cp*Ru(cod)Cl (4.4 mg, 0.011 mmol, 0.05 eq) was placed under N_2 in a 5 mL screw cap vial equipped with a magnetic stirring bar. Then, the vial was open to air and water (3.0 mL, 75 mM), isopropyl(phenylethynyl)sulfane (**2e**, 81.0 mg, 0.460 mmol, 2 eq) and 2-azidoethan-1-ol (**1d**, 20.0 mg, 0.230 mmol, 1 eq), were successively added (Note: during addition of water, thioalkyne and azide no efforts to exclude air were done). The vial was closed and the brown heterogeneous mixture was stirred at rt at 500 rpm and monitored by TLC (using a 8:2 Hexanes : EtOAc mixture), to follow azide conversion). Upon completion (17 h), CH_2Cl_2 (10 mL) was added and the mixture was immediately transferred to a separating funnel containing water (10 mL) The aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic phases were dried, concentrated and purified by column chromatography (Hexanes : EtOAc from 6:4 to 5:5) to yield 5-(isopropylthio)-1-phenethyl-4-phenyl-1*H*-1,2,3-triazole (**3de**), as a brown oil (55.7 mg, 0.210 mmol, 92%). $\mathbf{R_f}$ = 0.25 (Hexanes : EtOAc 1:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.12 (d, *J* = 7.2 Hz, 1H), 7.43 (t, *J* = 7.3 Hz, 2H), 7.37 (d, *J* = 7.1 Hz, 1H), 4.58 (t, *J* = 5.1 Hz, 2H), 4.16 (t, *J* = 5.1 Hz, 2H), 3.12 (hept, *J* = 6.7 Hz, 1H), 1.11 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.12 (C), 130.71 (C), 128.64 (CH), 128.56 (CH), 127.12 (CH), 126.28 (C), 61.28 (CH_2), 50.46 (CH_2), 41.05 (CH), 23.16 (CH_3). **LRMS** (*m/z, ESI*): 286.10 $(M+Na)^+$, 264.12 $(M+H)^+$, 222.07, 178.04, 149.03, 116.05. **HRMS-ESI** Calculated for $C_{13}H_{18}N_3OS$: 264.1165, found 264.1165.

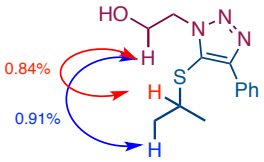
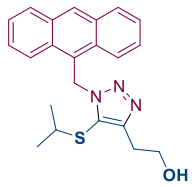


Figure S13. Significant nOe's observed for compound **3de**.

2-(1-(anthracen-9-ylmethyl)-5-(isopropylthio)-1H-1,2,3-triazol-4-yl)ethan-1-ol (3aa).



R_f = 0.30 (Hexanes:EtOAc 3:7). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.46 (dt, *J* = 9.0, 1.0 Hz, 2H), 8.03 (ddd, *J* = 8.4, 1.4, 0.7 Hz, 2H), 7.57 (ddd, *J* = 8.9, 6.6, 1.3 Hz, 2H), 7.49 (ddd, *J* = 8.4, 6.5, 1.1 Hz, 2H), 6.51 (s, 2H), 3.94 (q, *J* = 5.0 Hz, 2H), 3.15 (s, 1H), 2.93 (t, *J* = 5.8 Hz, 2H), 2.58 (hept, *J* = 6.6 Hz, 1H), 1.04 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 151.07 (C), 131.53 (C), 131.17 (C), 129.73 (CH), 129.36 (CH), 127.03 (CH), 126.28 (C), 125.25 (CH), 124.77 (C), 124.17 (CH), 61.47 (CH_2), 45.52 (CH_2), 41.00 (CH), 28.42 (CH_2), 23.02 (CH_3). **LRMS** (m/z, *ESI*): 400.15 $(M+Na)^+$, 191.10. **HRMS-ESI** Calculated for $C_{22}H_{24}N_3OS$: 378.1635, found 378.1634.

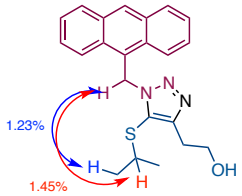
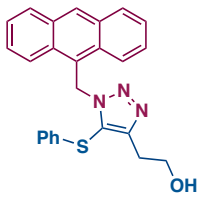


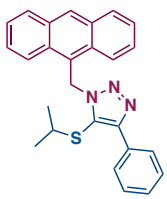
Figure S14. Significant nOe's observed for compound **3aa**.

2-(1-(Anthracen-9-ylmethyl)-5-(phenylthio)-1*H*-1,2,3-triazol-4-yl)ethan-1-ol (3ad)



Pale yellow solid. **R_f** = 0.29 (Hexanes:EtOAc 1:1). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.27 (d, *J* = 7.4 Hz, 2H), 7.94 (d, *J* = 9.6 Hz, 2H), 7.51 – 7.40 (m, 4H), 7.14 – 7.00 (m, 3H), 6.64 (d, *J* = 6.6 Hz, 2H), 6.43 (s, 2H), 3.93 (t, *J* = 5.8 Hz, 2H), 2.92 (t, *J* = 5.8 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 151.56 (C), 133.42 (C), 131.39 (C), 131.14 (C), 129.83 (CH), 129.31 (CH), 126.95 (CH), 126.57 (CH), 126.17 (CH), 125.04 (CH), 123.85 (CH), 123.69 (C), 61.32 (CH_2), 45.97 (CH_2), 28.25 (CH_2). **LRMS** (*m/z, ESI*): 434.13 $(M+Na)^+$, 191.08, 165.07, 152.06. **HRMS-ESI** Calculated for $C_{25}H_{21}N_3NaOS$: 434.1298, found 434.1299.

1-(Anthracen-9-ylmethyl)-5-(isopropylthio)-4-phenyl-1*H*-1,2,3-triazole (3ae)



Pale yellow solid. **R_f** = 0.47 (Hexanes:EtOAc 8:2). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.53 (s, 2H), 8.51 (s, 1H), 8.18 (d, *J* = 7.1 Hz, 2H), 8.04 (d, *J* = 8.5 Hz, 2H), 7.63 – 7.54 (m, 2H), 7.52 – 7.47 (m, 2H), 7.46 – 7.37 (m, 2H), 7.38 – 7.29 (m, 1H), 6.55 (s, 2H), 2.82 (hept, *J* = 6.7 Hz, 1H), 1.02 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.28 (C), 131.55 (C) 131.24 (C), 131.10 (C), 129.64 (CH), 129.33 (CH), 128.51 (CH), 128.35 (CH), 127.22 (CH), 126.92 (CH), 125.34 (C), 125.20 (CH), 124.99 (C), 124.34 (CH), 45.37 (CH_2), 41.19 (CH), 22.96 (CH_3). **LRMS** (*m/z, ESI*): 432.15 $(M+Na)^+$, 189.07, 165.07, 152.06. **HRMS-ESI** Calculated for $C_{26}H_{23}N_3NaS$: 432.1505, found 432.1506.

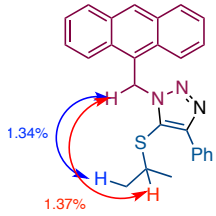
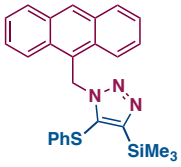


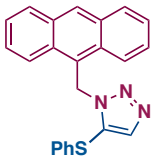
Figure S15. Significant nOe's observed for compound **3ae**.

1-(Anthracen-9-ylmethyl)-5-(phenylthio)-4-(trimethylsilyl)-1*H*-1,2,3-triazole (3ag)



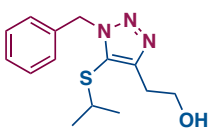
Pale yellow solid. **R_f** = 0.60 (Hexanes:EtOAc 7:3). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.34 (s, 2H), 8.31 (s, 1H), 7.97 – 7.87 (m, 2H), 7.52 – 7.36 (m, 4H), 7.01 (dt, *J* = 8.8, 6.7 Hz, 3H), 6.55 (d, *J* = 7.6 Hz, 2H), 6.41 (s, 2H), 0.28 (s, 9H).**^{13}C NMR** (75 MHz, $CDCl_3$) δ 153.50 (C), 134.55 (C), 131.40 (C), 131.25 (C), 131.15 (C), 129.66 (CH), 129.21 (CH), 129.07 (CH), 126.75 (CH), 126.06 (CH), 125.65 (CH), 124.96 (CH), 124.17 (CH), 45.37 (CH_2), -1.12 (CH_3). **LRMS** (*m/z, ESI*): 462.14 $(M+Na)^+$, 189.07, 165.07, 152.06. **HRMS-ESI** Calculated for $C_{26}H_{25}N_3NaSSi$: 462.1431, found 462.1433.

1-(Anthracen-9-ylmethyl)-5-(phenylthio)-1*H*-1,2,3-triazole (3ah)



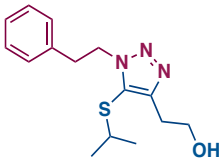
White solid. **R_f** = 0.55 (Hexanes:EtOAc 6:4). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.31 – 8.20 (m, 2H), 8.01 – 7.88 (m, 2H), 7.86 (s, 1H), 7.53 – 7.37 (m, 4H), 7.27 – 7.04 (m, 3H), 6.88 – 6.77 (m, 2H), 6.42 (s, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 140.64 (CH), 133.21 (C), 131.46 (C), 131.18 (C), 129.87 (CH), 129.39 (CH), 129.37 (CH), 127.54 (CH), 127.07 (CH), 127.00 (CH), 125.09 (CH), 123.85 (CH), 123.82 (C), 119.74 (C), 45.66 (CH_2). **LRMS** (*m/z, ESI*): 390.10 $(M+Na)^+$, 191.08. **HRMS-ESI** Calculated for $C_{23}H_{17}N_3NaS$: 390.1035, found 390.1034.

2-(1-Benzyl-5-(isopropylthio)-1*H*-1,2,3-triazol-4-yl)ethan-1-ol (3ba)



Pale yellow oil. **R_f** = 0.16 (Hexanes:EtOAc 5:5). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.34 – 7.27 (m, 5H), 5.61 (s, 2H), 3.98 (t, *J* = 5.8 Hz, 2H), 3.13 (s, 1H), 2.96 (t, *J* = 5.8 Hz, 2H), 2.74 (hept, *J* = 6.7 Hz, 1H), 1.09 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 150.98 (C), 135.34 (C), 128.89 (CH), 128.41 (CH), 127.85 (CH), 61.43 (CH_2), 52.12 (CH_2), 40.72 (CH), 28.62 (CH_2), 23.07 (CH). **LRMS** (*m/z, ESI*): 300.11 $(M+H)^+$, 278.13, 91.05. **HRMS-ESI** Calculated for $C_{14}H_{20}N_3OS$: 278.1322, found 278.1322.

2-(5-(Isopropylthio)-1-phenethyl-1*H*-1,2,3-triazol-4-yl)ethan-1-ol (3ca)



Pale yellow oil. **R_f** = 0.14 (Hexanes:EtOAc 1:1). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.25 – 7.13 (m, 3H), 7.07 (d, *J* = 7.3 Hz, 2H), 4.54 (t, *J* = 7.7 Hz, 2H), 3.90 (t, *J* = 6.0 Hz, 2H), 3.18 (t, *J* = 7.7 Hz, 2H), 2.88 (t, *J* = 5.9 Hz, 2H), 2.77 (m, 1H), 1.06 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 150.45 (C), 137.34 (C), 128.90 (CH), 128.84 (CH), 127.10 (CH), 126.63 (C), 61.55 (CH_2), 49.60 (CH_2), 40.97 (CH), 36.70 (CH_2), 28.56 (CH_2), 23.25 (CH_3). **LRMS** (*m/z, ESI*): 314.13 $(M+Na)^+$, 292.15 $(M+H)^+$, 250.10, 105.08. **HRMS-ESI** Calculated for $C_{15}H_{22}N_3OS$: 292.1478, found 292.1478.

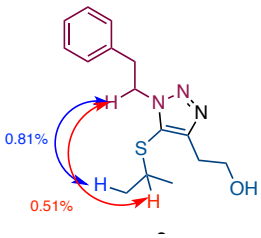
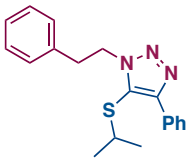


Figure S16. Significant nOe's observed for compound **3ca**.

5-(Isopropylthio)-1-phenethyl-4-phenyl-1*H*-1,2,3-triazole (3ce)



Light brown oil. $\mathbf{R_f}$ = 0.63 (Hexanes:EtOAc 7:3). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 7.0 Hz, 2H), 7.52 – 7.43 (m, 2H), 7.43 – 7.18 (m, 6H), 4.72 (t, *J* = 7.7 Hz, 2H), 3.31 (t, *J* = 7.8 Hz, 2H), 2.96 (hept, *J* = 6.7 Hz, 1H), 1.10 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.09 (C), 137.39 (C), 131.11 (C), 128.97 (CH), 128.85 (CH), 128.59 (CH), 128.39 (CH), 127.12 (CH), 127.09 (CH) 125.56 (C), 49.41 (CH_2), 40.94 (CH), 36.82 (CH_2), 23.14 (CH_3). **LRMS** (*m/z, ESI*): 346.13 $(M+Na)^+$, 324.15 $(M+H)^+$, 282.11, 105.07. **HRMS-ESI** Calculated for $C_{19}H_{22}N_3S$: 324.1529, found 324.1529.

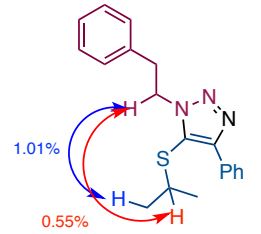
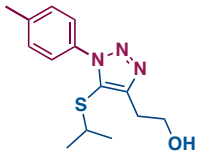


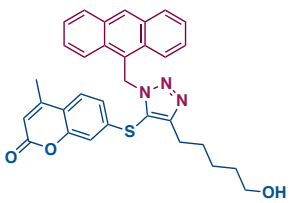
Figure S17. Significant nOe's observed for compound **3ce**.

2-(5-(Isopropylthio)-1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-ol (3ea)



Light brown oil. $\mathbf{R_f}$ = 0.25 (Hexanes:EtOAc 1:1). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.52 – 7.44 (m, 2H), 7.35 – 7.29 (m, 2H), 4.04 (t, *J* = 5.8 Hz, 2H), 3.04 (t, *J* = 5.9 Hz, 2H), 2.87 (hept, *J* = 6.7 Hz, 1H), 2.44 (s, 3H), 1.03 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 150.90 (C), 139.78 (C), 134.29 (C), 129.83 (CH), 125.41 (CH), 61.65 (CH_2), 40.38 (CH), 28.65 (CH_2), 23.09 (CH), 21.40 (CH). **LRMS** (*m/z, ESI*): 300.11 $(M+Na)^+$, 278.13 $(M+H)^+$, 157.09, 118.07. **HRMS-ESI** Calculated for $C_{14}H_{20}N_3OS$: 278.1322, found 278.1319.

7-((1-(Anthracen-9-ylmethyl)-4-(5-hydroxypentyl)-1*H*-1,2,3-triazol-5-yl)thio)-4-methyl-2*H*-chromen-2-one (3aj)



Pale yellow solid. $\mathbf{R_f}$ = 0.39 (Hexanes:EtOAc 1:9). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.41 (d, *J* = 8.9 Hz, 2H), 7.94 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 1H), 6.53 (s, 2H), 6.17 (s, 1H), 5.66 (d, *J* = 8.3 Hz, 1H), 5.62 (s, 1H), 3.56 (t, *J* = 6.5 Hz, 2H), 2.64 (t, *J* = 7.6 Hz, 2H), 2.28 (s, 3H), 1.79 (s, 1H), 1.67 (p, *J* = 7.6 Hz, 2H), 1.51 (p, *J* = 6.7 Hz, 2H), 1.43 – 1.29 (m, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 160.13 (C), 155.38 (C), 152.73 (C), 151.77 (C), 138.64 (C), 131.12 (C), 130.83 (C), 129.15 (CH), 128.83 (CH), 127.02 (CH), 125.11 (CH), 123.84 (CH), 123.01 (C), 119.53 (CH), 117.14 (C), 114.19 (CH), 112.13 (CH), 62.71 (CH_2), 47.15 (CH_2), 32.40 (CH_2), 28.90 (CH_2),

25.43 (CH_2), 25.12 (CH_2), 18.60 (CH_3). **LRMS** (*m/z, ESI*): 558.18 $(M+Na)^+$, 191.08, 165.07, 152.06. **HRMS-ESI** Calculated for $C_{32}H_{30}N_3O_3S$: 536.2002, found 536.2002.

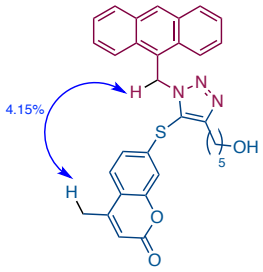
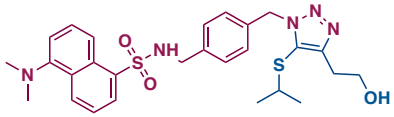


Figure S18. Significant nOe's observed for compound **3aj**.

5-(Dimethylamino)-*N*-(4-((4-(2-hydroxyethyl)-5-(isopropylthio)-1*H*-1,2,3-triazol-1-yl)methyl)benzyl) naphthalene-1-sulfonamide (3fa)



Pale yellow solid. $\mathbf{R_f}$ = 0.39 (Hexanes:EtOAc 2:8). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.56 (d, *J* = 8.5 Hz, 1H), 8.26 (dd, *J* = 16.3, 8.0 Hz, 2H), 7.52 (dt, *J* = 15.4, 8.0 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 1H), 7.09 (q, *J* = 8.0 Hz, 4H), 5.51 (s, 2H), 5.05 (s, 1H), 4.04 (d, *J* = 6.0 Hz, 2H), 3.96 (t, *J* = 6.1 Hz, 2H), 2.96 – 2.89 (m, 8H), 2.81 (hept, *J* = 6.7 Hz, 1H), 1.10 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.09 (C), 150.97 (C), 136.81 (C), 134.82 (C), 134.63 (C), 130.70 (CH), 129.96 (C), 129.93 (CH), 129.69 (C), 128.60 (CH), 128.35 (CH), 128.09 (CH), 126.54 (C), 123.30 (CH), 118.77 (CH), 115.38 (CH), 61.43 (CH_2), 51.65 (CH_2), 46.97 (CH_2), 45.57 (CH_3), 40.87 (CH), 28.57 (CH_2), 23.16 (CH_3). **LRMS** (*m/z, ESI*): 562.19 $(M+Na)^+$, 540.20 $(M+H)^+$, 353.13, 168.08. **HRMS-ESI** Calculated for $C_{27}H_{34}N_5O_3S_2$: 540.2098, found 540.2097.

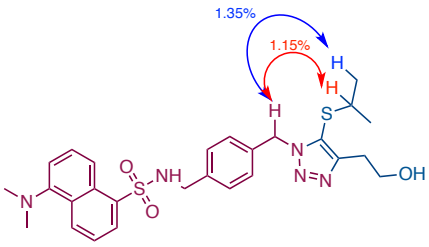
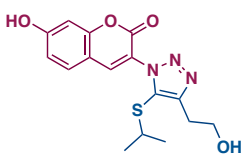


Figure S19. Significant nOe's observed for compound **3fa**.

7-Hydroxy-3-(4-(2-hydroxyethyl)-5-(isopropylthio)-1*H*-1,2,3-triazol-1-yl)-2H-chromen-2-one (3ga)



Brown solid. $\mathbf{R_f}$ = 0.19 Hexane:EtOAc (2:8).**^{1}H NMR** (500 MHz, CD_3OD) δ 8.27 (s, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 6.93 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 3.95 (t, *J* = 6.9 Hz, 2H), 3.23 (p, *J* = 6.6 Hz, 1H), 3.04 (t, *J* = 6.6 Hz, 2H), 1.16 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (126 MHz, CD_3OD) δ 165.14 (C), 159.31 (C), 157.56 (C), 150.52 (C), 144.69 (CH), 132.25 (CH), 131.37 (C), 120.08 (C), 115.65 (CH), 111.69 (C), 103.66 (CH), 61.76 (CH_2), 41.89 (CH), 29.97 (CH_2), 23.49 (CH_3). **LRMS** (*m/z, ESI*): 370.08 $(M+Na)^+$, 348.10 $(M+H)^+$, 214.05, 188.04, 177.04. **HRMS-ESI** Calculated for $C_{16}H_{17}N_3NaO_4S$: 370.0832, found 370.0835.

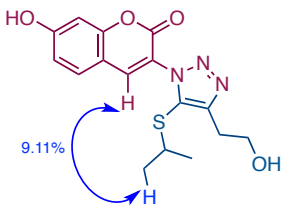
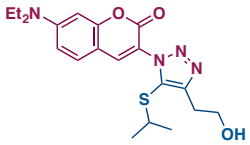


Figure S20. Significant nOe's observed for compound **3ga**.

7-(Diethylamino)-3-(4-(2-hydroxyethyl)-5-(isopropylthio)-1*H*-1,2,3-triazol-1-yl)-2H-chromen-2-one (3ha)



Dark green yellow solid. $\mathbf{R_f}$ = 0.64 (EtOAc). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.35 (d, *J* = 8.9 Hz, 1H), 6.65 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.55 (d, *J* = 2.4 Hz, 1H), 4.04 (t, *J* = 5.9 Hz, 2H), 3.45 (q, *J* = 7.1 Hz, 4H), 3.21 (hept, *J* = 6.7 Hz, 1H), 3.03 (t, *J* = 5.9 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 6H), 1.14 (dd, *J* = 6.7, 0.6 Hz, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 158.07 (C), 156.92 (C), 152.02 (C), 150.20 (C), 141.88 (CH), 130.13 (CH), 129.32 (C), 116.16 (C), 109.66 (CH), 106.69 (C), 97.20 (CH), 61.39 (CH_2), 45.00 (CH), 40.32 (CH_2), 28.51 (CH_2), 23.11 (CH_3), 12.37 (CH_3). **LRMS** (*m/z, ESI*): 425.16 $(M+Na)^+$, 403.18 $(M+H)^+$, 361.13, 300.15, 285.12, 243.11, 203.12. **HRMS-ESI** Calculated for $C_{20}H_{27}N_4O_3S$: 403.1798, found 403.1810.

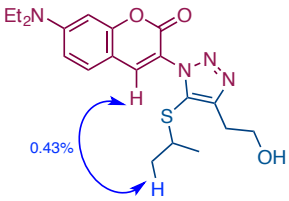
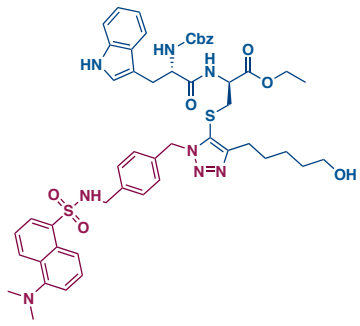


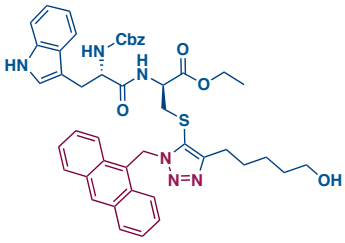
Figure S21. Significant nOe's observed for compound **3ha**.

Ethyl-*N*-(((Benzyloxy)carbonyl)-*L*-tryptophyl)-*S*-(1-(4-(((5-(dimethylamino)naphthalene)-1sulfonamido) methyl)benzyl)-4-(5-hydroxypentyl)-1*H*-1,2,3-triazol-5-yl)-*D*-cysteinate (3fk)



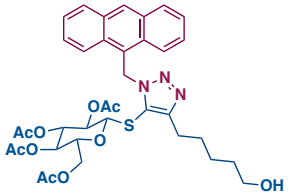
Yellow solid. $\mathbf{R_f}$ = 0.29 (Hexanes:EtOAc 2:8). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.62 – 8.53 (m, 2H), 8.38 (d, *J* = 8.6 Hz, 1H), 8.27 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.51 (dt, *J* = 14.3, 8.1 Hz, 2H), 7.33 – 7.27 (m, 6H), 7.22 – 7.10 (m, 2H), 7.12 – 6.98 (m, 4H), 6.95 (d, *J* = 7.9 Hz, 2H), 6.47 (d, *J* = 7.1 Hz, 1H), 6.15 (s, 1H), 5.75 (s, 1H), 5.49 – 5.30 (m, 2H), 5.17 – 5.04 (m, 2H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.27 (q, *J* = 6.3 Hz, 1H), 4.14 – 3.97 (m, 2H), 4.00 – 3.88 (m, 2H), 3.59 (t, *J* = 6.4 Hz, 2H), 3.24 – 3.12 (m, 2H), 2.92 (s, 6H), 2.63 (d, *J* = 6.5 Hz, 2H), 2.50 – 2.21 (m, 2H), 1.71 (p, *J* = 7.5 Hz, 2H), 1.62 – 1.50 (m, 2H), 1.44 – 1.31 (m, 2H), 1.18 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.66 (C), 169.29 (C), 156.25 (C), 152.92 (C), 137.14 (C), 136.42 (C), 136.26 (C), 135.08 (C), 134.89 (C), 130.48 (CH), 129.76 (C), 129.72 (CH), 128.77 (CH), 128.62 (CH), 128.36 (CH), 128.27 (CH), 128.09 (CH), 127.92 (CH), 127.35 (C), 124.94 (C), 123.65 (CH), 123.47 (CH), 122.28 (CH), 119.76 (CH), 118.67 (CH) , 115.46 (CH), 111.52 (CH), 109.94 (C), 67.18 (CH_2), 62.55 (CH_2), 62.38 (CH_2), 55.70 (CH), 52.24 (CH_2), 52.10 (CH), 46.89 (CH_2), 45.60 (CH_3), 37.50 (CH_2), 32.27 (CH_2), 28.58 (CH_2), 25.40 (CH_2), 25.23 (CH_2), 14.11 (CH_3). **LRMS** (*m/z, ESI*): 997.37 $(M+Na)^+$, 975.39 $(M+H)^+$, 931.40. **HRMS-ESI** Calculated for $C_{51}H_{59}N_8O_8S_2$: 975.3892, found 975.3888.

Ethyl-*S*-(1-(Anthracen-9-ylmethyl)-4-(5-hydroxypentyl)-1*H*-1,2,3-triazol-5-yl)-*N*-(((benzyloxy)carbonyl)-*L*-tryptophyl)-*D*-cysteinate (3ak)



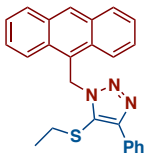
Pale yellow solid. $\mathbf{R_f}$ = 0.65 (Hexanes:EtOAc 1:9). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.30 (s, 1H), 8.27 (s, 2H), 7.92 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.46 – 7.33 (m, 4H), 7.29 – 7.17 (m, 7H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.97 (t, *J* = 7.4 Hz, 1H), 6.91 (s, 1H), 6.44 (d, *J* = 6.7 Hz, 1H), 6.30 (s, 2H), 5.44 (s, 1H), 4.99 (s, 2H), 4.42 (s, 1H), 4.32 (dd, *J* = 6.9, 4.9 Hz, 1H), 3.98 – 3.70 (m, 2H), 3.46 (t, *J* = 6.4 Hz, 2H), 3.21 – 3.04 (m, 2H), 2.64 – 2.54 (m, 1H), 2.50 – 2.40 (m, 2H), 1.85 (s, 1H), 1.54 (p, *J* = 7.6 Hz, 2H), 1.41 (p, *J* = 6.7 Hz, 2H), 1.26 – 1.16 (m, 2H), 1.04 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.49 (C), 169.16 (C), 152.12 (C), 136.42 (C), 136.22 (C), 131.51 (C), 131.13 (C), 129.73 (CH), 129.37 (CH), 128.65 (CH), 128.31 (CH), 128.19 (CH), 127.40 (C), 127.04 (CH), 125.27 (CH), 124.67 (C), 124.63 (C), 124.03 (CH), 123.47 (CH), 122.38 (CH), 119.84 (CH), 118.78 (CH), 111.44 (CH), 67.26 (CH_2), 62.65 (CH_2), 62.34 (CH_2), 55.65 (CH), 52.20 (CH), 45.64 (CH_2), 37.52 (CH_2), 32.20 (CH_2), 28.53 (CH_2), 25.28 (CH_2), 25.17 (CH_2), 14.08 (CH_3). **LRMS** (*m/z, ESI*): 835.32 $(M+Na)^+$, 813.34 $(M+H)^+$, 191.08, 165.07. **HRMS-ESI** Calculated for $C_{46}H_{49}N_6O_6S$: 813.3429, found 813.3431

(2*R*,3*R*,4*S*,5*R*,6*S*)-2-(Acetoxymethyl)-6-((1-(anthracen-9-ylmethyl)-4-(5-hydroxypentyl)-1*H*-1,2,3-triazol-5-yl)thio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (3al)



Pale yellow solid. $\mathbf{R_f}$ = 0.19 (Hexanes:EtOAc 3:7).**^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.41 (d, *J* = 8.8 Hz, 2H), 8.05 (d, *J* = 9.0 Hz, 2H), 7.59 – 7.52 (m, 2H), 7.51 – 7.46 (m, 2H), 6.64 – 6.44 (m, 2H), 5.13 – 4.94 (m, 3H), 4.16 – 4.07 (m, 3H), 3.61 (t, *J* = 6.4 Hz, 2H), 3.39 – 3.31 (m, 1H), 2.70 (td, *J* = 7.4, 2.8 Hz, 2H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.77 – 1.66 (m, 2H), 1.61 – 1.53 (m, 2H), 1.44 – 1.34 (m, 2H).**^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.61(C), 170.24 (C), 169.50 (C), 169.47 (C), 153.48 (C), 131.63 (C), 131.26 (C), 129.72 (CH), 129.44 (CH), 127.01 (CH), 125.30 (CH), 125.13 (C), 124.21 (CH), 121.87 (C), 86.08 (CH), 76.32 (CH), 73.54 (CH), 70.60 (CH), 67.95 (CH), 62.76 (CH_2), 61.98 (CH_2), 46.03 (CH_2), 32.43 (CH_2), 28.75 (CH_2), 25.48 (CH_2), 25.29 (CH_2), 20.81 (CH_3), 20.79 (CH_3), 20.72 (CH_3), 20.69 (CH_3). **LRMS** (*m/z, ESI*): 730.24 $(M+Na)^+$, 708.26 $(M+H)^+$, 191.09, 167.07. **HRMS-ESI** Calculated for $C_{36}H_{42}N_3O_{10}S$: 708.2585, found 708.2583.

