SUPPLEMENTARY INFORMATION
Part 1

Flavin-mediated dual oxidation controls an enzymatic Favorskii-type rearrangement

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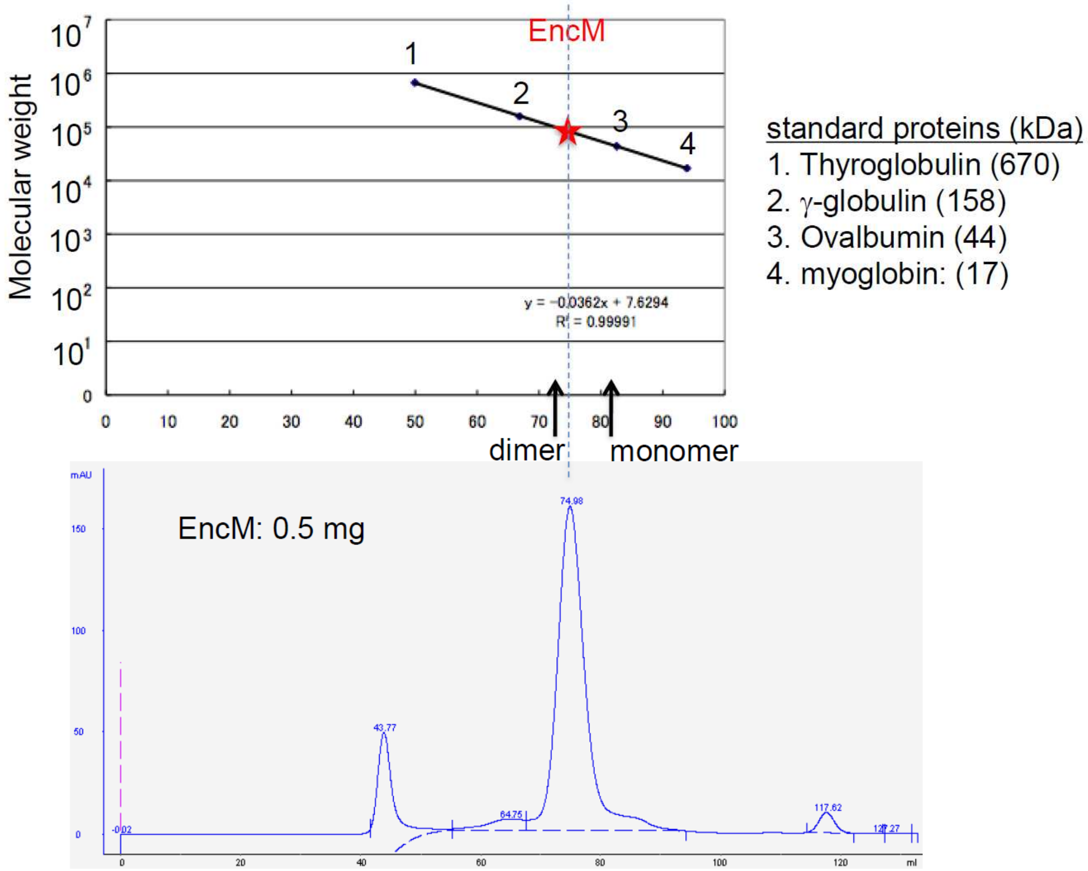
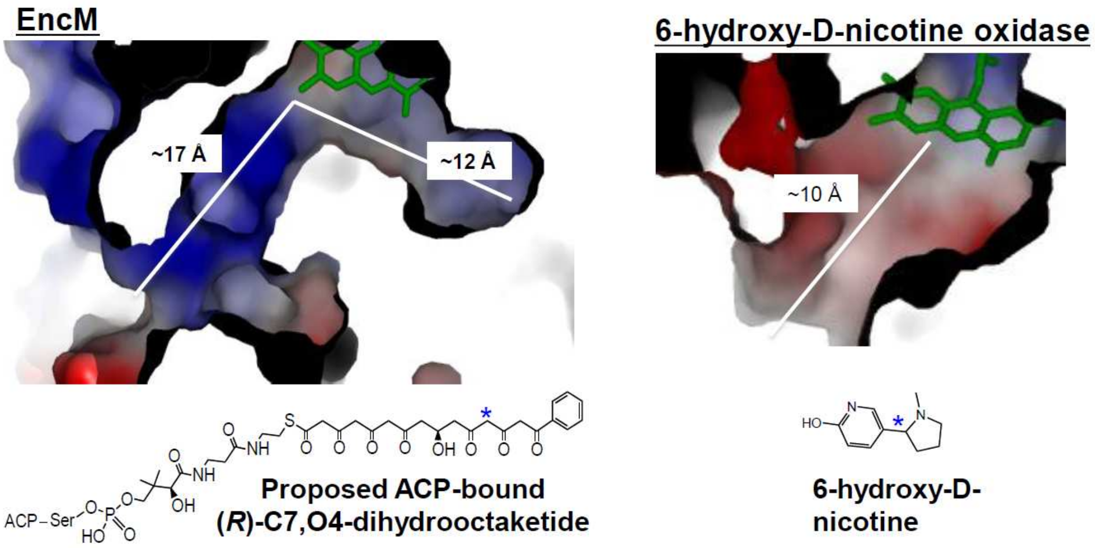


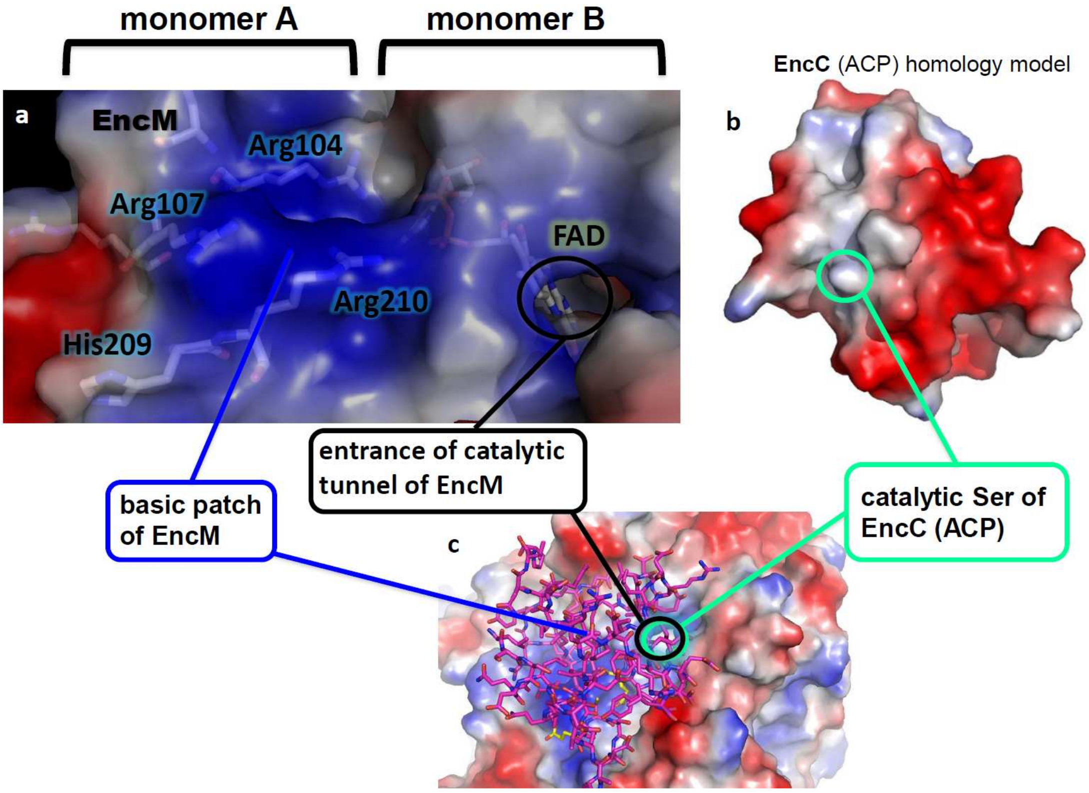
Supplementary Figure 1. 0.5 mg of EncM protein was loaded onto the HiLoad 26/60 Superdex 200 column equilibrated in buffer containing 20 mM TES-Na (pH 7.5), 0.15 M NaCl and 10 % glycerol. Eluted peaks were observed by monitoring the absorbance at 280 nm. The gel filtration column was calibrated using Bio-Rad standard proteins (thyroglobulin, 670 kDa; γ-globulin, 158 kDa; ovalbumin, 44 kDa; myoglobin, 17 kDa).



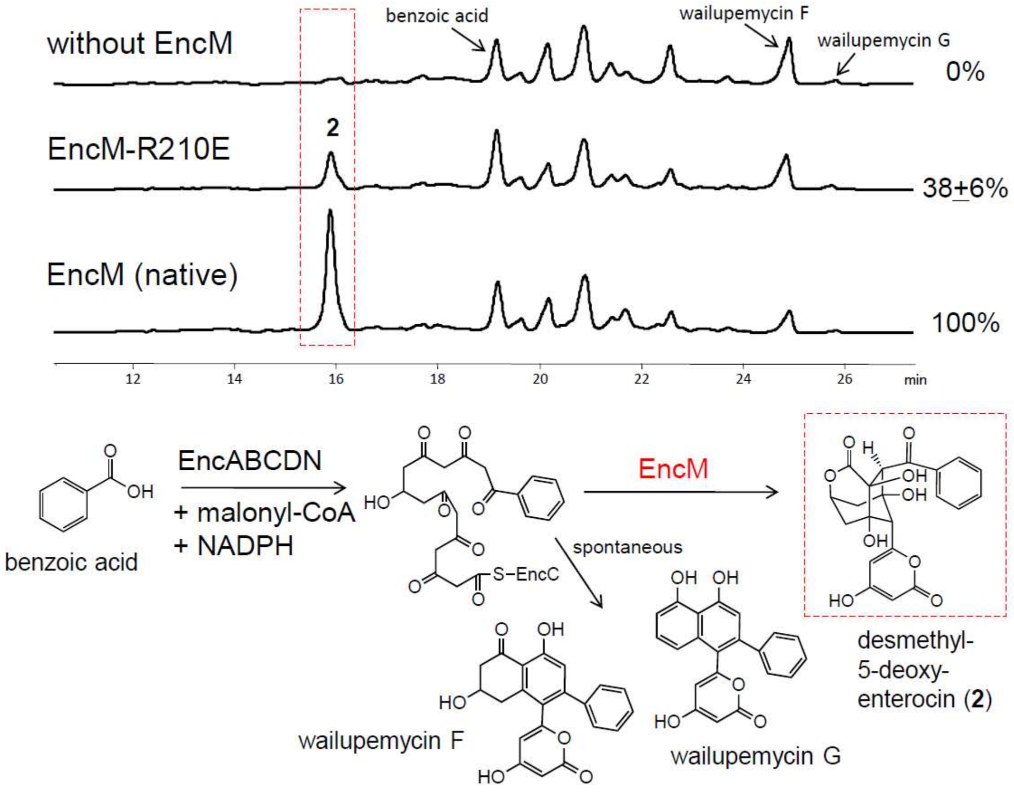
Proposed ACP-bound (*R*)-C7,O4-dihydrooctaketide

6-hydroxy-D-nicotine

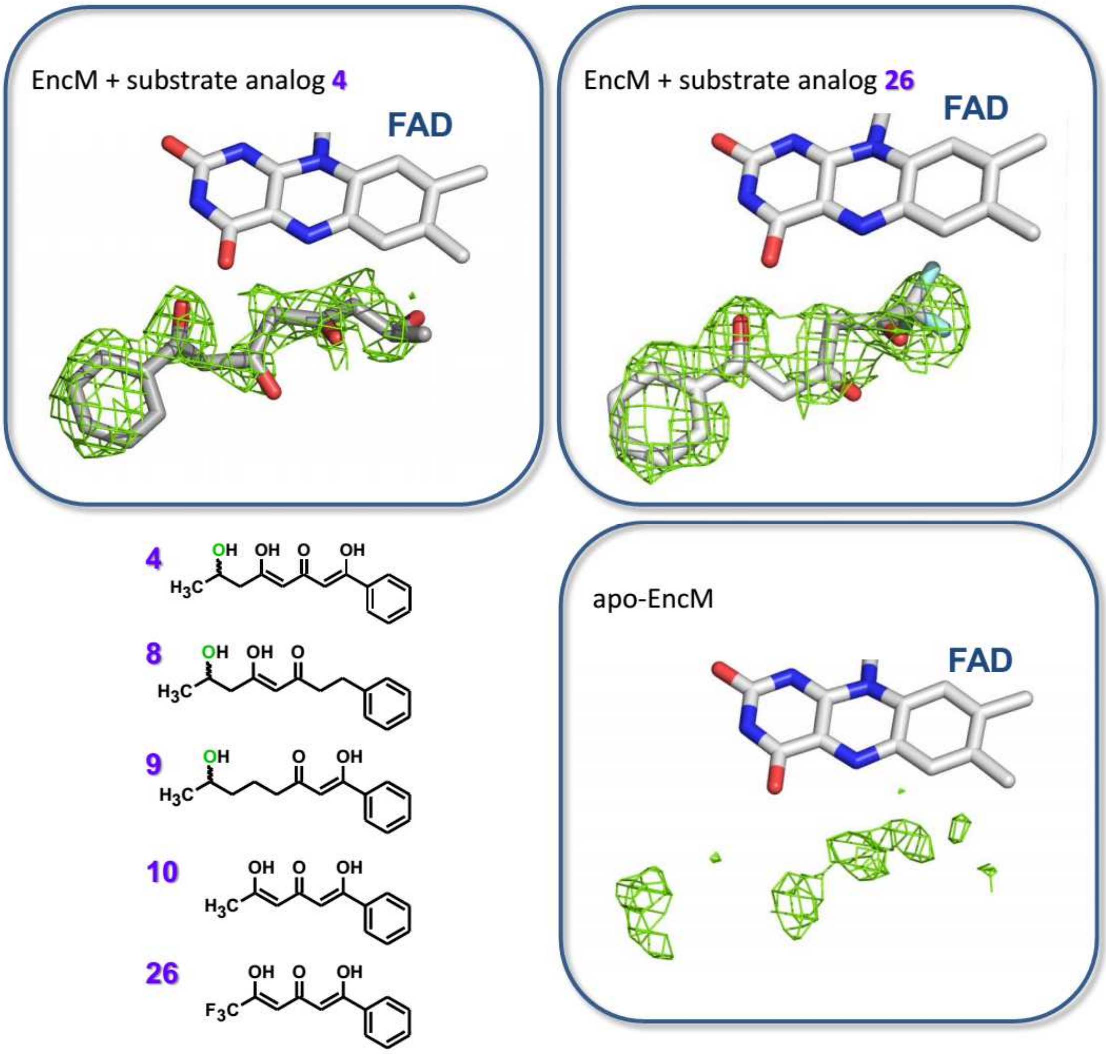
Supplementary Figure 2. Comparison of catalytic tunnels of EncM and the homologous 6-HDNO. The respective substrates are shown below. The blue asterisks indicate the site of proposed oxidation.



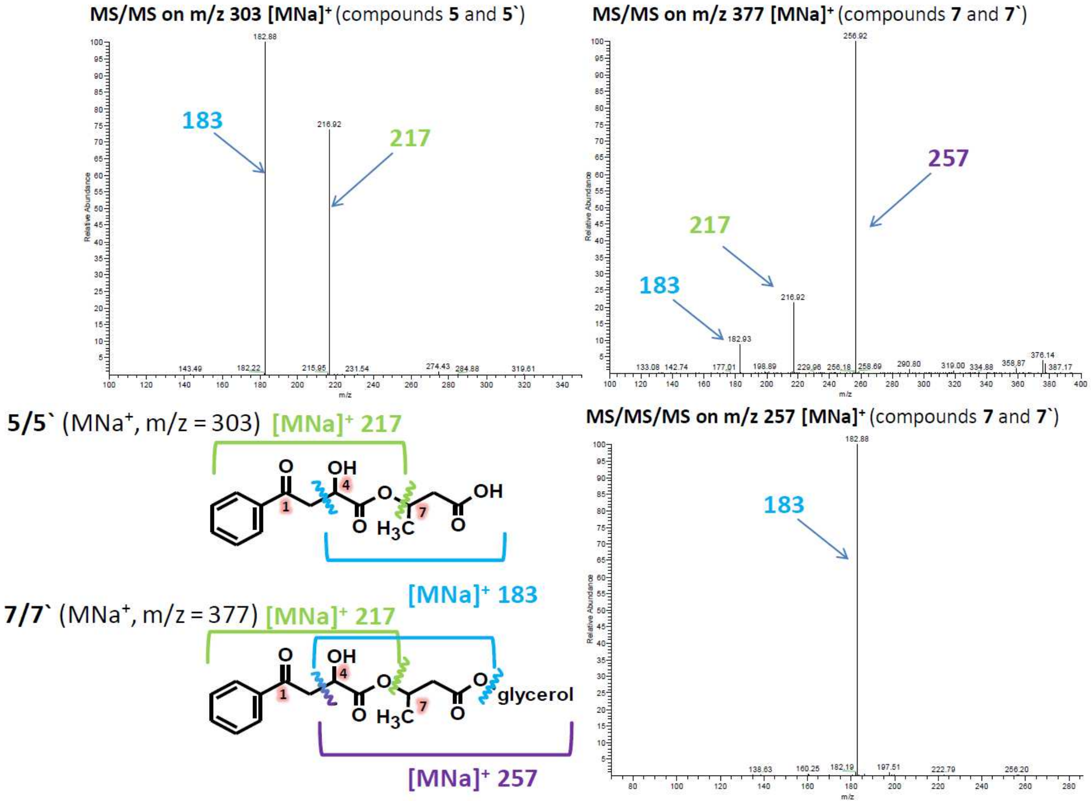
Supplementary Figure 3. **a,** Basic surface-patch and tunnel entrance of dimeric EncM (surface charge representation, blue color indicates positive charge, red color negative charge). The subunits reciprocally provide basic patches for their tunnel entrances. **b**, EncC homology model (surface charge representation). **c**, Energy-minimized docking simulation of the ACP EncC homology model (stick representation) and EncM. Salt bridge interactions were observed between basic residues of EncM and acidic residues of EncC, including Arg107 of EncM with Glu13 of EncC and Arg210 of EncM with Glu44 of EncC. The EncC serine residue, which binds the phosphopantheine moiety and the substrate (Ser39), points towards the tunnel entrance of EncM.



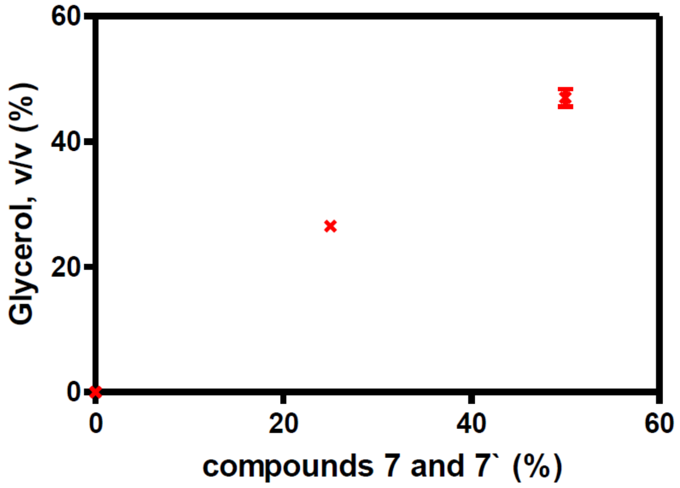
Supplementary Figure 4. *In vitro* reconstitution of the enterocin biosynthetic pathway using either native EncM or the basic patch mutant EncM-R210E. Additionally, a control assay without EncM is shown. Compounds were separated and analyzed by reversed-phase HPLC. In absence of EncM, only wailupemycins were produced. Addition of native EncM leads to predominant production of desmethyl-5-deoxyenterocin (**2**), whereas the EncM-R210E mutant is significantly less efficient in derailing aromatization (~40 % of **2** produced compared to native EncM).



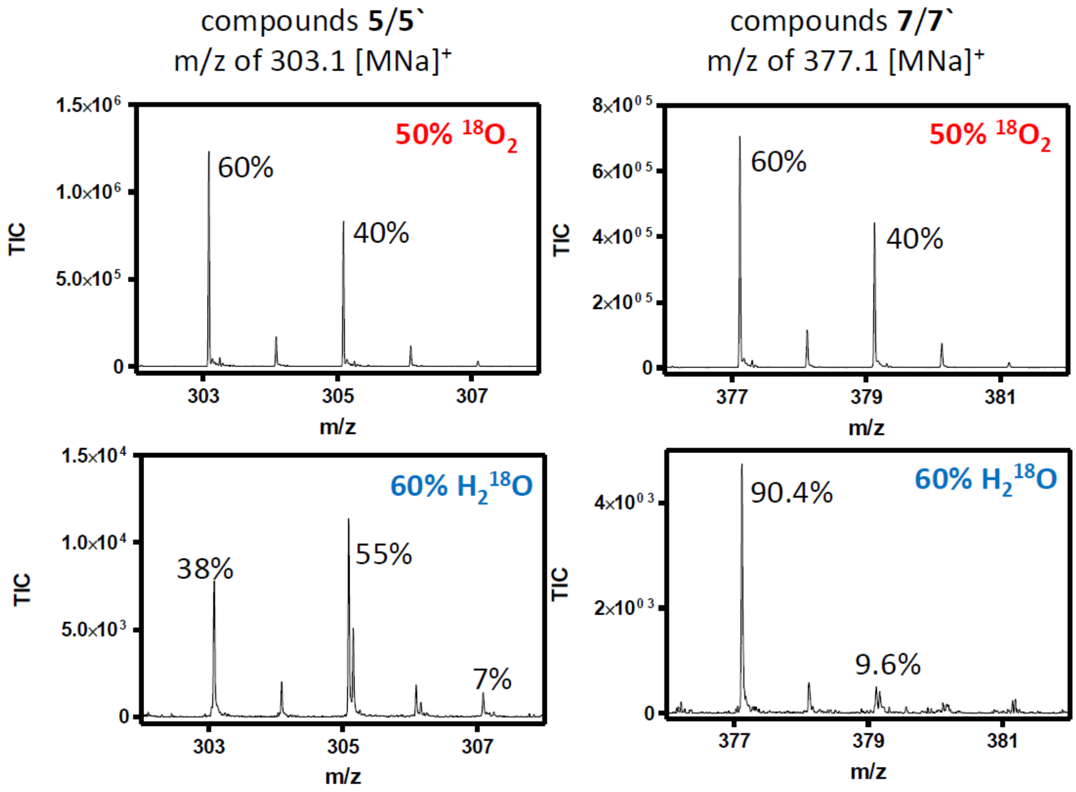
Supplementary Figure 5. Comparison of electron densities around the putative ligand binding site of EncM. FAD and the ligand atoms are shown in stick representation, with the carbon atoms colored grey. Shown in green mesh are the F_o-F_c difference maps of apo-EncM (crystallized in absence of substrate analogs), and of EncM co-crystallized with substrate analogs **4** or **26** calculated with the ligand atoms omitted prior to one round of refinement. Maps are contoured at 2.0σ. Mimetic binding can clearly be observed, although incomplete occupancy and elevated B-factors are likely responsible for the slightly disordered electron densities. Occupancies/B-factors for compound **4** were estimated as 0.79/33, respectively. Surrounding active site amino acids (occupancy = 1) show main-chain/side-chain B-factors of 8.7/23.8 (Glu355), 11.2/26.4 (Gln353), 9.3/18.6 (Tyr249) and 11.6/23.0 (Asn383). For compound **26**, occupancies/B-factors were estimated as 0.72/25. Surrounding active site amino acids show main-chain/side-chain B-factors of 12.6/24 (Glu355), 15.6/25.5 (Gln353), 12.7/21.7 (Tyr249) and 12.7/22.2 (Asn383). An overview of all substrate analogs used for structure-function analyses is shown in the bottom-left corner. Only **4** and **10** were transformed into products.