1-(Anthracen-9-ylmethyl)-5-(ethylthio)-4-phenyl-1*H*-1,2,3-triazole (3af)



Light Brown solid. $\mathbf{R_f}$ = 0.50 (Hexanes:EtOAc 8:2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.54 (d, *J* = 8.1 Hz, 3H), 8.14 (d, *J* = 7.3 Hz, 2H), 8.04 (d, *J* = 7.7 Hz, 2H), 7.62 – 7.54 (m, 2H), 7.53 – 7.45 (m, 2H), 7.45 – 7.38 (m, 2H), 7.41 – 7.30 (m, 1H), 6.58 (s, 2H), 2.36 (q, *J* = 7.5 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 148.75 (C), 131.40 (C), 131.05 (C), 130.88 (C), 1 29.53 (CH), 129.20 (CH), 128.49 (CH), 128.29 (CH), 126.94 (CH), 126.78 (CH), 125.34 (C), 125.09 (CH), 124.82 (C), 124.18 (CH), 45.16 (CH_2) , 30.32 (CH_2), 14.17 (CH_3). **LRMS** (*m/z, ESI*): 418.14 $(M+Na)^+$, 191.08. **HRMS-ESI** Calculated for $C_{25}H_{21}N_3NaS$: 418.1346, found 418.1349.

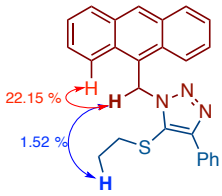
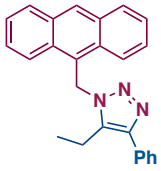


Figure S22. Significant nOe's observed for compound **3af**.

1-(Anthracen-9-ylmethyl)-5-ethyl-4-phenyl-1*H*-1,2,3-triazole (3ab)



Yellow solid. $\mathbf{R_f}$ = 0.60 (Hexanes:EtOAc 7:3). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.45 (d, *J* = 8.4 Hz, 2H), 8.06 (d, *J* = 8.4 Hz, 2H), 7.64 – 7.60 (m, 2H), 7.60 – 7.56 (m, 2H), 7.53 – 7.48 (m, 2H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 1H), 6.59 (s, 2H), 2.58 (q, *J* = 7.6 Hz, 2H), 0.57 (t, *J* = 7.6 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 131.83 (C), 131.54 (C), 131.08 (C), 129.85 (CH), 129.59 (CH), 128.71 (CH), 127.70 (CH), 127.39 (CH), 127.10 (CH), 125.35 (CH), 124.16 (C), 123.50 (CH), 119.73 (C), 46.35 (CH_2), 16.59 (CH_2), 12.95 (CH_3). **LRMS** (*m/z, ESI*): 386.16 $(M+Na)^+$, 191.08. **HRMS-ESI** Calculated for $C_{25}H_{21}N_3Na$: 386.1628, found 386.1627.

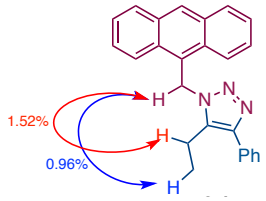
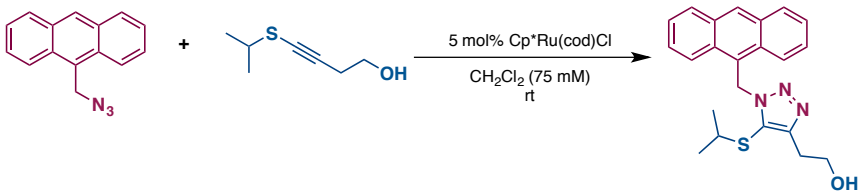


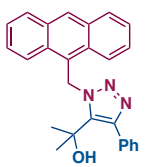
Figure S23. Significant nOe's observed for compound **3ab**.

10. General procedure for the cycloaddition in organic solvents (Exemplified for the preparation of **3aa** in CH_2Cl_2)



Cp*Ru(cod)Cl (2.4 mg, 0.006 mmol, 0.05 eq) was added under nitrogen to a dried Schlenk tube equipped with a magnetic stirring bar. CH_2Cl_2 (1.7 mL) was added followed by 4-(isopropylthio)but-3-yn-1-ol (**2a**, 37.1 mg, 0.257 mmol, 2 eq) and 9-(azidomethyl)anthracene (**1a**, 30.0 mg, 0.130 mmol, 1 eq). The brown mixture was stirred at rt under N_2 and monitored by TLC (Hexanes : EtOAc = 8:2). Upon completion (2 h) the crude was filtered through Florisil and eluted with EtOAc (5 x 1 mL). The crude was concentrated onto silica and purified by column chromatography (Hexanes:EtOAc from 6:4 to 2:8) to afford 2-(1-(anthracen-9-ylmethyl)-5-(isopropylthio)-1*H*-1,2,3-triazol-4-yl)ethan-1-ol (**3aa**) as a off-white solid (44.8 mg, 0.119 mmol, 92%).

2-(1-(Anthracen-9-ylmethyl)-4-phenyl-1*H*-1,2,3-triazol-5-yl)propan-2-ol (3ac)



Obtained from the reaction carried out in toluene (85% Yield). Light brown solid. $\mathbf{R_f}$ = 0.60 (Hexanes:EtOAc 1:1). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.40 (d, 2H), 8.13 (d, *J* = 7.4, 2.1 Hz, 2H), 7.61 – 7.47 (m, 4H), 7.45 – 7.35 (m, 3H), 7.36 – 7.28 (m, 2H), 6.75 (s, 2H), 6.10 (s, 1H, OH), 1.58 (s, 6H). **^{13}C NMR** (101, DMSO-d_6) δ 142.84 (C), 139.11 (C), 133.66 (C),

131.05 (C), 131.03 (C), 130.20 (CH), 128.89 (CH), 128.33 (CH), 128.05 (CH), 127.96 (CH), 126.64 (C), 126.62 (CH), 125.16 (CH), 124.61 (CH), 67.97 (C), 46.81 (CH_2), 31.16 (CH_3). **LRMS** (*m/z, ESI*): 416.17 $(M+Na)^+$, 191.09. **HRMS-ESI** Calculated for $C_{26}H_{24}N_3O$: 394.1914 , found 394.1916.

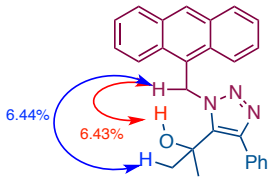
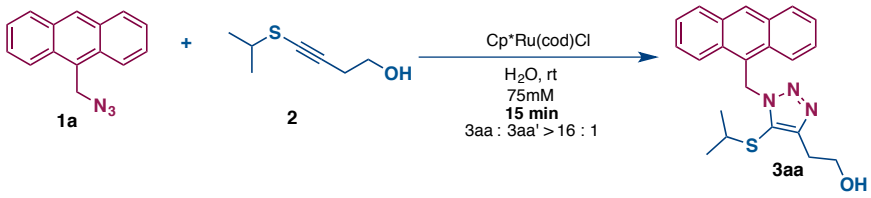


Figure S24. Significant nOe's observed for compound **3ac**.

11. Effect of the catalyst concentration on the reaction rate



Following the general procedure for the RuAtAC in water (page S17), the effect of the concentration of the catalyst using 5 mol%, 10 mol% , 20 mol% and 50 mol% of Cp*Ru(cod)Cl at short reaction times (15 min) was investigated:

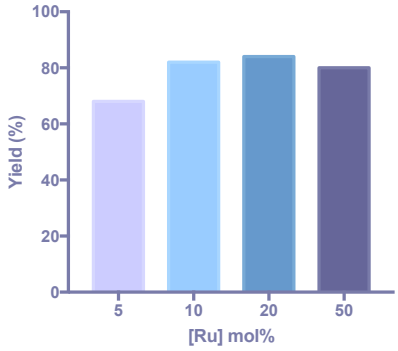


Figure S25. Reaction yield after 15 minutes using different catalyst concentration

12. Calculation of a rate constant

The rate constant for the reaction in CD_2Cl_2 was calulated using a reported procedure for a similar Cu-promoted reaction,[15] and the following second order rate equation:

$$\ln\left(\frac{[P]-n[A]_0}{n([P]-[A]_0)}\right)=(n-1)[A]_0kt$$

where n>1, [P] is the concentration of the product and $[A]_0$ the initial concentration of the azide.

The equation is of the type y = mx, in which y = $\frac{1}{[A]_0} ln\left(\frac{[P]-2[A]_0}{2([P]-[A]_0)}\right)$ and x = time, and the slope represents the rate constant k.

A CD_2Cl_2 solution of azide (75 mM) and thioalkyne (150 mM) was added into a nitrogen purged NMR tube. Cp*Ru(cod)Cl (0.225 mM, 0.3 mol%) was added under nitrogen, the NMR tube was immediately sealed and the reaction was followed by NMR at 25°C.

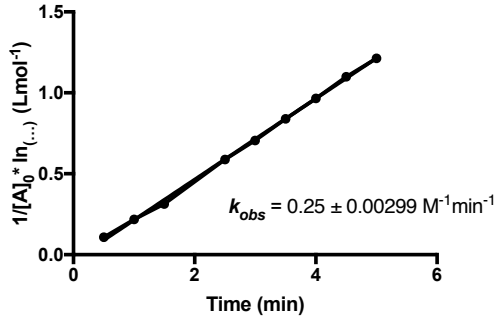


Figure S26. Representation of $\frac{1}{[A]_0} ln\left(\frac{[P]-2[A]_0}{2([P]-[A]_0)}\right)$ versus time

The above procedure was repeated using 2.25 mM (3 mol%) of the catalyst.

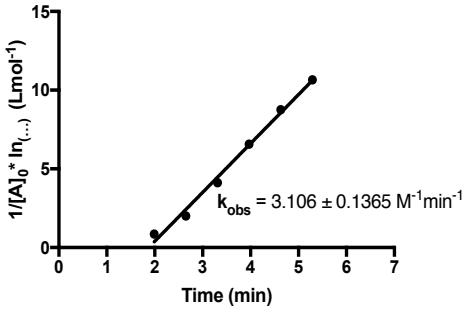


Figure S27. Calculated rate costant using 3 mol% of Cp*Ru(cod)

Reaction profile in water using 75 mM (1 eq), 150 mM (2 eq) and 300 mM (4 eq) of thioalkyne **2a**. ([Azide **1a**] = 75 mM, [Ru] = 2.25 mM).

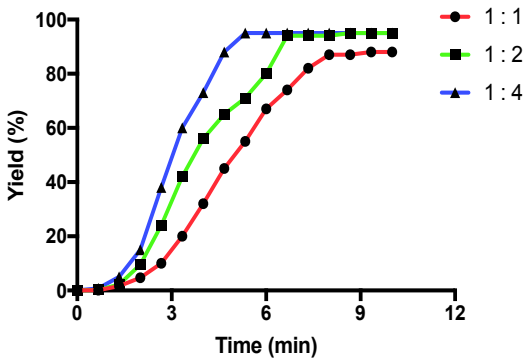


Figure S28. Effect of the thioalkyne concentration on reaction rate

13. RuAtAC reaction in bacterial cultures

Overnight cultures of bacteria (OD600=0,3) were pelleted by centrifugation (3000 xg, 5 min), washed once in PBS and resuspended in PBS containing 100 µM catalyst Cp*Ru(cod)Cl, 1 mM azide **1a** and 2 mM alkyne **2a**. As controls, we used solutions lacking the catalyst (Control reactants) or the alkyne **2a** (Control [Ru] + azide **1a**). These bacterial suspensions were incubated for 24 h at 37 ˚C. After this period, cell viability was checked by measuring OD600. Bacterial suspensions were pelleted by centrifugation (3000 xg, 5 min). The supernatant was collected for analysis (Extracellular PBS) and the bacterial pellet was resuspended in a solution of methanol/water (8:2) to extract the intracellular content. This methanol suspension was centrifuged at 14000 xg for 2 min to pellet the bacterial cells and the supernatant was collected for analysis (Methanol Extract). Both Extracellular PBS and Methanol Extract were analysed for the presence of product **3aa** by fluorescence in a Tecan 1000 plate reader with excitation and emission filters at 360/25 nm and 450/25 nm. Formation of **3aa** could also be confirmed by detection with HPLC/MS.

Viability analysis

The OD600 (absorbance at λ=600 nm) of samples of the different bacterial suspensions was measured in a Nanodrop ND-1000 spectrophotometer. No differences were found among the different experimental points (Table S5).

Table S5: Bacterial viability after RuAtAC. Values are averages of the OD600 obtained in 3 different experiments performed in duplicate.

	Average	Standard deviation
Control reactants	0,25	0,02
Control [Ru] + azide **1a**	0,26	0,03
RuAtAC	0,25	0,03

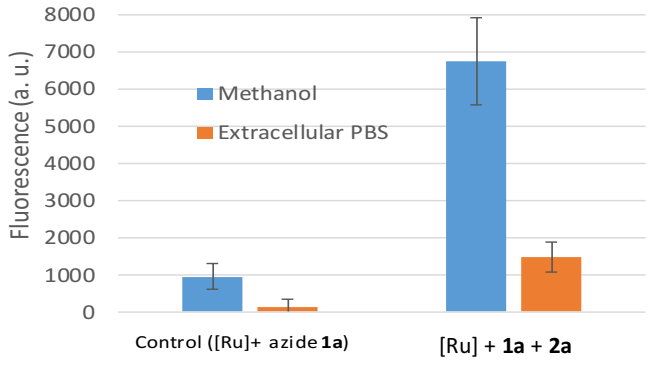


Figure S29.

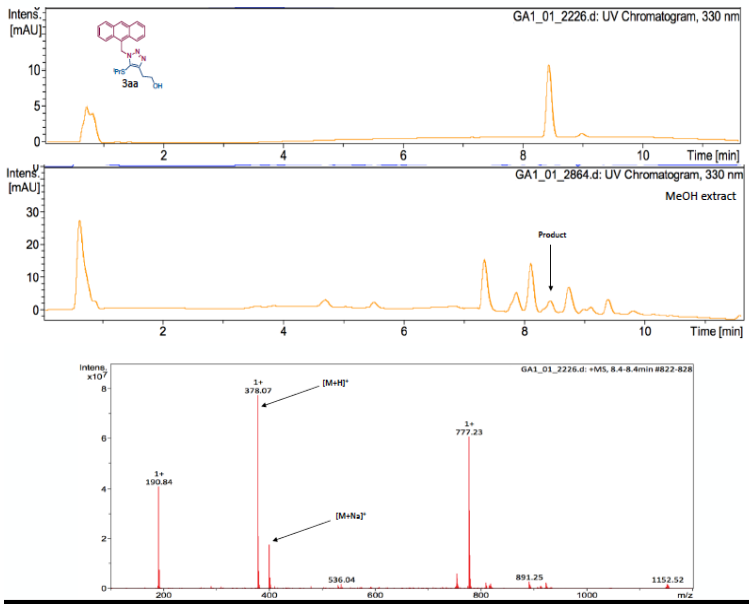


Figure S30. HPLC-chromatogram for the RuAtAC reaction in bacterial cultures.

14. Determination of NMR yields and regioselectivities for Table 1 and Table 3 of the main manuscript

The yield and regioisomeric ratios of all the reactions of Table 1, Table 3 and Table S3 were determined using 1,3,5-trimethoxybenzene as internal standard (1 eq or 0.33 eq.). NMR crudes with the corresponding determinations are provided below:

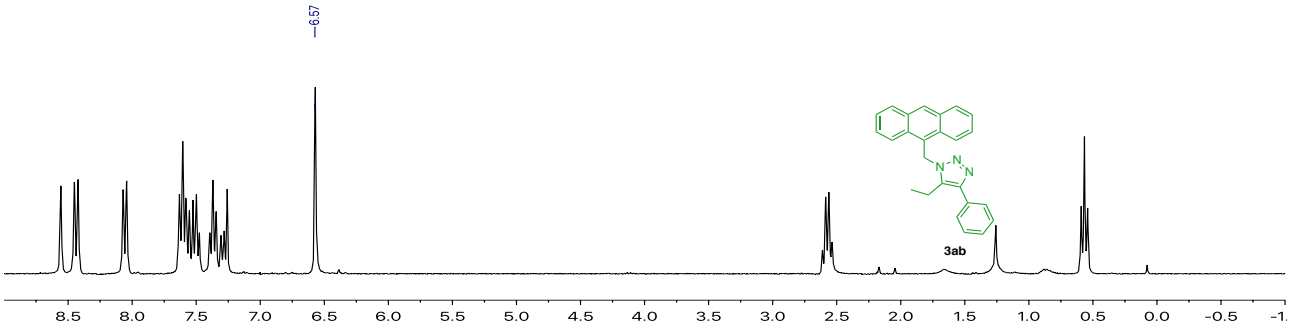


Figure S31. ^{1}H-NMR spectra of pure **3ab.**

Determination of the yield based on the 1H-NMR of the crude mixture for **Table 1**, **entry 11**

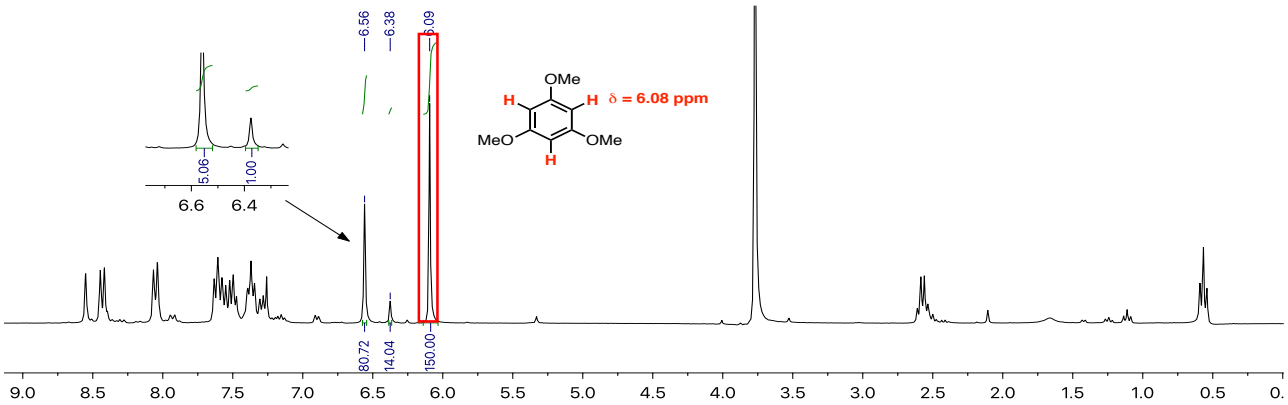


Figure S32 ^{1}H-NMR spectra of the crude mixture. Yield **3ab**: 81% , **3ab'**: 14%. Regioselection: **3ab** : **3ab'**=5:1

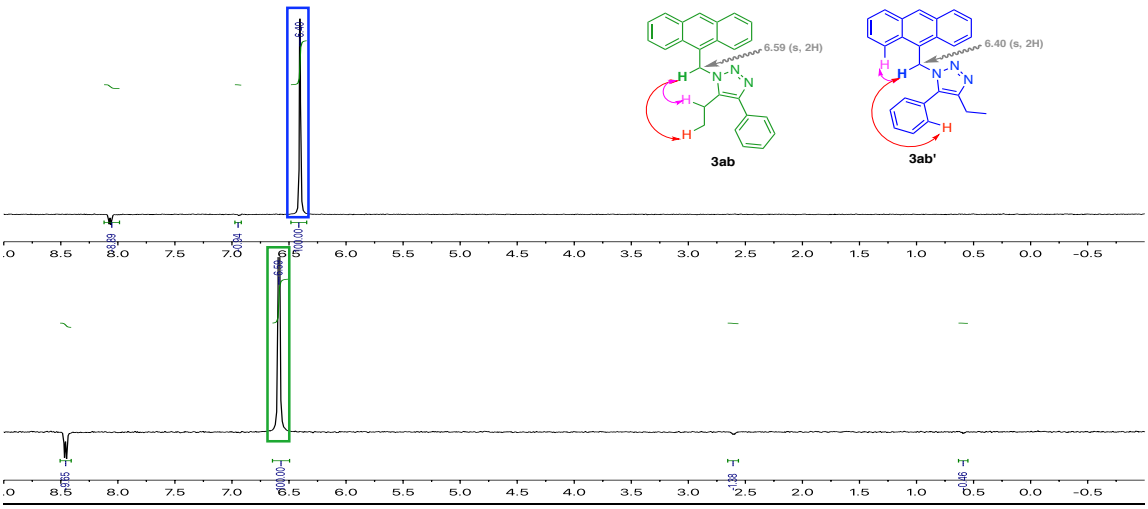


Figure S33. nOe spectra of the crude mixture of 3ab/3ab'.

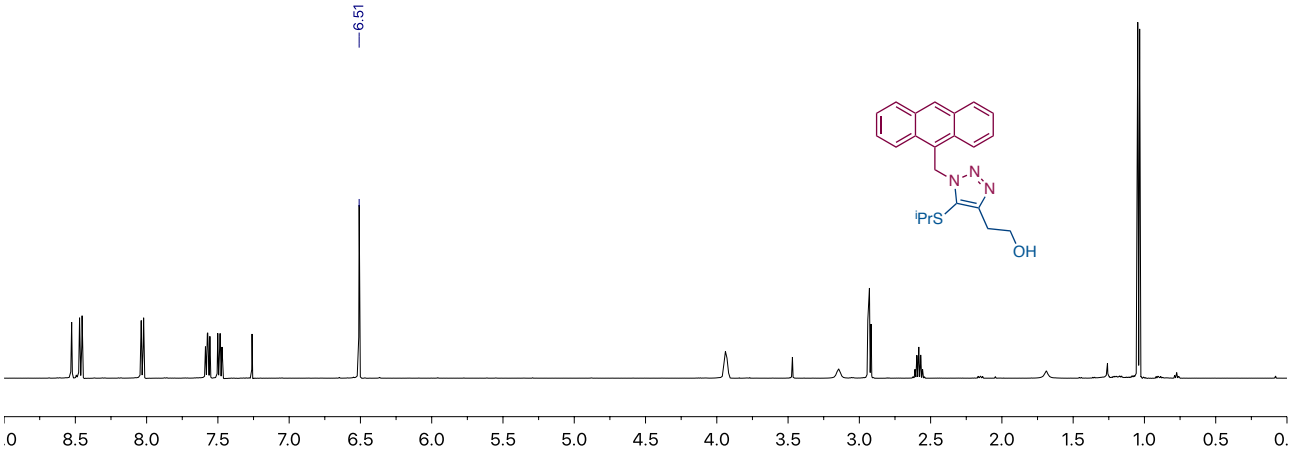


Figure S34. 1H-NMR spectra of pure 3aa

Determination of the yield based on the 1H-NMR of the crude mixture for **Table 1, entry 1**

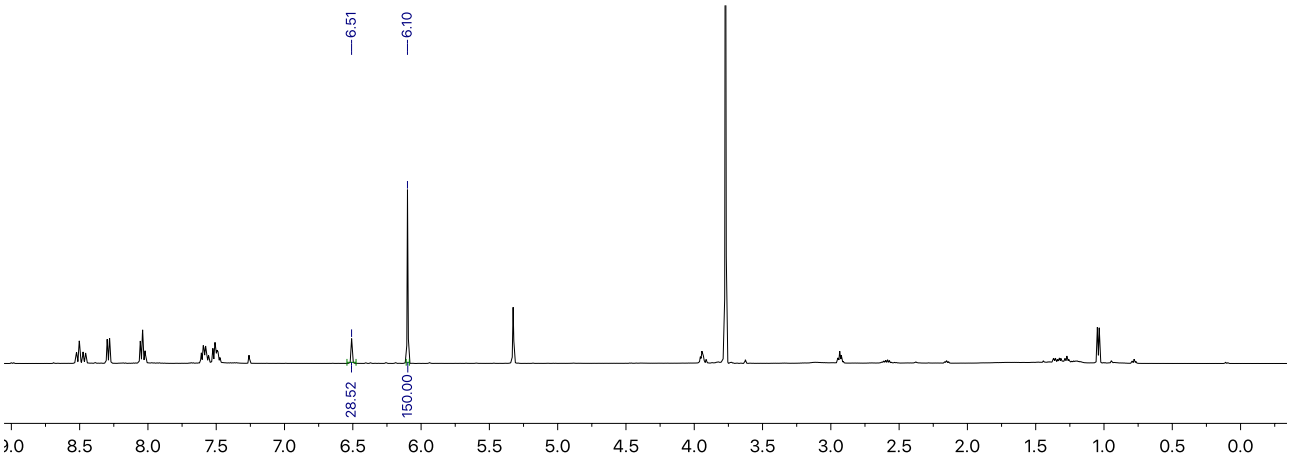


Figure S35. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : 29% ; **3aa'** : 0%. Regio. **3aa** : **3aa'** = 1 : 0.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 2**

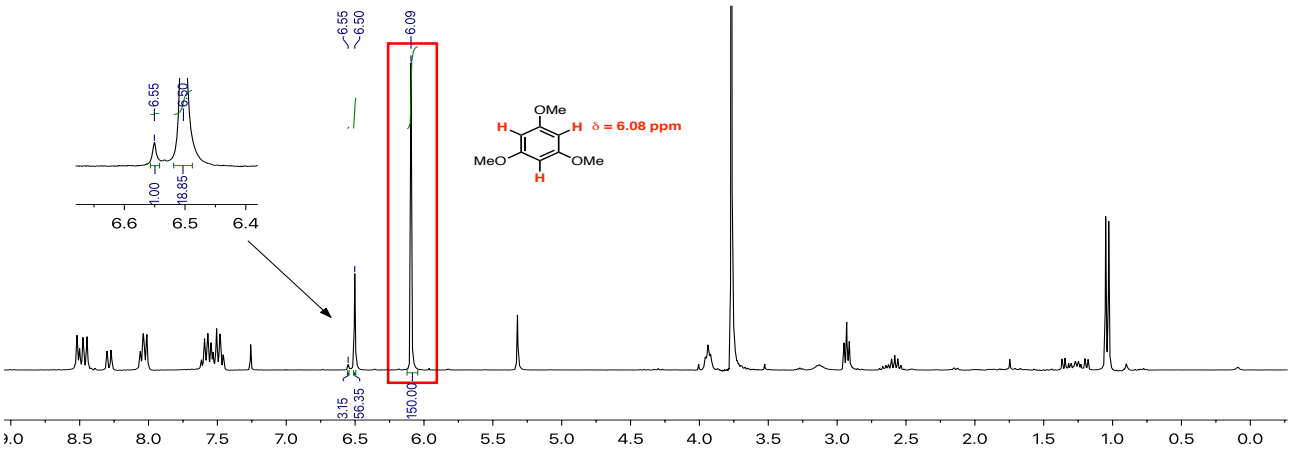


Figure S36. ^{1}H-NMR spectra of the crude mixture. Yield **3aa**:56% ; **3aa'**: 3%. Regio. **3aa** : **3aa'** = 19 : 1

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 3**

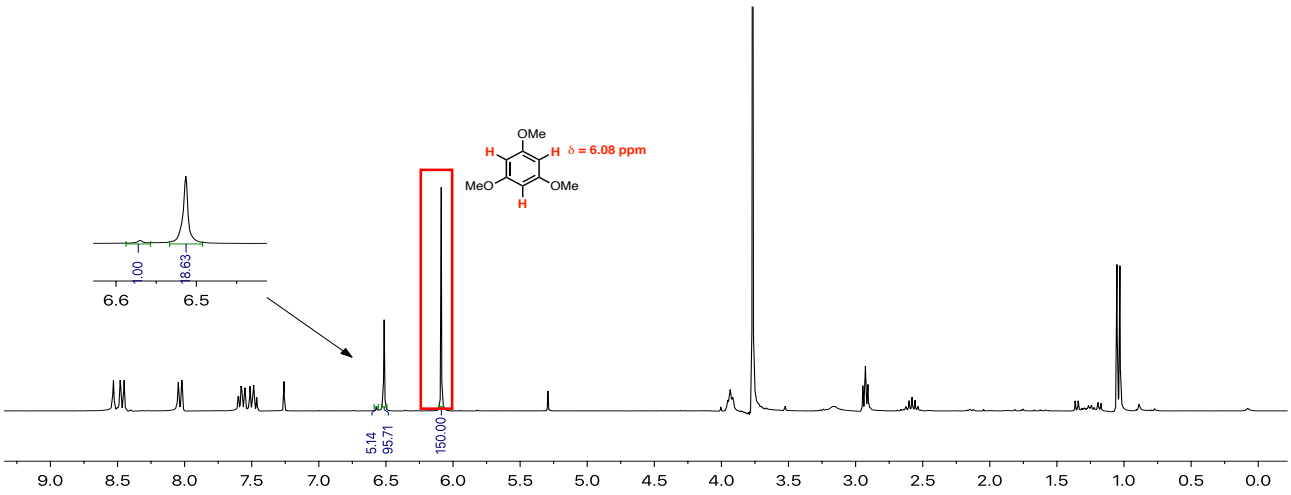


Figure S37. ^{1}H-NMR spectra of the crude mixture. Yield **3aa**: 95%. **3aa'**: 5% : Regio. **3aa : 3aa'** = 19 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 5**

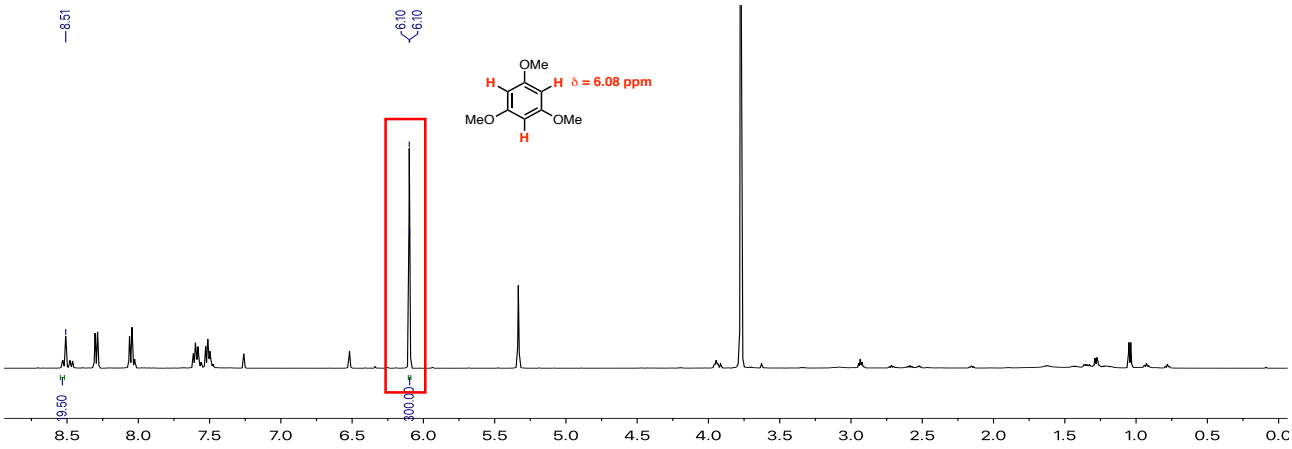


Figure S38. ^{1}H-NMR spectra of the crude mixture. Yield **3aa**: 20% **3aa'**: 0%. Regio. **3aa : 3aa'** = 1 : 0.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 6**

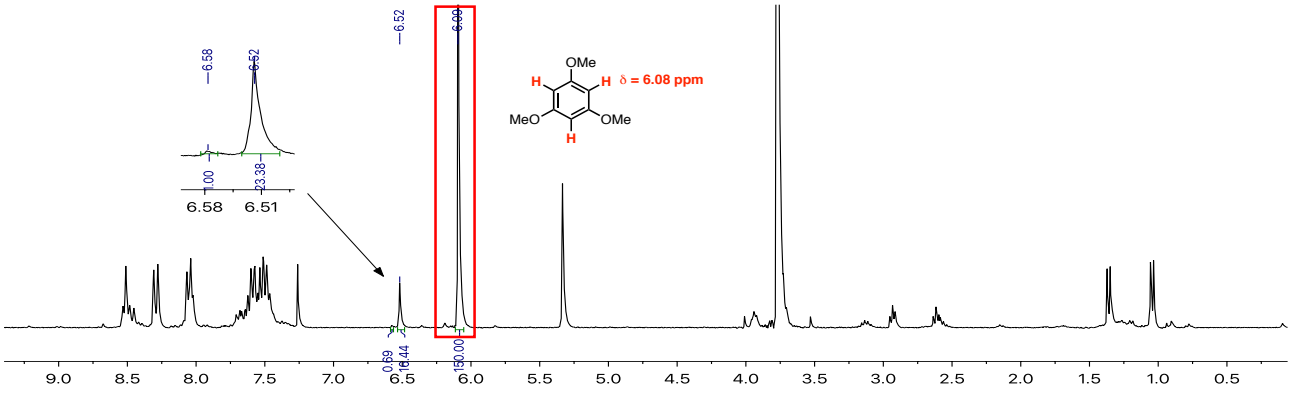


Figure S39. ^{1}H-NMR spectra of the crude mixture. Yield **3aa**: 16%, **3aa'**: 1% Regio. **3aa : 3aa'** = 23 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 7**

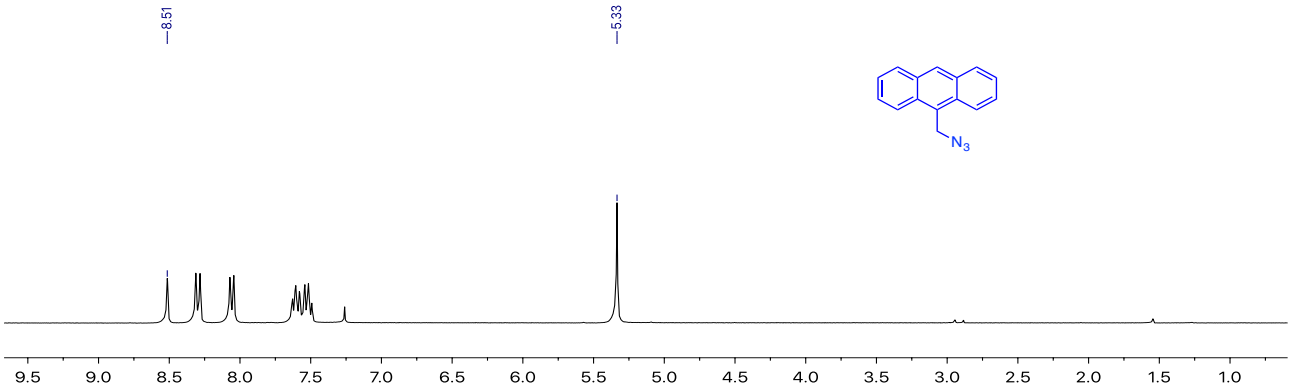


Figure S40. ^{1}H-NMR spectra of pure 9-(azidomethyl)anthracene **1a**.

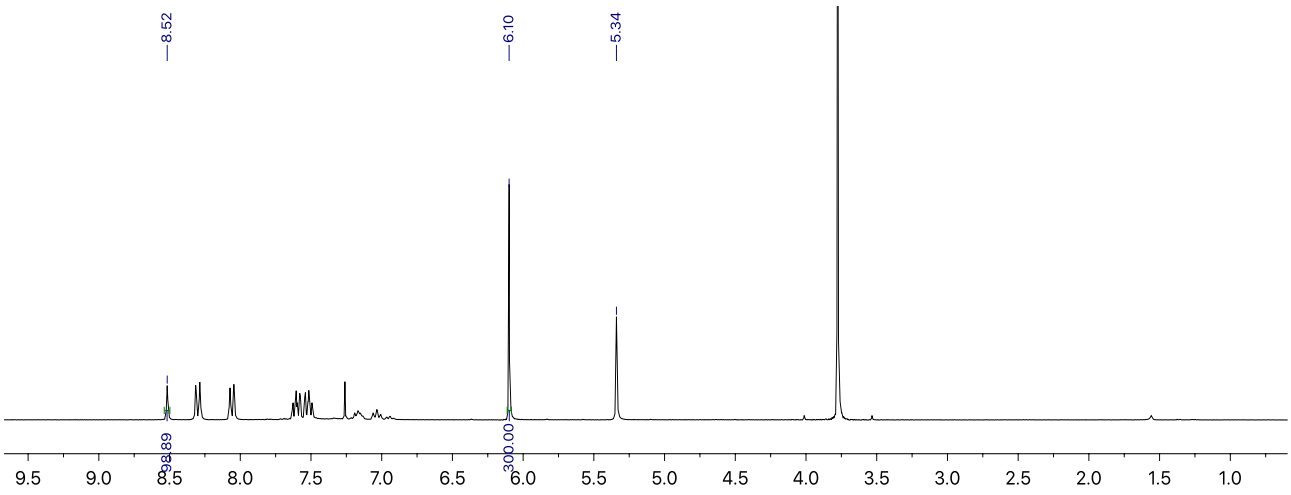


Figure S41. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : **3aa'** = 0%

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 8**

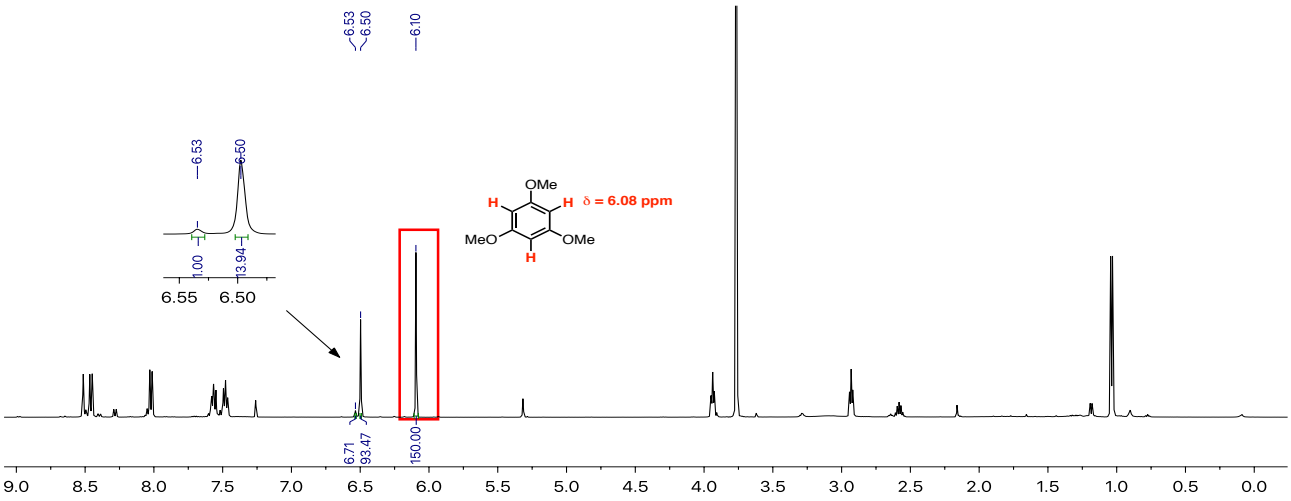


Figure S42. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : 93%, **3aa'**: 7%. Regio. **3aa : 3aa'** = 14 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 9**

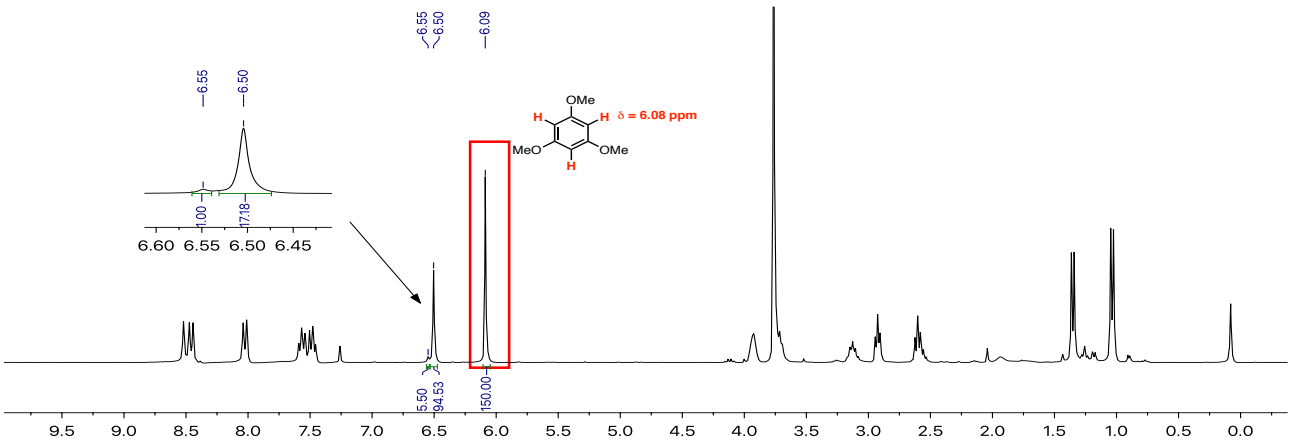


Figure S43. ^{1}H-NMR spectra of the crude mixture. Yield **3aa:** 94% ,**3aa'**: 5%. Regio. **3aa : 3aa'** = 17 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 1, entry 10**

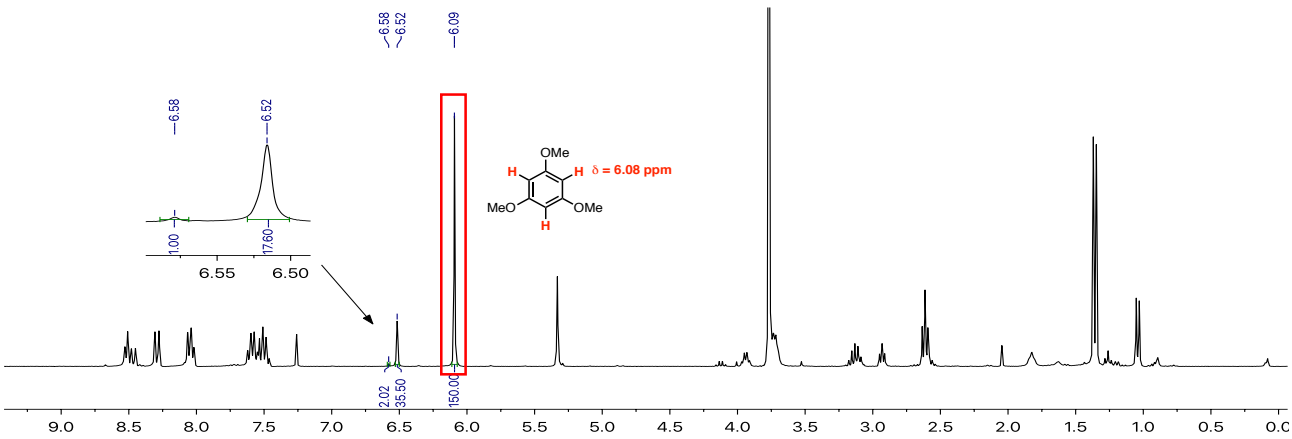


Figure S44. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : 35%, **3aa'**: 2%. Regio**. 3aa : 3aa'** = 18 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 3, entry 1**

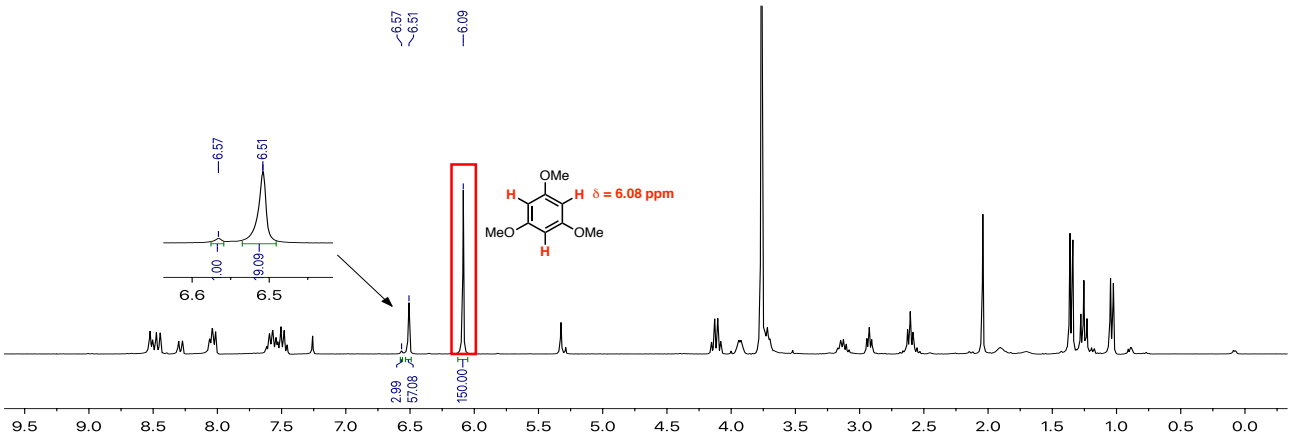


Figure S45. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : 57%, **3aa'** : 3%. Regio. **3aa : 3aa'** = 19 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 3, entry 3**

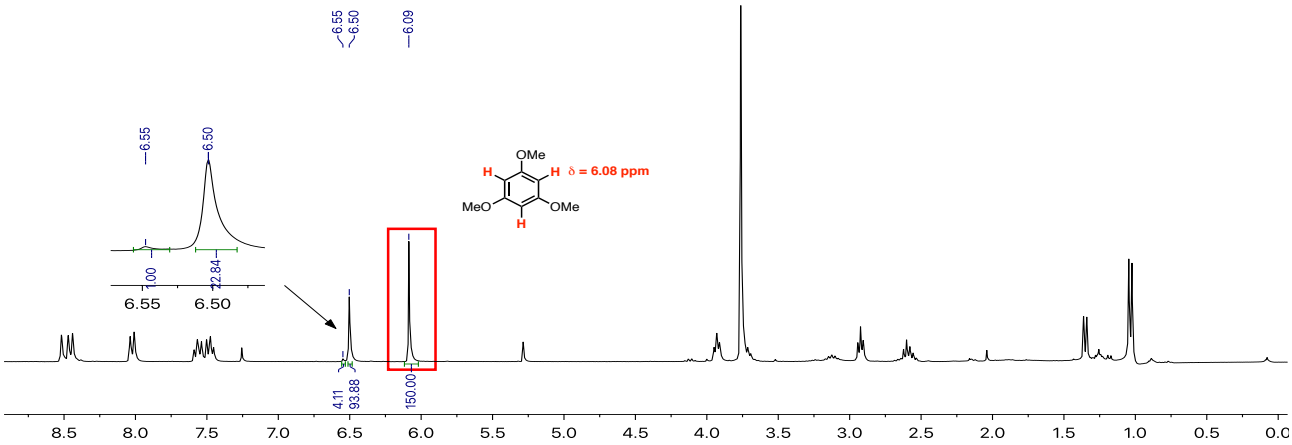


Figure S46. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : 94%, **3aa'**: 4%. Regio. **3aa : 3aa'** = 23 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 3, entry 4**

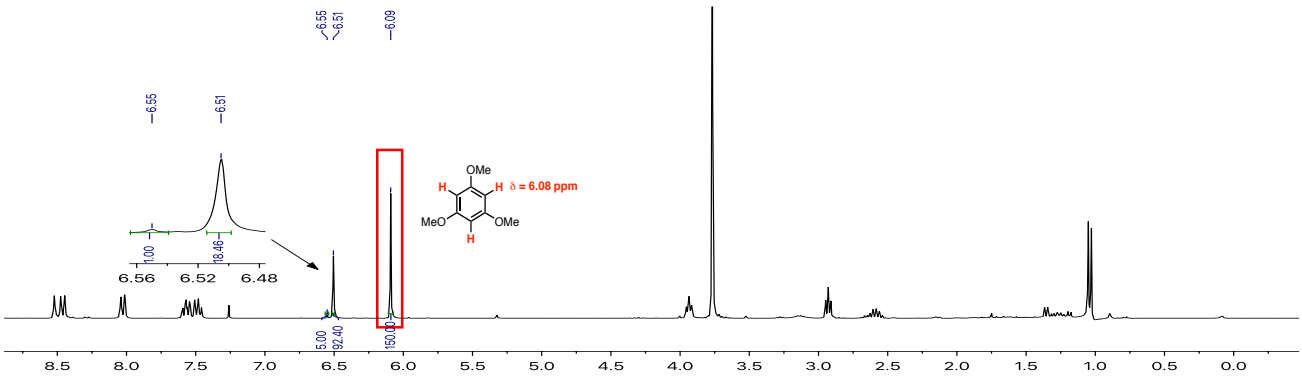


Figure S47. ^{1}H-NMR spectra of the crude mixture. Yield **3aa**: 92% , **3aa'**: 5%. Regio. **3aa : 3aa'** = 18 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 3, entry 6**

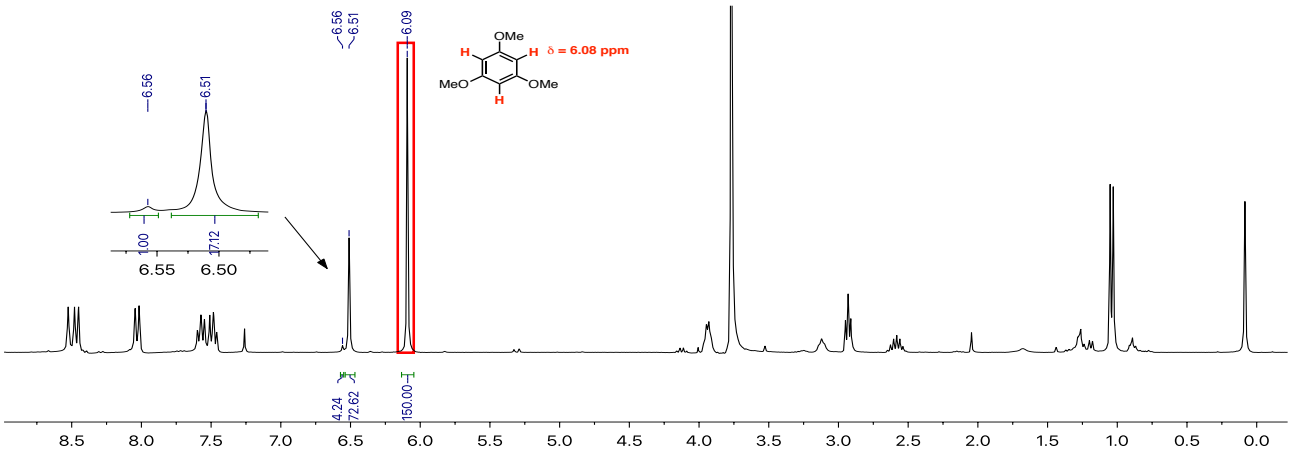


Figure S48. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : 73% , **3aa'**: 4%. Regio.**3aa : 3aa'** = 17 : 1.

Determination of the yield based on the ^{1}H-NMR of the crude mixture for **Table 3, entry 7**

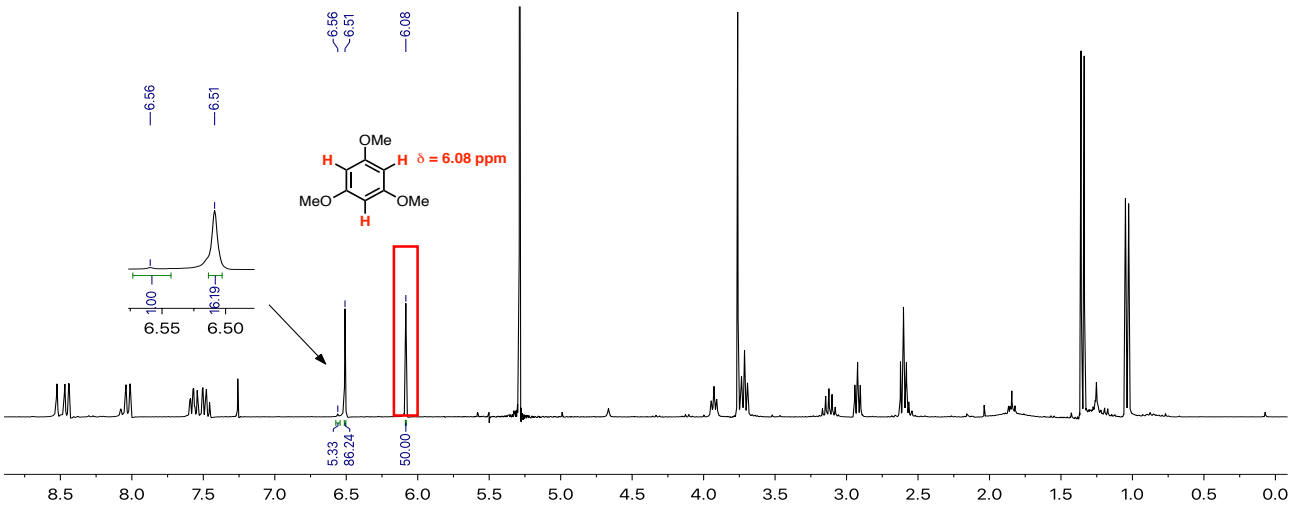


Figure S49. ^{1}H-NMR spectra of the crude mixture. Yield **3aa** : 86% , **3aa'**: 5%. Regio. **3aa : 3aa'** = 16 : 1

15. References

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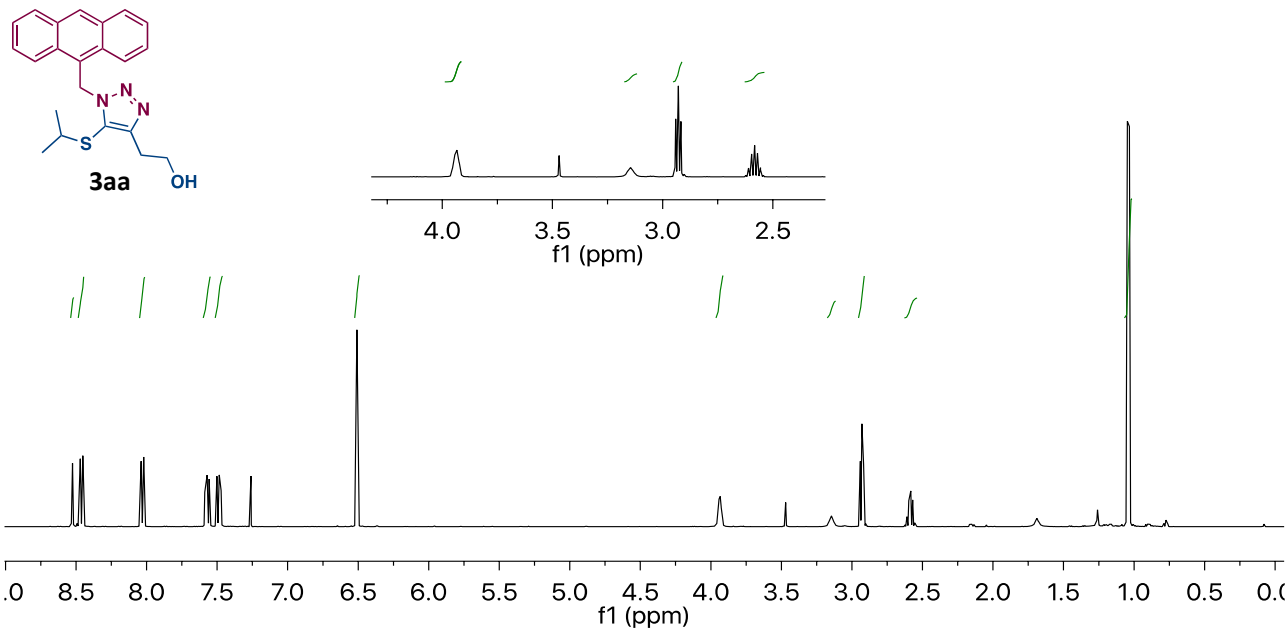
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3.0
2.5
f1 (ppm)
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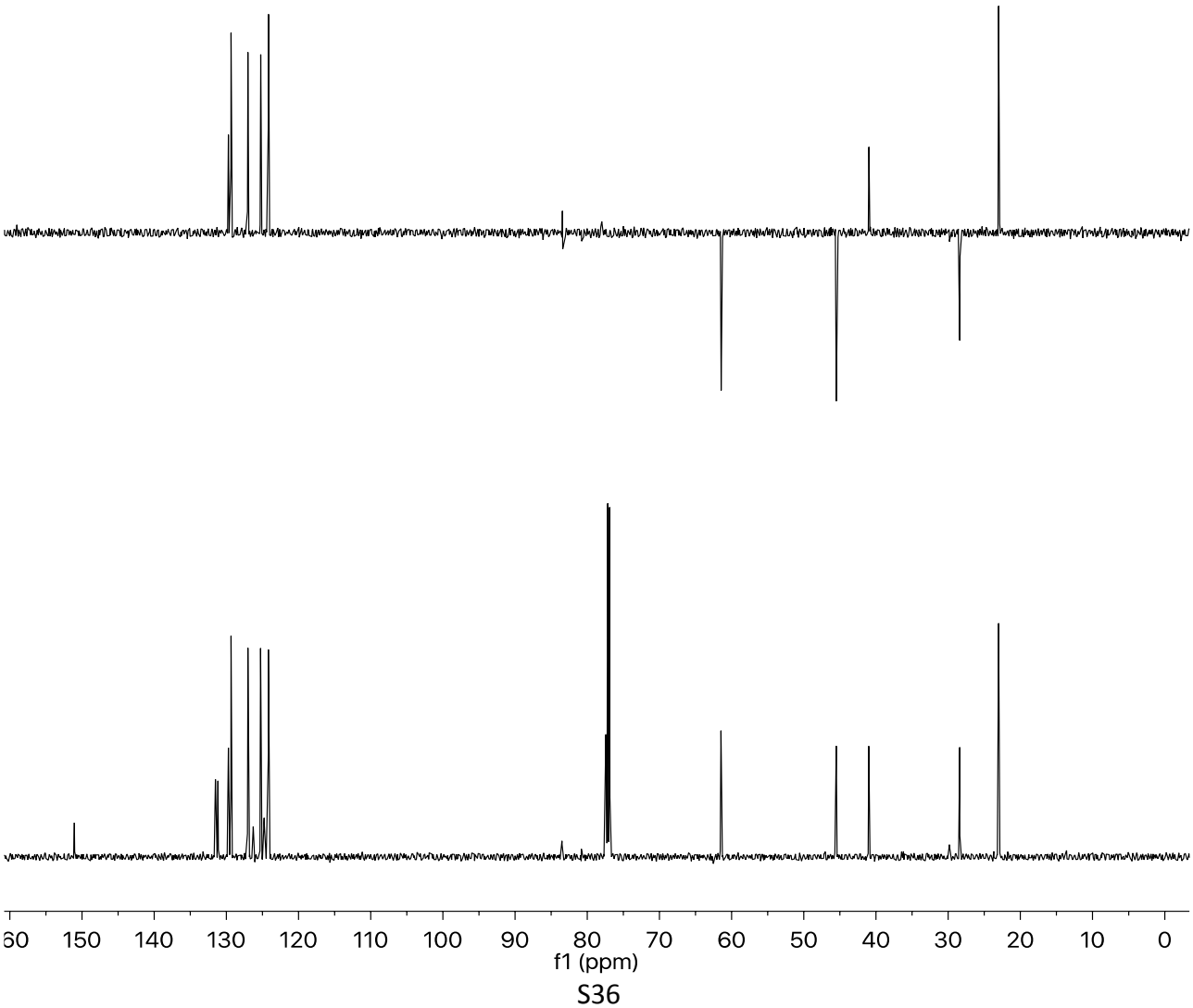
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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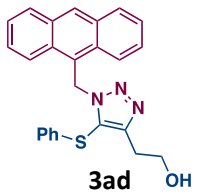
f1 (ppm)