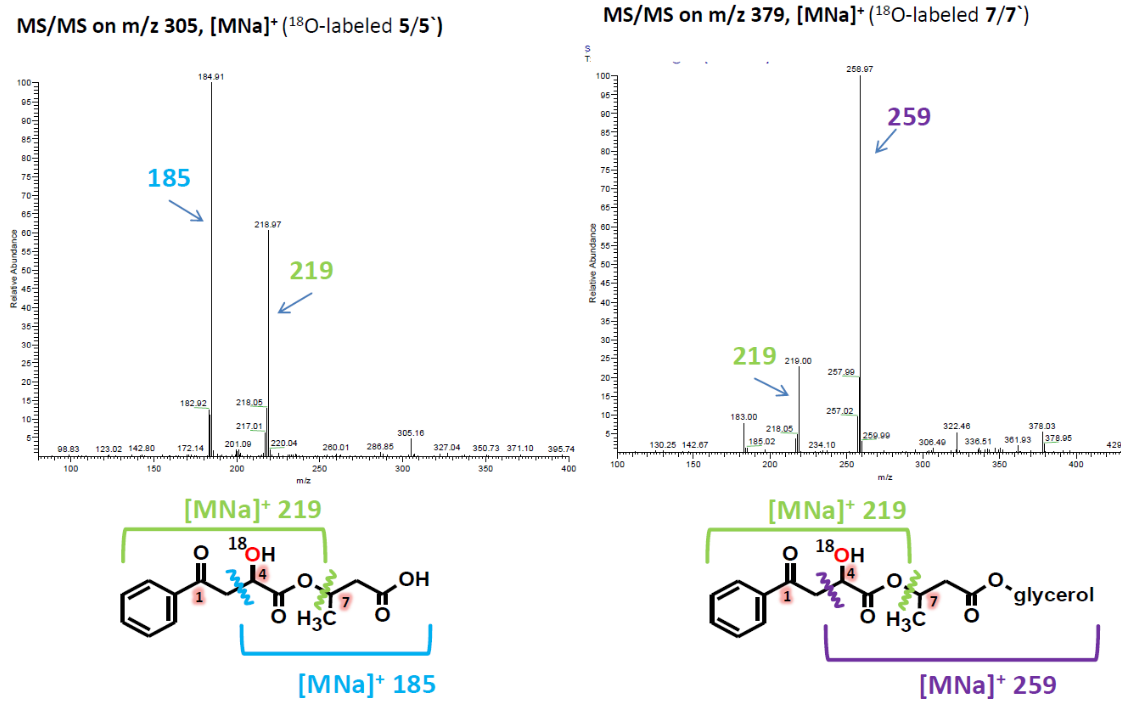
Supplementary Figure 6. Positive ion mode ESI MS and MS^n measurements of products **5/5’** and **7/7’** (see Fig. 3b). HR-MS measurements of the derivatives were consistent with the proposed structures with m/z of $[MNa]^+$ = 303.0837 (theoretical: 303.0839, Δppm 0.75) and $[MNa]^+$ = 377.1208 (theoretical: 377.1207, Δppm 0.3), respectively.



Supplementary Figure 7. Observed relative amounts of compounds **7/7’** (in % of total products **5/5’** + **7/7’**, quantified by RP-HPLC) in presence of 0, 25, and 50 % glycerol (v/v %) (see Fig. 3b for compounds and reactions).



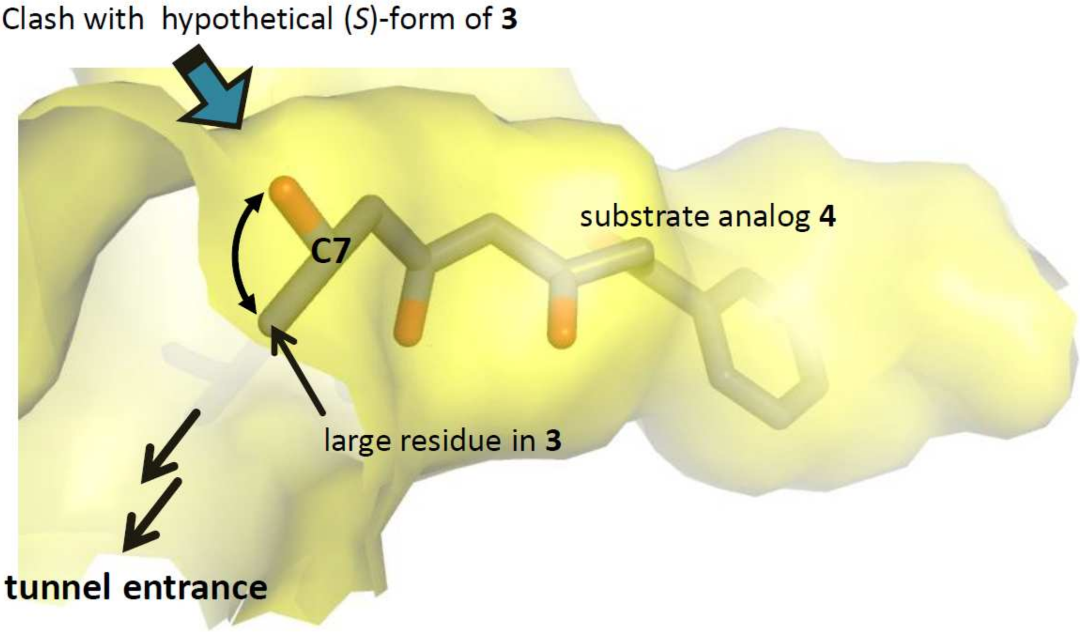
Supplementary Figure 8. Positive ion mode ESI MS measurements of compounds **5/5’** and **7/7’** produced by EncM in presence of 50 % $^{18}O_2$ gas (1:1 mixture of $^{16}O_2$ and $^{18}O_2$) or 60 % $H_2{}^{18}O$ (v/v), respectively. When ~50 % $^{18}O_2$ was added, 40 % of both compounds had a single ^{18}O-atom incorporated, clearly demonstrating the monooxygenase activity of EncM (see also Supplementary Fig. 9). When labeled water was used, very short incubation times and immediate freezing of the samples (until MS analysis) were required, as ketones (which are present in both substrate and the products) spontaneously exchange oxygen with water via the keto-hydrates. The results clearly show that **7/7’** were unlabeled. The minor amounts (<10 %) of single ^{18}O-labeled **7/7’** arose from the inevitable spontaneous exchange with water. On the other hand, 55 % of **5/5’** had a single ^{18}O-atom incorporated. The minor fraction harboring a second ^{18}O-atom (7 %) resulted again from spontaneous exchange with water. Longer incubation times caused increasing amounts of double or multiply labeled products (not shown). The data suggests that the postulated lactone **6** is directly formed via intramolecular attack of the C7-hydroxyl (rather than by cyclopropanone hydrolysis and subsequent lactonization), before derivatization with either water or glycerol to yield **5/5’** and **7/7’**, respectively (see Fig. 3b for reactions and intermediates).



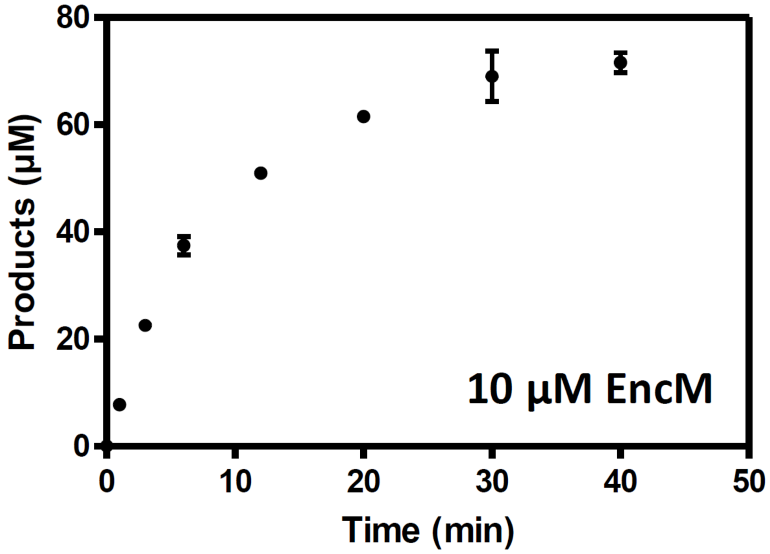
MS/MS on m/z 305, [MNa]$^+$ (^{18}O-labeled **5/5`**)

MS/MS on m/z 379, [MNa]$^+$ (^{18}O-labeled **7/7`**)

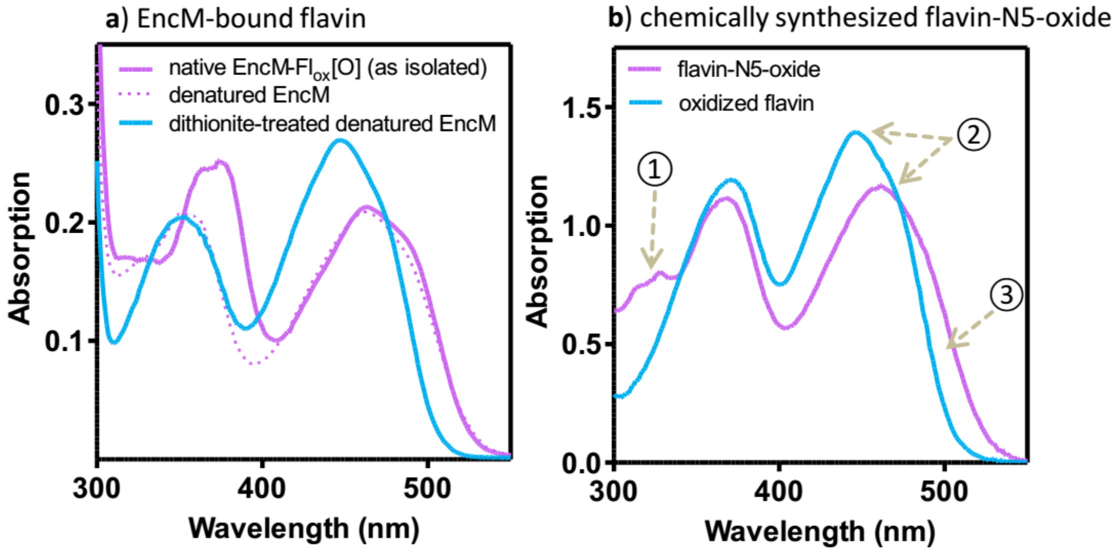
Supplementary Figure 9. Positive ion mode ESI MS/MS measurements of single ^{18}O-labeled compounds **7/7’** (label derived from $^{18}O_2$, see Supplementary Fig. 8). The C4-OH most likely harbors the ^{18}O, as both observed main fragments of **5/5’** and **7/7’** were labeled, in agreement with the postulated mechanism shown in Fig. 3b.



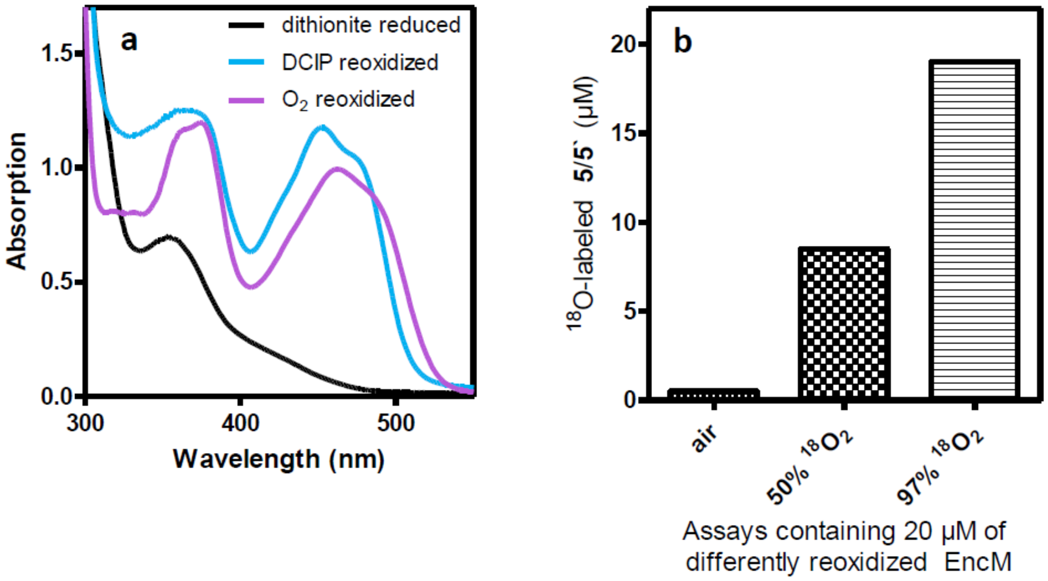
Supplementary Figure 10. Internal cavity (yellow) of EncM with bound substrate analog **4**. Both enantiomers of **4** fit into the tunnel and, consistently, are converted by EncM. Shown is the (*S*)-form of **4** that corresponds, however, to the (*R*)-form of the larger natural substrate **3** according to nomenclature (due to a change in substituent priority). Only the (*R*)-form of **3** can thus be accommodated.



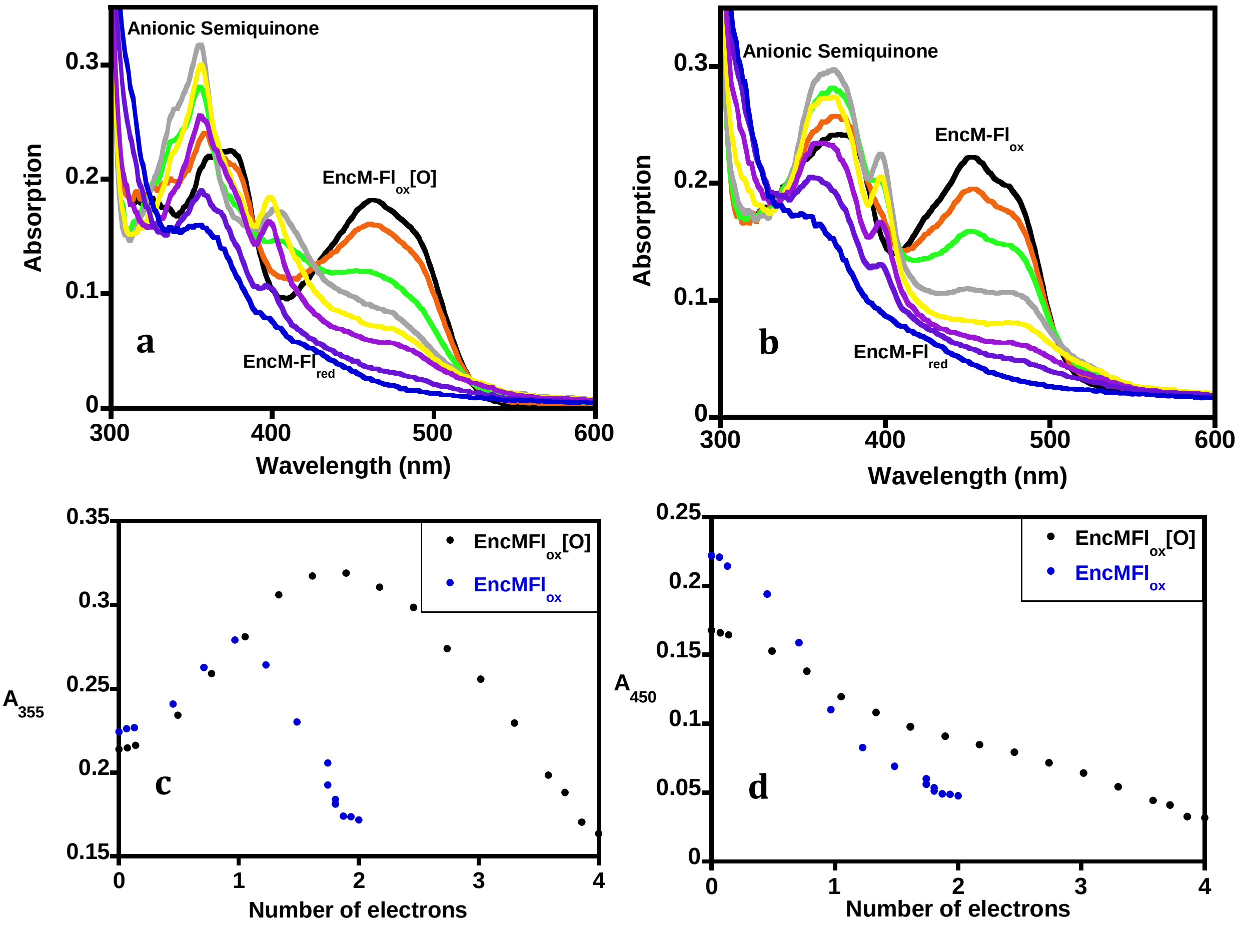
Supplementary Figure 11. Time-course of EncM-catalyzed formation of **5/5'**. The reaction mix contained 10 μM EncM and was started by the addition of 0.7 mM **4**. Samples were sequentially quenched at shown time points prior to product quantification by RP-HPLC. The curve shows decrease of EncM activity over time. Inactivation went along with distinct spectral changes (see Fig. 3c). The initial enzymatic activity was ≈25 mU/mg.



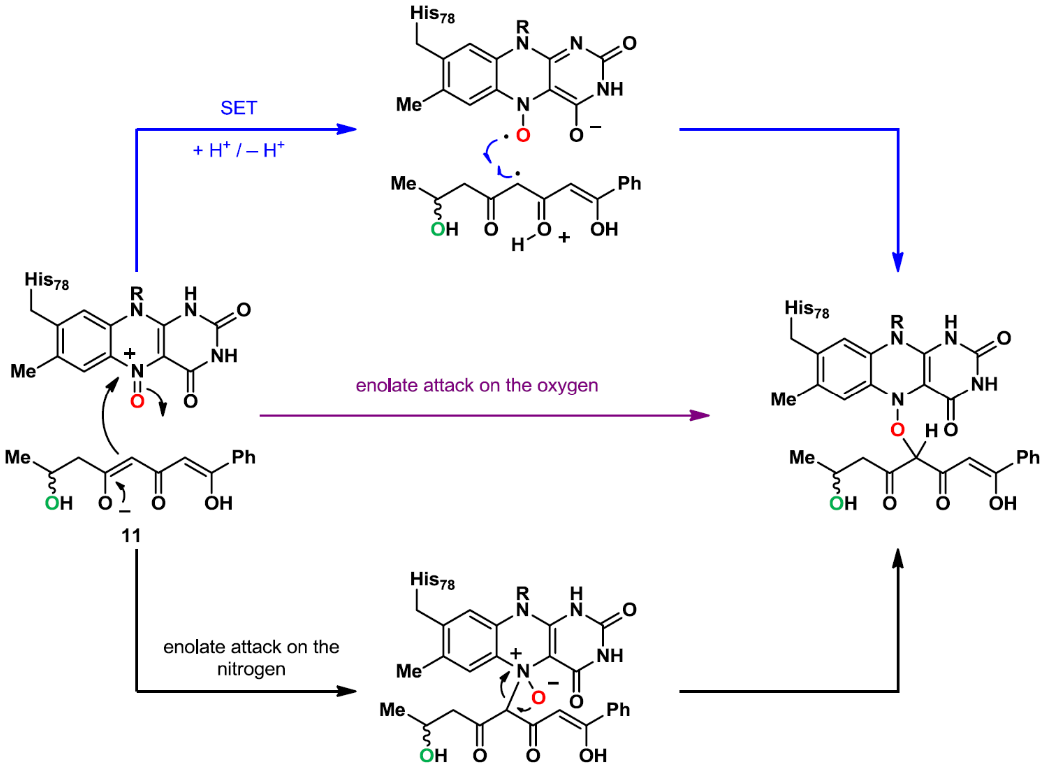
Supplementary Figure 12. a, UV-Vis spectrum of EncM-bound flavin as isolated (= EncM-Fl$_{ox}$[O]) (purple curve). Upon denaturation, the typical UV-Vis signals of EncM-Fl$_{ox}$[O] were retained in the 460 nm region (dashed purple curve), implying that the oxygen species [O] causing the spectral perturbances is associated with the flavin cofactor itself, rather than the enzymatic environment. After reduction of denatured EncM with dithionite and subsequent reoxidation of the flavin with O$_2$ (blue curve), [O] could not be restored in contrast to an analogous experiment with native enzyme (see Supplementary Fig. 13). Note that the flavin cofactor remained covalently attached to denatured EncM. **b**, UV-Vis spectrum of chemically synthesized flavin-N5-oxide (**30**) that could be analogously converted to oxidized flavin by treatment with dithionite and reoxidation with O$_2$. The spectra of (denatured) EncM-Fl$_{ox}$[O] and synthesized flavin-N5-oxide are highly similar and share the following features: *1*, an absorption "bridge" in the 300-340 nm region; *2*, the extinction coefficient in the 450 nm region is decreased by around 20 % compared to oxidized flavin; *2* and *3*, a red-shift of the 450 nm absorption maxima.



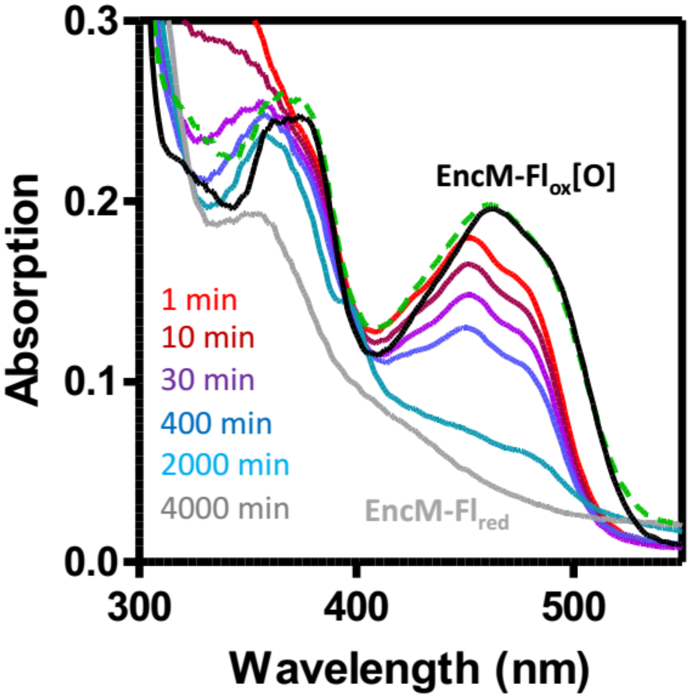
Supplementary Figure 13. **a**, UV-Vis spectrum of EncM (100 µM, recorded in an anaerobic chamber) after complete flavin reduction with dithionite (black curve). $FADH_2$ was subsequently reoxidized through addition of the chemical oxidant 2,6-dichlorophenolindophenol (DCIP) (blue curve, resulting in the spectrum of EncM-Fl_{ox} see also Fig. 3c) or molecular oxygen ($^{16}O_2$ or $^{18}O_2$, purple curve, resulting in the spectrum of EncM-Fl_{ox}[O], see also Fig. 3c). **b**, Aliquots of dithionite-reduced EncM (each aliquot containing 20 µM EncM-bound flavin) were reoxidized with either DCIP, air, ~50 % $^{18}O_2$ (1:1 mixture of $^{18}O_2$ and $^{16}O_2$), or ~97 % $^{18}O_2$ (followed by thorough removal of unreacted $^{18}O_2$), and then incubated with **4** under aerobic conditions. Samples were subsequently analyzed by LC-MS. DCIP-oxidized EncM (i.e., EncM-Fl_{ox}) was inactive and unable to initiate the catalytic cycle (no products were detected) (see Fig. 3b for proposed catalysis). In all other samples, **4** was completely converted to **5/5'**. 8.5 µM and 19 µM ^{18}O-labeled **5** was found in the EncM samples reoxidized with ~50 % $^{18}O_2$ and ~97 % $^{18}O_2$, respectively, whereas no significant ^{18}O-enrichment (<0.5 %) was observed in the products of the control reaction with air-oxidized EncM. The results thus confirm that each flavin binds an oxygenating species in its stable active-state, allowing for hydroxylation of 1 substrate molecule.



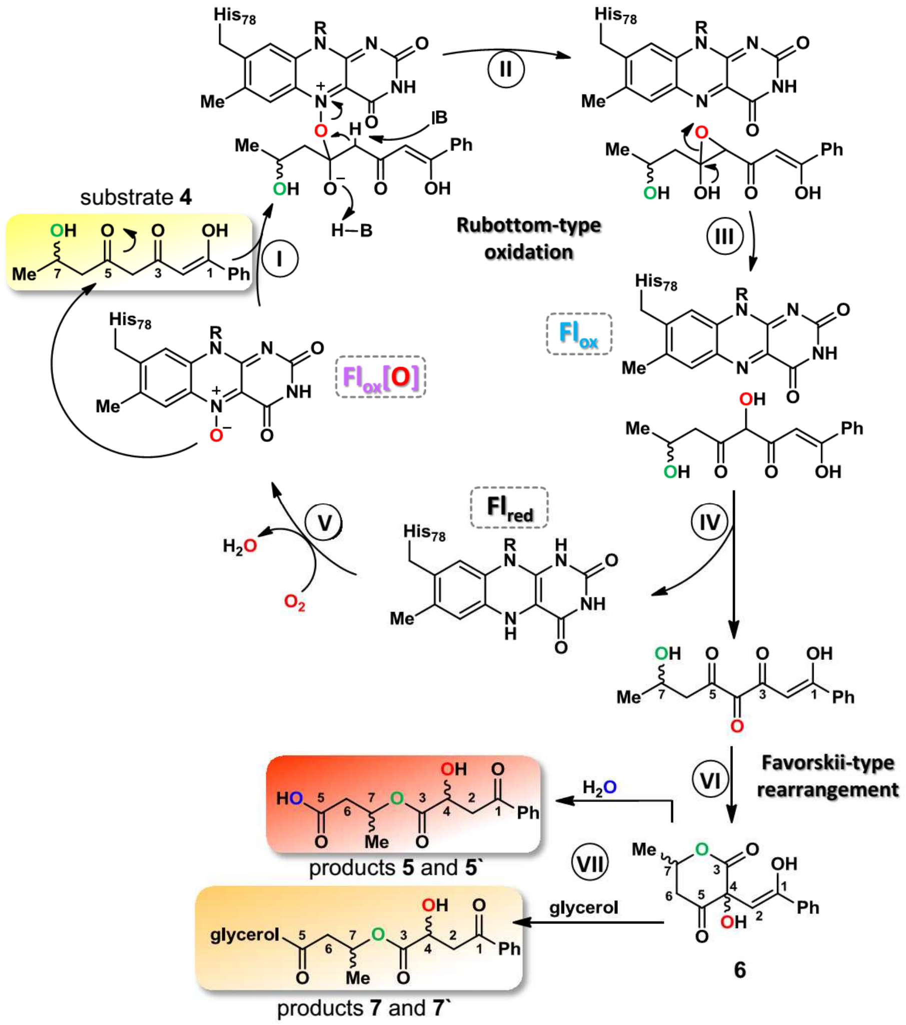
Supplementary Figure 14. UV-Vis spectra of EncM-Fl$_{ox}$[O] (**a**) and EncM-Fl$_{ox}$ (**b**) anaerobically titrated with a calibrated dithionite solution. **c** and **d** show overlays of the absorbance at 355 nm and 450 nm, respectively, against the number of electrons added relative to the EncM concentration. EncM-Fl$_{ox}$[O] required four electrons to be reduced to the flavin hydroquinone (EncM-Fl$_{red}$) – twice the number of electrons expected for an FAD-containing protein – consistent with a flavin N5-oxide. The anionic semiquinones observed during the titrations are common among flavoproteins where the flavin is covalently linked to the protein via the C8-methyl of the isoalloxazine.



Supplementary Figure 15. Potential pathways for the oxygenation of enolate **11** by the oxoammonium species.



Supplementary Figure 16. Time-course of EncM UV-Vis spectral changes during anaerobic single turnover of **4**. Addition of 20 µM **4** to 20 µM EncM-Fl$_{ox}$[O] (black curve) led to complete reduction of the flavin cofactor (EncM-Fl$_{red}$, grey curve). Oxygenation and partial reduction occured rapidly, whereas complete flavin reduction required significantly longer incubation times, supporting a stepwise dual oxidation as depicted in steps III-V, Fig. 3b. After complete reduction, the enzyme was exposed to air which resulted in restoration of the EncM-Fl$_{ox}$[O] spectrum (green dashed curve). As a control, EncM-Fl$_{ox}$[O] was similarly incubated and monitored anaerobically in the absence of substrate over a time period of 72h. No spectral changes were observed (not shown), confirming that flavin reduction is substrate-dependent.



Supplementary Figure 17. Alternative EncM mechanism in which the N5-oxide acts as a stable nucleophile and adds to the C5 ketone of **4** (step I). The putative site of enzymatic attack, C5 of **4**, is appropriately positioned at 3.2 Å proximity to N5 of the flavin (see Fig. 2c). A general base (possibly Glu355) could deprotonate C4 (step II) and the newly formed enolate would displace the flavin moiety leading to a hydroxy oxirane intermediate in an enzymatic Rubottom-type oxidation (steps II, III). Rearrangement of the epoxy alcohol followed by proton transfer would yield the C4-hydroxyl intermediate (step III). Subsequent dehydrogenation of the hydroxyl group then produces reduced flavin (step IV) and a ketone that enables the Favorskii-type rearrangement (step VI) and subsequent *retro*-Claisen cleavage (step VII). Reaction of reduced flavin with molecular oxygen then regenerates the flavin N-oxide (step V).

Supplementary Table 1. Data collection, phasing, and refinement statistics (molecular replacement). Compounds used for co-crystallization are shown in Supplementary Fig. 5.

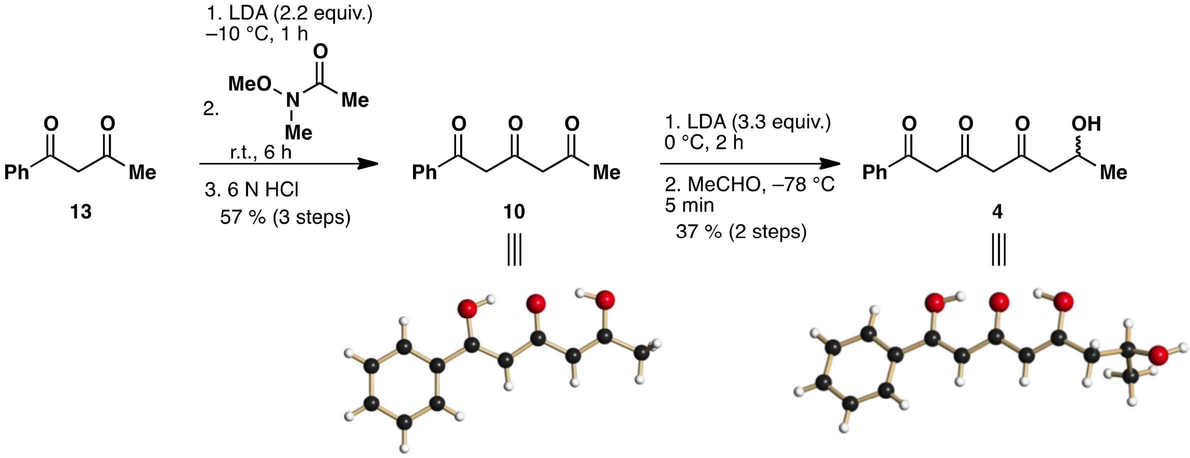
	EncM apo	EncM with **26**	EncM with **4**
Data collection			
Space group	$P2_1$	$P2_1$	$P2_1$
Cell dimensions			
a, b, c (Å)	71.41, 85.28, 79.67	71.25, 85.18, 79.36	71.07, 85.12, 79.34
α, β, γ (°)	90.00, 99.84, 90.00	90.00, 99.47, 90.00	90.00, 99.58, 90.00
Resolution (Å)	50.0-1.95(1.98-1.95)	50.0-1.82(1.85-1.82)	42.6-1.80(1.90-1.80)
R_{merge} (%)	13.7(53.1)	12.0(71.7)	8.3(39.2)
$I / \sigma I$	13.1(2.0)	20.7(1.9)	9.2(3.1)
Completeness (%)	99.5(94.4)	99.9(97.0)	99.9(99.7)
Redundancy	5.5(4.8)	6.0(4.3)	4.0(4.0)
Refinement			
Resolution (Å)	50.0-1.95	50.0-1.82	42.6-1.80
No. reflections	65,215	79,007	81,869
R_{work} / R_{free}	19.1/24.3	15.3/19.2	15.8/19.6
No. atoms			
Protein	7,008	7,014	7,008
FAD	106	106	106
Substrate analog	0	36	36
Water	630	651	724
B-factors			
Protein	16.8	19.4	16.9
FAD	9.8	11.8	9.8
Substrate analog	-	24.6	32.5
Water	25.3	29.7	29.1
Occupancies			
Substrate analog	-	0.72	0.79
R.m.s. deviations			
Bond lengths (Å)	0.019	0.020	0.022
Bond angles (°)	2.071	2.076	2.161

Spectroscopic data of enzymatic products 5 and 5':

5 (retention time 24.7 min):
^{1}H NMR (600 MHz, CD_3CN): δ 7.95 (d, *J* = 7.2 Hz, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.51 (t, *J* = 7.9 Hz, 2H), 5.25 – 5.22 (m, 1H), 4.58 (dd, *J* = 6.9 Hz, 4.5 Hz, 1H), 3.42 – 3.33 (m, 2H), 2.60 – 2.50 (m, 2H), 1.27 (d, *J* = 6.0 Hz, 3H) ppm.
^{13}C-NMR (151 MHz, CD_3CN) δ 198.36, 173.89, 171.84, 137.77, 134.32, 129.63, 128.99, 69.33, 68.03, 43.33, 40.51, 19.85 ppm.
IR (neat): $\tilde{\nu}$ = 3445, 2925, 1730, 1682, 1597, 1449, 1366, 1195, 1138, 1101, 1052, 758, 689 cm^{-1}.
UV/vis: λ_{max} 246 nm
HRMS (ESI-TOF): calc'd for $C_{14}H_{16}O_6$ $[M+Na]^+$ 303.0839, found 303.0837.

5' (retention time 25.3 min):
^{1}H NMR (600 MHz, CD_3CN) δ 7.95 (d, *J* = 7.2 Hz, 2H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 2H), 5.26 – 5.22 (m, 1H), 4.53 (dd, *J* = 7.0 Hz, 4.6 Hz, 1H), 3.45 – 3.36 (m, 2H), 2.60 – 2.50 (m, 2H), 1.23 (d, *J* = 6.3 Hz, 3H) ppm.
^{13}C NMR (151 MHz, CD_3CN) δ 198.4, 173.8, 171.9, 137.8, 134.3, 129.6, 129.0, 69.3, 68.0, 43.2, 40.5, 19.8 ppm.
IR (neat): $\tilde{\nu}$ = 3448, 2927, 1731, 1682, 1597, 1449, 1368, 1200, 1138, 1103, 1052, 759, 689 cm^{-1}.
UV/vis: λ_{max} 246 nm
HRMS (ESI-TOF): calc'd for $C_{14}H_{16}O_6$ $[M+Na]^+$ 303.0839, found 303.0836.

Synthesis of substrate analogs 4, 8, 9, and 10:



13 10 4

10: **10** was prepared according to a literature procedure. [A. Oster, T. & M. Harris, T. Acetylations of strongly basic and nucleophilic enolate anions with *N*-methoxy-*N*-methylacetamide. *Tetrahedron Lett.* **24,** 1851-1854, (1983).] All the flasks were flame-dried prior to use. A mixture of diisopropylamine (0.67 g, 0.93 mL, 2.2 equiv.) in THF (50 mL) was cooled to –78 °C under argon and ^{n}BuLi (3.1 mL, C = 2.3 M, 2.4 equiv.) was slowly added. The LDA solution was warmed to –10 °C and a solution of 1-benzoylacetone (**13**) (0.50 g, 1.0 equiv.) in THF (40 mL) under argon was added dropwise via a cannula. The red mixture was left at –10 °C for 1 h, then *N*-methoxy-*N*-methylacetamide (0.34 g, 0.35 mL, 1.1 equiv.) was added in one portion and the reaction was stirred at room temperature for another 5 h. Solvent was then concentrated *in vacuo* and the residue was suspended in 100 mL of Et_2O and acidified to pH = 1 at 0 °C with 6 mL of HCl (6N). The phases were separated, and the aqueous phase was extracted twice with DCM (2 × 50 mL). The combined organic layers were dried over $MgSO_4$. Evaporation *in vacuo* resulted in a dark orange, viscous liquid (0.73 g). Column chromatography (deactivated SiO_2 with 1 % Et_3N in Et_2O, 0:10 to 3:7 EtOAc:hexanes) provided yellow crystals (0.35 g, 57 %) that can be recrystallized by diffusion of hexanes into a solution of **10** in Et_2O.

Melting point: 101–102 °C.
TLC: R_f = 0.34–0.37 (3:7 EtOAc:hexanes, UV active, stains orange upon *p*-anisaldehyde staining).
NMR showed a complex mixture of keto-enol tautomers.
^{1}H NMR (600 MHz, $CDCl_3$, major peaks): δ 8.00 – 7.98 (m), 7.89 – 7.87 (m), 7.82 – 7.80 (m), 7.62 – 7.41 (m), 6.24 (s), 5.83 (s), 5.64 (s), 5.33 (s), 4.24 (s), 3.97 (s), 3.79 (s), 3.56 (s), 2.35 (s), 2.05 (s), 2.03 (s) ppm.
^{13}C NMR (151 MHz, $CDCl_3$, major peaks): δ 194.1, 178.8, 173.8, 133.9, 132.9, 131.7, 128.9, 128.7, 127.3, 126.5, 99.7, 97.4, 96.0, 55.0, 22.1 ppm.
IR (neat): $\tilde{\nu}$ = 1604, 1573, 1492, 1466, 1385, 1260, 1211, 1149, 1085, 1014, 928, 899, 820, 786, 727, 690 cm^{-1}.
HRMS (ESI-TOF): calc'd for $C_{12}H_{12}O_3$ [M + H$^+$] 205.0859, found 205.0853.

X-ray crystallographic data for **10**:

Supplementary Table 2. Crystal data and structure refinement for CCDC 922821.

Identification code	CCDC 922821	
Empirical formula	$C_{12}H_{12}O_3$	
Formula weight	204.22	
Temperature	123(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/n	
Unit cell dimensions	a = 8.0279(15) Å	α= 90°
	b = 5.5200(10) Å	β= 99.237(2)°
	c = 22.457(4) Å	γ = 90°
Volume	982.3(3) Å^3	
Z	4	
Density (calculated)	1.381 g/cm^3	
Absorption coefficient	0.099 mm^{-1}	
F(000)	432	
Crystal size	0.52 × 0.38 × 0.10 mm^3	
Crystal color, habit	Colorless plate	
Theta range for data collection	1.84 to 25.45°	
Index ranges	-9<=h<=9, -6<=k<=6, -27<=l<=18	
Reflections collected	9559	
Independent reflections	1818 [R(int) = 0.0326]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	Multi-scan	
Max. and min. transmission	0.9902 and 0.9503	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	1818 / 0 / 154	
Goodness-of-fit on F2	1.016	
Final R indices [I>2sigma(I)]	R1 = 0.0342, wR2 = 0.0953	
R indices (all data)	R1 = 0.0407, wR2 = 0.1006	
Extinction coefficient	0.007(2)	
Largest diff. peak and hole	0.223 and -0.221 e Å^{-3}	

Supplementary Table 3. Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters (Å^2 × 10^3) for CCDC 922821. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
O(1)	7644(1)	1206(2)	-792(1)	26(1)
O(2)	6456(1)	2977(2)	98(1)	22(1)
O(3)	5316(1)	4668(2)	1000(1)	23(1)
C(1)	8847(2)	-2704(2)	-755(1)	24(1)
C(2)	8013(2)	-760(2)	-451(1)	20(1)
C(3)	7640(2)	-933(2)	118(1)	20(1)
C(4)	6839(1)	1013(2)	391(1)	18(1)
C(5)	6491(2)	755(2)	997(1)	19(1)
C(6)	5762(1)	2569(2)	1277(1)	17(1)
C(7)	5390(1)	2446(2)	1902(1)	18(1)
C(8)	5965(2)	521(2)	2283(1)	20(1)
C(9)	5623(2)	467(2)	2871(1)	21(1)
C(10)	4706(2)	2321(2)	3082(1)	21(1)
C(11)	4128(2)	4236(2)	2705(1)	22(1)
C(12)	4474(2)	4314(2)	2118(1)	20(1)

Supplementary Table 4. Bond lengths [Å] and angles [°] for CCDC 922821.

O(1)-C(2)	1.3344(15)
O(1)-H(1)	0.96(2)
O(2)-C(4)	1.2801(15)
O(3)-C(6)	1.3368(15)
O(3)-H(3)	0.94(2)
C(1)-C(2)	1.4882(17)
C(1)-H(1A)	0.9800
C(1)-H(1B)	0.9800
C(1)-H(1C)	0.9800
C(2)-C(3)	1.3600(17)
C(3)-C(4)	1.4392(18)
C(3)-H(3)	0.975(15)
C(4)-C(5)	1.4396(17)
C(5)-C(6)	1.3631(17)
C(5)-H(5)	0.956(15)
C(6)-C(7)	1.4816(17)
C(7)-C(8)	1.3956(17)
C(7)-C(12)	1.3986(17)
C(8)-C(9)	1.3909(17)

C(8)-H(8A)	0.9500
C(9)-C(10)	1.3876(18)
C(9)-H(9A)	0.9500
C(10)-C(11)	1.3860(18)
C(10)-H(10A)	0.9500
C(11)-C(12)	1.3910(17)
C(11)-H(11A)	0.9500
C(12)-H(12A)	0.9500

C(2)-O(1)-H(1)	104.8(11)
C(6)-O(3)-H(3)	104.6(12)
C(2)-C(1)-H(1A)	109.5
C(2)-C(1)-H(1B)	109.5
H(1A)-C(1)-H(1B)	109.5
C(2)-C(1)-H(1C)	109.5
H(1A)-C(1)-H(1C)	109.5
H(1B)-C(1)-H(1C)	109.5
O(1)-C(2)-C(3)	122.34(11)
O(1)-C(2)-C(1)	113.38(10)
C(3)-C(2)-C(1)	124.27(12)
C(2)-C(3)-C(4)	121.90(12)
C(2)-C(3)-H(3)	117.9(8)
C(4)-C(3)-H(3)	120.2(8)
O(2)-C(4)-C(5)	120.21(11)
O(2)-C(4)-C(3)	119.96(11)
C(5)-C(4)-C(3)	119.82(11)
C(6)-C(5)-C(4)	121.74(11)
C(6)-C(5)-H(5)	121.5(8)
C(4)-C(5)-H(5)	116.7(8)
O(3)-C(6)-C(5)	121.66(11)
O(3)-C(6)-C(7)	113.62(10)
C(5)-C(6)-C(7)	124.72(11)
C(8)-C(7)-C(12)	119.22(11)
C(8)-C(7)-C(6)	121.25(11)
C(12)-C(7)-C(6)	119.52(11)
C(9)-C(8)-C(7)	120.17(11)
C(9)-C(8)-H(8A)	119.9
C(7)-C(8)-H(8A)	119.9
C(10)-C(9)-C(8)	120.29(11)
C(10)-C(9)-H(9A)	119.9
C(8)-C(9)-H(9A)	119.9
C(11)-C(10)-C(9)	119.90(11)
C(11)-C(10)-H(10A)	120.0
C(9)-C(10)-H(10A)	120.0

C(10)-C(11)-C(12)	120.20(11)
C(10)-C(11)-H(11A)	119.9
C(12)-C(11)-H(11A)	119.9
C(11)-C(12)-C(7)	120.22(11)
C(11)-C(12)-H(12A)	119.9
C(7)-C(12)-H(12A)	119.9

Supplementary Table 5. Anisotropic displacement parameters (Å^{2}x 10^3) for CCDC 922821. The anisotropic displacement factor exponent takes the form: -2 π 2[h2 a*2U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
O(1)	30(1)	26(1)	23(1)	4(1)	11(1)	3(1)
O(2)	26(1)	20(1)	22(1)	4(1)	7(1)	3(1)
O(3)	32(1)	20(1)	21(1)	4(1)	8(1)	6(1)
C(1)	23(1)	27(1)	22(1)	-4(1)	5(1)	-1(1)
C(2)	16(1)	21(1)	22(1)	-2(1)	2(1)	-4(1)
C(3)	20(1)	19(1)	21(1)	0(1)	3(1)	-1(1)
C(4)	14(1)	20(1)	20(1)	1(1)	1(1)	-3(1)
C(5)	20(1)	18(1)	19(1)	1(1)	2(1)	0(1)
C(6)	14(1)	17(1)	20(1)	1(1)	0(1)	-2(1)
C(7)	15(1)	18(1)	19(1)	-1(1)	1(1)	-3(1)
C(8)	18(1)	19(1)	22(1)	0(1)	4(1)	0(1)
C(9)	20(1)	23(1)	21(1)	3(1)	3(1)	-1(1)
C(10)	20(1)	26(1)	18(1)	-1(1)	5(1)	-4(1)
C(11)	20(1)	23(1)	24(1)	-5(1)	5(1)	0(1)
C(12)	19(1)	18(1)	21(1)	0(1)	2(1)	0(1)

Supplementary Table 6. Hydrogen coordinates (× 10^4) and isotropic displacement parameters (Å^2 × 10^3) for CCDC 922821.