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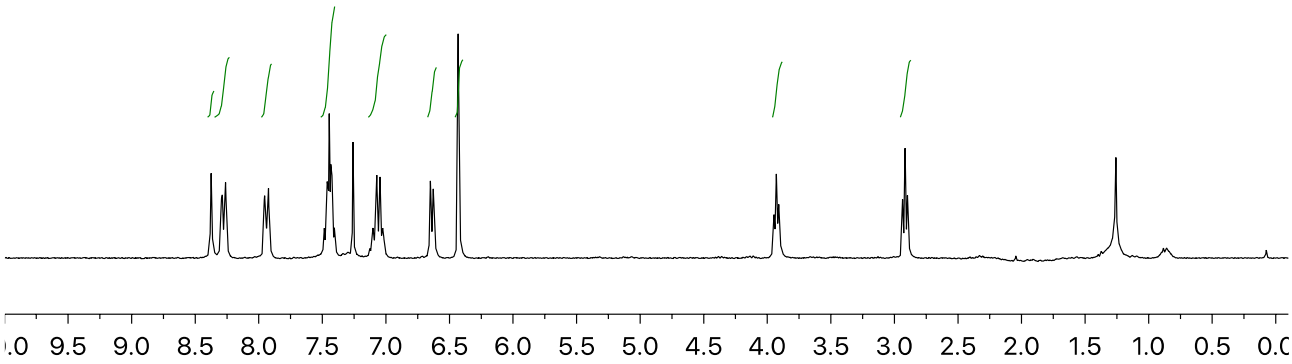
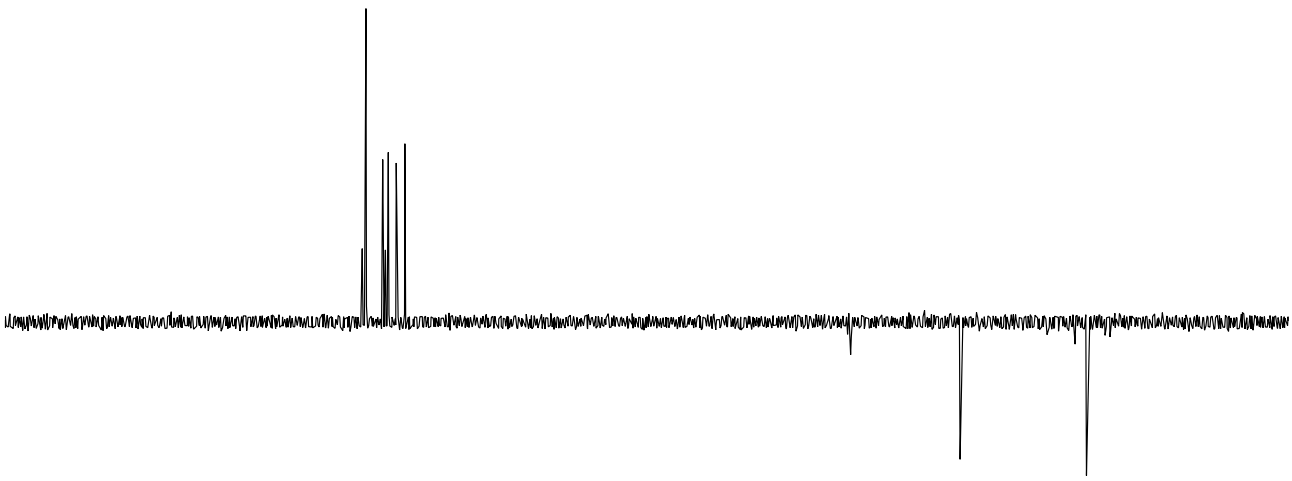
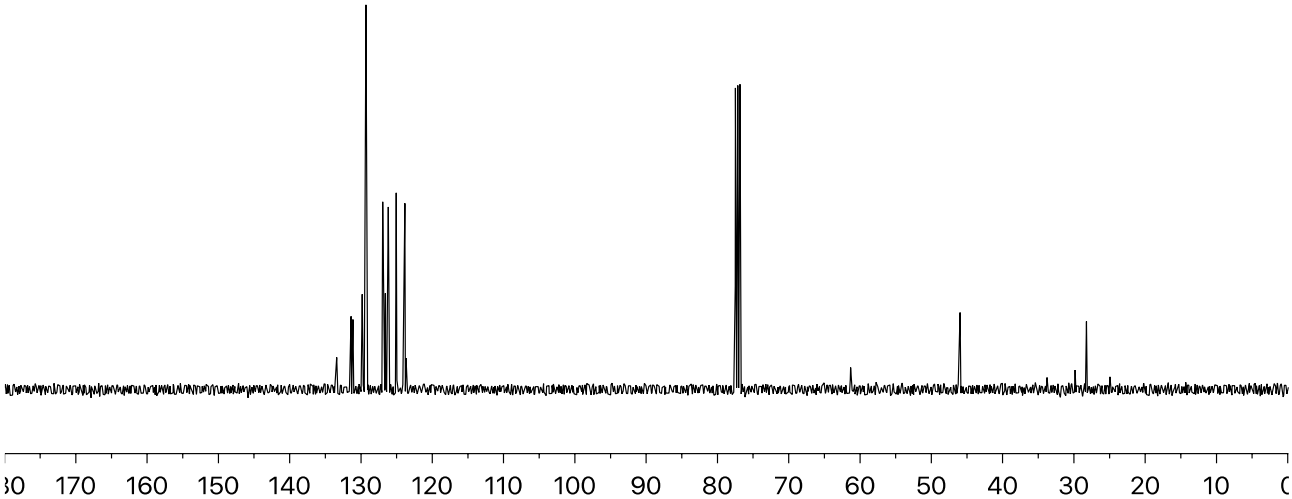
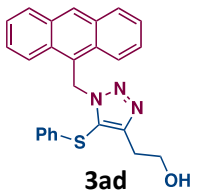
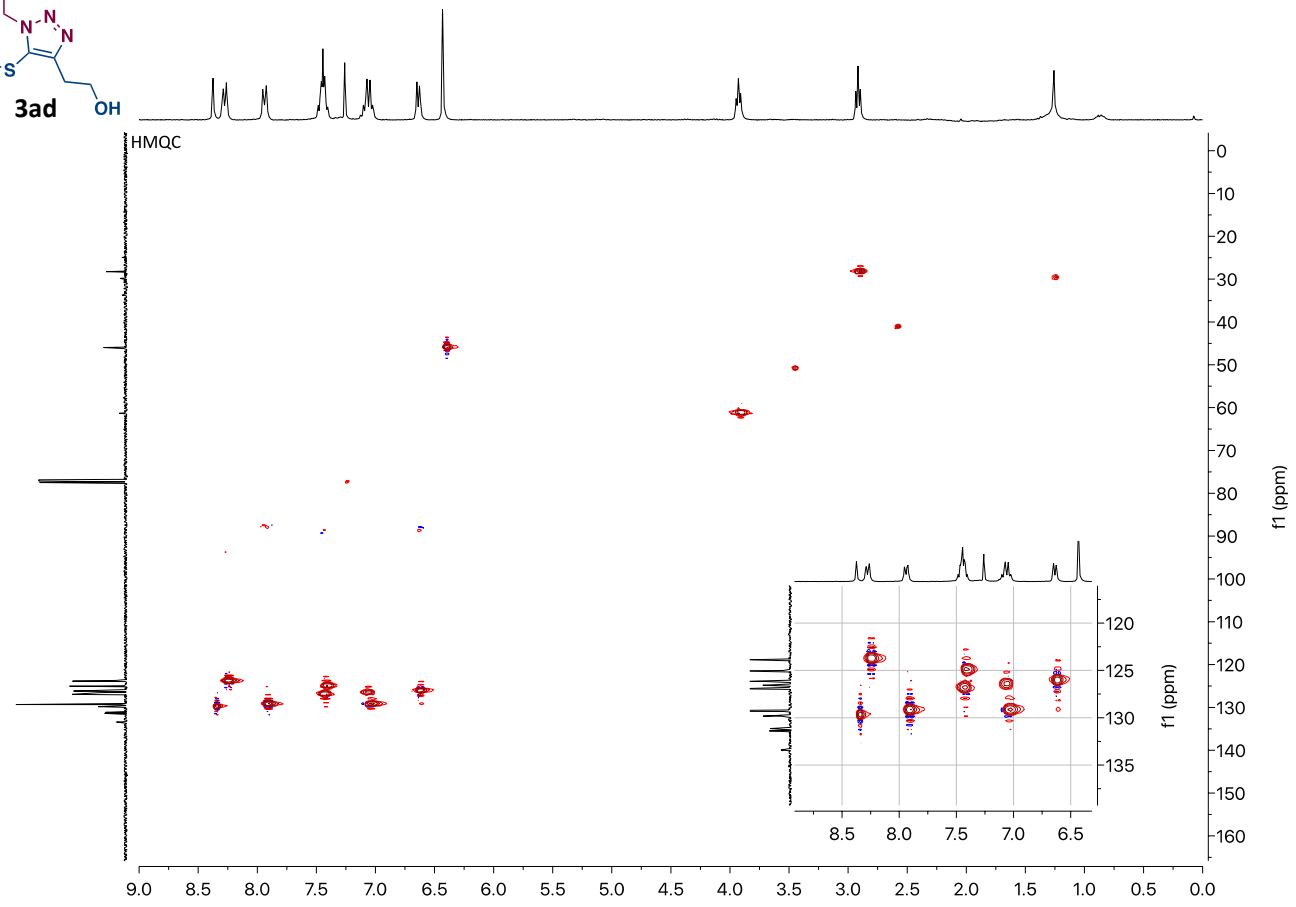


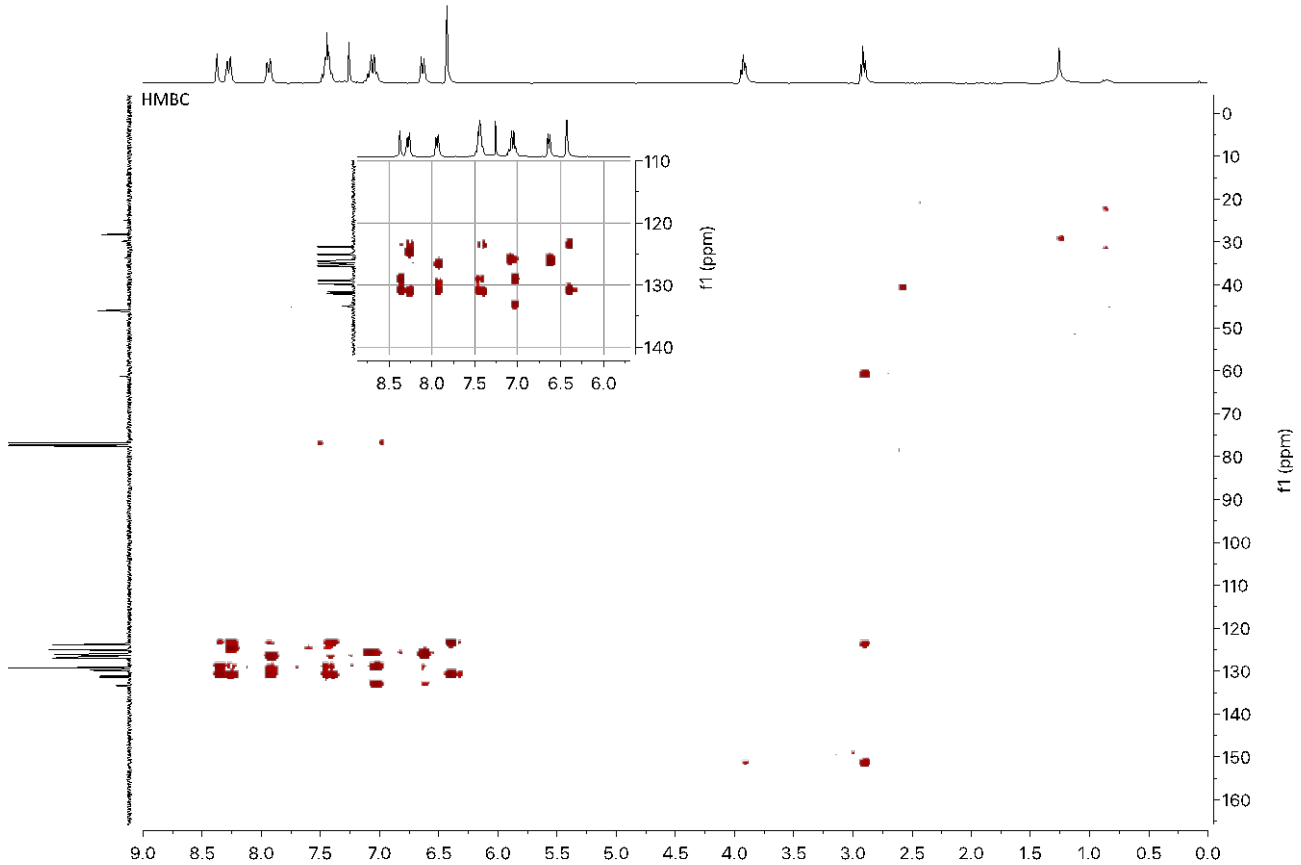


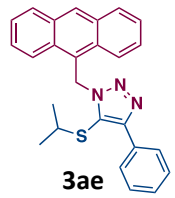




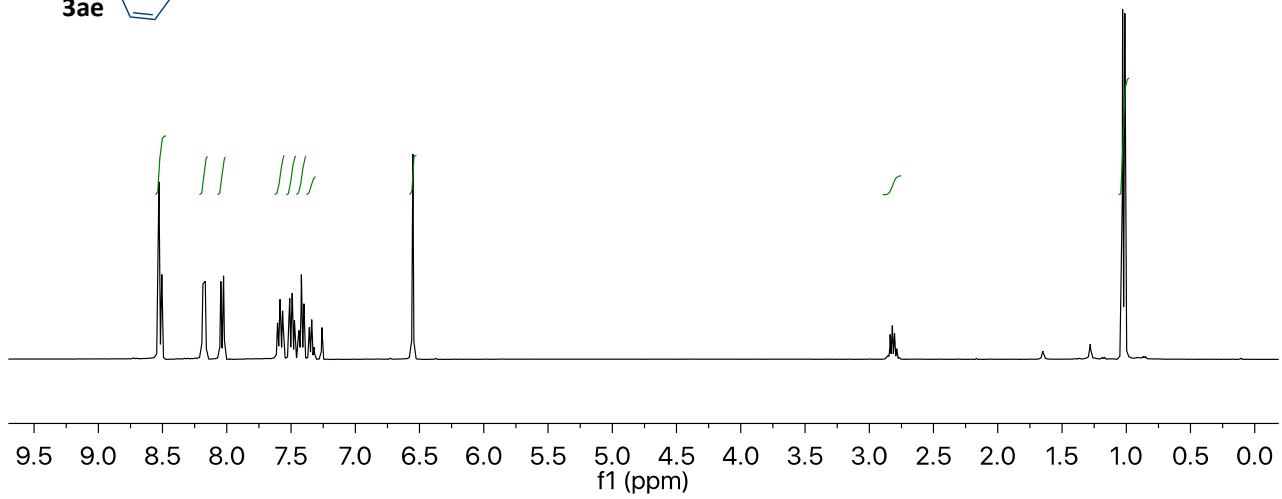
3ad







3ae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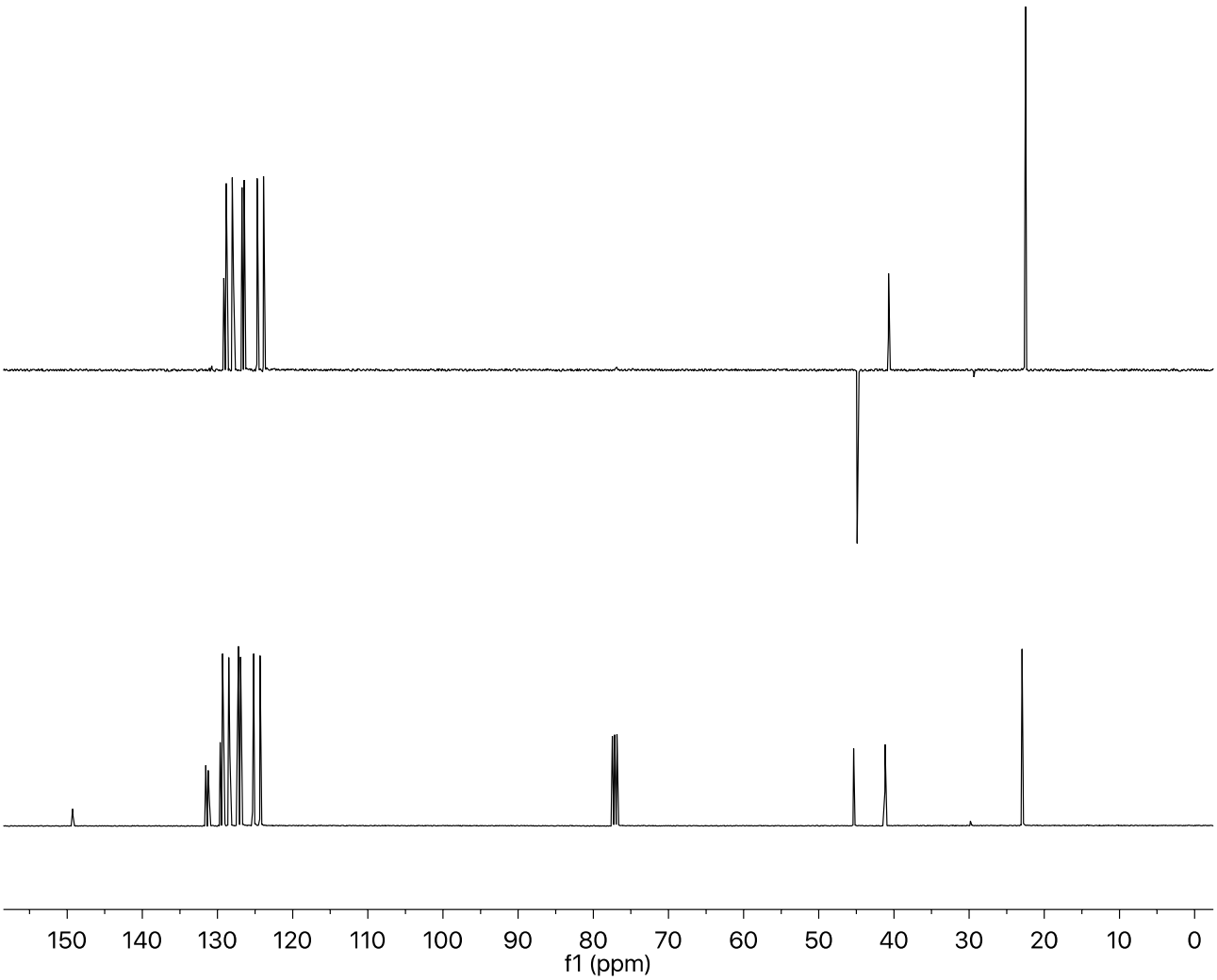
f1 (ppm)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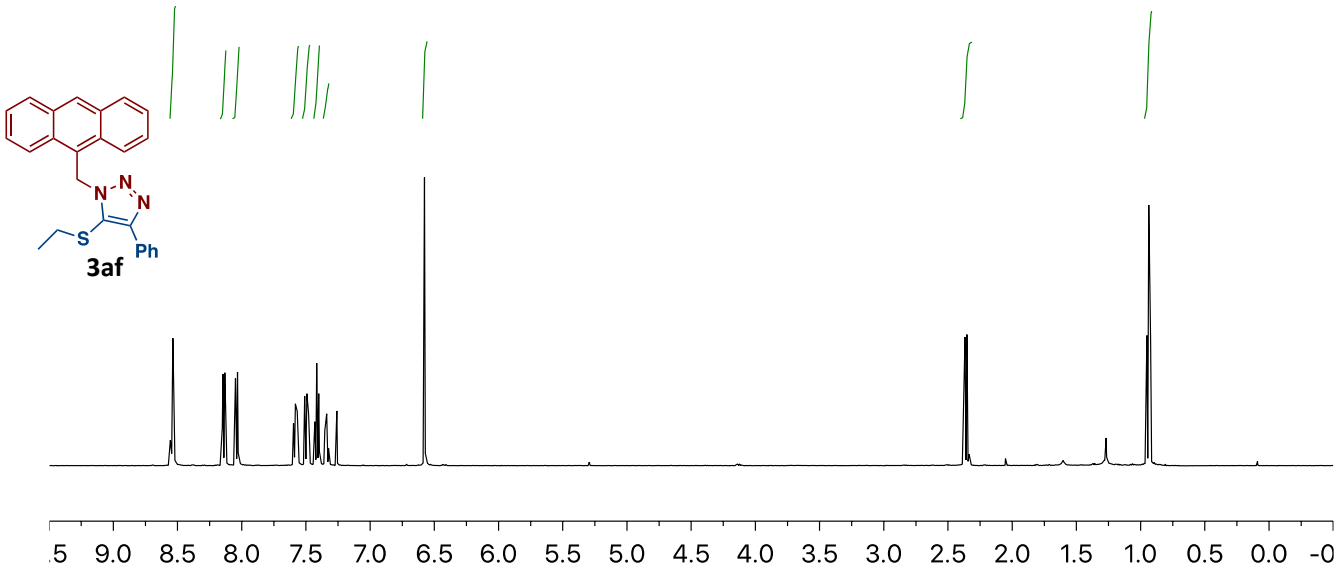
f1 (ppm)


N
N
N
S
Ph

3af

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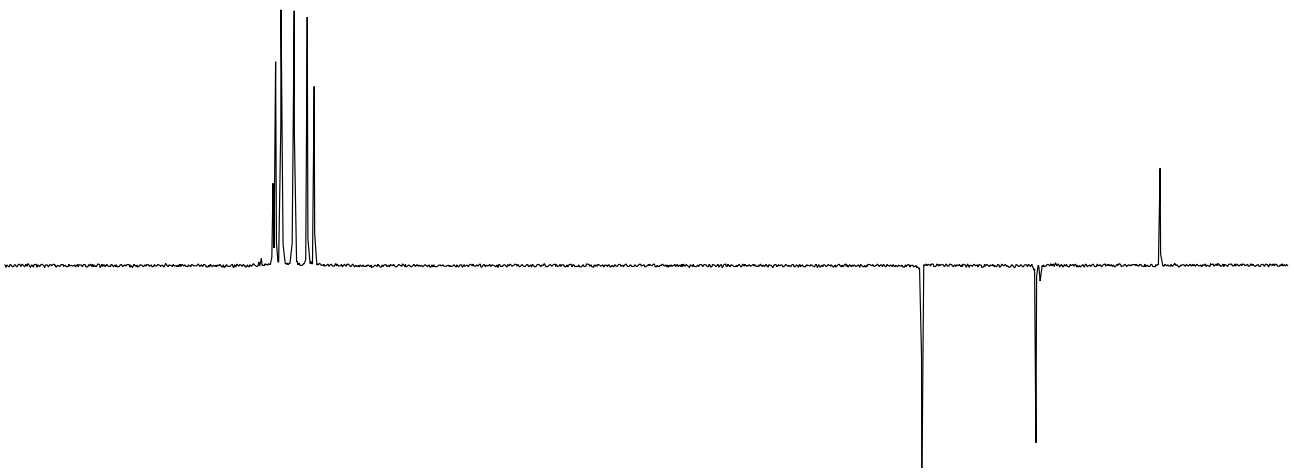

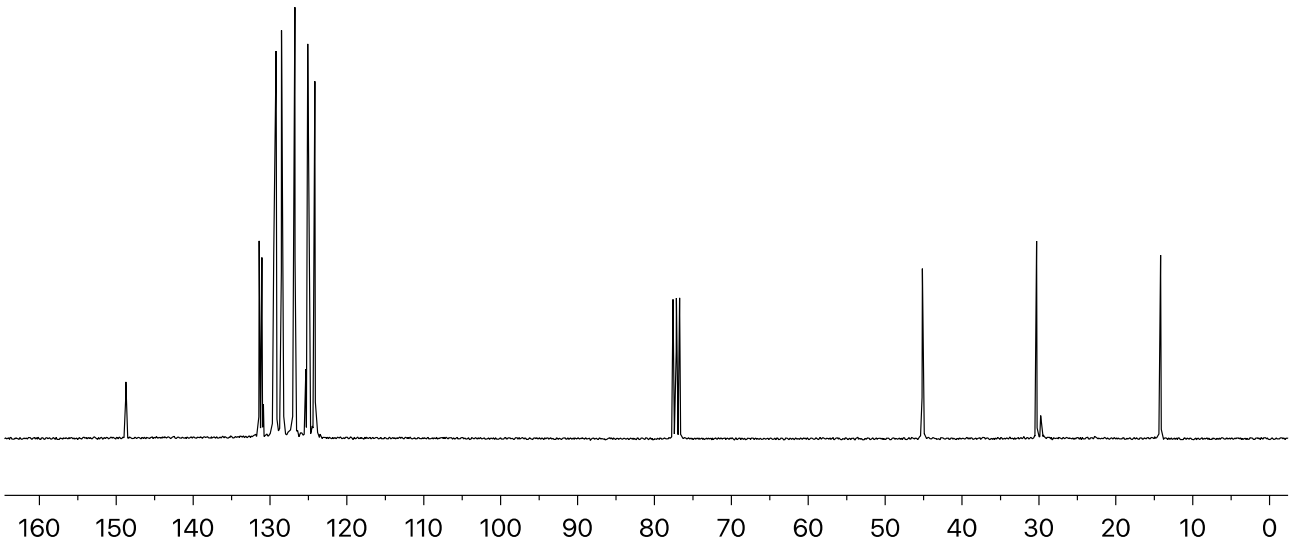
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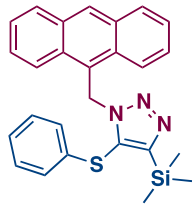
N
N
N
S
Si

3ag

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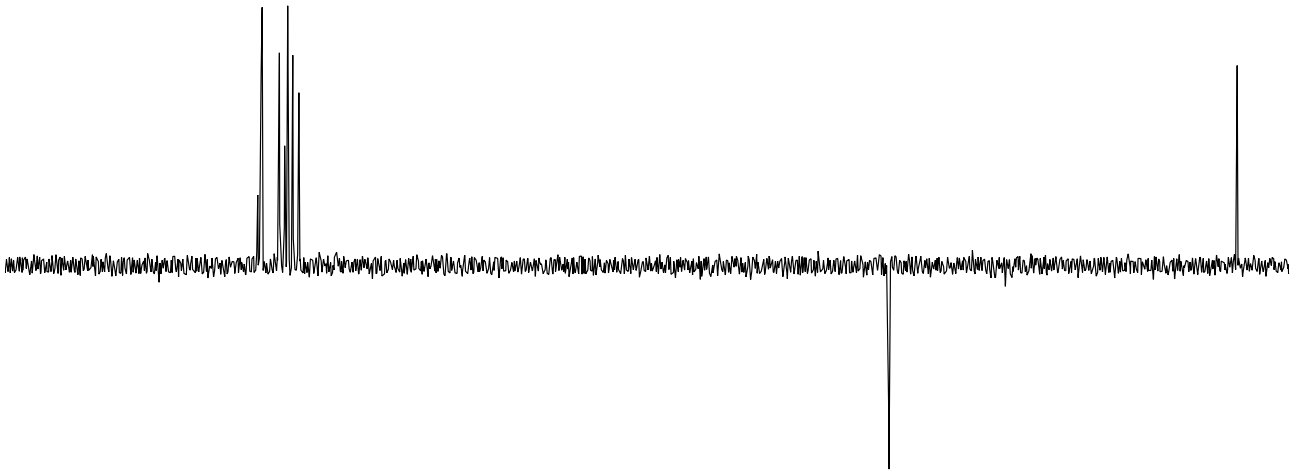