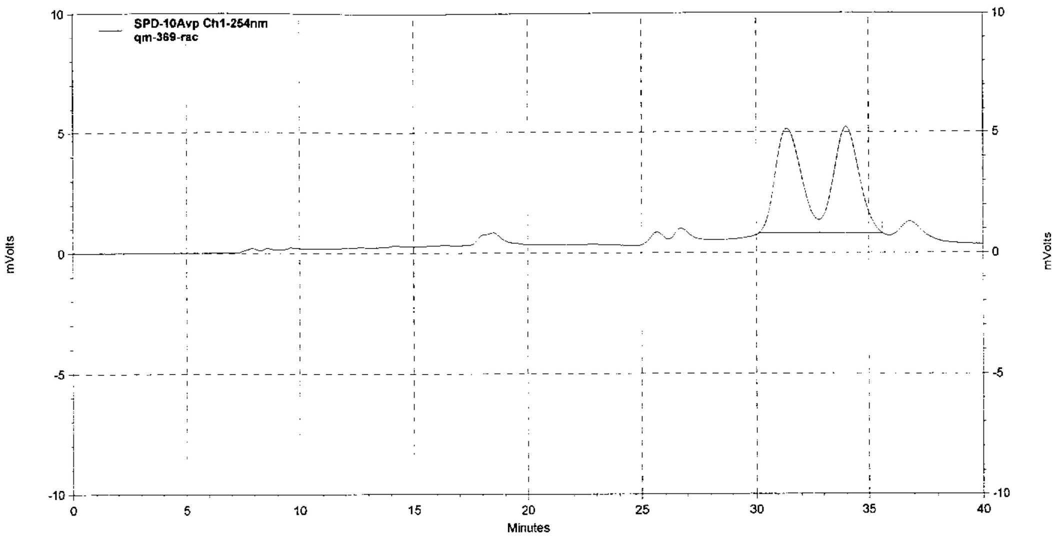
	x	y	z	U(eq)
H(1)	7090(20)	2270(30)	-551(9)	54(5)
H(3)	5570(30)	4470(40)	610(9)	63(6)
H(1A)	9842	-2042	-899	35
H(1B)	8055	-3334	-1098	35
H(1C)	9193	-4019	-468	35
H(3)	7919(18)	-2440(30)	337(6)	23(4)
H(5)	6770(18)	-770(30)	1191(6)	23(4)
H(8A)	6590	-755	2141	24
H(9A)	6019	-845	3129	26
H(10A)	4475	2277	3483	26

H(11A)	3494	5500	2849	26
H(12A)	4086	5640	1864	24

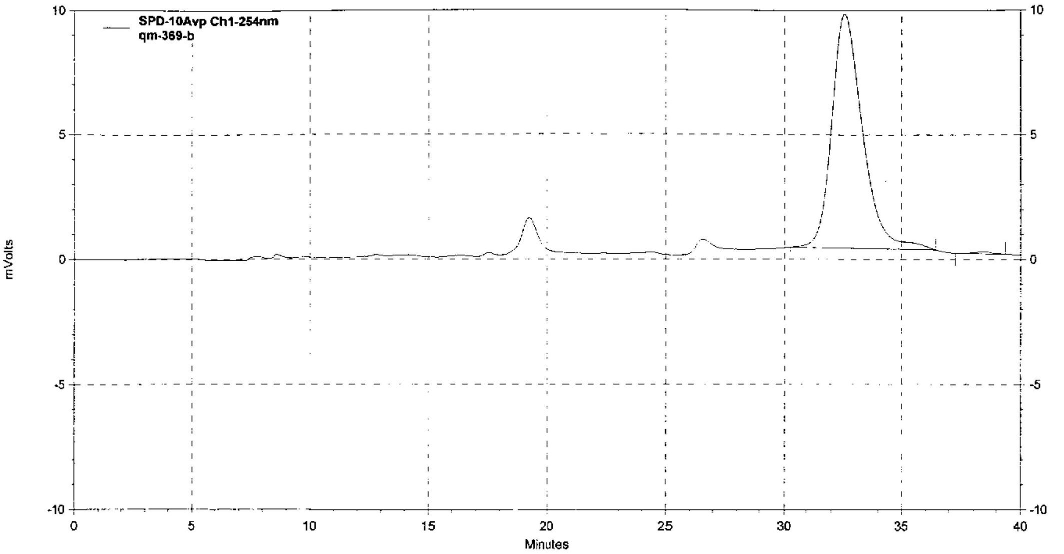
4: The preparation of **4** was adapted from a literature procedure. [Evans, D. A., Chapman, K. T. & Carreira, E. M. Directed reduction of β-hydroxy ketones employing tetramethylammonium triacetoxyborohydride. *J. Am. Chem. Soc.* **110,** 3560-3578, (1988).] All the flasks were flame-dried prior to use. A mixture of diisopropylamine (0.21 g, 0.29 mL, 3.4 equiv.) in THF (3 mL) was cooled to –78 °C under argon and ^{n}BuLi (1.1 mL, C = 1.8 M, 3.3 equiv.) was slowly added. The LDA solution was warmed to 0 °C and a solution of **10** (0.12 g, 1.0 equiv.) in THF (2 mL) under argon was added dropwise via a cannula within 10 min. The mixture was stirred at 0 °C for 2 h and turned dark red. Then, the reaction was cooled to –78 °C and freshly distilled acetaldehyde (0.11 g, 0.13 mL, 4 equiv.) was added in one portion. The mixture was stirred for 5 min and then quenched with $NaHSO_4$ (1 M). Et_2O (20 mL) was added and the phases were separated. The aqueous phase was extracted twice with Et_2O (2 × 20 mL). The combined organic layers were diluted with hexanes (60 mL) and dried over Na_2SO_4. Evaporation *in vacuo* resulted in a dark orange, viscous liquid (ca. 0.3 g). Quick column chromatography (deactivated SiO_2 with 1 % Et_3N in Et_2O, 0:10 to 1:1 EtOAc:hexanes) provided an orange solid (0.055 g, 37 %) that can be recrystallized by diffusion of hexanes into a solution of **4** in Et_2O at 4 °C.

Melting point: 146–149 °C
TLC: R_f = 0.19–0.22 (4:6 EtOAc:hexanes, UV active, stains orange upon *p*-anisaldehyde staining). NMR showed a complex mixture of keto-enol tautomers.
^{1}H NMR (600 MHz, $CDCl_3$, major peaks): δ 7.99 – 7.95 (m), 7.89 – 7.88 (m), 7.82 – 7.81 (m), 7.64 – 7.60(m), 7.56 – 7.54 (m), 7.51 – 7.42 (m), 6.23 (s), 5.86 (s), 5.66 (s), 5.38 (s), 4.30 – 4.20 (m), 3.97 (m), 3.58 (s), 3.14 – 3.12 (m), 2.81 – 2.68 (m), 2.65 – 2.63 (m), 2.48 – 2.35 (m), 2.22 – 2.16 (m), 1.28 (d, *J* = 6.0 Hz), 1.25 – 1.20 (m) ppm.
^{13}C NMR (151 MHz, $CDCl_3$, major peaks): δ 205.8, 194.8, 189.8, 184.1, 179.3, 175.0, 134.9, 134.8, 134.7, 134.4, 133.7, 132.6, 129.8, 129.7, 129.7, 129.6, 129.6, 129.5, 129.1, 128.0, 127.3, 102.4, 101.4, 99.7, 98.2, 96.8, 78.1, 77.8, 77.7, 77.6, 66.7, 66.5, 65.9, 64.6, 55.5, 52.2, 52.1, 49.8, 49.4, 47.7, 46.8, 45.8, 24.0, 23.8, 23.2, 22.5 ppm.
IR (neat): $\tilde{\nu}$ = 3400, 2971, 1716, 1592, 1571, 1494, 1450, 1395, 1273, 1202, 1149, 1080, 946, 769, 690 cm^{-1}.
HRMS (ESI-TOF): calc'd for $C_{14}H_{16}O_4$ [M + H^{+}] 249.1121, found 249.1129.
Optical rotation: (+)-**4** $[\alpha]_D^{20}$ (c 0.4, $CHCl_3$) = +16°, (–)-**4** $[\alpha]_D^{20}$ (c 0.4, $CHCl_3$) = –16°.

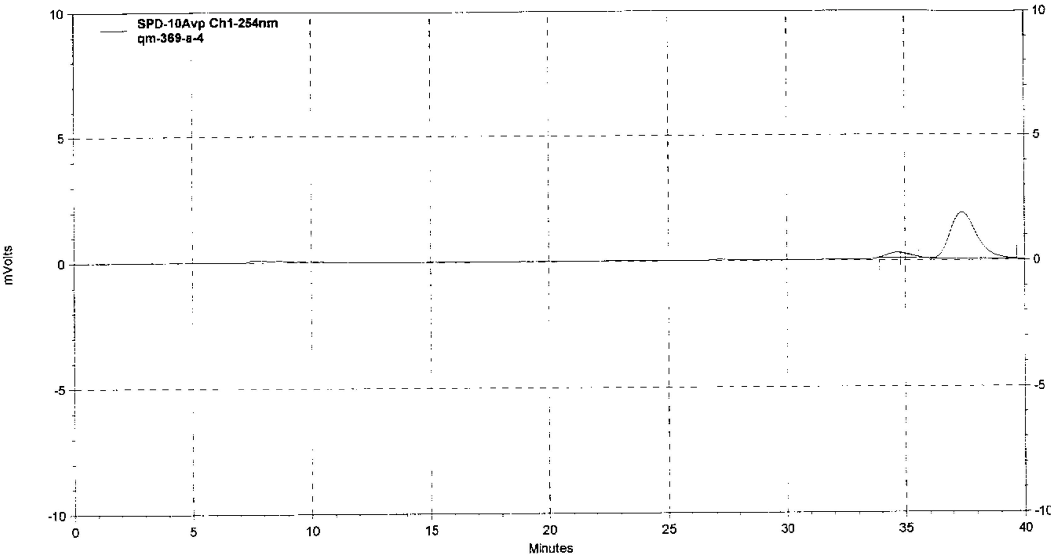
Enantiopure **4** was obtained by resolution with chiral HPLC (α = 1.12).
Column: Daicel Chiralcel® OJ
Dimensions: 2 × 250 mm
Eluent: 25 % ^{i}PrOH:hexanes
Flow rate: 0.7 mL/min



Supplementary Figure 17. Chromatogram of (+/–)-**4.**



Supplementary Figure 18. Chromatogram of (–)-**4** after HPLC resolution.



Supplementary Figure 19. Chromatogram of (+)-**4** after HPLC resolution.

X-ray crystallographic data for **4**:

Supplementary Table 7. Crystal data and structure refinement for CCDC 922822.

Identification code	CCDC 922822	
Empirical formula	$C_{14}H_{16}O_4$	
Formula weight	247.76	
Temperature	123(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	a = 54.130(16) Å	α= 90°
	b = 5.6103(17) Å	β= 99.508(4)°
	c = 16.288(5) Å	γ = 90°
Volume	4879(3) $Å^3$	
Z	16	
Density (calculated)	1.349 g/cm^3	
Absorption coefficient	0.099 mm^{-1}	
F(000)	2104	
Crystal size	0.35 × 0.24 × 0.07 mm^3	
Crystal color, habit	Colorless plate	
Theta range for data collection	1.53 to 25.40°	
Index ranges	-64<=h<=64, -6<=k<=6, -9<=l<=19	
Reflections collected	19682	
Independent reflections	4465 [R(int) = 0.0460]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F2	

Data / restraints / parameters	4465 / 2 / 362
Goodness-of-fit on F2	1.055
Final R indices [I>2sigma(I)]	R1 = 0.0519, wR2 = 0.1307
R indices (all data)	R1 = 0.0758, wR2 = 0.1484
Extinction coefficient	0.00064(17)
Largest diff. peak and hole	0.299 and -0.274 e Å^{-3}

Supplementary Table 8. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters (Å^2 $\times 10^3$)for CCDC 922822. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
O(1')	1552(1)	3251(3)	1182(1)	35(1)
O(1)	-1516(1)	11039(3)	-4381(1)	43(1)
O(2')	1157(1)	4219(3)	205(1)	32(1)
O(2)	-1151(1)	10589(3)	-3234(1)	46(1)
O(3')	769(1)	5283(3)	-810(1)	36(1)
O(3)	-788(1)	10123(3)	-2071(1)	52(1)
O(4)	-277(1)	5954(5)	-658(1)	79(1)
O(4')	344(1)	9070(6)	39(2)	44(1)
O(4")	230(1)	6799(8)	-595(3)	52(2)
C(1')	1802(1)	8293(4)	2469(1)	30(1)
C(1)	-1936(1)	5914(4)	-4582(1)	32(1)
C(2')	2020(1)	8858(4)	3007(1)	31(1)
C(2)	-2168(1)	5346(4)	-5034(1)	34(1)
C(3')	2221(1)	7313(4)	3112(1)	32(1)
C(3)	-2288(1)	6903(4)	-5631(1)	33(1)
C(4)	-2172(1)	9012(4)	-5784(1)	34(1)
C(4')	2203(1)	5162(4)	2688(1)	32(1)
C(5)	-1941(1)	9594(4)	-5335(1)	33(1)
C(5')	1985(1)	4580(4)	2158(1)	29(1)
C(6')	1783(1)	6146(4)	2034(1)	27(1)
C(6)	-1821(1)	8069(4)	-4722(1)	29(1)
C(7')	1559(1)	5536(4)	1423(1)	27(1)
C(7)	-1582(1)	8816(4)	-4219(1)	31(1)
C(8)	-1438(1)	7489(4)	-3623(1)	31(1)
C(8')	1377(1)	7093(4)	1094(1)	29(1)
C(9')	1172(1)	6388(4)	462(1)	28(1)
C(9)	-1216(1)	8424(4)	-3127(1)	35(1)
C(10')	990(1)	8082(4)	101(1)	34(1)
C(10)	-1068(1)	7007(4)	-2502(1)	36(1)
C(11)	-862(1)	7870(5)	-2005(1)	41(1)
C(11')	799(1)	7491(4)	-511(1)	34(1)

C(12)	-699(1)	6522(6)	-1342(1)	50(1)
C(12')	606(1)	9189(4)	-926(2)	41(1)
C(13)	-424(1)	6538(5)	-1438(1)	41(1)
C(13')	348(1)	8780(5)	-770(2)	62(1)
C(14')	167(1)	10538(5)	-1258(2)	69(1)
C(14)	-369(1)	4804(5)	-2079(2)	49(1)

Supplementary Table 9. Bond lengths [Å] and angles [°] for CCDC 922822.

O(1')-C(7')	1.339(3)
O(1')-H(1')	1.04(3)
O(1)-C(7)	1.335(3)
O(1)-H(1)	1.01(4)
O(2')-C(9')	1.285(3)
O(2)-C(9)	1.285(3)
O(3')-C(11')	1.331(3)
O(3')-H(3')	1.06(3)
O(3)-C(11)	1.336(3)
O(3)-H(3)	1.04(4)
O(4)-C(13)	1.422(3)
O(4)-H(4)	0.9799(11)
O(4')-C(13')	1.333(4)
O(4')-O(4")	1.691(6)
O(4')-H(4')	0.9800(11)
O(4")-C(13')	1.338(5)
O(4")-H(4')	0.83(2)
C(1')-C(2')	1.387(3)
C(1')-C(6')	1.393(3)
C(1')-H(1'A)	0.9500
C(1)-C(2)	1.386(3)
C(1)-C(6)	1.394(3)
C(1)-H(1A)	0.9500
C(2')-C(3')	1.380(3)
C(2')-H(2'A)	0.9500
C(2)-C(3)	1.386(3)
C(2)-H(2A)	0.9500
C(3')-C(4')	1.385(3)
C(3')-H(3'A)	0.9500
C(3)-C(4)	1.382(3)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.377(3)
C(4)-H(4A)	0.9500
C(4')-C(5')	1.383(3)

C(4')-H(4'A)	0.9500
C(5)-C(6)	1.393(3)
C(5)-H(5A)	0.9500
C(5')-C(6')	1.392(3)
C(5')-H(5'A)	0.9500
C(6')-C(7')	1.477(3)
C(6)-C(7)	1.474(3)
C(7')-C(8')	1.358(3)
C(7)-C(8)	1.362(3)
C(8)-C(9)	1.429(3)
C(8)-H(8A)	0.9500
C(8')-C(9')	1.437(3)
C(8')-H(8'A)	0.9500
C(9')-C(10')	1.425(3)
C(9)-C(10)	1.430(3)
C(10')-C(11')	1.354(3)
C(10')-H(10A)	0.9500
C(10)-C(11)	1.352(3)
C(10)-H(10B)	0.9500
C(11)-C(12)	1.485(4)
C(11')-C(12')	1.490(3)
C(12)-C(13)	1.525(3)
C(12)-H(12A)	0.9900
C(12)-H(12B)	0.9900
C(12')-C(13')	1.477(4)
C(12')-H(12C)	0.9900
C(12')-H(12D)	0.9900
C(13)-C(14)	1.491(4)
C(13)-H(13A)	1.0000
C(13')-C(14')	1.521(4)
C(13')-H(4')	1.33(3)
C(14')-H(14A)	0.9800
C(14')-H(14B)	0.9800
C(14')-H(14C)	0.9800
C(14)-H(14D)	0.9800
C(14)-H(14E)	0.9800
C(14)-H(14F)	0.9800

C(7')-O(1')-H(1')	101.5(19)
C(7)-O(1)-H(1)	102(2)
C(11')-O(3')-H(3')	103.9(17)
C(11)-O(3)-H(3)	105(2)
C(13)-O(4)-H(4)	119(4)
C(13')-O(4')-O(4")	50.9(2)

C(13')-O(4')-H(4')	68.1(18)
O(4")-O(4')-H(4')	19.6(18)
C(13')-O(4")-O(4')	50.6(2)
C(13')-O(4")-H(4')	71.1(18)
O(4')-O(4")-H(4')	23.2(16)
C(2')-C(1')-C(6')	120.2(2)
C(2')-C(1')-H(1'A)	119.9
C(6')-C(1')-H(1'A)	119.9
C(2)-C(1)-C(6)	120.1(2)
C(2)-C(1)-H(1A)	119.9
C(6)-C(1)-H(1A)	119.9
C(3')-C(2')-C(1')	120.5(2)
C(3')-C(2')-H(2'A)	119.7
C(1')-C(2')-H(2'A)	119.7
C(1)-C(2)-C(3)	120.4(2)
C(1)-C(2)-H(2A)	119.8
C(3)-C(2)-H(2A)	119.8
C(2')-C(3')-C(4')	119.7(2)
C(2')-C(3')-H(3'A)	120.1
C(4')-C(3')-H(3'A)	120.1
C(4)-C(3)-C(2)	119.5(2)
C(4)-C(3)-H(3A)	120.3
C(2)-C(3)-H(3A)	120.3
C(5)-C(4)-C(3)	120.4(2)
C(5)-C(4)-H(4A)	119.8
C(3)-C(4)-H(4A)	119.8
C(5')-C(4')-C(3')	120.0(2)
C(5')-C(4')-H(4'A)	120.0
C(3')-C(4')-H(4'A)	120.0
C(4)-C(5)-C(6)	120.7(2)
C(4)-C(5)-H(5A)	119.7
C(6)-C(5)-H(5A)	119.7
C(4')-C(5')-C(6')	120.8(2)
C(4')-C(5')-H(5'A)	119.6
C(6')-C(5')-H(5'A)	119.6
C(5')-C(6')-C(1')	118.8(2)
C(5')-C(6')-C(7')	119.34(19)
C(1')-C(6')-C(7')	121.80(19)
C(5)-C(6)-C(1)	118.8(2)
C(5)-C(6)-C(7)	119.2(2)
C(1)-C(6)-C(7)	122.0(2)
O(1')-C(7')-C(8')	121.23(19)
O(1')-C(7')-C(6')	113.43(18)
C(8')-C(7')-C(6')	125.30(19)

O(1)-C(7)-C(8)	120.7(2)
O(1)-C(7)-C(6)	113.2(2)
C(8)-C(7)-C(6)	126.0(2)
C(7)-C(8)-C(9)	122.0(2)
C(7)-C(8)-H(8A)	119.0
C(9)-C(8)-H(8A)	119.0
C(7')-C(8')-C(9')	121.83(19)
C(7')-C(8')-H(8'A)	119.1
C(9')-C(8')-H(8'A)	119.1
O(2')-C(9')-C(10')	119.65(19)
O(2')-C(9')-C(8')	119.62(19)
C(10')-C(9')-C(8')	120.71(19)
O(2)-C(9)-C(8)	119.6(2)
O(2)-C(9)-C(10)	119.1(2)
C(8)-C(9)-C(10)	121.3(2)
C(11')-C(10')-C(9')	122.2(2)
C(11')-C(10')-H(10A)	118.9
C(9')-C(10')-H(10A)	118.9
C(11)-C(10)-C(9)	122.5(2)
C(11)-C(10)-H(10B)	118.8
C(9)-C(10)-H(10B)	118.8
O(3)-C(11)-C(10)	121.3(2)
O(3)-C(11)-C(12)	112.9(2)
C(10)-C(11)-C(12)	125.8(2)
O(3')-C(11')-C(10')	121.9(2)
O(3')-C(11')-C(12')	113.31(19)
C(10')-C(11')-C(12')	124.8(2)
C(11)-C(12)-C(13)	113.4(2)
C(11)-C(12)-H(12A)	108.9
C(13)-C(12)-H(12A)	108.9
C(11)-C(12)-H(12B)	108.9
C(13)-C(12)-H(12B)	108.9
H(12A)-C(12)-H(12B)	107.7
C(13')-C(12')-C(11')	115.7(2)
C(13')-C(12')-H(12C)	108.3
C(11')-C(12')-H(12C)	108.3
C(13')-C(12')-H(12D)	108.4
C(11')-C(12')-H(12D)	108.4
H(12C)-C(12')-H(12D)	107.4
O(4)-C(13)-C(14)	109.3(2)
O(4)-C(13)-C(12)	108.48(18)
C(14)-C(13)-C(12)	112.2(2)
O(4)-C(13)-H(13A)	108.9
C(14)-C(13)-H(13A)	108.9