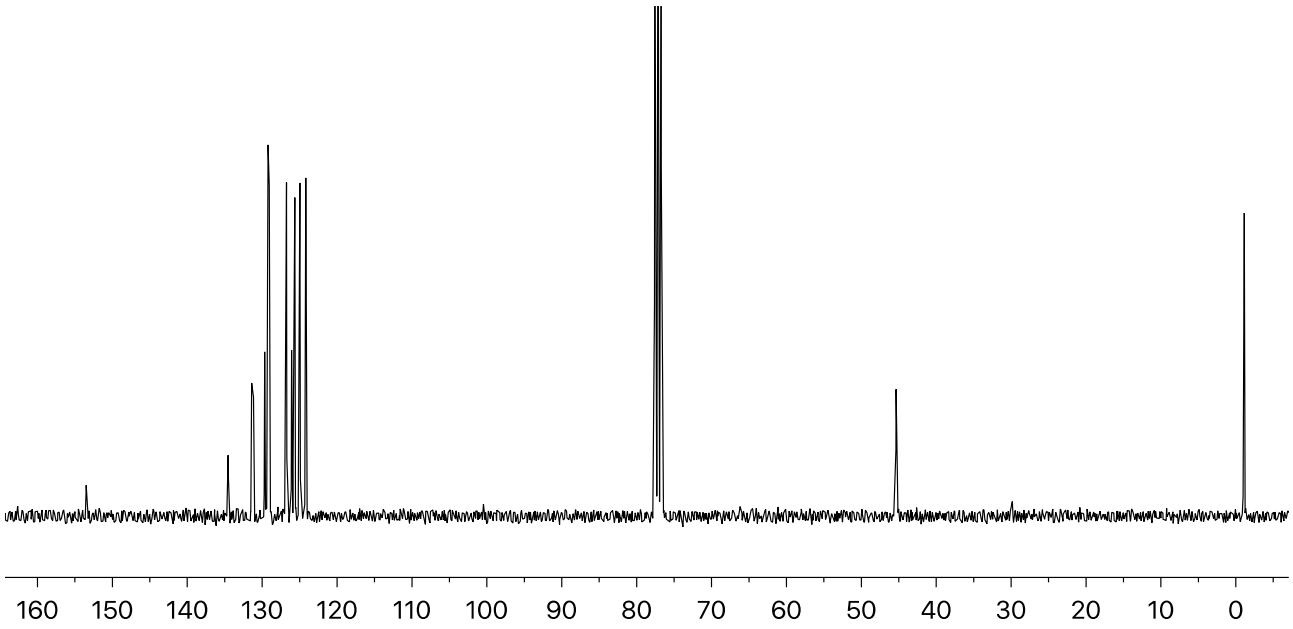
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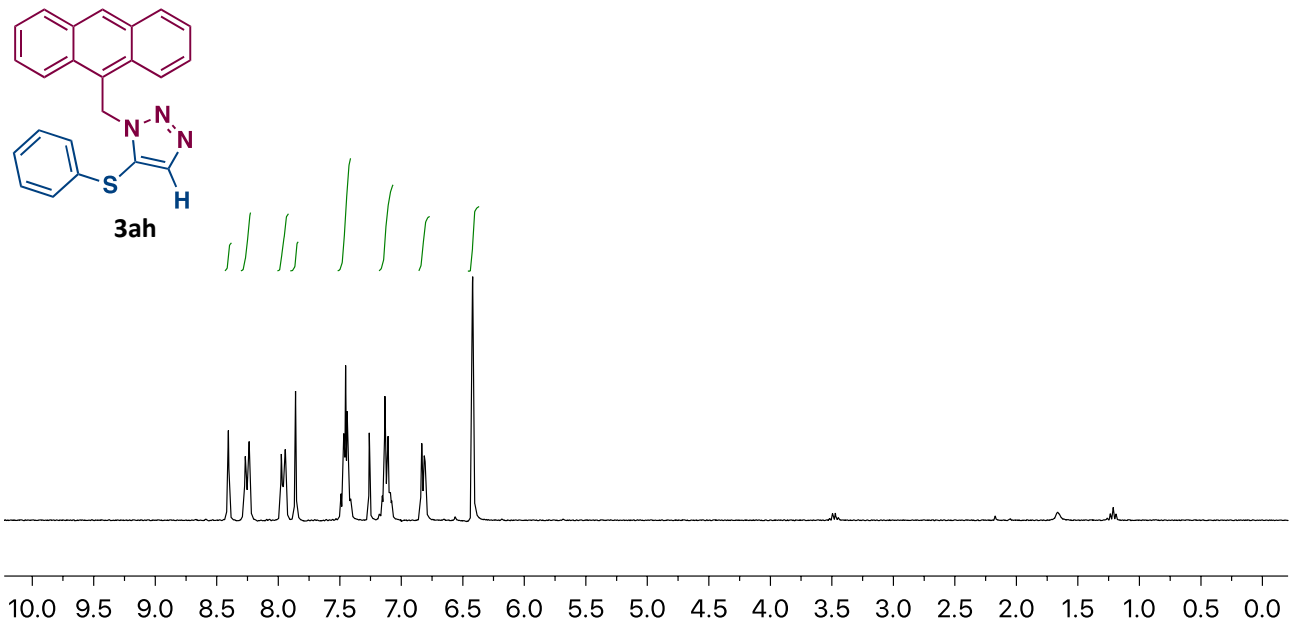
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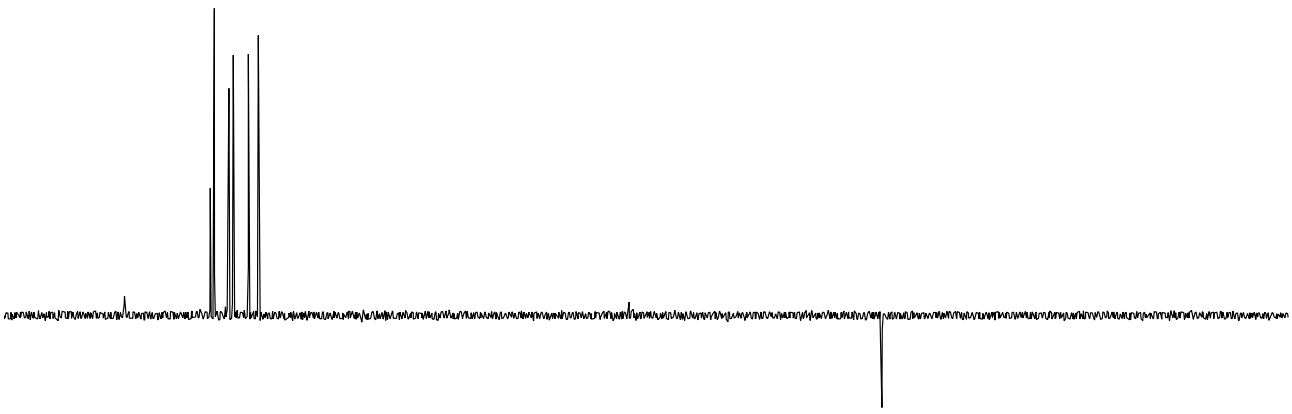

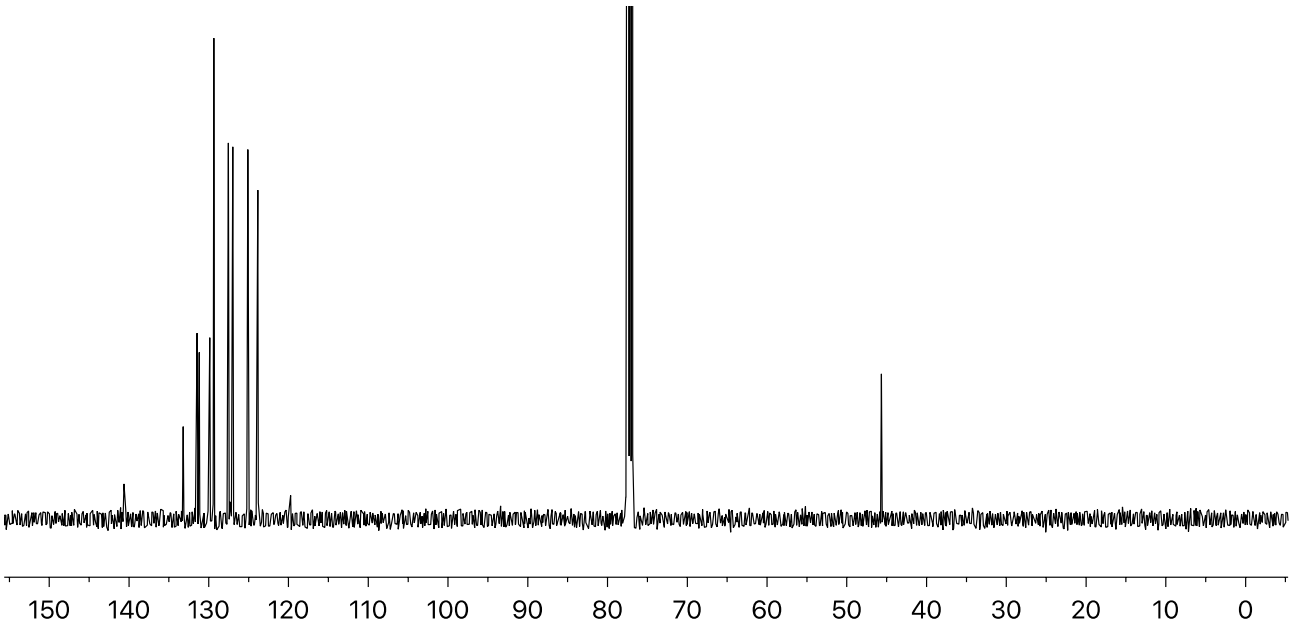
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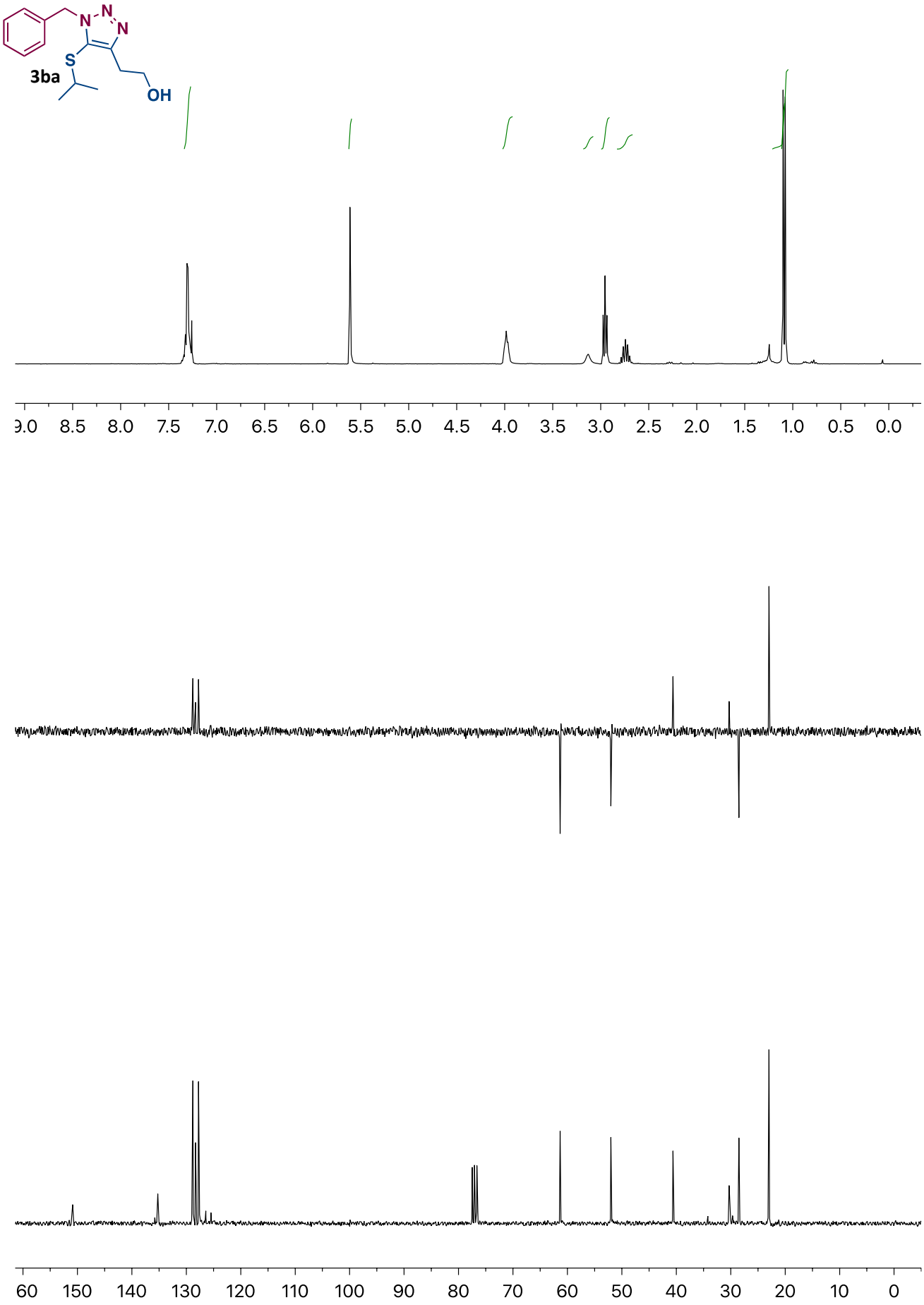
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N
S
OH
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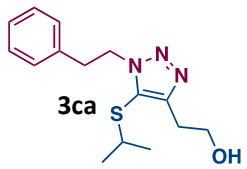
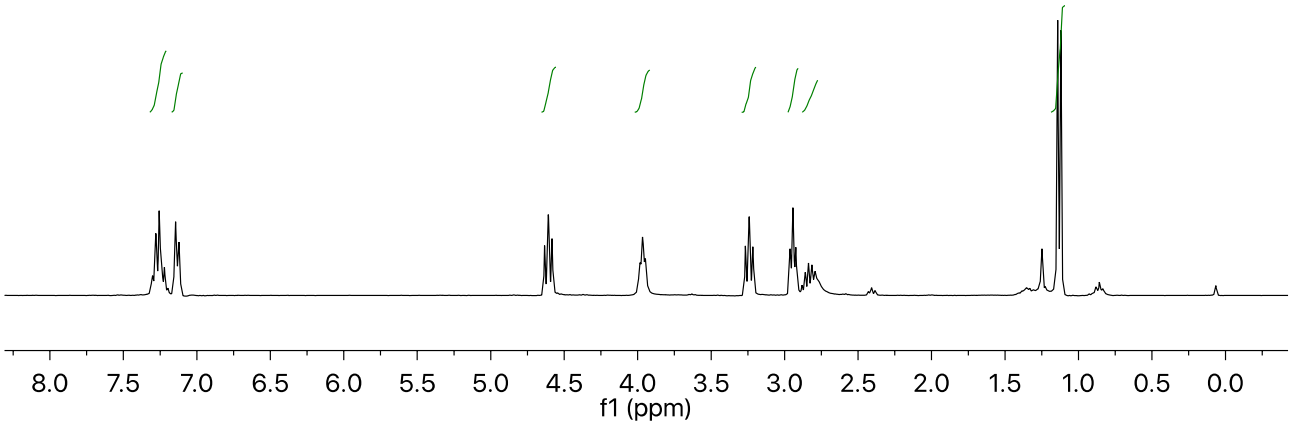
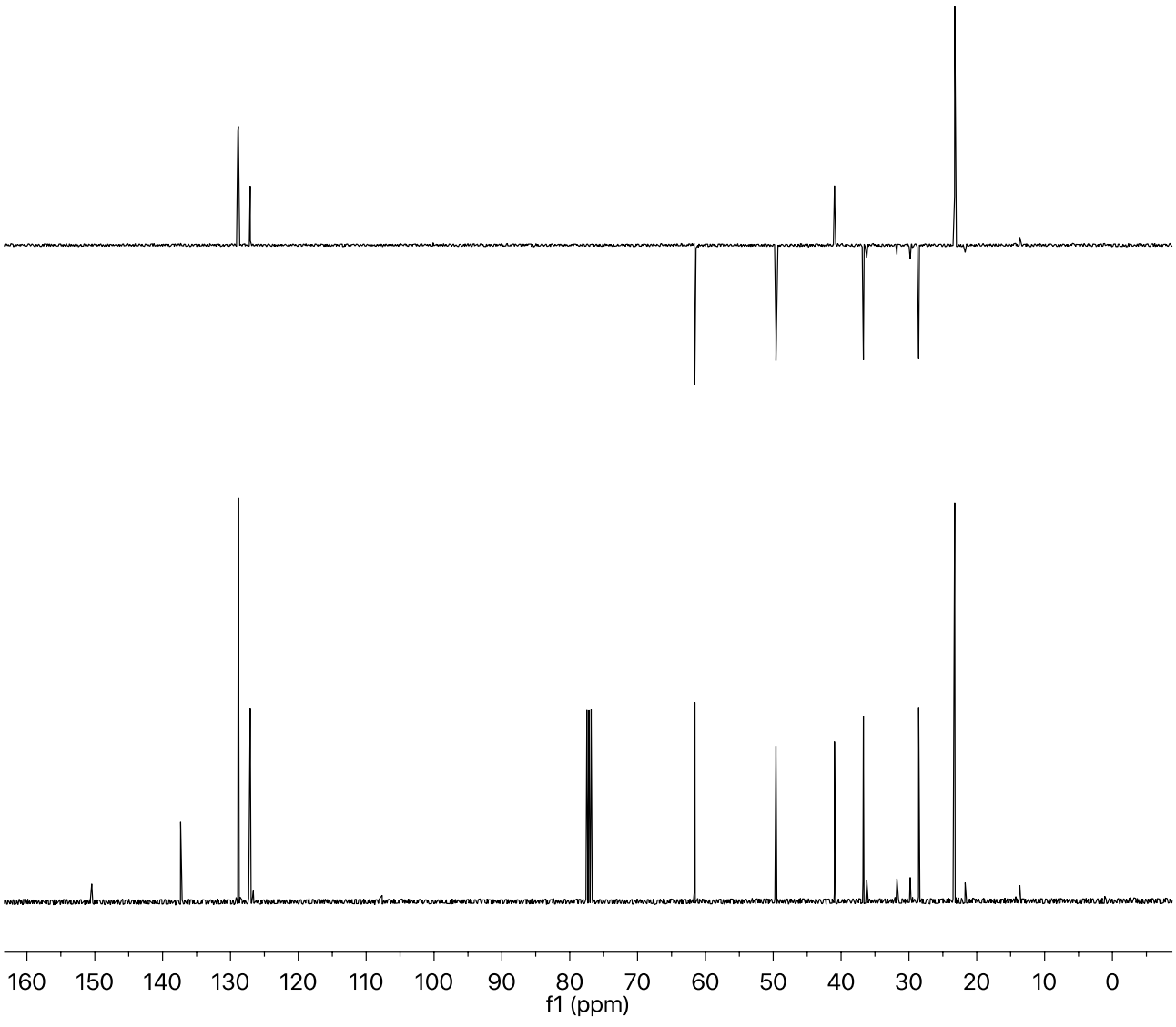
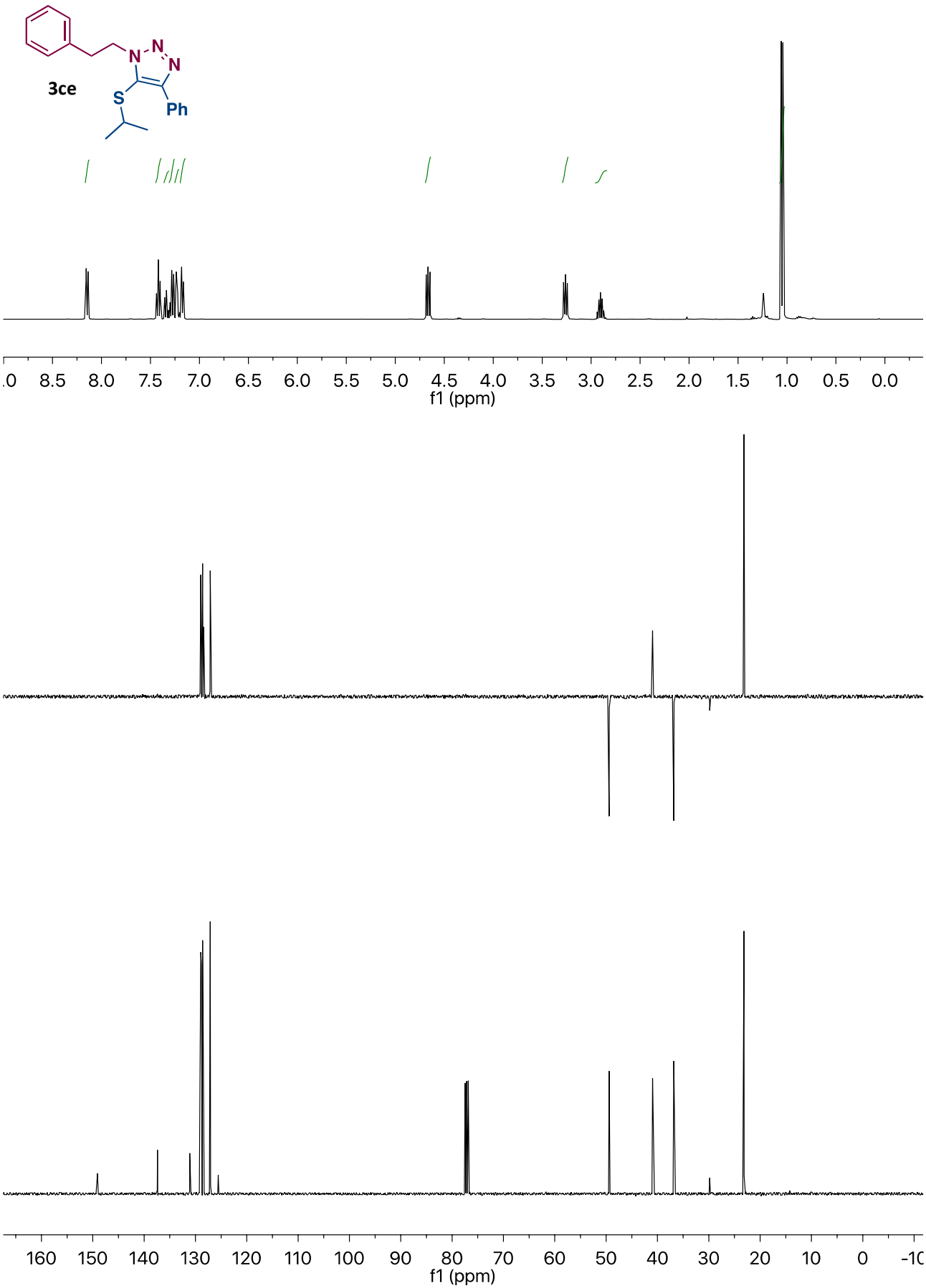
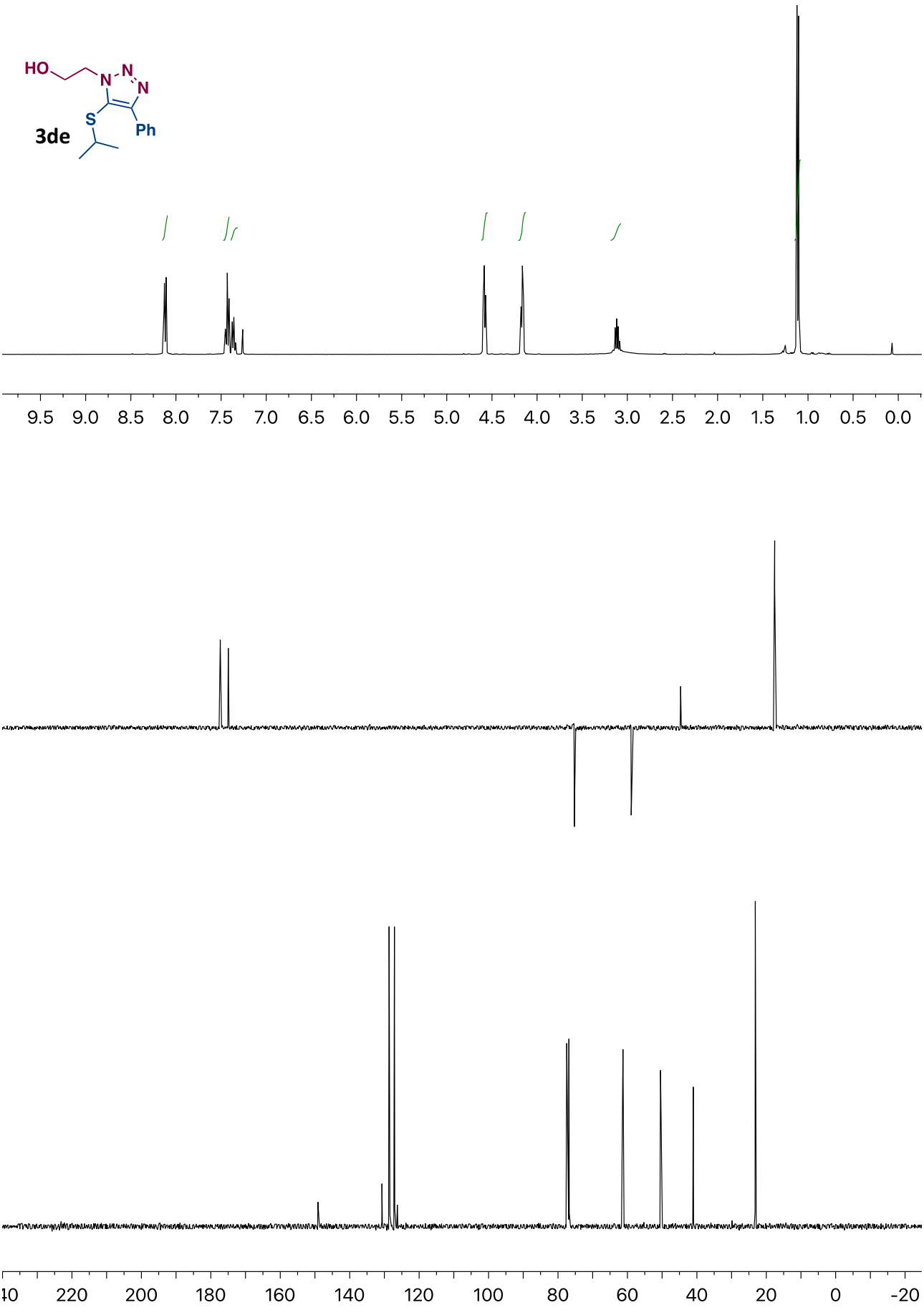
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
60
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

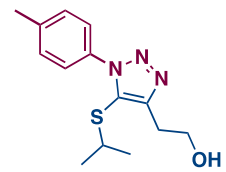

3ca
N
N
N
S
OH


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

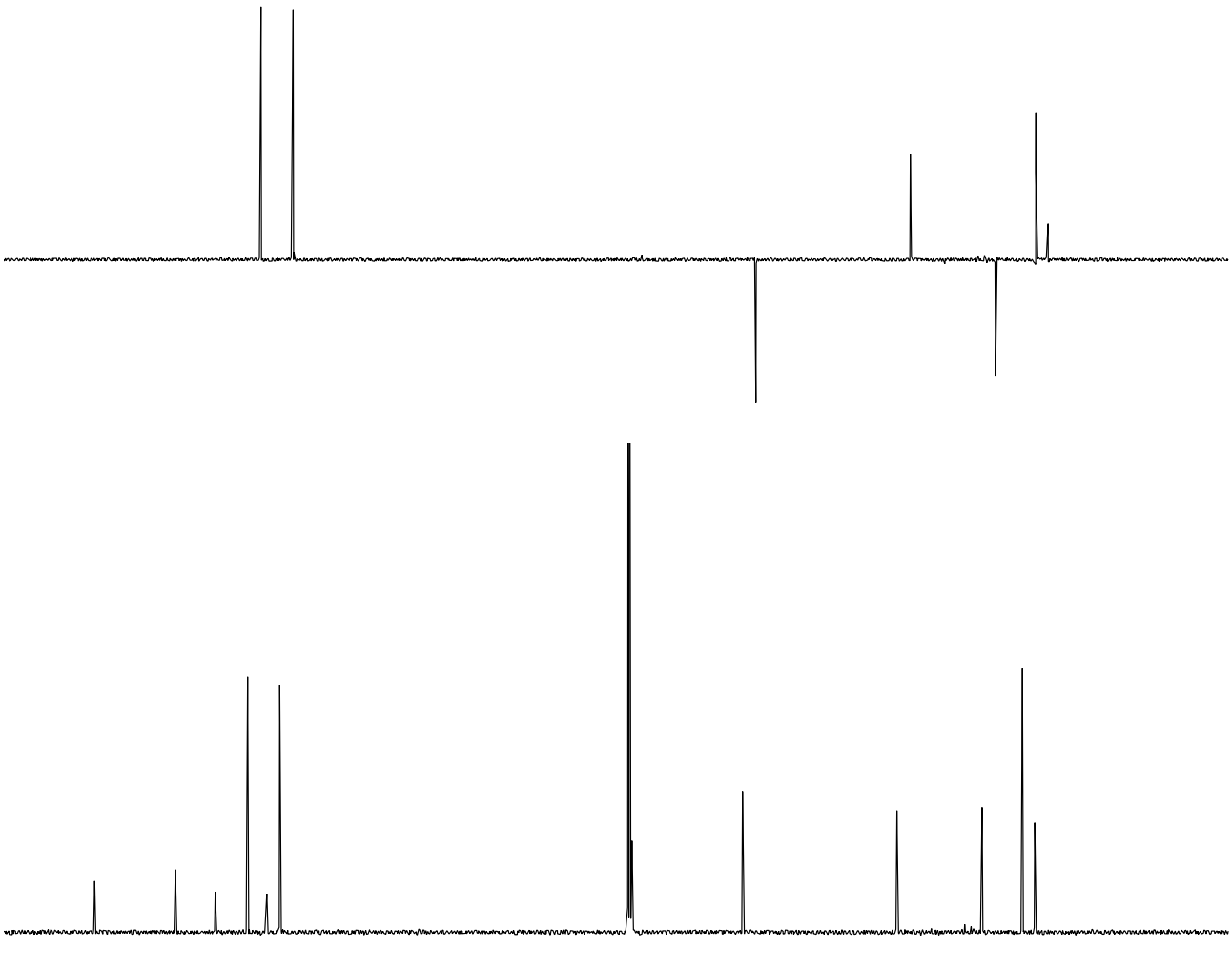

3ce
N
N
N
S
Ph
.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1(
f1 (ppm)