C(12)-C(13)-H(13A)	108.9
O(4')-C(13')-O(4")	78.6(3)
O(4')-C(13')-C(12')	108.9(3)
O(4")-C(13')-C(12')	131.8(3)
O(4')-C(13')-C(14')	108.7(3)
O(4")-C(13')-C(14')	111.0(3)
C(12')-C(13')-C(14')	111.0(2)
O(4')-C(13')-H(4')	43.2(5)
O(4")-C(13')-H(4')	36.5(6)
C(12')-C(13')-H(4')	137.9(12)
C(14')-C(13')-H(4')	108.7(13)
C(13')-C(14')-H(14A)	109.5
C(13')-C(14')-H(14B)	109.5
H(14A)-C(14')-H(14B)	109.5
C(13')-C(14')-H(14C)	109.5
H(14A)-C(14')-H(14C)	109.5
H(14B)-C(14')-H(14C)	109.5
C(13)-C(14)-H(14D)	109.5
C(13)-C(14)-H(14E)	109.5
H(14D)-C(14)-H(14E)	109.5
C(13)-C(14)-H(14F)	109.5
H(14D)-C(14)-H(14F)	109.5
H(14E)-C(14)-H(14F)	109.5

Supplementary Table 10. Anisotropic displacement parameters (Å^{2}x 10^3) for CCDC 922822. The anisotropic displacement factor exponent takes the form: -2 π 2[h2 a*2U11 + ... + 2 h k a* b* U12]

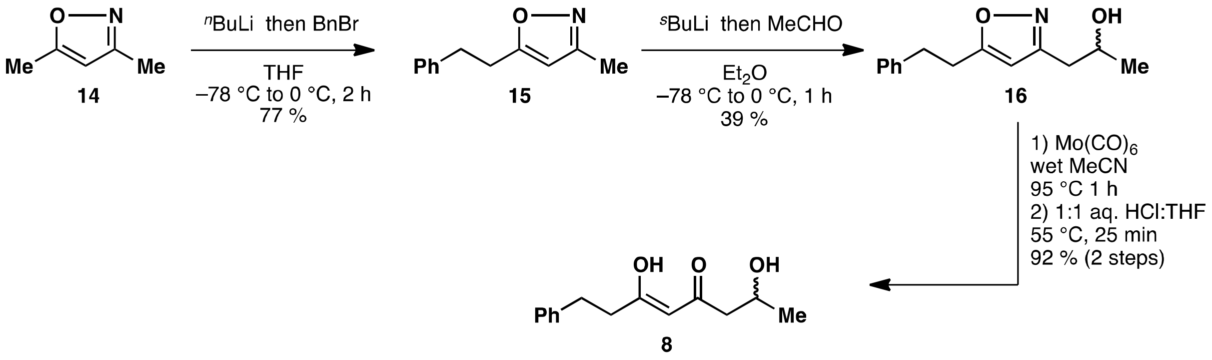
	U11	U22	U33	U23	U13	U12
O(1')	33(1)	22(1)	49(1)	-6(1)	0(1)	3(1)
O(1)	34(1)	26(1)	66(1)	6(1)	4(1)	-4(1)
O(2')	28(1)	25(1)	42(1)	-5(1)	4(1)	-2(1)
O(2)	34(1)	30(1)	71(1)	-7(1)	3(1)	-5(1)
O(3')	35(1)	29(1)	43(1)	-3(1)	-1(1)	-3(1)
O(3)	38(1)	50(1)	68(1)	-20(1)	4(1)	-10(1)
O(4)	31(1)	167(3)	34(1)	4(1)	-4(1)	-13(1)
O(4')	37(2)	53(2)	41(2)	-1(1)	6(1)	0(2)
O(4")	32(3)	41(3)	75(3)	15(3)	-17(2)	-13(2)
C(1')	31(1)	25(1)	33(1)	1(1)	7(1)	1(1)
C(1)	31(1)	28(1)	37(1)	1(1)	6(1)	0(1)
C(2')	37(1)	25(1)	32(1)	-1(1)	6(1)	-3(1)
C(2)	35(1)	29(1)	40(1)	-3(1)	7(1)	-5(1)

C(3')	34(1)	31(1)	30(1)	2(1)	3(1)	-5(1)
C(3)	30(1)	34(1)	35(1)	-6(1)	4(1)	0(1)
C(4)	34(1)	30(1)	37(1)	3(1)	6(1)	2(1)
C(4')	28(1)	32(1)	35(1)	3(1)	3(1)	4(1)
C(5)	31(1)	28(1)	42(1)	2(1)	11(1)	0(1)
C(5')	31(1)	24(1)	33(1)	1(1)	6(1)	2(1)
C(6')	28(1)	24(1)	30(1)	2(1)	7(1)	-1(1)
C(6)	27(1)	26(1)	35(1)	-2(1)	11(1)	1(1)
C(7')	28(1)	22(1)	33(1)	0(1)	11(1)	-2(1)
C(7)	27(1)	25(1)	42(1)	-3(1)	12(1)	0(1)
C(8)	28(1)	27(1)	39(1)	-3(1)	10(1)	-4(1)
C(8')	29(1)	20(1)	37(1)	-2(1)	7(1)	-1(1)
C(9')	26(1)	25(1)	34(1)	0(1)	8(1)	-3(1)
C(9)	31(1)	31(1)	45(1)	-9(1)	13(1)	-1(1)
C(10')	34(1)	23(1)	43(1)	-1(1)	4(1)	-1(1)
C(10)	31(1)	41(1)	37(1)	-5(1)	8(1)	-7(1)
C(11)	34(1)	53(2)	38(1)	-12(1)	14(1)	-10(1)
C(11')	31(1)	28(1)	41(1)	3(1)	4(1)	-3(1)
C(12)	35(2)	85(2)	30(1)	-3(1)	5(1)	-15(1)
C(12')	44(2)	30(1)	46(1)	3(1)	-6(1)	0(1)
C(13)	34(1)	56(2)	34(1)	3(1)	5(1)	-11(1)
C(13')	33(2)	51(2)	99(3)	36(2)	6(2)	3(1)
C(14')	33(2)	56(2)	111(3)	34(2)	-7(2)	-1(1)
C(14)	40(2)	48(2)	56(2)	1(1)	-2(1)	8(1)

Supplementary Table 11. Hydrogen coordinates (× 10^4) and isotropic displacement parameters (Å^2 × 10^3) for CCDC 922822.

	x	y	z	U(eq)
H(1'A)	1665	9373	2398	35
H(1A)	-1854	4834	-4176	38
H(2'A)	2031	10319	3305	38
H(2A)	-2246	3880	-4934	41
H(3'A)	2372	7722	3473	38
H(3A)	-2449	6524	-5933	40
H(4A)	-2251	10067	-6202	41
H(4'A)	2341	4087	2763	38
H(5A)	-1863	11051	-5445	40
H(5'A)	1973	3095	1874	35
H(8A)	-1486	5893	-3535	37
H(8'A)	1385	8693	1288	34
H(10A)	1003	9680	296	40
H(10B)	-1115	5397	-2434	43

H(12A)	-758	4850	-1347	60
H(12B)	-715	7216	-795	60
H(12C)	657	10823	-740	50
H(12D)	605	9115	-1534	50
H(13A)	-377	8175	-1603	50
H(14A)	-2	10254	-1136	103
H(14B)	166	10322	-1855	103
H(14C)	218	12169	-1097	103
H(14D)	-194	4963	-2152	73
H(14E)	-478	5129	-2608	73
H(14F)	-399	3179	-1897	73
H(3')	925(6)	4340(60)	-478(19)	76(10)
H(1')	1391(6)	3210(60)	740(20)	91(11)
H(1)	-1361(7)	11270(70)	-3950(20)	99(12)
H(3)	-920(7)	10850(70)	-2540(20)	102(13)
H(4')	237(5)	7750(30)	-196(17)	50(10)
H(4)	-96(2)	6230(120)	-570(40)	250(30)



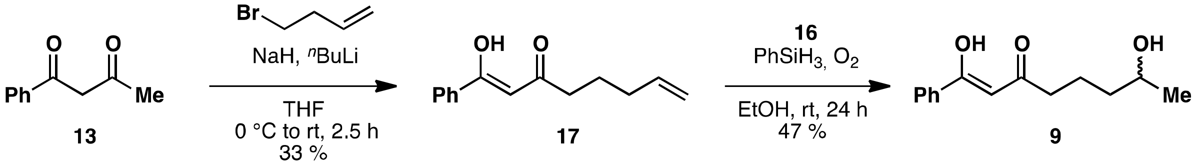
15 was synthesized according to a literature procedure. [Brunelle, D. J. Isoxazoles as β-diketone synthons-selective anion formation on 3,5-dialkylisoxazoles. *Tetrahedron Lett.* **22,** 3699-3702, (1981).]

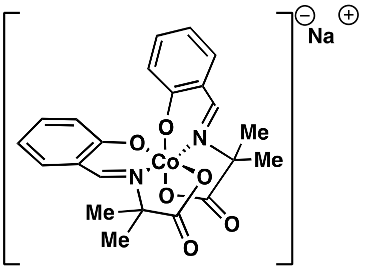
16: All the flasks were flame-dried prior to use. A mixture of **15** (1.47 g, 1 equiv.) in Et_2O (50 mL) was cooled to –78 °C under argon and ^{s}BuLi (7.3 mL, C = 1.3 M, 1.2 equiv.) was slowly added dropwise. The mixture was stirred over 30 min and turned yellow. Freshly distilled acetaldehyde (1.04 g, 1.32 mL, 3 equiv.) was added in one portion and the mixture was slowly warmed to 0 °C in 30 min. The reaction was quenched by adding 20 mL of saturated NH_4Cl and the phases were separated. The aqueous phase was extracted twice with Et_2O (2 × 20 mL). The combined organic layers were dried over $MgSO_4$. Evaporation *in vacuo* resulted in a dark orange, viscous liquid (2.08 g). This crude product constituted a mixture of 3 regioisomers and was very difficult to separate; consequently, a small sample of this crude product (2.5 % of the total mass) was purified by preparative TLC (3:7 EtOAc:hexanes, eluted 4 times) to give pure **16** (16 mg, 39 % yield after extrapolation).

TLC: R_f = 0.20–0.23 (3:7 EtOAc:hexanes, UV active, stains off-white upon *p*-anisaldehyde staining).
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.30 – 7.28 (m, 2H), 7.23 – 7.20 (m, 1H), 7.18 – 7.17 (m, 2H), 5.83 (s, 1H), 4.18 – 4.13 (m, 1H), 3.04 – 3.00 (m, 4H), 2.80 – 2.70 (m, 2H), 1.26 (d, *J* = 6.2 Hz, 3H) ppm.
^{13}C NMR (151 MHz, $CDCl_3$) δ 172.5, 161.5, 140.2, 128.7, 128.4, 126.6, 101.9, 66.4, 35.9, 33.7, 28.6, 23.0 ppm.
IR (neat): $\tilde{\nu}$ = 3395, 2968, 2928, 1601, 1454, 1421, 1118, 1077, 941, 801, 749, 698 cm^{-1}.
HRMS (ESI-TOF): calc'd for $C_{14}H_{17}NO_2$ [M + H$^+$] 232.1338, found 232.1330.

8: **16** (5.0 mg, 1 equiv.) and $Mo(CO)_6$ (6.0 mg, 1 equiv.) were dissolved in MeCN (0.5 mL) and 1 drop of water was added. The mixture was stirred at 95 °C for 90 min, then cooled down to room temperature and the solvent was concentrated *in vacuo*. The residues were filtrated over a small celite plug (around 2 cm high, eluted with 5 mL of EtOAc). After removal of the solvent *in vacuo*, the vinyloguous amide intermediate was taken up in THF (0.5 mL) and HCl (1N, 0.5 mL) and the mixture was stirred at 55 °C for 25 min then poured into water (10 mL). EtOAc (20 mL) was added and the phases were separated. The aqueous phase was extracted twice with EtOAc (2 × 10 mL). The combined organic layers were diluted with hexanes (ca 1:1 hexanes:EtOAc) and dried over Na_2SO_4. Evaporation *in vacuo* resulted in a yellow viscous liquid (ca. 7 mg). Purification by preparative TLC (deactivated with 1 % Et_3N in hexanes, 4:6 EtOAc:hexanes, eluted once) afforded pure **8** (4.6 mg, 92 %) as a colorless oil.

TLC: R_f = 0.37–0.46 (3:7 EtOAc:hexanes, UV active, stains orange upon *p*-anisaldehyde staining).
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.30 – 7.27 (m, 2H), 7.23 – 7.20 (m, 1H), 7.18 – 7.17 (m, 2H), 5.29 (s, 1H), 4.49 – 4.43 (m, 1H), 2.89 – 2.87 (m, 2H), 2.57 – 2.53 (m, 2H), 2.39 – 2.37 (m, 2H), 1.44 (d, *J* = 6.4 Hz, 3H) ppm.
^{13}C NMR (151 MHz, $CDCl_3$) δ 193.2, 176.6, 140.3, 128.7, 128.4, 126.5, 104.5, 75.8, 42.8, 36.7, 32.7, 20.6 ppm.
IR (neat): $\tilde{\nu}$ = 2926, 1663, 1600, 1454, 1385, 1335, 1270, 1241, 1198, 1105, 1040, 941, 807, 749, 699 cm^{-1}.
HRMS (ESI-TOF): calc'd for $C_{14}H_{18}O_3$ [M – H_2O + H$^+$] 217.1223, found 217.1225.





18

17: To a suspension of NaH (98 mg, 60 % dispersion in mineral oil, 2 equiv.) in 4 mL of THF cooled to 0 °C was added a solution of 1-benzoylacetone (**13**) (200 mg, 1 equiv.) in 1 mL of THF. The mixture was stirred at 0 °C for 10 min, ^{n}BuLi (0.6 mL, C = 2.1 M, 1 equiv.) was added dropwise and the mixture was stirred for another 10 min. 4-Bromo-1-butene (0.17 g, 0.13 mL, 1 equiv.) was added dropwise and the mixture was stirred at 0 °C for another 10 min then for 2 h at room temperature. The reaction was cooled to 0 °C and quenched slowly with HCl (3N, 5 mL). Saturated NH_4Cl (10 mL) and Et_2O (15 mL) were added and the phases were separated. The aqueous phase was extracted twice with Et_2O (2 × 10 mL). The combined organic layers were dried over $MgSO_4$. Evaporation *in vacuo* resulted in a dark orange viscous liquid (ca. 240 mg). Column chromatography (hexanes) provided a pale oil (89 mg, 33 %)

The spectroscopic data for this compound were identical to those reported in the literature. [Lin, S., Song, C.-X., Cai, G.-X., Wang, W.-H. & Shi, Z.-J. Intra/intermolecular direct allylic alkylation via Pd(II)-Catalyzed allylic C–H Activation. *J. Am. Chem. Soc.* **130,** 12901-12903, (2008).]

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88 – 7.86 (m, 2H), 7.53 – 7.50 (m, 1H), 7.46 – 7.43 (m, 2H), 6.17 (s, 1H), 5.84 – 5.87 (m, 1H), 5.06 – 4.99 (m, 2H), 2.45 – 2.42 (m, 2H), 2.15 – 2.12 (m, 2H), 1.82 – 1.77 (m, 2H) ppm.

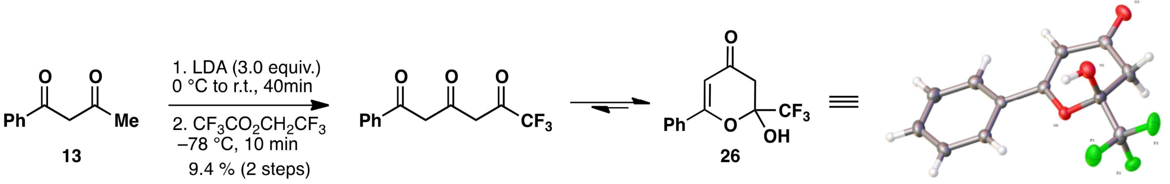
^{13}C NMR (151 MHz, $CDCl_3$): δ 196.8, 183.6, 137.9, 135.1, 132.3, 128.7, 127.1, 115.5, 96.3, 38.6, 33.3, 25.0 ppm.

18 was synthesized according to a literature procedure (procedure A) and used without further purification. [Waser, J., Gaspar, B., Nambu, H. & Carreira, E. M. Hydrazines and azides via the metal-catalyzed hydrohydrazination and hydroazidation of olefins. *J. Am. Chem. Soc.* **128,** 11693-11712, (2006).]

9: Under O_2 (1 atm), **17** (8.0 mg, 1 equiv.) was dissolved in EtOH (0.5 mL) presaturated with O_2 for 30 min under sonication. **18** (2.0 mg, 0.1 equiv.) was added followed by $PhSiH_3$ (8.8 mg, 10 μL, 2.2 equiv.). The mixture was stirred at room temperature under an O_2 atmosphere for 24 h., sat. $Na_2S_2O_3$ (0.5 mL) was added and the mixture was vigorously stirred for 2 h. Sat. NaCl (1 mL) was then added, followed by EtOAc (5 mL) and the phases were separated. The aqueous phase was extracted twice with EtOAc (2 × 10 mL). The combined organic layers were diluted with hexanes (25 mL) and dried over Na_2SO_4. Evaporation *in vacuo* resulted in an orange viscous liquid (ca. 8.0 mg). Purification by preparative TLC (deactivated with 1 % Et_3N in hexanes, 4:6 EtOAc:hexanes, eluted once) afforded pure **9** (4.1 mg, 47 %) as a colorless oil.

TLC: R_f = 0.21–0.28 (3:7 EtOAc:hexanes, UV active, stains orange upon *p*-anisaldehyde staining).
^{1}H NMR (600 MHz, $CDCl_3$, major tautomer): δ 7.92 – 7.84 (m, 2H), 7.55 – 7.50 (m, 1H), 7.49 – 7.43 (m, 2H), 6.18 (s, 1H), 3.85 – 3.82 (m, 1H), 2.49 – 2.46 (m, 2H), 1.85 –1.73 (m, 2H), 1.53 (m, 2H), 1.22 (d, *J* = 6.1 Hz, 3H) ppm.
^{13}C NMR (151 MHz, $CDCl_3$) δ 193.1, 183.3, 135.0, 132.4, 128.8, 127.1, 96.3, 67.8, 39.2, 38.8, 23.7, 21.9 ppm.
IR (neat): $\tilde{\nu}$ = 3416, 2921, 2851, 1720, 1673, 1599, 1574, 1459, 1374, 1263, 1182, 1074, 1014, 802, 765, 692 cm^{-1}.
HRMS (ESI-TOF): calc'd for $C_{14}H_{18}O_3$ [$M - H_2O + H^+$] 217.1223, found 217.1228.

Synthesis of substrate analog 26:



A solution of benzoyl acetone (**13**) (0.5 g, 1.0 equiv.) in THF (2 mL) was treated with a solution of lithium diisopropylamide (LDA) in THF (15.7 ml, 0.6 M, 3.0 equiv.). The mixture was stirred at 0 °C for 10 min, warmed to room temperature and stirred for 30 min, then cooled to –78 °C. 2,2,2-trifluoroethyl trifluoroacetate (0.412 ml, 1.0 equiv.) was added and the mixture was stirred at –78 °C for 10 min and then quenched with water (10 mL). After acidification by addition of HCl (2N, 10 mL), the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (3:1 EtOAc:hexanes) to afford **26** (70 mg, 9.4 %) that can be recrystallized in EtOAc:hexanes.

Melting point: 82–84 °C
TLC: R_f = 0.26 – 0.36 (3:7 EtOAc:hexanes, UV active).
NMR showed a complex mixture of keto-enol tautomers.
^{1}H NMR (600 MHz, $CDCl_3$, major peaks): δ 8.01 – 7.43 (m), 7.08 (s), 6.85 (d, *J* = 2.2 Hz), 6.76 (d, *J* = 2.2 Hz), 6.45 (s), 6.18 (s), 6.07 (s), 5.87 (s), 5.72 (s), 3.85 (br), 7.08 (s), 3.04 – 2.88 (m), 2.17 (s), ppm.
^{13}C NMR (151 MHz, $CDCl_3$, major peaks): δ 206.1, 205.0, 203.7, 133.0, 132.4, 129.4, 129.3, 129.0, 127.1, 126.6, 126.2, 97.0 ppm.

^{13}F NMR ($CDCl_3$) δ 104.6 ppm.
IR (neat): $\tilde{\nu}$ = 2360, 2340, 1667, 1604, 1190, 687 cm^{-1}.
HRMS (ESI-TOF): calc'd for $C_{12}H_9F_3O_3$ [M – H_2O + H^+] 241.0476, found 241.0479.

X-ray crystallographic data for **26**:
Supplementary Table 12. Crystal data and structure refinement for CCDC 949270.

Identification code	CCDC 949270	
Empirical formula	$C_{36}H_{27}F_9O_9$	
Molecular formula	$3(C_{12}H_9F_3O_3)$	
Formula weight	774.57	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21/c 1	
Unit dimensions	a = 6.0819(10) Å	α = 90°.
	b = 17.503(3) Å	β = 91.626(2)°.
	c = 30.597(5) Å	γ = 90°.
Volume	3255.8(10) Å^3	
Z	4	
Density (calculated)	1.580 Mg/m^3	
Absorption coefficient	0.146 mm^{-1}	
F(000)	1584	
Crystal size	0.137 x 0.115 x 0.108 mm^3	
Crystal color, habit	Colorless Block	
Theta range for data collection	2.664 to 26.308°.	
Index ranges	-7<=h<=7, -21<=k<=21, -38<=l<=38	
Reflections collected	43185	
Independent reflections	6612 [R(int) = 0.0465]	
Completeness to theta = 25.000°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7454 and 0.7122	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	6612 / 0 / 490	
Goodness-of-fit on F2	1.049	
Final R indices [I>2sigma(I)]	R1 = 0.0633, wR2 = 0.1555	
R indices (all data)	R1 = 0.0783, wR2 = 0.1650	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.732 and -0.623 e.Å^{-3}	

Supplementary Table 13. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 103) for CCDC 949270. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
F(1)	7786(3)	2917(1)	120(1)	39(1)
F(2)	4702(3)	3313(1)	-145(1)	31(1)
F(3)	5226(3)	3274(1)	553(1)	38(1)
O(1)	5829(4)	1723(1)	549(1)	31(1)
O(2)	5330(3)	1768(1)	-203(1)	19(1)
O(3)	-362(3)	1056(1)	321(1)	27(1)
C(1)	5593(5)	2907(2)	177(1)	26(1)
C(2)	4729(5)	2097(2)	197(1)	26(1)
C(3)	2324(5)	2045(2)	275(1)	26(1)
C(4)	1405(5)	1261(2)	174(1)	22(1)
C(5)	2638(5)	800(2)	-123(1)	21(1)
C(6)	4462(5)	1068(2)	-310(1)	19(1)
C(7)	5748(5)	680(2)	-646(1)	18(1)
C(8)	7759(5)	990(2)	-770(1)	21(1)
C(9)	9040(5)	613(2)	-1068(1)	23(1)
C(10)	8360(5)	-74(2)	-1241(1)	28(1)
C(11)	6351(6)	-380(2)	-1128(1)	29(1)
C(12)	5044(5)	-1(2)	-832(1)	24(1)
F(1")	2058(4)	4496(1)	278(1)	39(1)
F(2")	-808(3)	4902(1)	590(1)	39(1)
F(3")	1854(4)	5668(1)	472(1)	45(1)
O(1")	1386(4)	5268(1)	1346(1)	36(1)
O(2")	1715(3)	4001(1)	1114(1)	18(1)
O(3")	7557(3)	4715(1)	1637(1)	31(1)
C(1")	1381(5)	4952(2)	589(1)	26(1)
C(2")	2385(6)	4767(2)	1036(1)	31(1)
C(3")	4767(5)	4888(2)	1080(1)	27(1)
C(4")	5757(5)	4509(2)	1480(1)	24(1)
C(5")	4561(5)	3875(2)	1653(1)	23(1)
C(6")	2672(5)	3627(2)	1461(1)	18(1)
C(7")	1397(5)	2949(2)	1584(1)	17(1)
C(8")	-650(5)	2806(2)	1383(1)	22(1)
C(9")	-1884(5)	2180(2)	1500(1)	25(1)
C(10")	-1084(6)	1680(2)	1820(1)	26(1)
C(11")	948(6)	1812(2)	2018(1)	27(1)
C(12")	2201(5)	2436(2)	1903(1)	24(1)
F(1')	1510(3)	2310(1)	-839(1)	29(1)
F(2')	4402(3)	2760(1)	-1129(1)	32(1)
F(3')	1746(3)	3510(1)	-968(1)	34(1)

O(1')	2068(4)	3246(1)	-1868(1)	27(1)
O(2')	1814(3)	1953(1)	-1696(1)	17(1)
O(3')	-4024(3)	2788(1)	-2168(1)	26(1)
C(1')	2209(5)	2824(2)	-1126(1)	21(1)
C(2')	1169(5)	2710(2)	-1582(1)	22(1)
C(3')	-1265(5)	2815(2)	-1594(1)	22(1)
C(4')	-2263(5)	2531(2)	-2020(1)	21(1)
C(5')	-1117(5)	1918(2)	-2228(1)	20(1)
C(6')	776(5)	1631(2)	-2052(1)	17(1)
C(7')	1933(5)	949(2)	-2207(1)	18(1)
C(8')	4069(5)	781(2)	-2055(1)	21(1)
C(9')	5150(5)	141(2)	-2209(1)	27(1)
C(10')	4119(6)	-340(2)	-2506(1)	31(1)
C(11')	1986(6)	-190(2)	-2649(1)	32(1)
C(12')	899(5)	453(2)	-2506(1)	26(1)

Supplementary Table 14. Bond lengths [Å] and angles [°] for CCDC 949270.

F(1)-C(1)	1.350(4)
F(2)-C(1)	1.318(4)
F(3)-C(1)	1.342(4)
O(1)-H(1)	0.8400
O(1)-C(2)	1.412(4)
O(2)-C(2)	1.411(3)
O(2)-C(6)	1.371(3)
O(3)-C(4)	1.231(4)
C(2)-C(1)	1.513(5)
C(2)-C(3)	1.491(4)
C(3)-H(3A)	0.9900
C(3)-H(3B)	0.9900
C(4)-C(3)	1.511(5)
C(5)-C(4)	1.441(4)
C(5)-H(5)	0.9500
C(6)-C(5)	1.348(4)
C(7)-C(6)	1.475(4)
C(7)-C(12)	1.383(4)
C(8)-C(7)	1.400(4)
C(8)-H(8)	0.9500
C(9)-C(8)	1.385(4)
C(9)-H(9)	0.9500
C(10)-C(9)	1.372(5)
C(10)-H(10)	0.9500
C(10)-C(11)	1.387(5)
C(11)-H(11)	0.9500
C(11)-C(12)	1.391(4)
C(12)-H(12)	0.9500
F(1")-C(1")	1.317(4)
F(2")-C(1")	1.334(4)
F(3")-C(1")	1.337(4)
O(1")-H(1")	0.8400
O(1")-C(2")	1.437(4)
O(2")-C(2")	1.424(4)
O(2")-C(6")	1.365(3)
O(3")-C(4")	1.236(4)
C(1")-C(2")	1.519(4)
C(2")-C(3")	1.467(4)
C(3")-H(3"A)	0.9900
C(3")-H(3"B)	0.9900
C(4")-C(3")	1.503(5)
C(5")-C(4")	1.436(4)
C(5")-H(5")	0.9500
C(6")-C(5")	1.347(4)
C(6")-C(7")	1.472(4)
C(7")-C(8")	1.396(4)
C(7")-C(12")	1.405(4)
C(8")-H(8")	0.9500
C(9")-C(8")	1.382(4)
C(9")-H(9")	0.9500
C(10")-C(9")	1.390(5)
C(10")-H(10")	0.9500

C(11")-C(10")	1.380(5)
C(11")-H(11")	0.9500
C(12")-C(11")	1.382(5)
C(12")-H(12")	0.9500
F(1')-C(1')	1.336(3)
F(2')-C(1')	1.339(3)
F(3')-C(1')	1.330(3)
O(1')-H(1')	0.8400
O(1')-C(2')	1.405(4)
O(2')-C(2')	1.428(3)
O(2')-C(6')	1.366(3)
O(3')-C(4')	1.236(4)
C(1')-C(2')	1.526(4)
C(2')-C(3')	1.492(4)
C(3')-H(3'A)	0.9900
C(3')-H(3'B)	0.9900
C(4')-C(3')	1.506(4)
C(5')-C(4')	1.437(4)
C(5')-H(5')	0.9500
C(6')-C(5')	1.354(4)
C(6')-C(7')	1.470(4)
C(7')-C(8')	1.399(4)
C(7')-C(12')	1.399(4)
C(8')-H(8')	0.9500
C(8')-C(9')	1.389(4)
C(9')-H(9')	0.9500
C(9')-C(10')	1.376(5)
C(10')-H(10')	0.9500
C(10')-C(11')	1.382(5)
C(11')-H(11')	0.9500
C(12')-C(11')	1.382(5)
C(12')-H(12')	0.9500
C(2)-O(1)-H(1)	109.5
C(6)-O(2)-C(2)	117.7(2)
F(1)-C(1)-C(2)	111.3(3)
F(2)-C(1)-F(1)	106.4(3)
F(2)-C(1)-F(3)	107.9(3)
F(2)-C(1)-C(2)	113.5(3)
F(3)-C(1)-F(1)	107.0(2)
F(3)-C(1)-C(2)	110.4(3)
O(1)-C(2)-C(1)	107.9(3)
O(1)-C(2)-C(3)	107.2(3)
O(2)-C(2)-O(1)	110.1(2)
O(2)-C(2)-C(1)	104.4(2)

O(2)-C(2)-C(3)	113.1(3)
C(3)-C(2)-C(1)	114.0(3)
C(2)-C(3)-H(3A)	109.1
C(2)-C(3)-H(3B)	109.1
C(2)-C(3)-C(4)	112.4(3)
H(3A)-C(3)-H(3B)	107.8
C(4)-C(3)-H(3A)	109.1
C(4)-C(3)-H(3B)	109.1
O(3)-C(4)-C(3)	120.8(3)
O(3)-C(4)-C(5)	122.9(3)
C(5)-C(4)-C(3)	116.2(3)
C(4)-C(5)-H(5)	119.3
C(6)-C(5)-C(4)	121.5(3)
C(6)-C(5)-H(5)	119.3
O(2)-C(6)-C(7)	111.7(2)
C(5)-C(6)-O(2)	121.8(3)
C(5)-C(6)-C(7)	126.5(3)
C(8)-C(7)-C(6)	119.6(3)
C(12)-C(7)-C(6)	121.3(3)
C(12)-C(7)-C(8)	119.1(3)
C(7)-C(8)-H(8)	119.8
C(9)-C(8)-C(7)	120.3(3)
C(9)-C(8)-H(8)	119.8
C(8)-C(9)-H(9)	119.9
C(10)-C(9)-C(8)	120.2(3)
C(10)-C(9)-H(9)	119.9
C(9)-C(10)-H(10)	120.0
C(9)-C(10)-C(11)	120.0(3)
C(11)-C(10)-H(10)	120.0
C(10)-C(11)-H(11)	119.9
C(10)-C(11)-C(12)	120.2(3)
C(12)-C(11)-H(11)	119.9
C(7)-C(12)-C(11)	120.2(3)
C(7)-C(12)-H(12)	119.9
C(11)-C(12)-H(12)	119.9
C(2")-O(1")-H(1")	109.5
C(6")-O(2")-C(2")	117.6(2)
F(1")-C(1")-F(2")	107.2(3)
F(1")-C(1")-F(3")	107.5(3)
F(1")-C(1")-C(2")	113.4(3)
F(2")-C(1")-F(3")	106.5(3)
F(2")-C(1")-C(2")	111.1(3)
F(3")-C(1")-C(2")	110.9(3)
O(1")-C(2")-C(1")	107.3(3)

O(1")-C(2")-C(3")	106.7(3)
O(2")-C(2")-O(1")	109.7(2)
O(2")-C(2")-C(1")	103.9(2)
O(2")-C(2")-C(3")	114.0(3)
C(3")-C(2")-C(1")	115.0(3)
C(2")-C(3")-H(3"A)	109.1
C(2")-C(3")-H(3"B)	109.1
C(2")-C(3")-C(4")	112.5(3)
H(3"A)-C(3")-H(3"B)	107.8
C(4")-C(3")-H(3"A)	109.1
C(4")-C(3")-H(3"B)	109.1
O(3")-C(4")-C(3")	121.2(3)
O(3")-C(4")-C(5")	122.3(3)
C(5")-C(4")-C(3")	116.5(3)
C(4")-C(5")-H(5")	119.2
C(6")-C(5")-C(4")	121.5(3)
C(6")-C(5")-H(5")	119.2
O(2")-C(6")-C(7")	111.6(2)
C(5")-C(6")-O(2")	121.6(3)
C(5")-C(6")-C(7")	126.7(3)
C(8")-C(7")-C(6")	120.1(3)
C(8")-C(7")-C(12")	118.7(3)
C(12")-C(7")-C(6")	121.1(3)
C(7")-C(8")-H(8")	119.6
C(9")-C(8")-C(7")	120.7(3)
C(9")-C(8")-H(8")	119.6
C(8")-C(9")-H(9")	120.0
C(8")-C(9")-C(10")	120.1(3)
C(10")-C(9")-H(9")	120.0
C(9")-C(10")-H(10")	120.2
C(11")-C(10")-C(9")	119.6(3)
C(11")-C(10")-H(10")	120.2
C(10")-C(11")-H(11")	119.5
C(10")-C(11")-C(12")	121.0(3)
C(12")-C(11")-H(11")	119.5
C(7")-C(12")-H(12")	120.1
C(11")-C(12")-C(7")	119.8(3)
C(11")-C(12")-H(12")	120.1
C(2')-O(1')-H(1')	109.5
C(6')-O(2')-C(2')	117.1(2)
F(1')-C(1')-F(2')	106.5(2)
F(1')-C(1')-C(2')	112.5(2)
F(2')-C(1')-C(2')	111.7(2)
F(3')-C(1')-F(1')	107.1(2)
F(3')-C(1')-F(2')	107.4(2)
F(3')-C(1')-C(2')	111.4(2)
O(1')-C(2')-O(2')	110.7(2)
O(1')-C(2')-C(1')	108.8(2)
O(1')-C(2')-C(3')	107.7(2)
O(2')-C(2')-C(1')	103.6(2)
O(2')-C(2')-C(3')	112.8(2)
C(3')-C(2')-C(1')	113.1(2)
C(2')-C(3')-H(3'A)	109.5
C(2')-C(3')-H(3'B)	109.5
C(2')-C(3')-C(4')	110.9(2)
H(3'A)-C(3')-H(3'B)	108.0
C(4')-C(3')-H(3'A)	109.5
C(4')-C(3')-H(3'B)	109.5
O(3')-C(4')-C(3')	121.4(3)
O(3')-C(4')-C(5')	122.4(3)
C(5')-C(4')-C(3')	116.1(3)
C(4')-C(5')-H(5')	119.4
C(6')-C(5')-C(4')	121.3(3)
C(6')-C(5')-H(5')	119.4
O(2')-C(6')-C(7')	112.2(2)
C(5')-C(6')-O(2')	122.1(3)
C(5')-C(6')-C(7')	125.7(3)
C(8')-C(7')-C(6')	120.8(3)
C(12')-C(7')-C(6')	120.3(3)
C(12')-C(7')-C(8')	118.9(3)
C(7')-C(8')-H(8')	120.0
C(9')-C(8')-C(7')	120.1(3)
C(9')-C(8')-H(8')	120.0
C(8')-C(9')-H(9')	119.8
C(10')-C(9')-C(8')	120.4(3)
C(10')-C(9')-H(9')	119.8
C(9')-C(10')-H(10')	120.0
C(9')-C(10')-C(11')	119.9(3)
C(11')-C(10')-H(10')	120.0
C(10')-C(11')-H(11')	119.7
C(12')-C(11')-C(10')	120.5(3)
C(12')-C(11')-H(11')	119.7
C(7')-C(12')-H(12')	119.9
C(11')-C(12')-C(7')	120.2(3)
C(11')-C(12')-H(12')	119.9

Supplementary Table 15. Anisotropic displacement parameters (Å^{2}x 10^3) for CCDC 949270. The anisotropic displacement factor exponent takes the form: -2p2[h2 a*2U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
F(1)	24(1)	36(1)	58(1)	-12(1)	-1(1)	0(1)
F(2)	37(1)	22(1)	33(1)	5(1)	4(1)	2(1)
F(3)	46(1)	34(1)	33(1)	-18(1)	-4(1)	11(1)
O(1)	30(1)	36(1)	28(1)	0(1)	3(1)	4(1)
O(2)	21(1)	19(1)	17(1)	-3(1)	3(1)	-1(1)
O(3)	17(1)	38(1)	27(1)	4(1)	3(1)	2(1)
C(1)	23(2)	27(2)	29(2)	-8(1)	-3(1)	2(1)
C(2)	34(2)	22(2)	23(2)	-2(1)	8(1)	3(1)
C(3)	20(2)	37(2)	22(2)	-7(1)	2(1)	4(1)
C(4)	18(1)	30(2)	18(1)	3(1)	-1(1)	3(1)
C(5)	22(2)	22(2)	20(1)	1(1)	0(1)	0(1)
C(6)	21(1)	18(1)	17(1)	2(1)	-3(1)	2(1)
C(7)	19(1)	22(1)	14(1)	2(1)	0(1)	5(1)
C(8)	22(2)	21(2)	18(1)	0(1)	1(1)	1(1)
C(9)	23(2)	30(2)	17(1)	1(1)	4(1)	2(1)
C(10)	35(2)	28(2)	20(2)	-4(1)	6(1)	8(1)
C(11)	40(2)	22(2)	23(2)	-6(1)	3(1)	0(1)
C(12)	26(2)	26(2)	21(1)	1(1)	1(1)	-2(1)
F(1")	53(1)	48(1)	17(1)	1(1)	1(1)	17(1)
F(2")	33(1)	44(1)	41(1)	17(1)	5(1)	8(1)
F(3")	57(1)	34(1)	43(1)	17(1)	2(1)	-12(1)
O(1")	35(1)	34(1)	39(1)	-4(1)	2(1)	0(1)
O(2")	19(1)	19(1)	17(1)	0(1)	-1(1)	-3(1)
O(3")	19(1)	44(1)	30(1)	-11(1)	0(1)	-8(1)
C(1")	28(2)	23(2)	26(2)	6(1)	4(1)	-2(1)
C(2")	39(2)	33(2)	19(2)	6(1)	-2(1)	-16(2)
C(3")	20(2)	22(2)	37(2)	-6(1)	2(1)	-1(1)
C(4")	20(2)	27(2)	26(2)	-12(1)	4(1)	-2(1)
C(5")	21(2)	30(2)	18(1)	-4(1)	1(1)	1(1)
C(6")	19(1)	23(2)	13(1)	-5(1)	3(1)	2(1)
C(7")	21(1)	20(1)	12(1)	-3(1)	2(1)	1(1)
C(8")	24(2)	24(2)	18(1)	2(1)	0(1)	-1(1)
C(9")	25(2)	23(2)	27(2)	-2(1)	2(1)	-4(1)
C(10")	37(2)	17(1)	26(2)	-2(1)	10(1)	-2(1)
C(11")	40(2)	22(2)	20(2)	2(1)	4(1)	5(1)
C(12")	27(2)	26(2)	18(1)	-1(1)	-1(1)	5(1)
F(1')	35(1)	28(1)	22(1)	4(1)	0(1)	-6(1)
F(2')	21(1)	45(1)	30(1)	-6(1)	1(1)	-3(1)

F(3')	46(1)	24(1)	33(1)	-8(1)	-3(1)	5(1)
O(1')	23(1)	24(1)	34(1)	6(1)	4(1)	0(1)
O(2')	17(1)	14(1)	20(1)	0(1)	0(1)	2(1)
O(3')	17(1)	31(1)	31(1)	9(1)	2(1)	4(1)
C(1')	21(2)	19(1)	24(2)	-1(1)	2(1)	0(1)
C(2')	25(2)	18(1)	22(1)	-1(1)	0(1)	6(1)
C(3')	17(1)	17(1)	32(2)	-1(1)	2(1)	1(1)
C(4')	17(1)	20(2)	26(2)	9(1)	4(1)	-3(1)
C(5')	18(1)	23(2)	20(1)	2(1)	1(1)	-2(1)
C(6')	18(1)	17(1)	17(1)	2(1)	3(1)	-2(1)
C(7')	21(1)	16(1)	18(1)	2(1)	5(1)	-2(1)
C(8')	23(2)	18(1)	24(2)	1(1)	3(1)	0(1)
C(9')	29(2)	22(2)	31(2)	5(1)	7(1)	4(1)
C(10')	44(2)	18(2)	32(2)	-1(1)	15(2)	4(1)
C(11')	44(2)	25(2)	27(2)	-8(1)	5(1)	-7(2)
C(12')	26(2)	26(2)	24(2)	-4(1)	2(1)	-4(1)

Supplementary Table 16. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for CCDC 949270.

	x	y	z	U(eq)
H(1)	7118	1616	478	46
H(3A)	1539	2428	90	31
H(3B)	2056	2171	584	31
H(5)	2153	295	-188	25
H(8)	8246	1463	-648	25
H(9)	10394	830	-1153	28
H(10)	9265	-339	-1438	33
H(11)	5866	-849	-1253	34
H(12)	3663	-210	-757	29
H(1")	109	5114	1395	54
H(3"A)	5069	5443	1095	32
H(3"B)	5478	4684	818	32
H(5")	5115	3624	1909	27
H(8")	-1202	3143	1162	26
H(9")	-3280	2091	1363	30
H(10")	-1932	1250	1902	32
H(11")	1494	1470	2236	33
H(12")	3603	2517	2040	28
H(1')	3387	3134	-1911	41
H(3'A)	-1615	3364	-1558	26

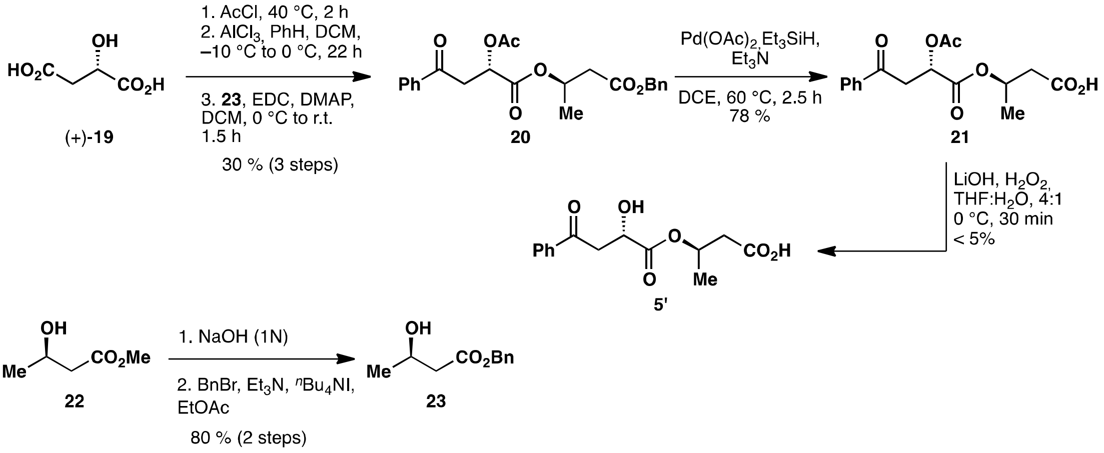
H(3'B)	-1911	2532	-1349	26
H(5')	-1699	1711	-2494	24
H(8')	4780	1105	-1845	26
H(9')	6610	34	-2109	33
H(10')	4873	-774	-2613	37
H(11')	1262	-531	-2847	38
H(12')	-554	558	-2611	31

Supplementary Table 17. Hydrogen bonds for CCDC 949270 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(3)#1	0.84	1.89	2.703(3)	161.6
O(1")-H(1")...O(3")#2	0.84	1.87	2.698(3)	166.3
O(1')-H(1')...O(3')#1	0.84	1.88	2.695(3)	163.0

Symmetry transformations used to generate equivalent atoms:
#1 x+1,y,z   #2 x-1,y,z

Synthesis of product 5′ and common intermediates 24 and 25:



23 was prepared in two steps and 80 % yield according to literature precedent: Griesbeck, A. & Seebach, D. Einfache Umwandlung von(–)-(R)-3-Hydroxybuttersäure in das (+)-(S)-Enantiomere und dessen Lacton(–)-(S)-4-Methyloxetan-2-on. *Helv. Chim. Acta* **70,** 1320-1325, (1987); Martin, O. R. *et al.* Synthesis and Immunobiological Activity of an Original Series of Acyclic Lipid A Mimics Based on a Pseudodipeptide Backbone. *J. Med. Chem.* **49,** 6000-6014, (2006).

The spectroscopic data for this compound were identical to those reported in the literature. [Shiomi, T., Adachi, T., Toribatake, K., Zhou, L. & Nishiyama, H. Asymmetric [β]-boration of [α], [β]-unsaturated carbonyl compounds promoted by chiral rhodium-bisoxazolinylphenyl catalysts. *Chem. Commun.* 5987-5989, (2009).]

^{1}H NMR (600 MHz, $CDCl_3$): d 7.33-7.40 (m, 5H), 5.16 (s, 2H), 4.22 (m, 1H), 2.92 (broad, 1H), 2.44-2.59 (m, 2H), 1.24 (d, *J* = 6.3 Hz, 3H) ppm.
^{13}C NMR (151 MHz, $CDCl_3$) δ 172.3, 135.7, 128.8, 128.6, 128.4, 66.7. 64.4, 42.9, 22.6. ppm.