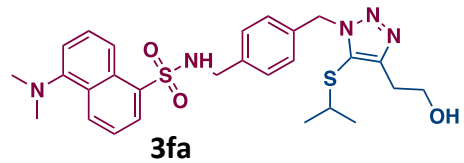

HO
N
N
N
S
Ph
3de
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
220 200 180 160 140 120 100 80 60 40 20 0 -20


N
N
N
S
OH

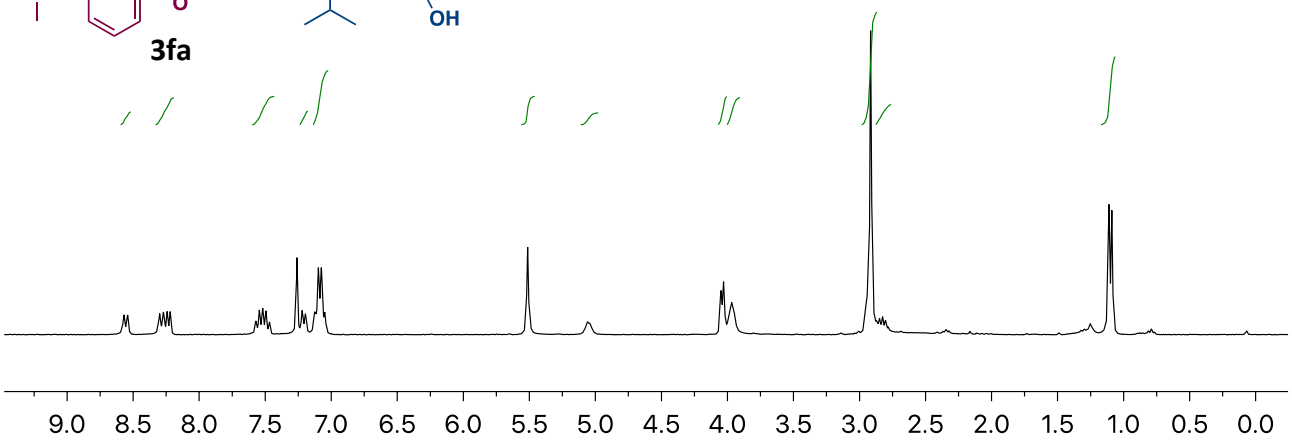
3ea

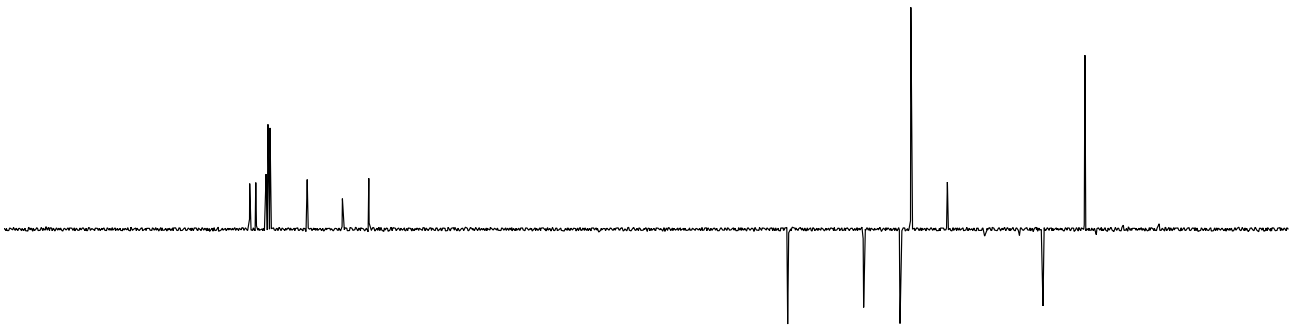
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

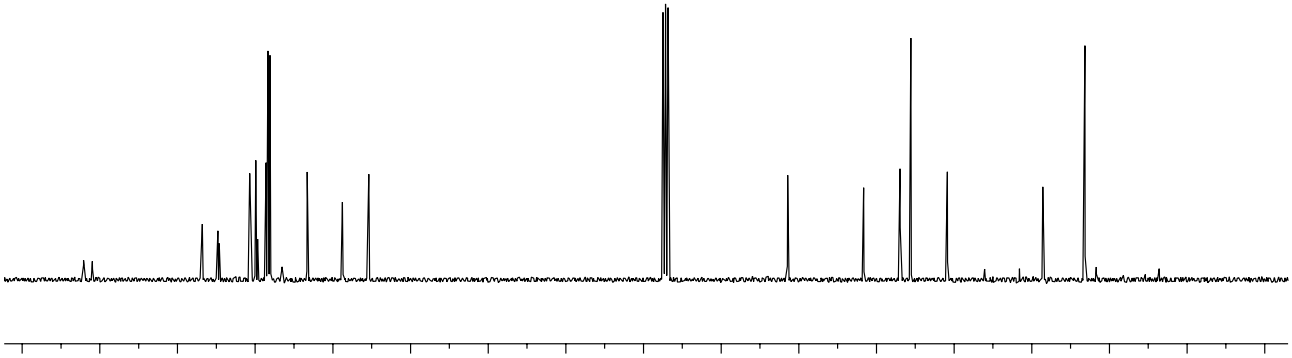

160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

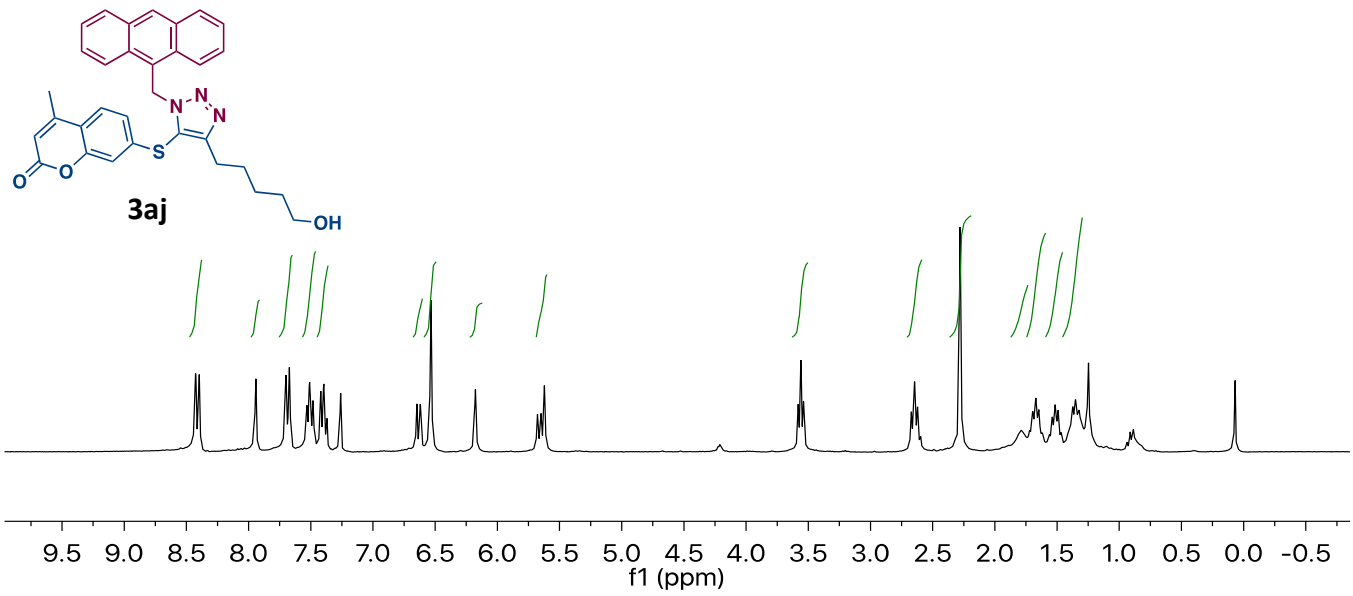

O
NH
S
O
N
N
N
S
OH

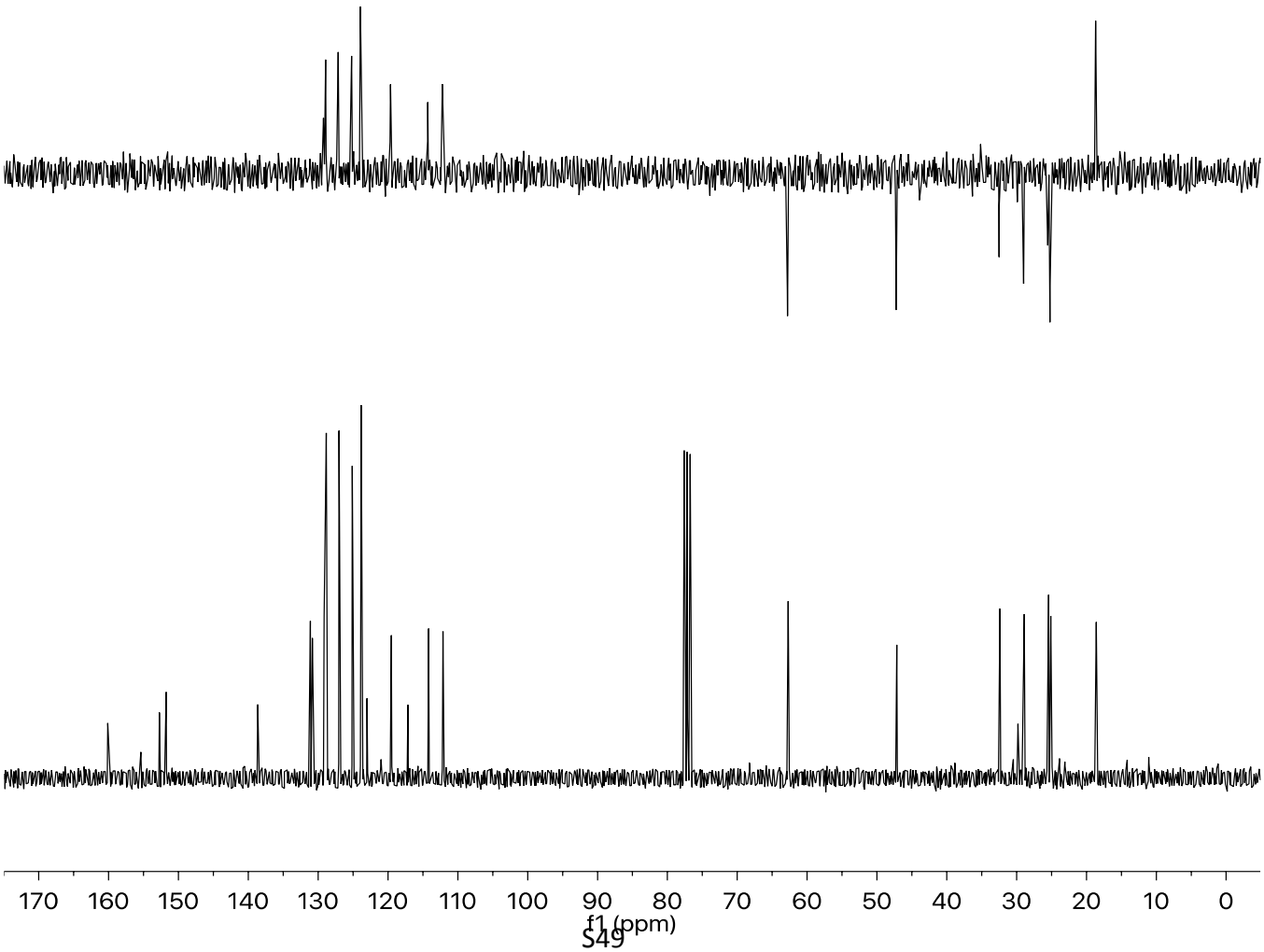
3fa

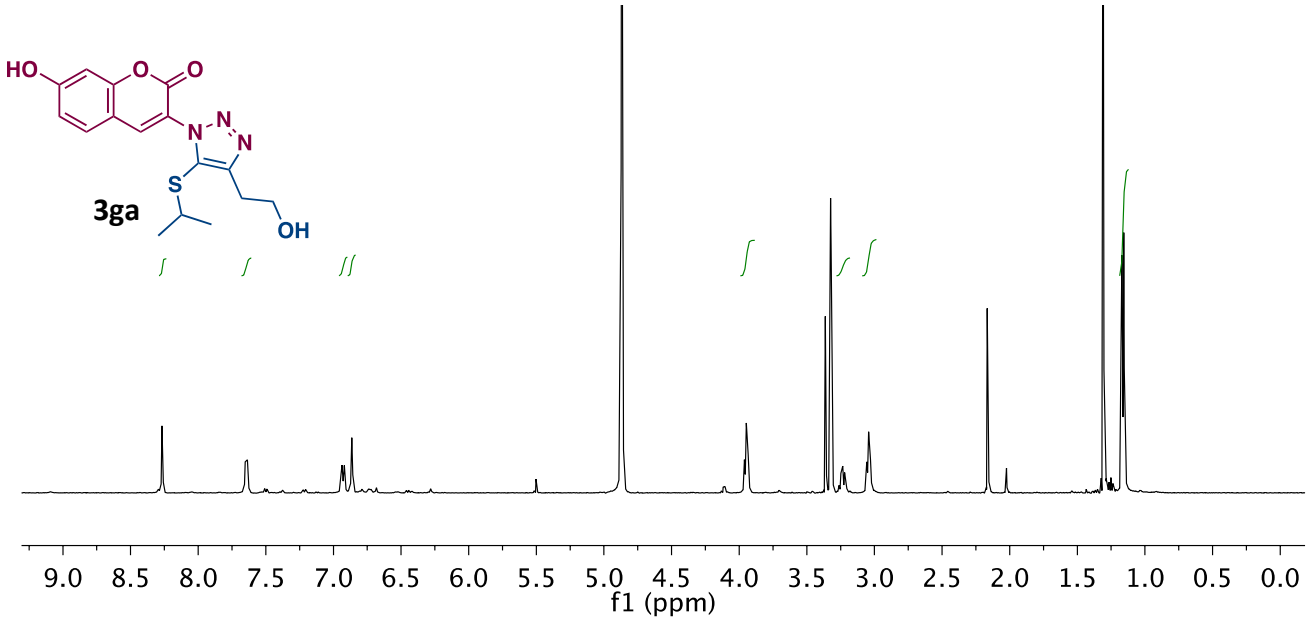

9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

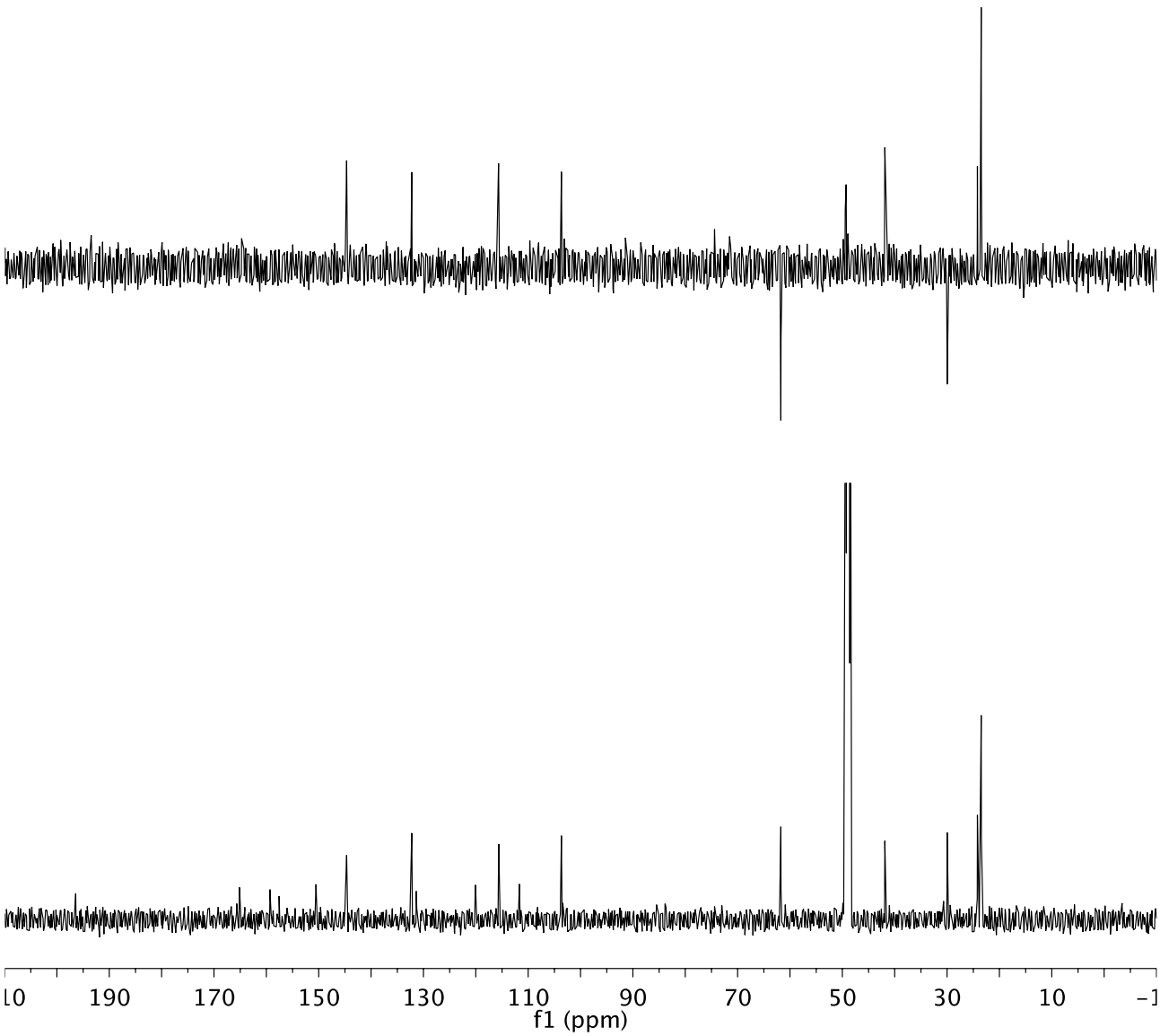


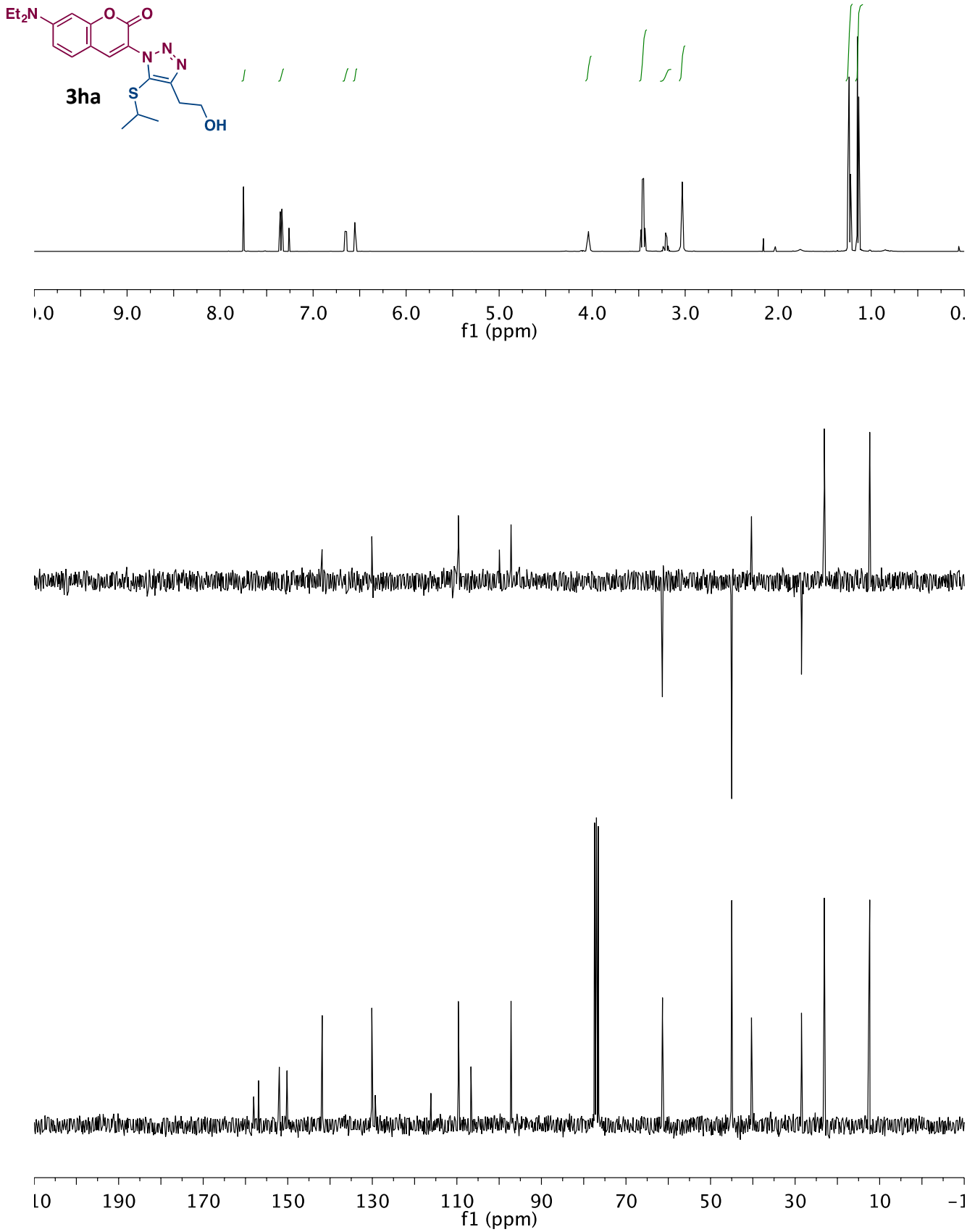

160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

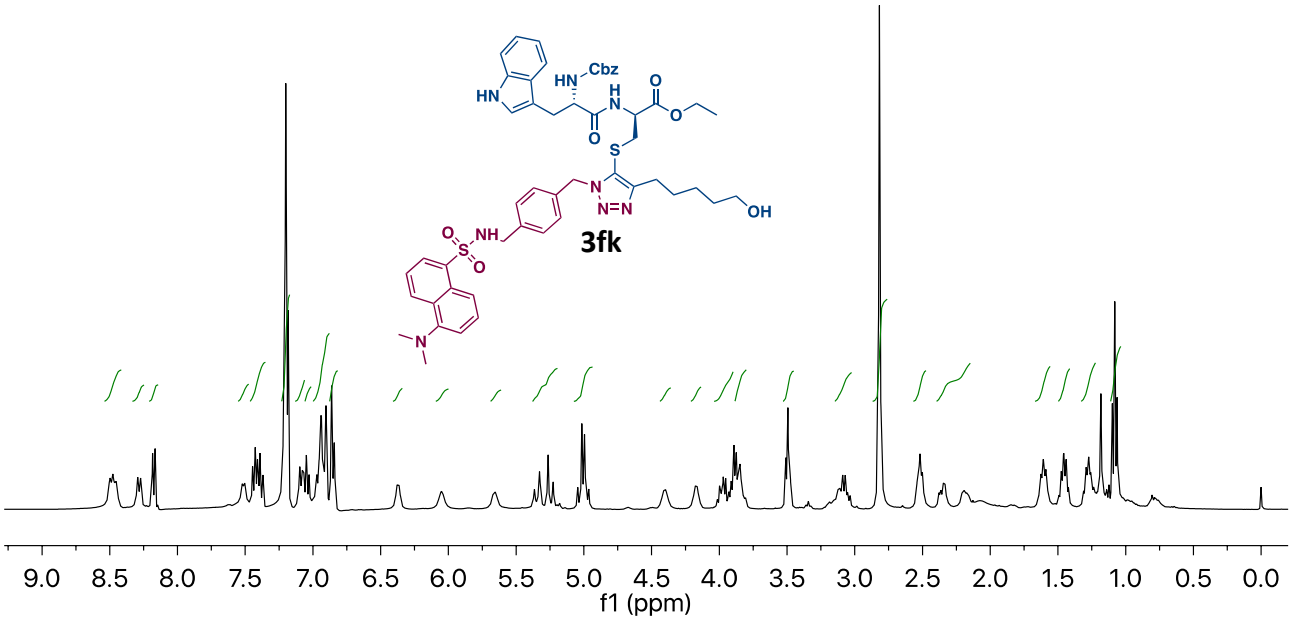

3aj
OH
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

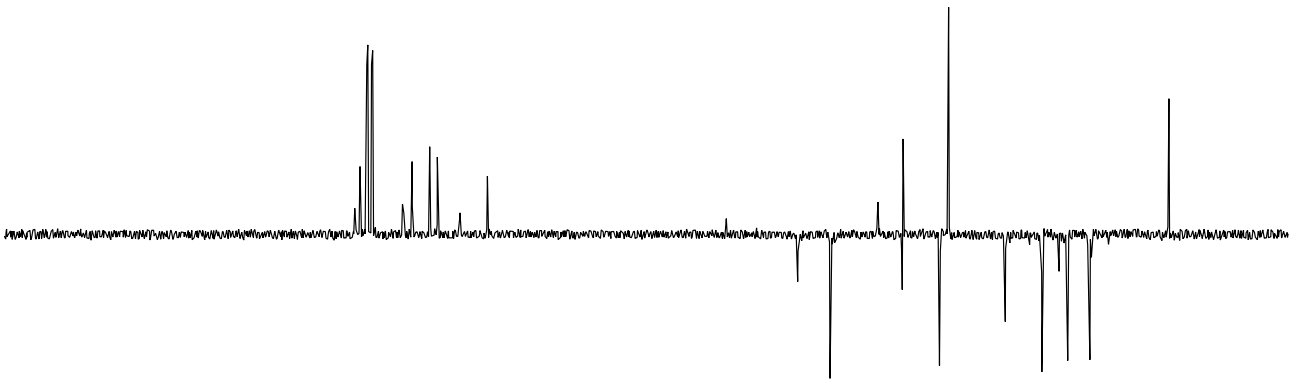

HO
O
O
N
N
N
S
OH
3ga
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

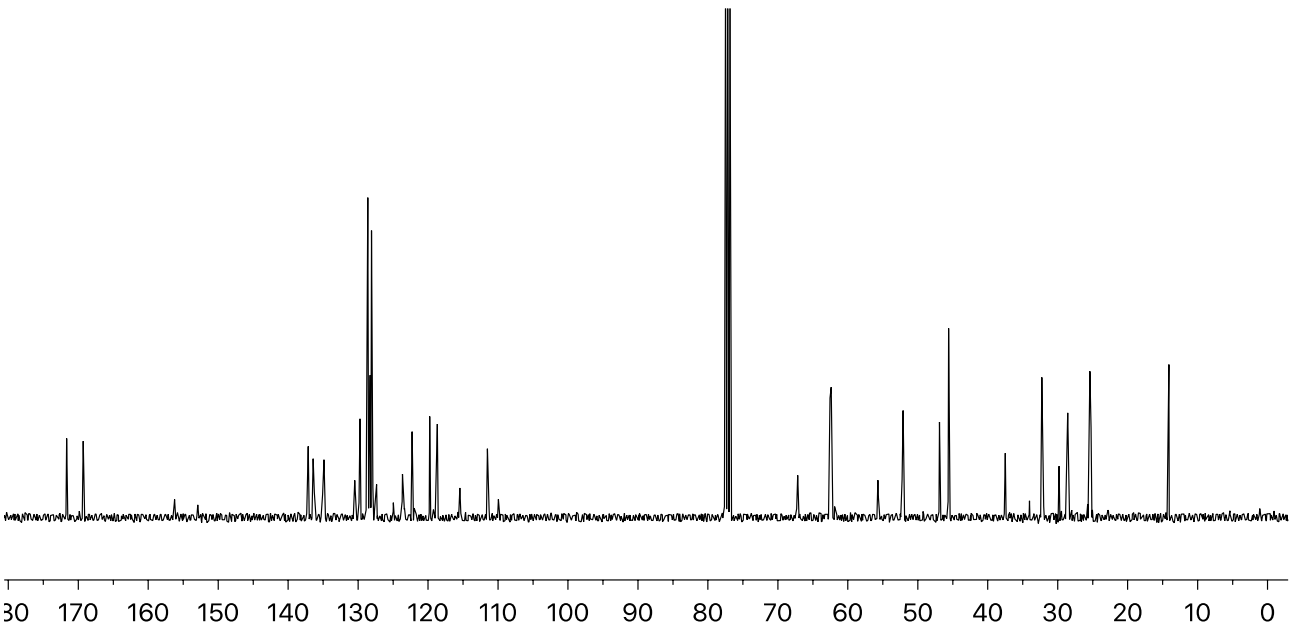

190 170 150 130 110 90 70 50 30 10
f1 (ppm)