20: L-Malic acid ((+)-**19**) (1.08 g, 1 equiv.) and acetyl chloride (5mL) were stirred at 40 °C for 2 h. Acetyl chloride was removed *in vacuo* and the residues were dissolved in DCM (15 mL). Benzene (0.78 g, 0. 89 mL, 1 equiv.) was added and the mixture was cooled to –10 °C. $AlCl_3$ (2.66 g, 2 equiv.) was slowly transferred to the reaction flask with 25 mL of DCM. The mixture was stirred at 4 °C for 22 h and then quenched at 0 °C with HCl (6N, 15 mL). The phases were separated and the aqueous phase was extracted twice with DCM (2 × 30 mL). The combined organic layers were diluted with hexanes (100 mL) and dried over Na_2SO_4. Evaporation *in vacuo* resulted in a yellow solid (ca. 1.38 g) that was dried under high vacuum overnight. 10 mg of this solid (1.1 equiv.), DMAP (0.5 mg, 0.1 equiv.) and **23** (7.0 mg, 1 equiv.) were dissolved in DCM (0.5 mL) and the mixture was cooled to 0 °C. EDC (7.0 mg, 1.2 equiv.) was added in one portion. The mixture was stirred at 0 °C for 5 min, then at room temperature for 1.5 h. The reaction was quenched with HCl (1N, 1 mL) and DCM (5 mL) was added. The phases were separated and the aqueous phase was extracted twice with DCM (2 × 5 mL). The combined organic layers were diluted with hexanes (15 mL) and dried over Na_2SO_4. Evaporation *in vacuo* resulted in a yellow oil (ca. 15 mg). Quick purification by preparative TLC (4:6 EtOAc:hexanes, eluted once) afforded almost pure **20** (8.0 mg, 30 %) as a colorless oil. The minor impurity resulted from elimination of the OAc group on the silica gel during the purification and could not be totally removed.

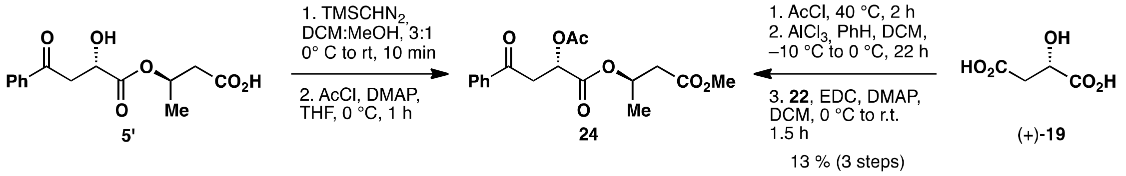
TLC: R_f = 0.37–0.46 (3:7 EtOAc:hexanes, UV active, stains blue upon *p*-anisaldehyde staining).
^{1}H NMR (600 MHz, CD_3CN): δ 7.93 (d, *J* = 7.1 Hz, 2H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.36 – 7.31 (m, 5H), 5.61 (dd, *J* = 7.9, 3.7 Hz, 1H), 5.38 (q, *J*=6.5 Hz, 1H), 5.16 – 5.12 (m, 2H), 3.54 – 3.40 (m, 2H), 2.76 – 2.57 (m, 2H), 2.08 (s, 3H), 1.31 (d, *J* = 6.3 Hz, 3H) ppm.
^{13}C-NMR (151 MHz, CD_3CN) δ 194.9, 170.1, 169.9, 169.1, 136.3, 135.8, 133.8, 128.9, 128.7, 128.5, 128.4, 128.3, 69.1, 68.1, 66.7, 40.8, 39.6, 20.7, 19.8 ppm.
IR (neat): $\tilde{\nu}$ = 3356, 2921, 2851, 1740, 1689, 1597, 1449, 1375, 1261, 1212, 1182, 1052, 799, 754, 690 cm^{-1}.
HRMS (ESI-TOF):): calc'd for $C_{23}H_{24}O_7$ $[M+H]^+$ 413.1595, found 413.1591.

21: $Pd(OAc)_2$ (18 mg, 0.5 equiv.) was dissolved in DCE (3 mL), Et_3SiH (37 mg, 50 µL, 2 equiv.) and Et_3N (26 mg, 36 µL, 1.6 equiv.) were added and the black mixture was stirred at room temperature under argon for 15 min. A portion (0.3 mL) of the mixture was transferred into a flask containing **20** (6 mg, 1 equiv.) under argon and the reaction was heated at 60 °C for 2.5 h. The mixture was cooled to room temperature and filtered over a small celite plug (ca. 3 cm high in a pipette, eluted with EtOAc). HCl (3N, 0.5 mL) and EtOAc (2 mL) were added and the phases were separated and the aqueous phase was extracted twice with EtOAc (2 × 5 mL). The combined organic layers were diluted with hexanes (10 mL) and dried over Na_2SO_4. Evaporation *in vacuo* resulted in a yellow oil (ca. 8 mg). The residues were dissolved in $MeCN:MeOH:H_2O$ (1:1:2, 0.8 mL), microfiltered and purified via semi-preparative HPLC reverse-phase HPLC (10–40 % $MeCN:H_2O$–0.07 % TFA gradient over 20 min then 40 % $MeCN:H_2O$–0.07 % TFA isocratic) (retention time 30 min). **21** was obtained as an amorphous white solid (4.0 mg, 78 %).

^{1}H NMR (600 MHz, CD_3CN): δ 7.97– 7.94 (m, 2H), 7.61 – 7.60 (m, 1H), 7.50 – 7.48 (m, 2H), 5.61 – 5.59 (m, 1H), 5.40 – 5.38 (m, 1H), 3.61 – 3.43 (m, 2H), 2.72 – 2.59 (m, 2H), 2.10 (s, 3H), 1.35 – 1.33 (m, 3H) ppm.
^{13}C-NMR (151 MHz, CD_3CN) δ 195.0, 173.3, 170.7, 169.2, 136.3, 133.9, 128.9, 128.3, 68.8, 68.3, 40.2, 39.6, 20.7, 19.7 ppm.
IR (neat): $\tilde{\nu}$ = 2924, 2854, 1740, 1689, 1597, 1449, 1374, 1287, 1212, 1140, 1050, 1002, 757, 690 cm^{-1}.
HRMS (ESI-TOF):): calc'd for $C_{16}H_{18}O_7$ $[M+H]^+$ 323.1125, found 323.1122.

5': **21** (0.5 mg, 1 equiv.) was dissolved in THF (0.1 mL) and cooled to 0 °C. $LiOH\cdot H_2O$ (10.7 mg, 144 equiv.) was dissolved in H_2O (2 mL) and H_2O_2 (35 %, 28 µL, 176 equiv.) was added at 0 °C. An aliquot of the stock solution (25 µL) was added to the solution of **21** in THF at 0 °C and the mixture was stirred for 30 min. Then, at 0 ºC a solution of Na_2SO_3 (0.15 M, 0.1 mL, 8.3 equiv.) was added to quench the excess peroxide. After stirring at 0 ºC for 15 min, the mixture was buffered to pH 9-10 with sat. $NaHCO_3$ and the THF was evaporated under reduced pressure. The aqueous phase was acidified to pH 1-2 with HCl (3N) and the product was extracted with EtOAc (3 x 4 mL). The combined organic layers were diluted with hexanes (10 mL) and dried over Na_2SO_4. Evaporation *in vacuo* afforded a yellow oil. Analysis of the crude sample by LCMS proved the presence of **5'** (same retention time and mass spectrum as the sample synthesized by EncM) albeit in very low yield (< 5 %).

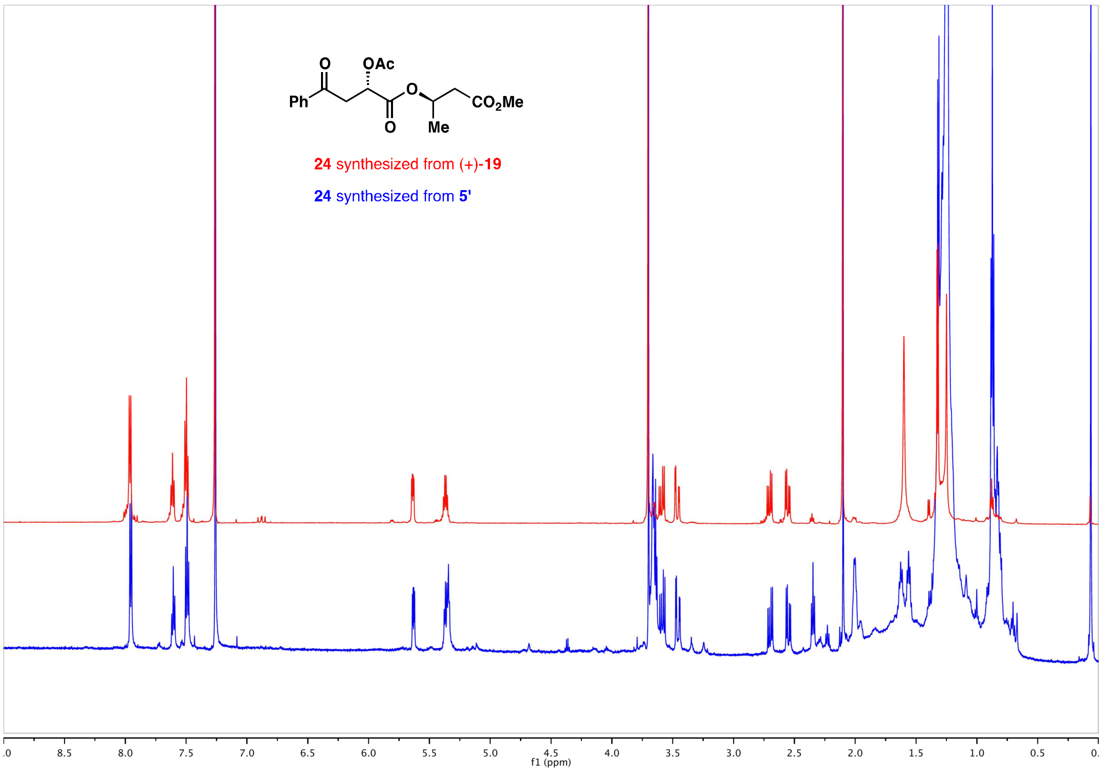
The very low yield of the last step rendered isolation of pure synthetic **5'** difficult. Since none of the conditions tried gave a better result, **5'** synthesized by EncM was converted in two steps to **24** to be compared with a synthetic sample of **24** derived from L-malic acid.



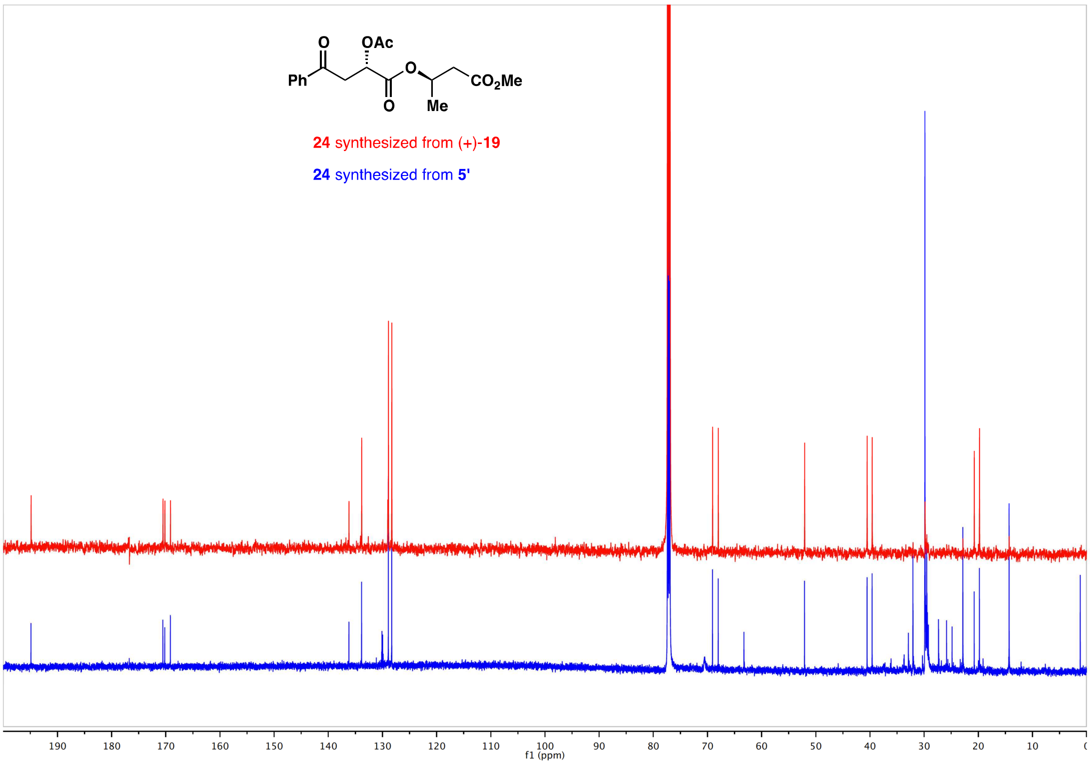
24 from (+)-**19**: **24** was made from L-malic ((+)-**19**) acid using the same sequence as **20**, except that **22** was used instead of **23** in the third step. **24** was obtained as a colorless oil (3.0 mg, 13 %) after quick purification by preparative TLC (4:6 EtOAc:hexanes, eluted once).

TLC: R_f = 0.27–0.37 (3:7 EtOAc:hexanes, UV active, stains yellow upon *p*-anisaldehyde staining).
^{1}H NMR (600 MHz, CD_3CN): δ 7.96 (d, *J* = 7.4 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.49 (t, *J* = 7.7 Hz, 2H), 5.63 (dd, *J* = 8.0, 3.6 Hz, 1H), 5.36 (q, *J*=6.5 Hz, 1H), 3.70 (s, 3H), 3.61 – 3.44 (m, 2H), 2.72 – 2.53 (m, 2H), 2.10 (s, 3H), 1.31 (d, *J* = 6.3 Hz, 3H) ppm.
^{13}C-NMR (151 MHz, CD_3CN) δ 194.9, 170.5, 170.2, 169.1, 136.2, 133.8, 128.9, 128.3, 69.1, 68.0, 52.1, 40.5, 39.6, 20.7, 19.8 ppm.
IR (neat): $\tilde{\nu}$ = 2923, 2852, 1740, 1690, 1597, 1449, 1373, 1286, 1211, 1102, 1054, 1002, 757, 690 cm^{-1}.
HRMS (ESI-TOF):): calc'd for $C_{17}H_{20}O_7$ $[M+H]^+$ 337.1287, found 337.1289.

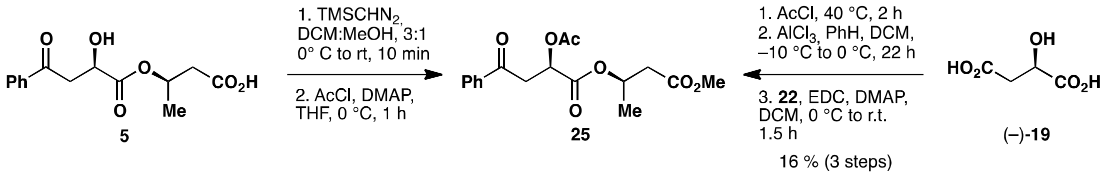
24 from **5'**: **5'** (0.4 mg, 1 equiv.) was dissolved in a mixture of DCM (0.3 mL) and MeOH (0.1 mL) and cooled to 0 °C. Trimethylsilyldiazomethane (7 μL, C = 2.0 M in Et_2O, 10 equiv.) was added and the mixture was warmed to room temperature for 10 min. Then, the solvents and the trimethylsilyldiazomethane were blown away under nitrogen. DCM (0.1 mL) and DMAP (1 crystal, ca. 0.3 mg) were added and the solution was cooled to 0 °C. Acetyl chloride (11 mg, 10 μL, 100 equiv.) was dissolved in DCM (5 mL) and an aliquot (0.05 mL) of the solution was added dropwise to the reaction. The mixture was stirred at 0 °C for 1 h and quenched with HCl (3N, 0.3 mL). EtOAc (2 mL) was added, the phases were separated, and the aqueous phase was extracted once with EtOAc (2 mL) and twice with DCM (2 x 2 mL). The combined organic layers were diluted with hexanes (5 mL) and dried over Na_2SO_4. Evaporation *in vacuo* resulted in a yellow oil of almost pure **24** (*vide infra*).



Supplementary Figure 20. ^{1}H NMR spectra of **24** synthesized from (+)-**19** and **5'**.



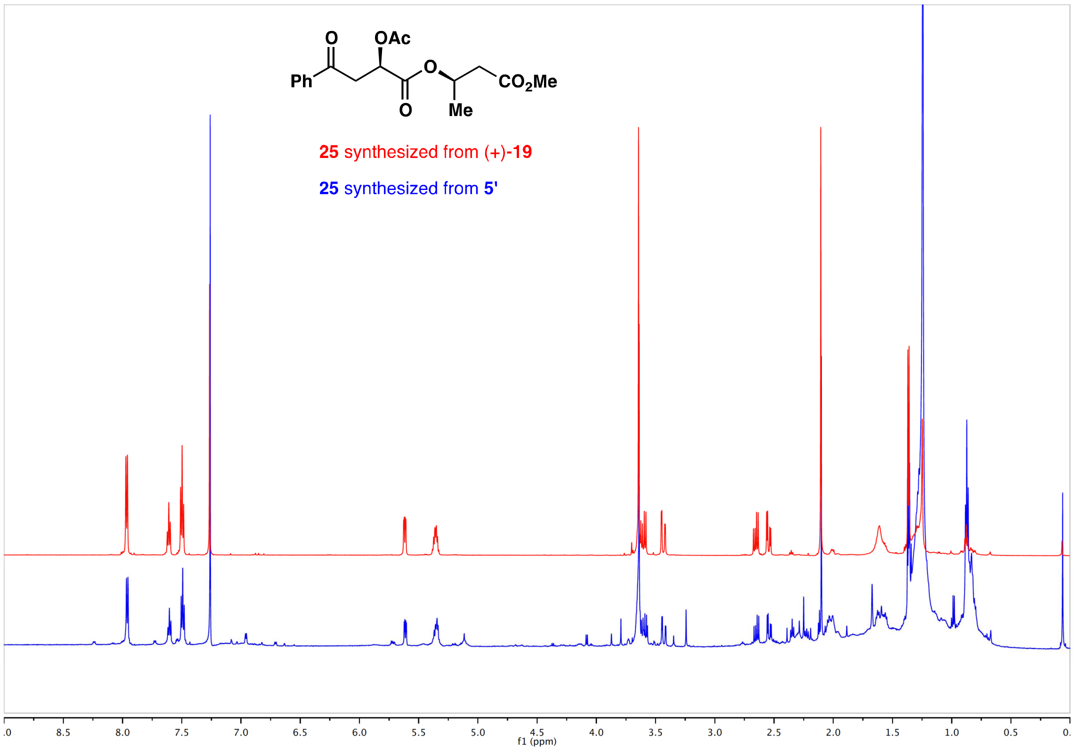
Supplementary Figure 21. ^{13}C NMR spectra of **24** synthesized from (+)-**19** and **5'**.



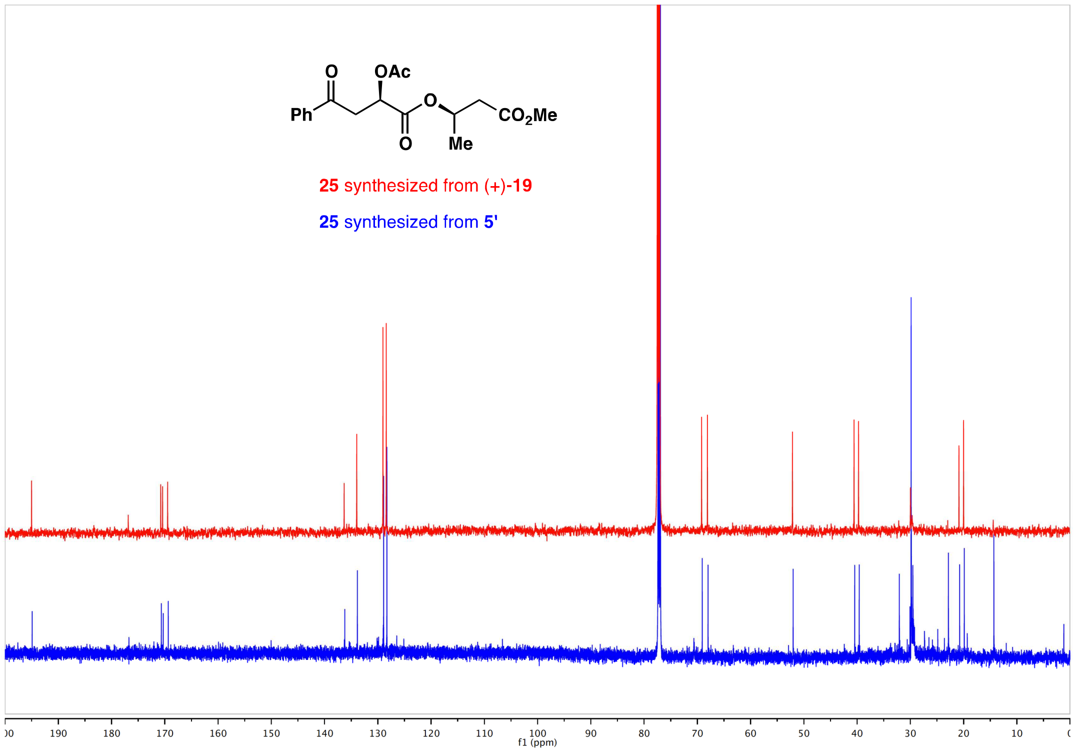
25 from (–)-**19**: **25** was made from L-malic ((–)-**19**) acid using the same sequence as **24**, except that (–)-**19** was used instead of (+)-**19** in the first step. **25** was obtained as a colorless oil (5.0 mg, 16 %) after quick purification by preparative TLC (4:6 EtOAc:hexanes, eluted once).

TLC: R_f = 0.27–0.37 (3:7 EtOAc:hexanes, UV active, stains yellow upon *p*-anisaldehyde staining).
^{1}H NMR (600 MHz, CD_3CN): δ 7.96 (d, *J* = 7.4 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.49 (t, *J* = 7.7 Hz, 2H), 5.61 (dd, *J* = 8.0, 3.6 Hz, 1H), 5.35 (q, *J*=6.5 Hz, 1H), 3.64 (s, 3H), 3.62 – 3.41 (m, 2H), 2.67 – 2.52 (m, 2H), 2.10 (s, 3H), 1.36 (d, *J* = 6.3 Hz, 3H) ppm.
^{13}C-NMR (151 MHz, CD_3CN) δ 194.9, 170.6, 170.3, 169.3, 136.2, 133.8, 128.9, 128.3, 69.1, 68.0, 52.0, 40.4, 39.6, 20.7, 19.9 ppm.
IR (neat): $\tilde{\nu}$ = 2924, 2852, 1738, 1690, 1597, 1449, 1373, 1282, 1212, 1202, 1054, 757, 690 cm^{-1}.
HRMS (ESI-TOF):): calc'd for $C_{17}H_{20}O_7$ $[M+H]^+$ 337.1287, found 337.1289.