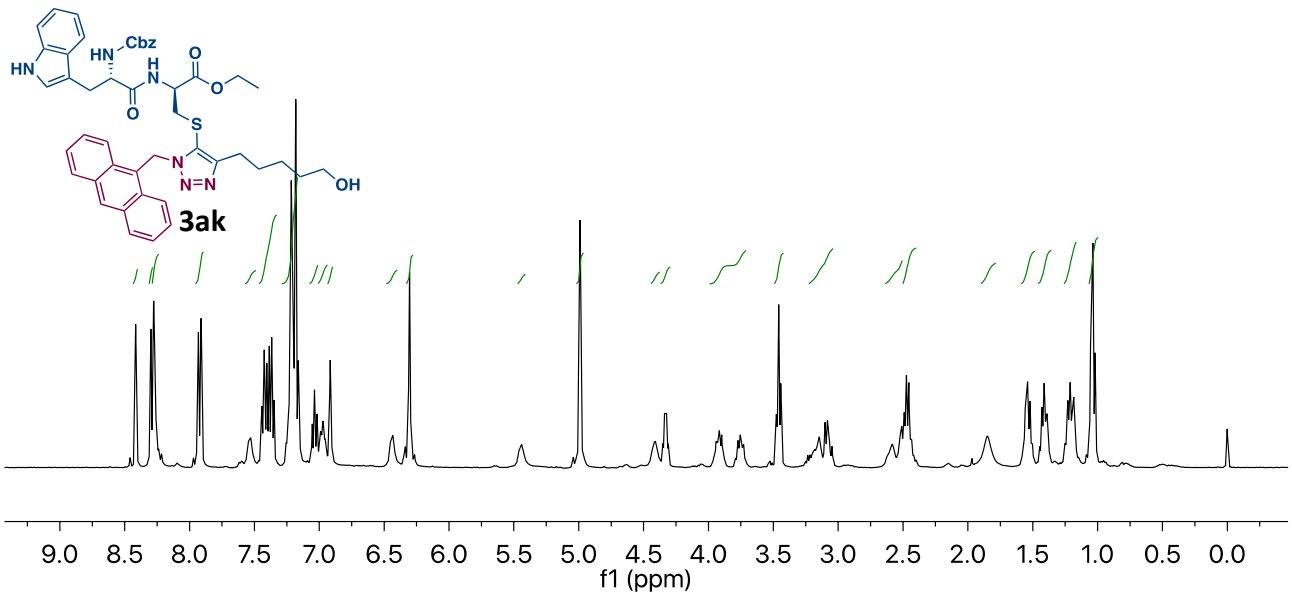

Et2N
O
O
N
N
N
S
OH
3ha
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
f1 (ppm)
190
170
150
130
110
90
70
50
30
10
f1 (ppm)

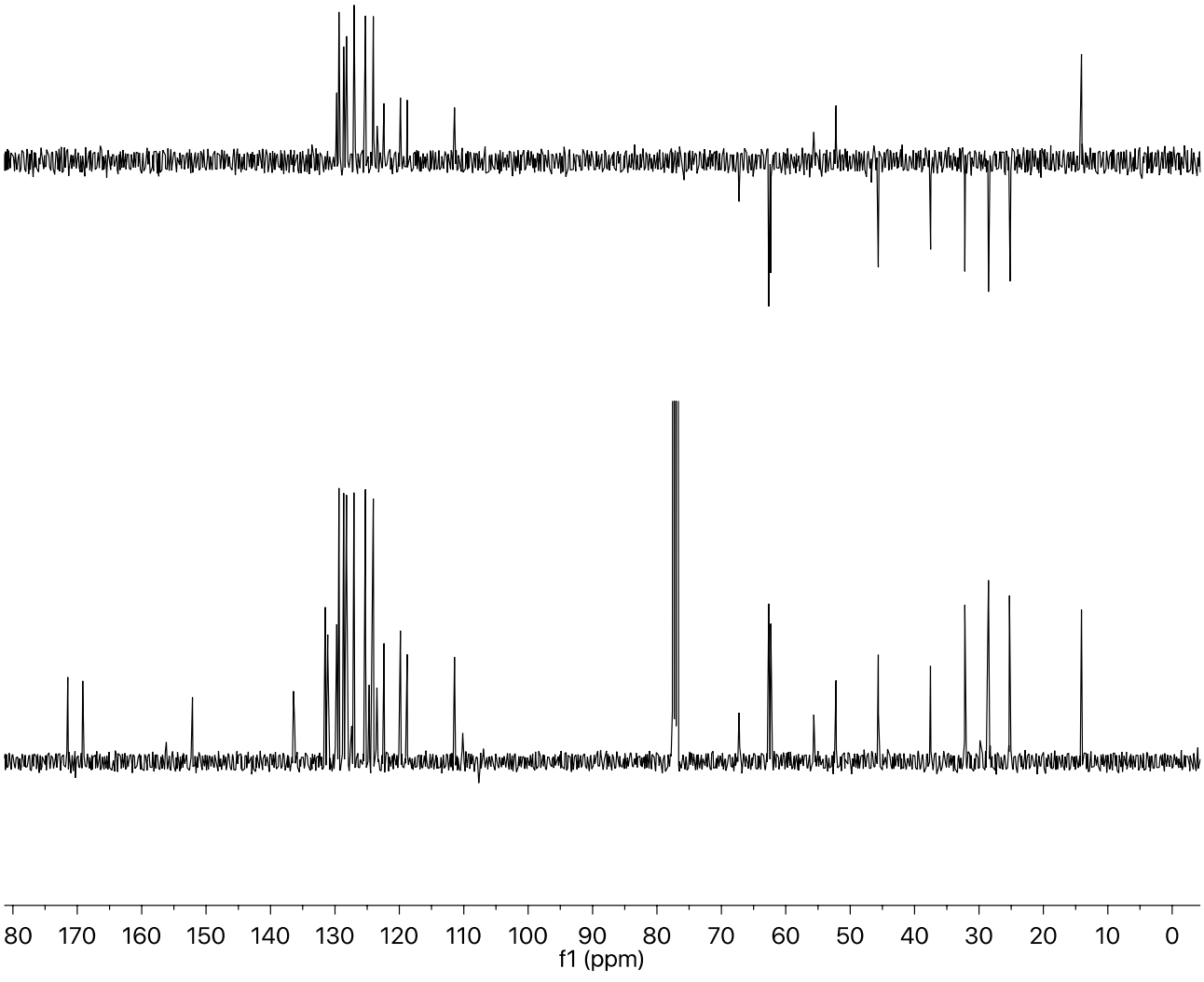

Cbz
HN
HN
H
N
O
O
O
O
S
N
N=N
OH
NH
O
S
O
N
3fk

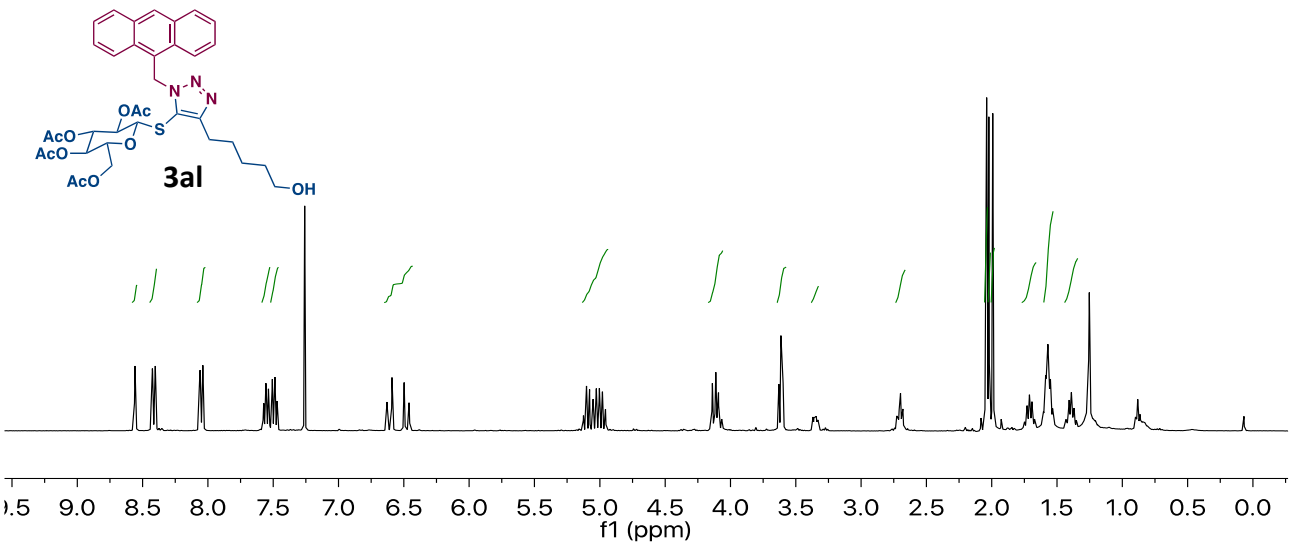
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

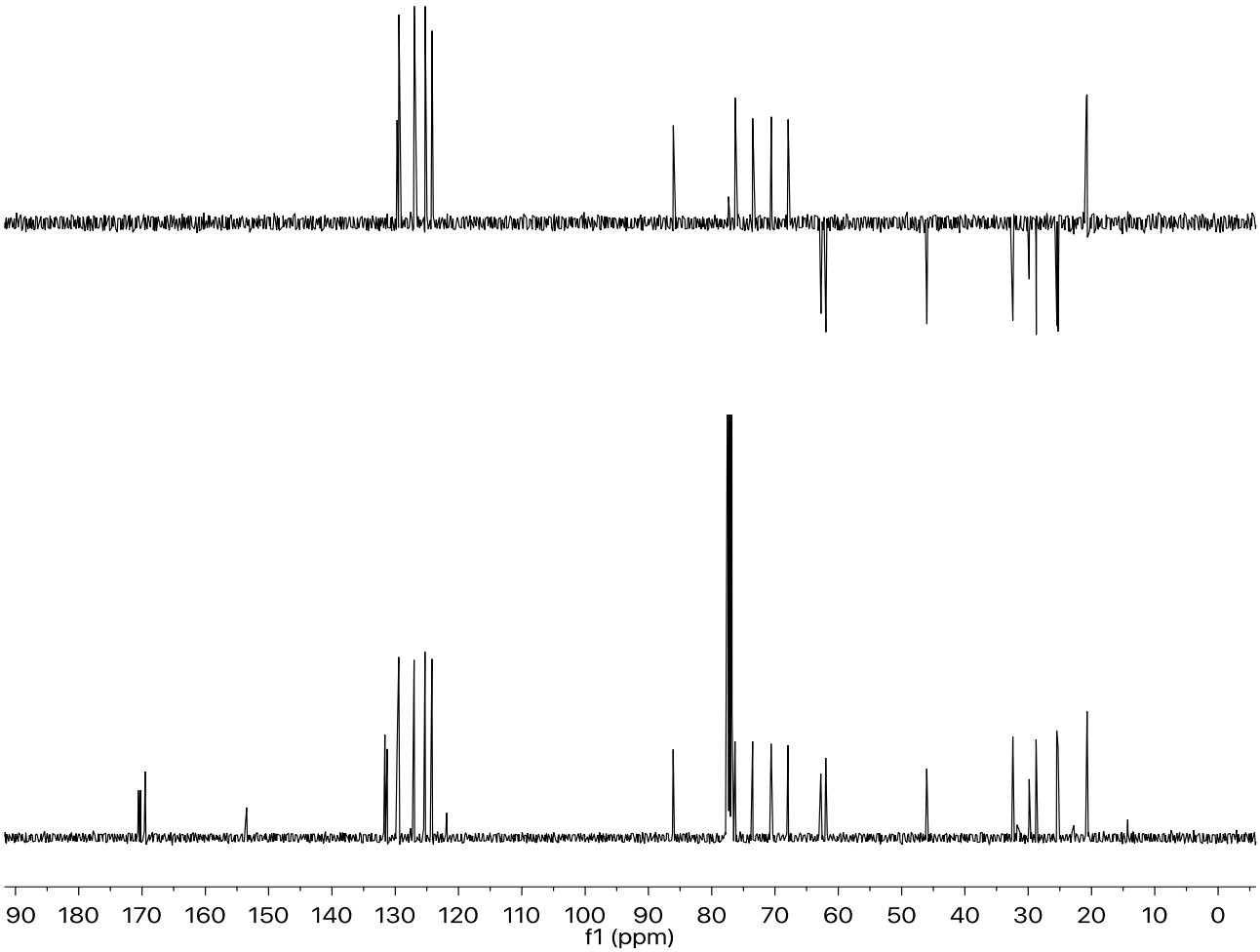


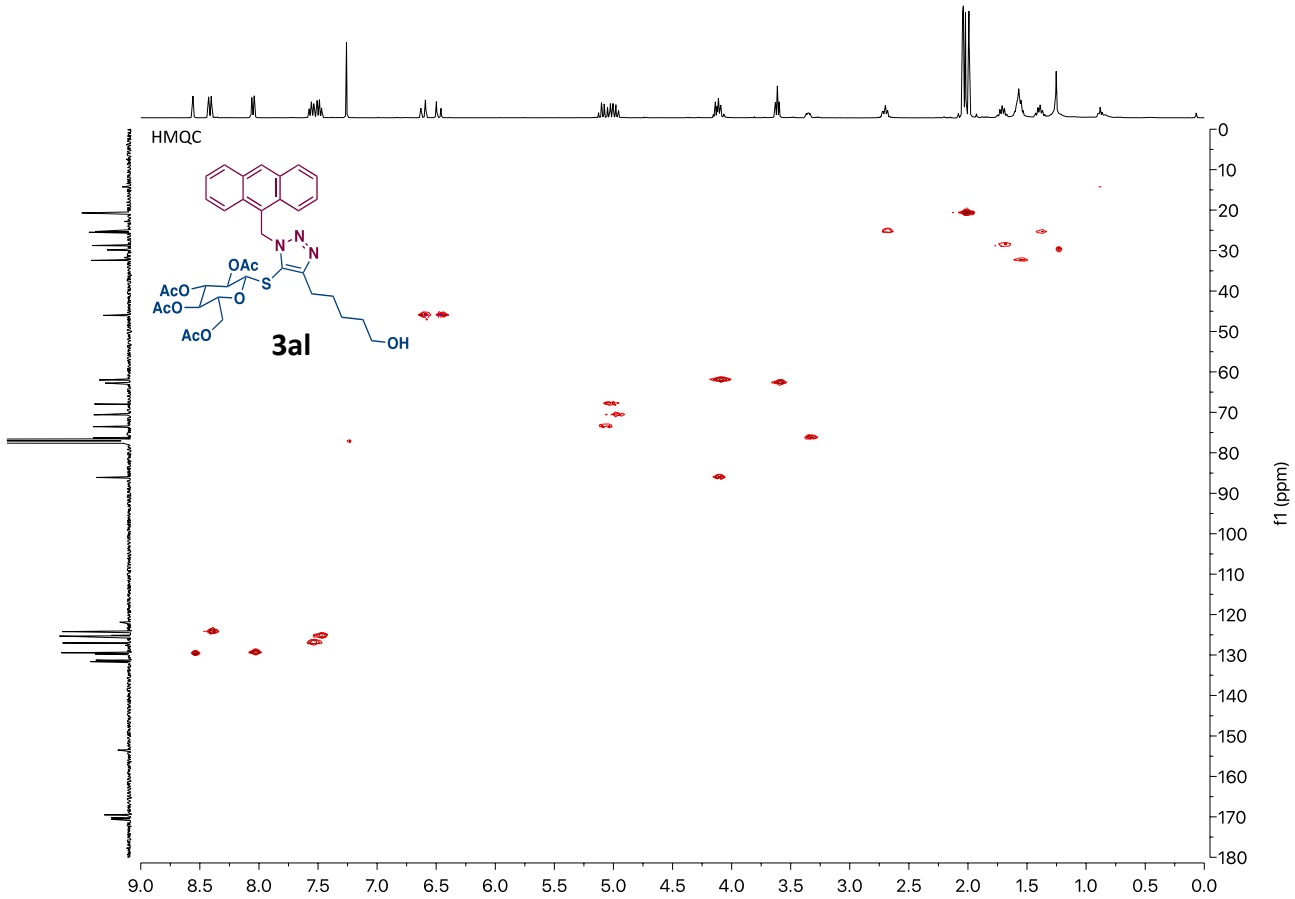

30 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

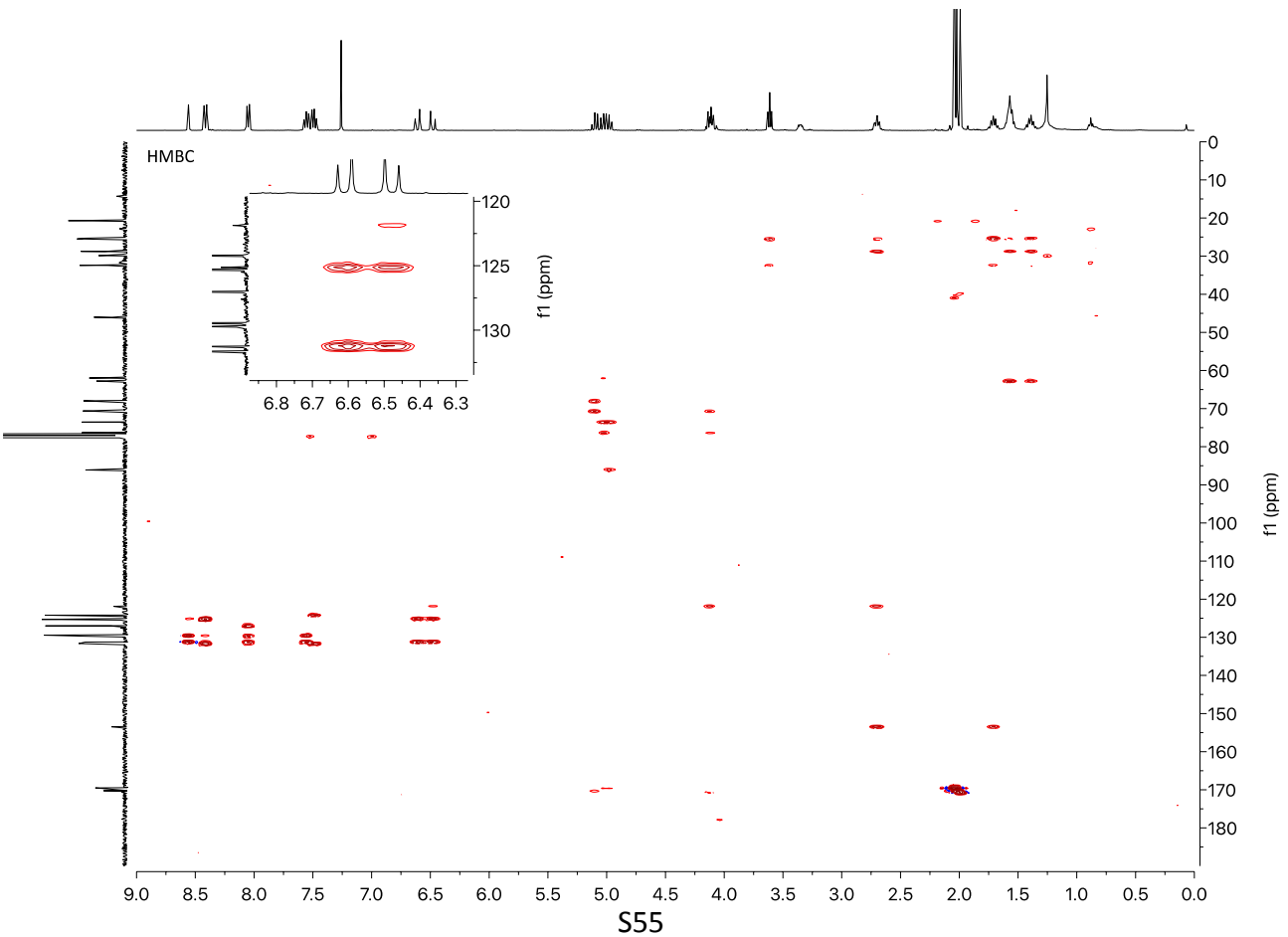

HN
HN
Cbz
H
N
O
O
O
S
N
N=N
OH
3ak
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

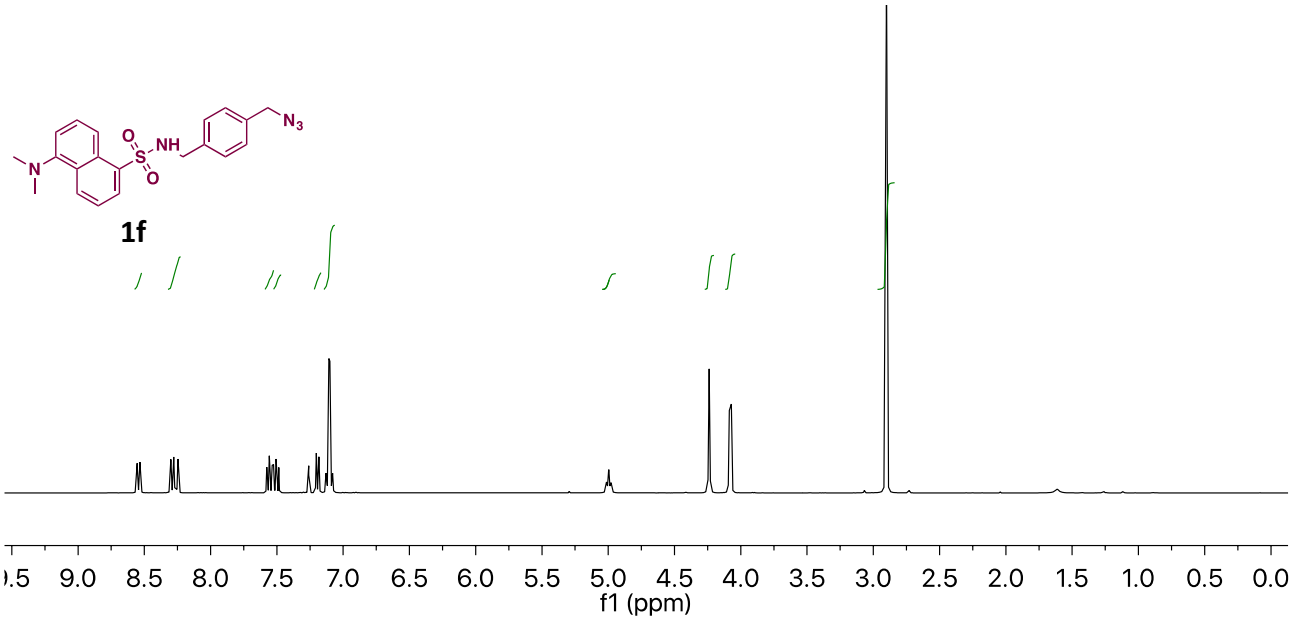

80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

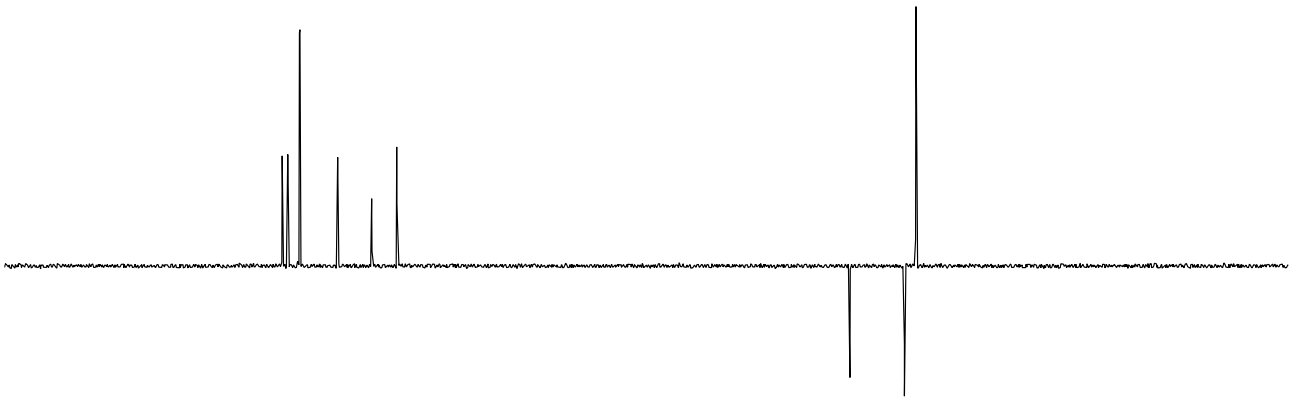

OAc
AcO
AcO
AcO
O
S
N
N
N
OH
3al
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

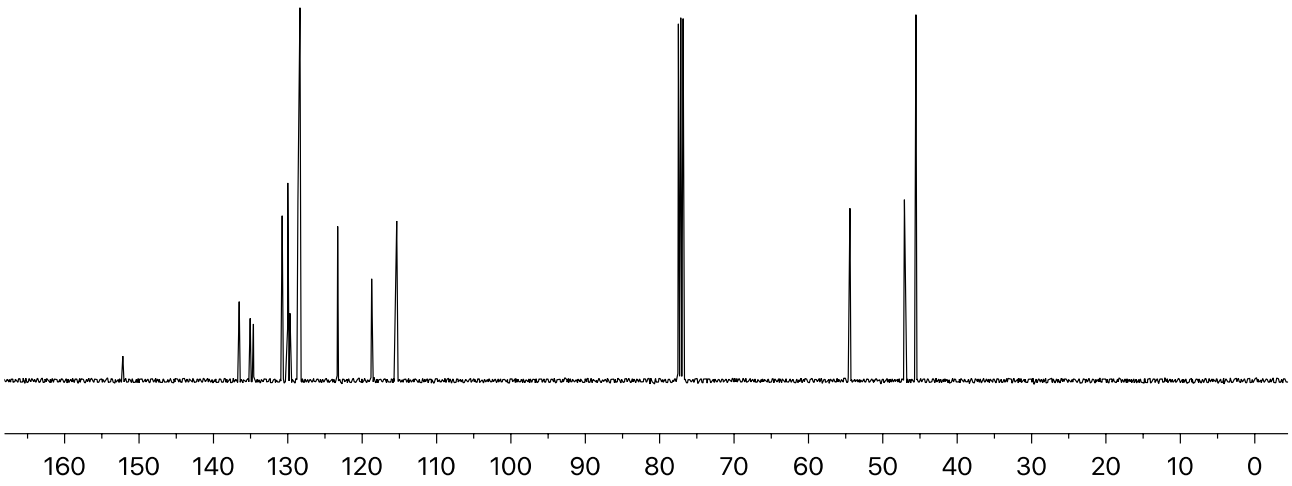

90 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

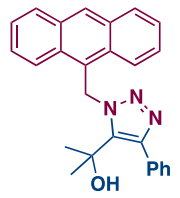

HMQC
OAc
AcO
AcO
O
S
N
N
N
AcO
OH
3al
f1 (ppm)


HMBC
f1 (ppm)
f1 (ppm)

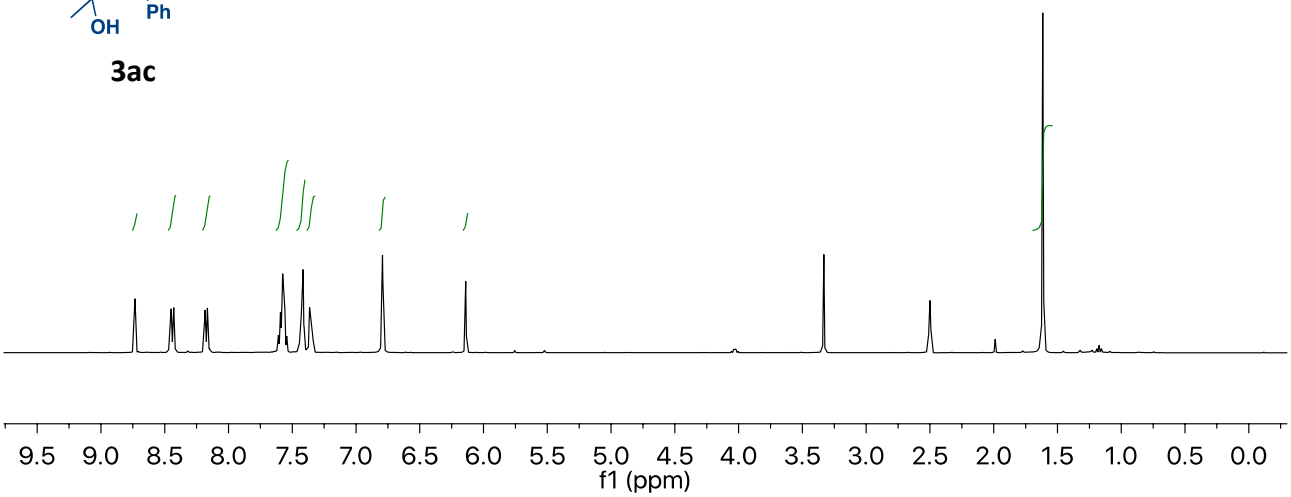

N
O
S
O
NH
N3
1f
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

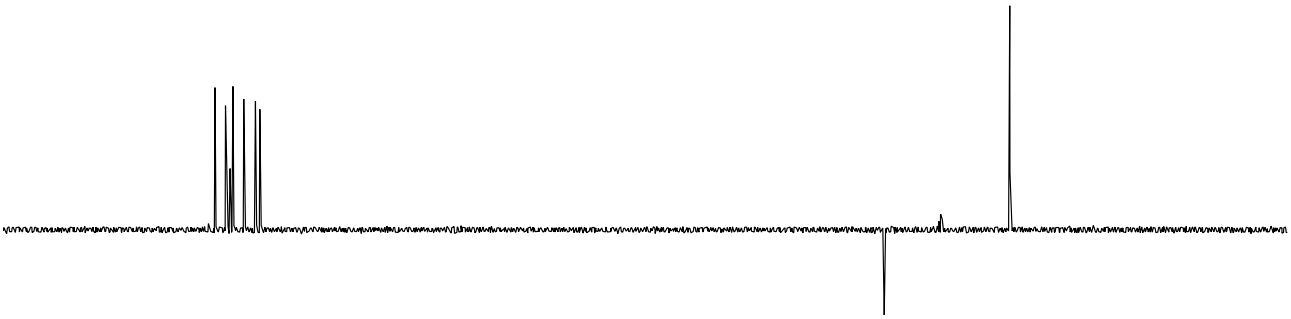


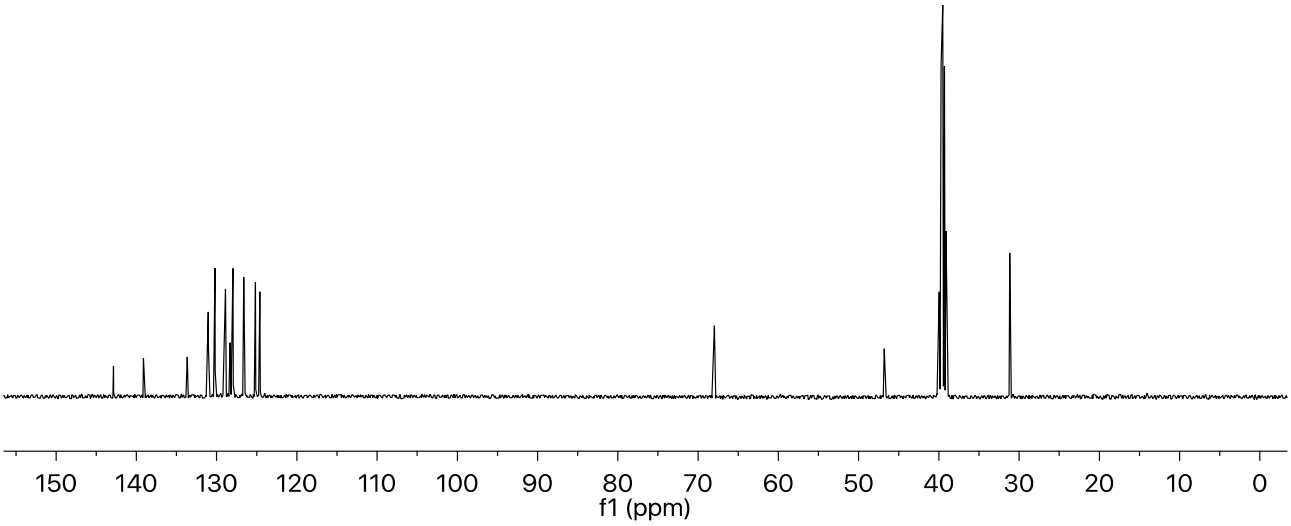

160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

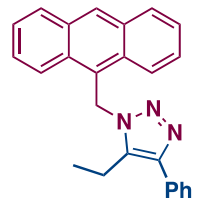

N
N
N
Ph
OH

3ac

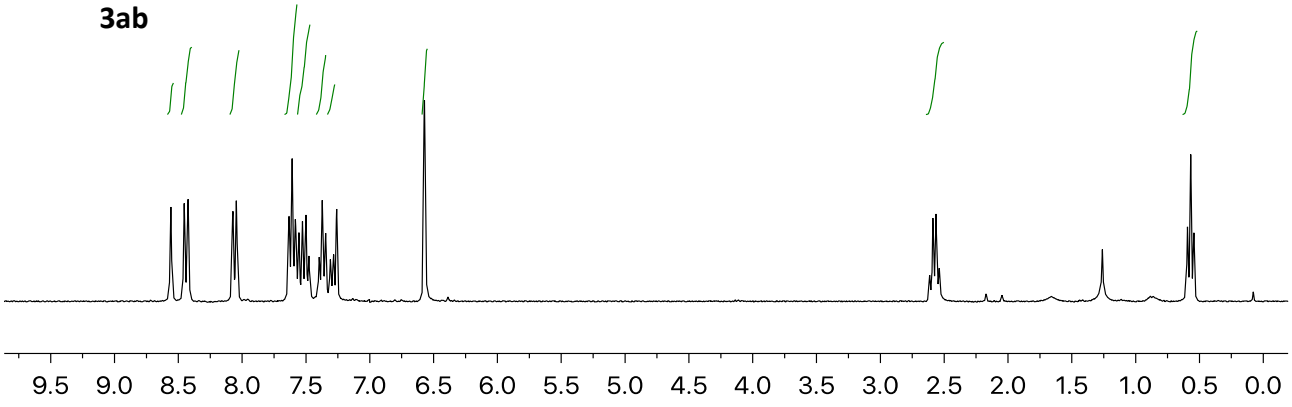

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

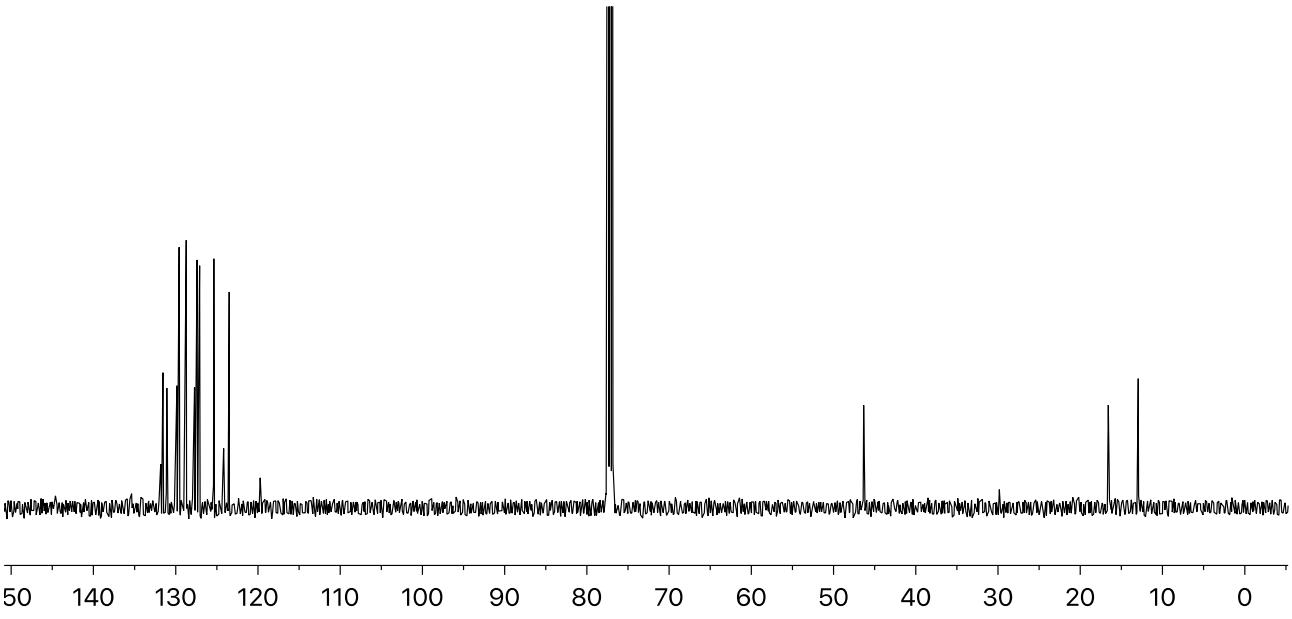


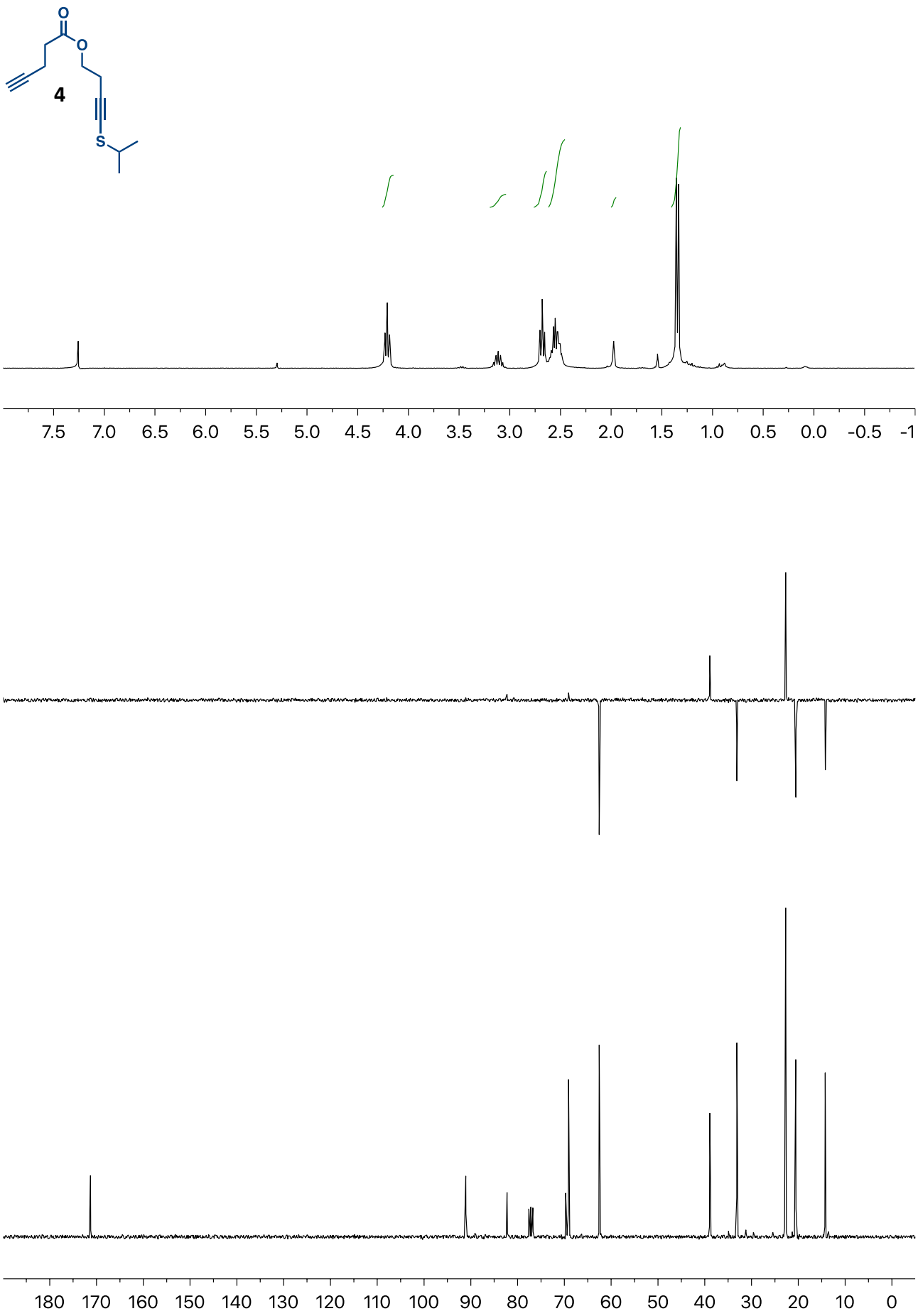

150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

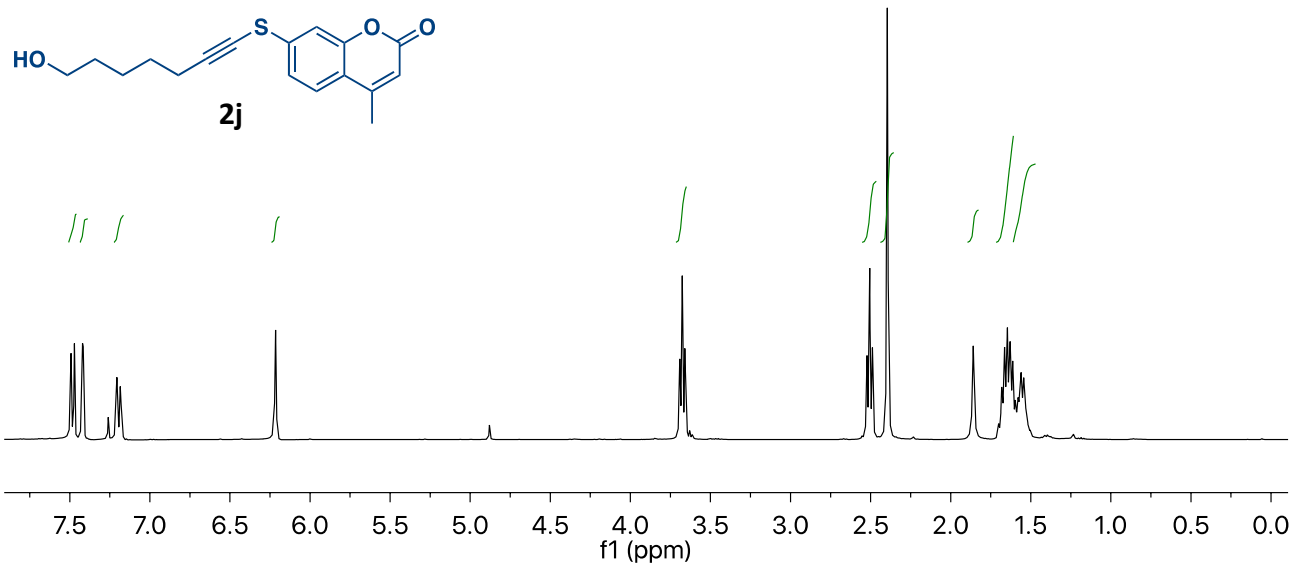

N
N
N
Ph

3ab

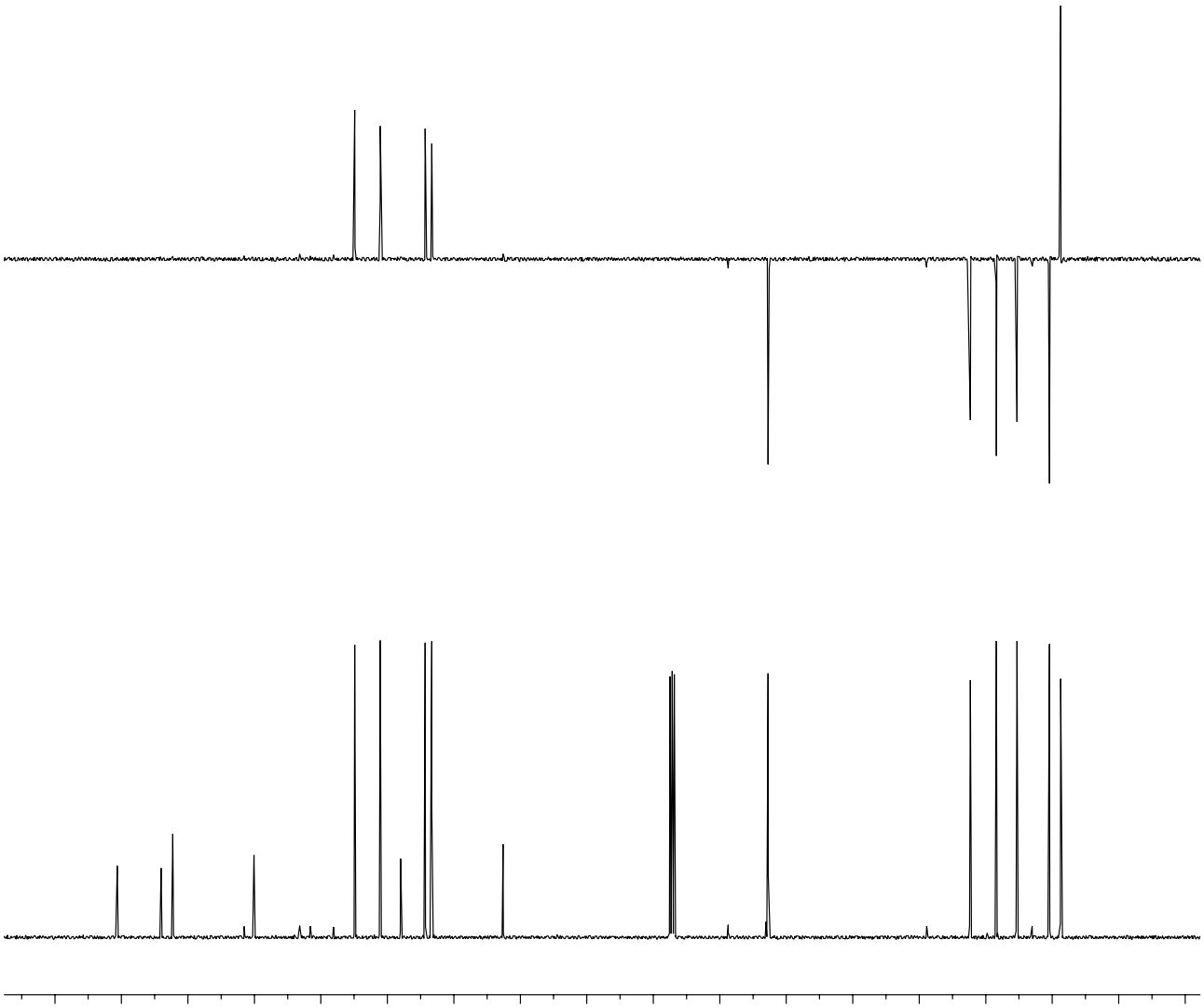

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

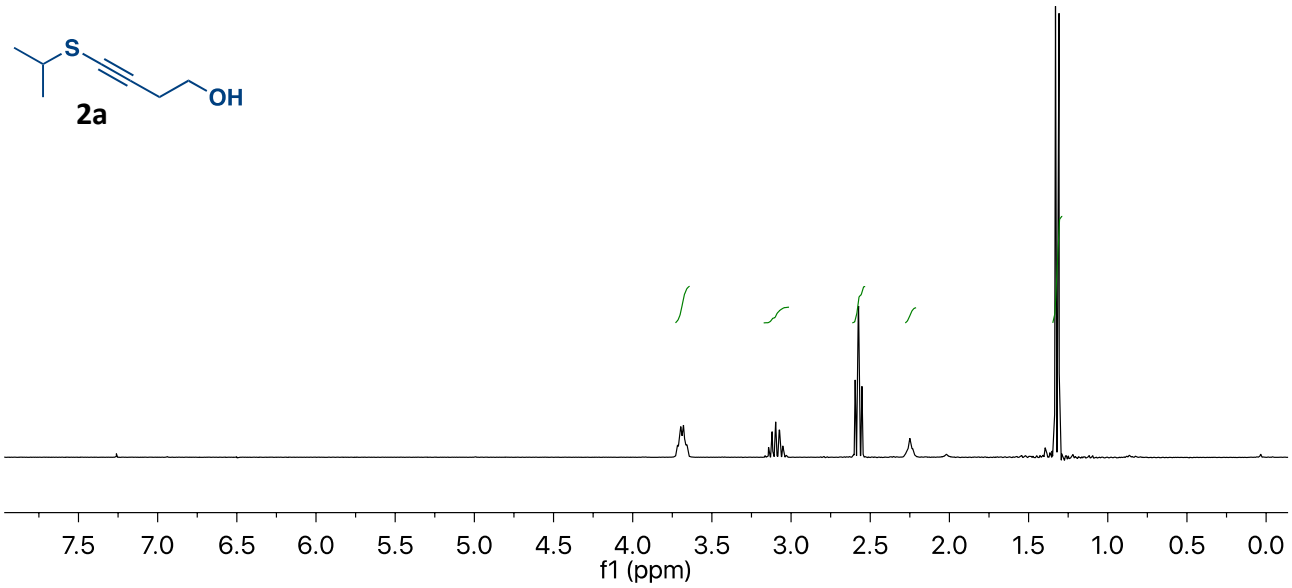

50 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

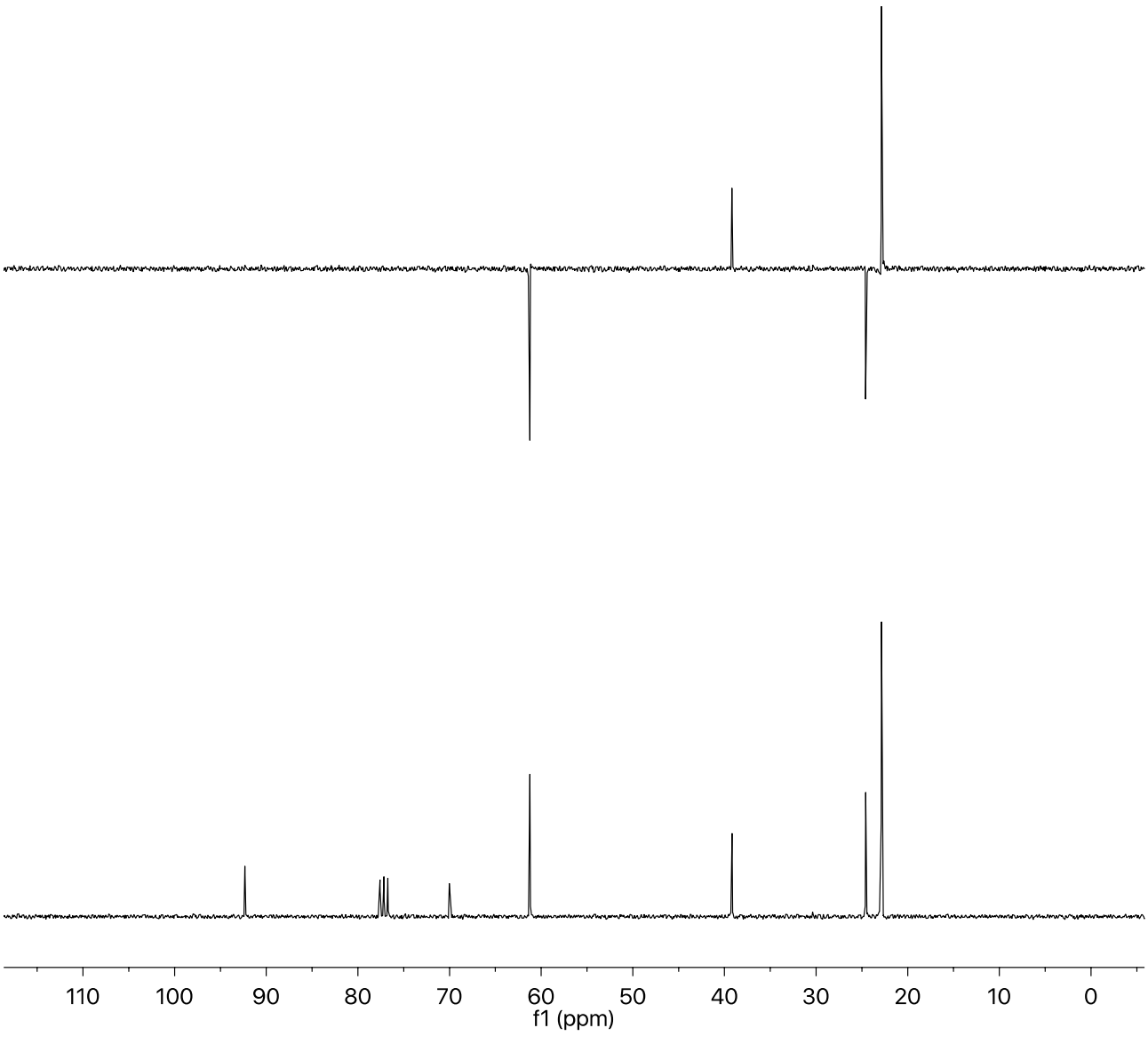

O
O
S
4
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

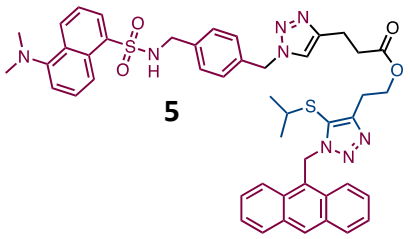

HO
S
O
O
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

2j

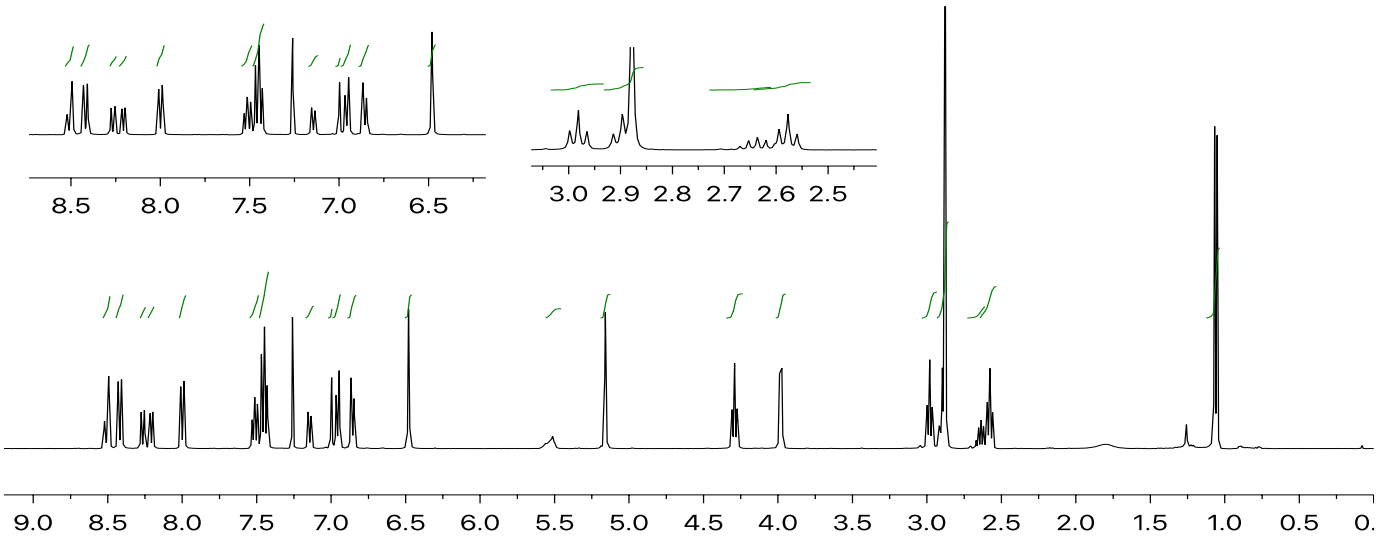

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


S
OH
2a
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



5


8.5
8.0
7.5
7.0
6.5
3.0
2.9
2.8
2.7
2.6
2.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
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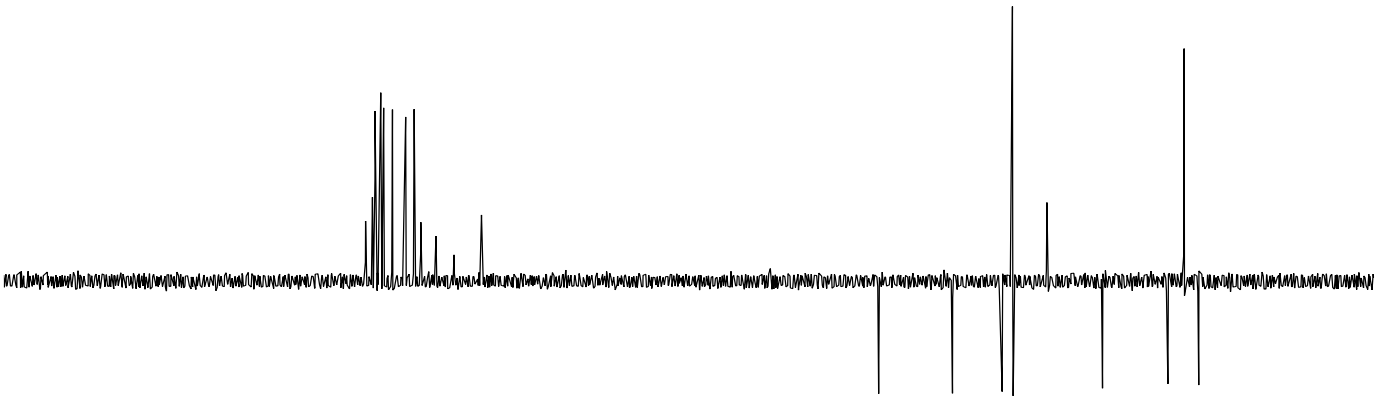

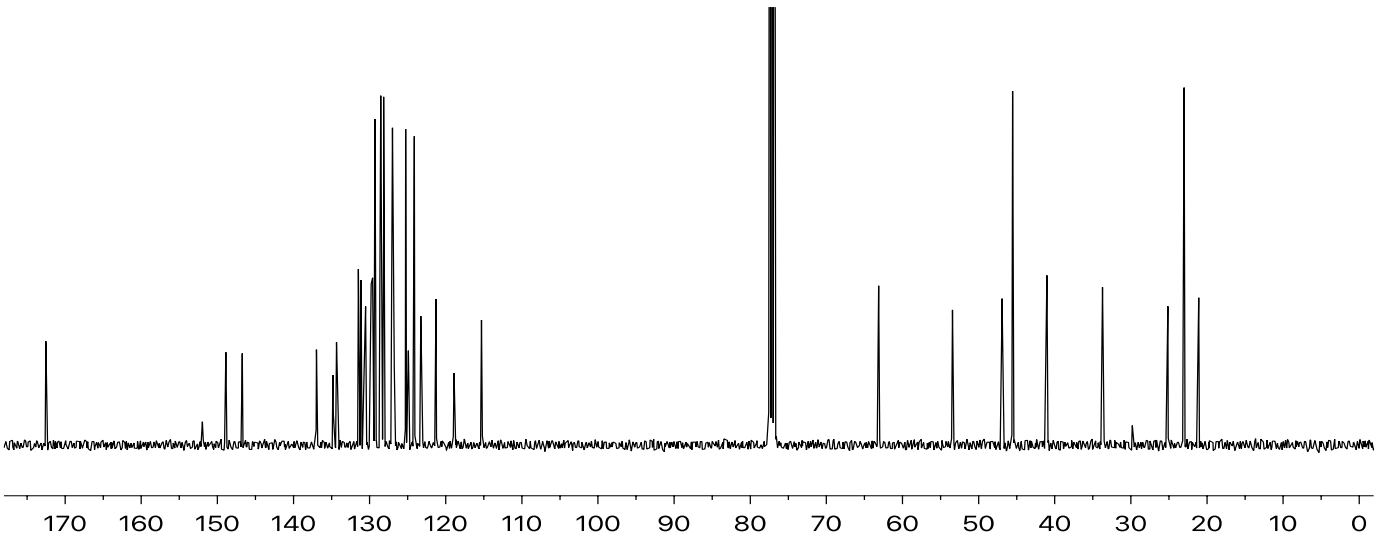
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