25 from **5**: **25** was obtained using the same two steps sequence as **24** using **5** instead of **5'** (*vide infra*).



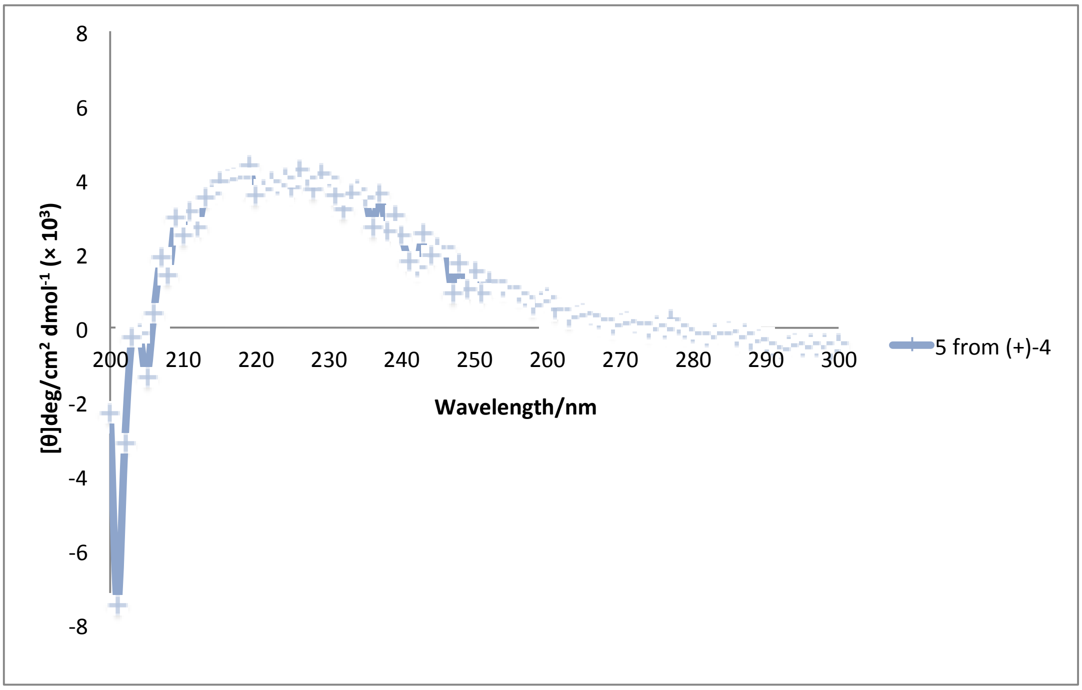
Supplementary Figure 22. ^{1}H NMR spectra of **25** synthesized from (–)-**19** and **5**.



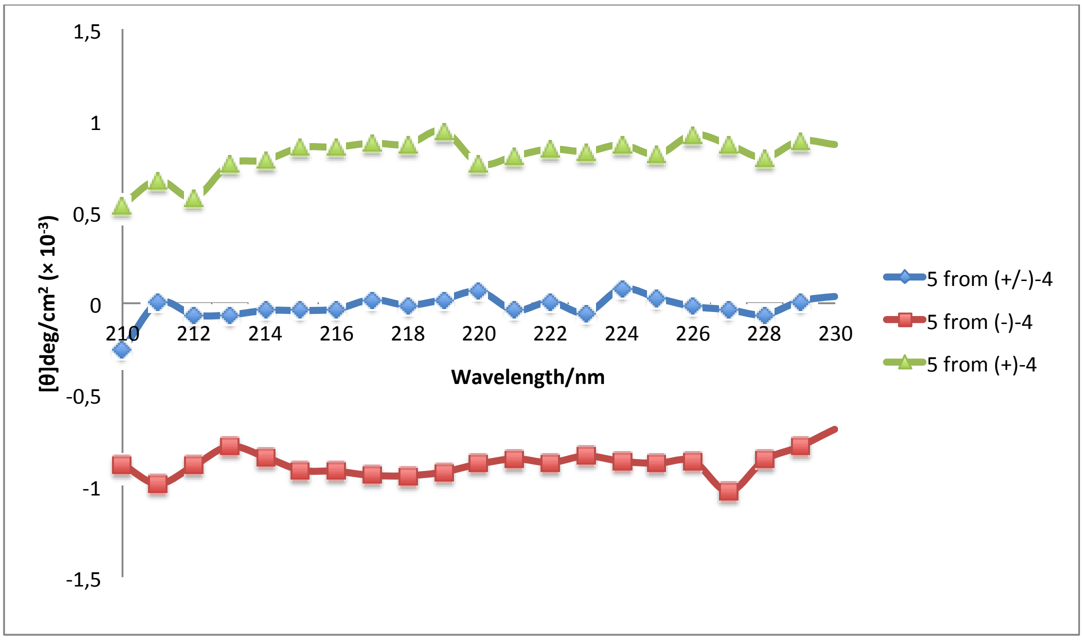
Supplementary Figure 23. ^{13}C NMR spectra of **25** synthesized from (–)-**19** and **5**.

Circular dichroism experiments with racemic and enantiopure 5 and 5’.

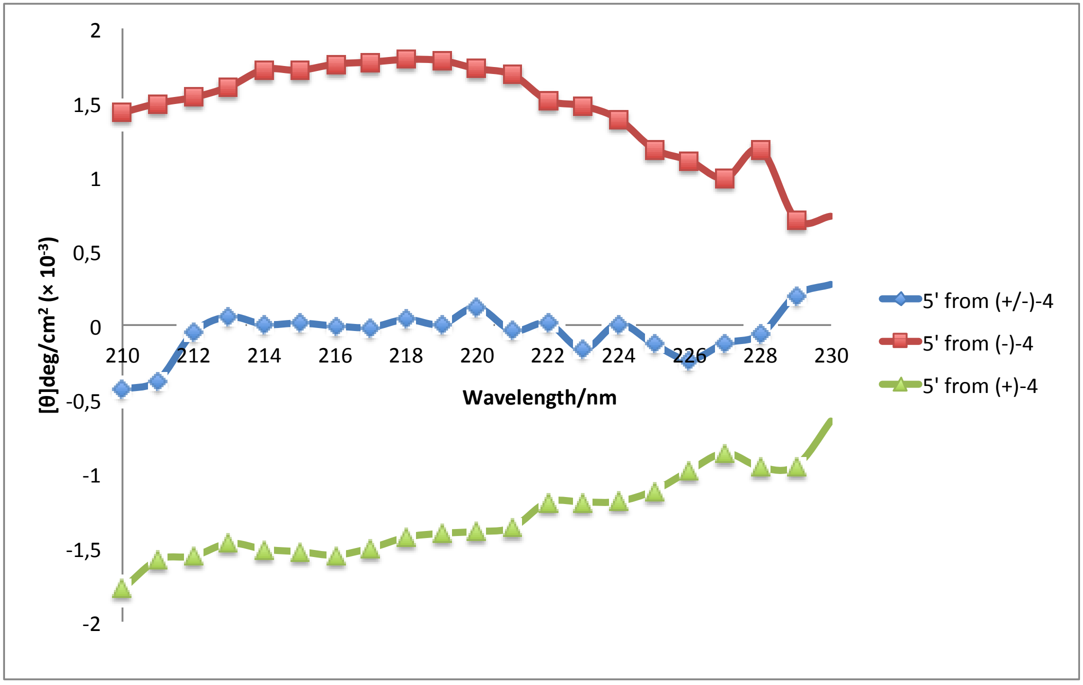
Circular dichroism spectroscopy (CD) measurements were recorded with wavelengths between 200 and 300 nm using 0.3 to 0.6 mg of **5** and **5’** in 0.3 mL of HPLC grade MeCN. A blank experiment using only MeCN was performed first, and two scans were realized for every sample. An example is shown in Supplementary Fig. 24. Over 250 nm, the ellipticity of all the products was quasi null. Moreover, for some samples, the [200-210] and [230-250] regions presented some measurement values with too much uncertainty, and, consequently, these regions were disregarded. The normalized circular dichroism spectra for diastereoisomers **5** and **5’**, enzymatically synthesized from racemic and enantiopure **4**, are shown in Supplementary Fig. 25 and Supplementary Fig. 26, respectively. The comparison of the CD spectra for **5** and **5’** proves that the chirality of the substrates is maintained during the transformation by EncM.



Supplementary Figure 24. Circular dichroism spectrum for **5,** enzymatically prepared from (+)-**4**.

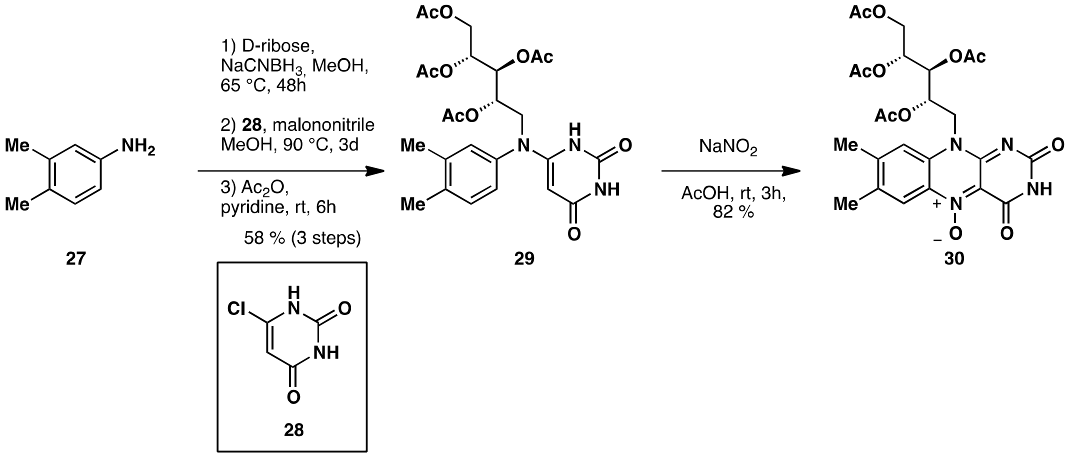


Supplementary Figure 25. Normalized circular dichroism spectrum for **5,** enzymatically prepared from (+/–), (–) and (+)-**4**.



Supplementary Figure 26. Normalized circular dichroism spectrum for **5’,** enzymatically prepared from (+/–), (–) and (+)-**4**.

Synthesis of flavin-N5-oxide 30:



29: In a flame-dried flask, aniline **27** (95 mg, 1.0 equiv.), D-ribose (353 mg, 3.0 equiv.), and sodium cyanoborohydride (99 mg, 2.0 equiv.) were dissolved in methanol (5 mL), and the mixture was heated to 65 °C for 48 h. Solvent was removed under reduced pressure, then sat. $NaHCO_3$ (2 mL) was added before the mixture was extracted with EtOAc (6 × 5 mL). The combined organic layers were washed with brine and dried with $MgSO_4$, and the solvent was removed under reduced pressure to yield an off-white solid (180 mg). **27** (125 mg, 1.2 equiv.) and malononitrile (40 mg, 0.9 equiv.) were added and the flask was dried under vacuum. Under argon atmosphere, dry MeOH (5mL) was added and the mixture was refluxed for 3 days. The reaction was cooled to r.t. and MeOH was concentrated *in vacuo*. The residues were dissolved in pyridine (4 mL) and acetic anhydride (0.43 g, 0.4 mL, 6 equiv.). The resulting mixture was allowed to stir at r.t. for 6 h. The solvent was then removed under reduced pressure with toluene, and the residues were dissolved in DCM (50 mL) and washed with water (3 × 20 mL) and brine (20 mL). The organic extracts were dried with $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Column chromatography (0:10 to 0.5:9.5 MeOH/DCM) provided a yellow solid (242 mg, 58 %).

The spectroscopic data for this compound were identical to those reported in the literature. [Mansurova, M., Koay, M. S. & Gärtner, W. Synthesis and Electrochemical Properties of Structurally Modified Flavin Compounds. *Eur. J. Org. Chem.* **2008,** 5401-5406, (2008).

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.88 (br, 1H), 7.40 (br, 1H), 7.24 – 7.22 (m, 1H), 6.95 – 6.91 (m, 2H), 5.36 – 5.32 (m, 1H), 5.27 – 5.24 (m, 1H), 5.13 – 5.09 (m, 1H), 4.97 (s, 1H), 4.27 (dd, *J* = 12.3, 3.0 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.73 (dd, *J* = 15.3, 2.7 Hz, 1H), 2.29 (s, 3H), 2.28 (s, 3H), 2.11 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.89 (s, 3H) ppm.

^{13}C NMR (151 MHz, $CDCl_3$): δ 170.7, 170.1, 169.7, 169.6, 164.7, 153.6, 150.2, 140.1, 138.8, 136.7, 132.1, 129.1, 125.5, 77.0, 70.2, 69.5, 69.4, 61.9, 52.0, 21.1, 20.9, 20.7, 20.7, 20.0, 19.6 ppm.

30: $NaNO_2$ (8 mg, 5.0 equiv.) was added to a solution of **29** (13 mg, 1.0 equiv.) in acetic acid (0.2 mL) in the dark. The mixture was stirred at r.t. for 3 h, and then water (0.15 mL) was added. The suspension was quenched for an additional 3 h before the solvents were removed under reduced pressure. The crude orange solid was washed with water (0.4 mL), and dried. **30** was obtained as an amorphous orange solid (11.0 mg, 82 %) after purification by preparative TLC (0.5:9.5 MeOH:DCM, eluted once).

Melting point: 224–225 °C (decomposition).
TLC: R_f = 0.11–0.14 (0.5:9.5 MeOH:DCM, stains orange).
^{1}H NMR (600 MHz, CD_3OD): δ 8.20 (br, 1H), 7.57 (br, 1H), 5.66 (br, 1H), 5.53 – 5.53 (m, 1H), 5.43 – 5.42 (m, 2H), 4.51 – 4.49 (m, 1H), 4.26 (dd, J = 12.3, 6.2 Hz, 1H), 3.65 – 3.64 (m, 2H), 2.57 (s, 3H), 2.46 (s, 3H), 2.20 (s, 3H), 2.19 (s, 3H), 2.03 (s, 3H), 1.75 (br, 3H) ppm.
^{13}C NMR (151 MHz, CD_3OD): δ 172.2, 171.8, 171.7, 171.5, 158.9, 157.3, 155.60 149.1, 138.6, 134.6, 125.9, 121.7, 118.7, 71.7, 71.6, 70.8, 63.0, 45.9, 21.0, 20.9, 20.7, 20.6, 20.4, 19.5 ppm.
IR (neat): $\tilde{\nu}$ = 2925, 2853, 2360, 2340, 1742, 1703, 1656, 1404, 1371, 1217, 1042, 780, 619, 454 cm^{-1}.
HRMS (ESI-TOF): calc’d for $C_{25}H_{28}N_4O_{11}$ [M + H^+] 561.1833, found 561.1840.

Flavin-mediated dual oxidation controls an enzymatic Favorskii-type rearrangement

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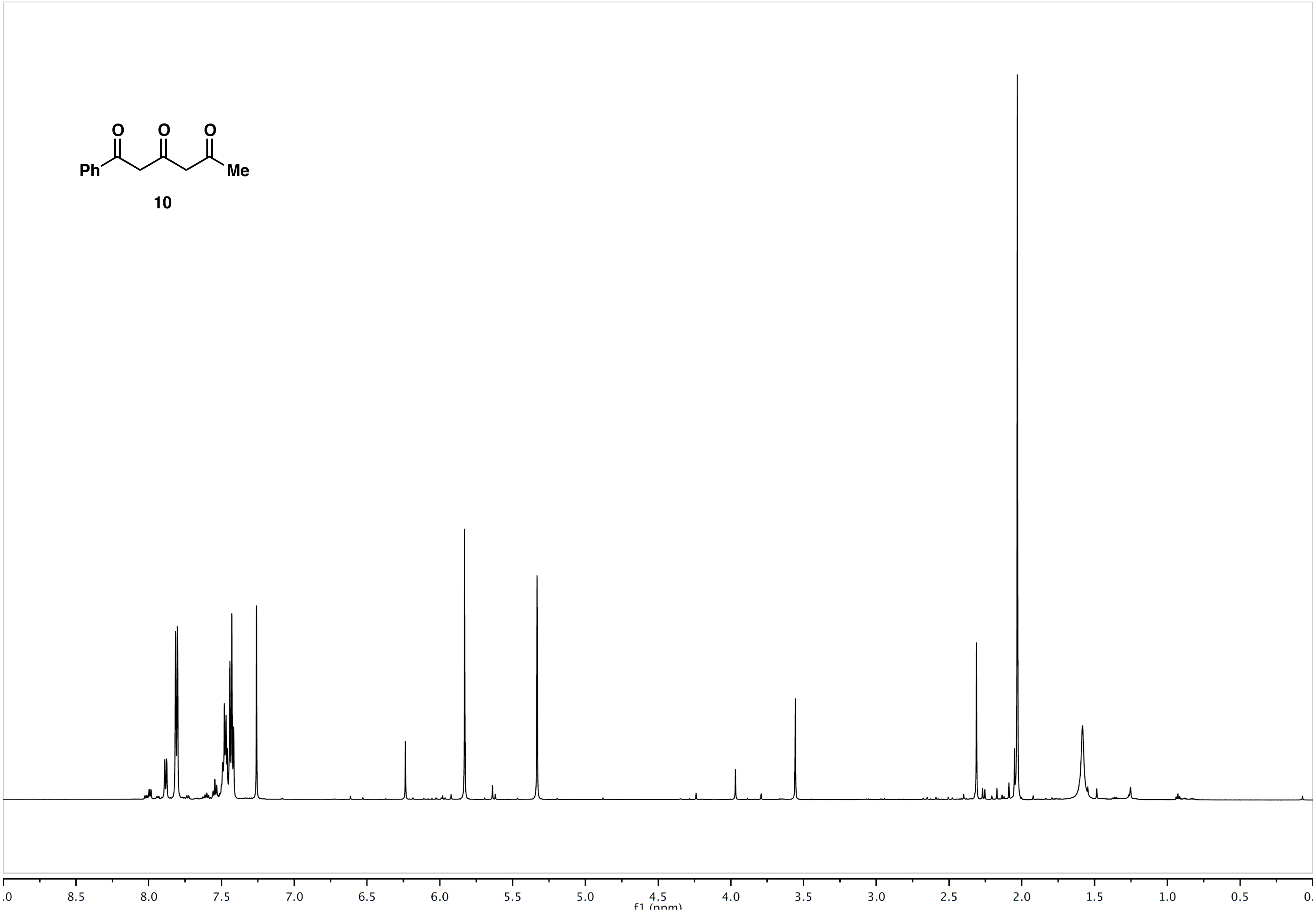
**These authors contributed equally to the work*

SUPPLEMENTARY INFORMATION

Part 2: NMR Spectra of Synthesized Compounds

Table of Contents



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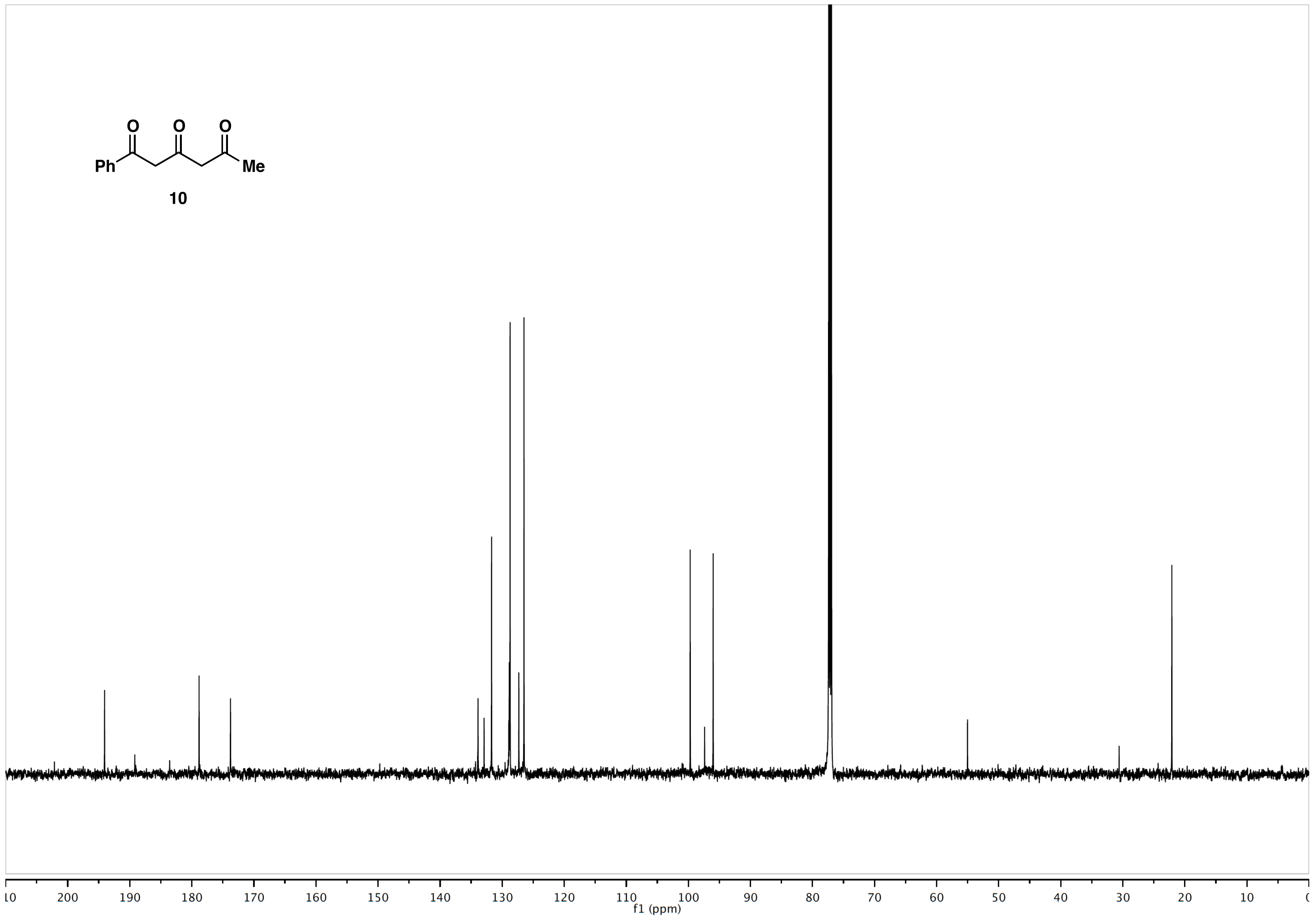
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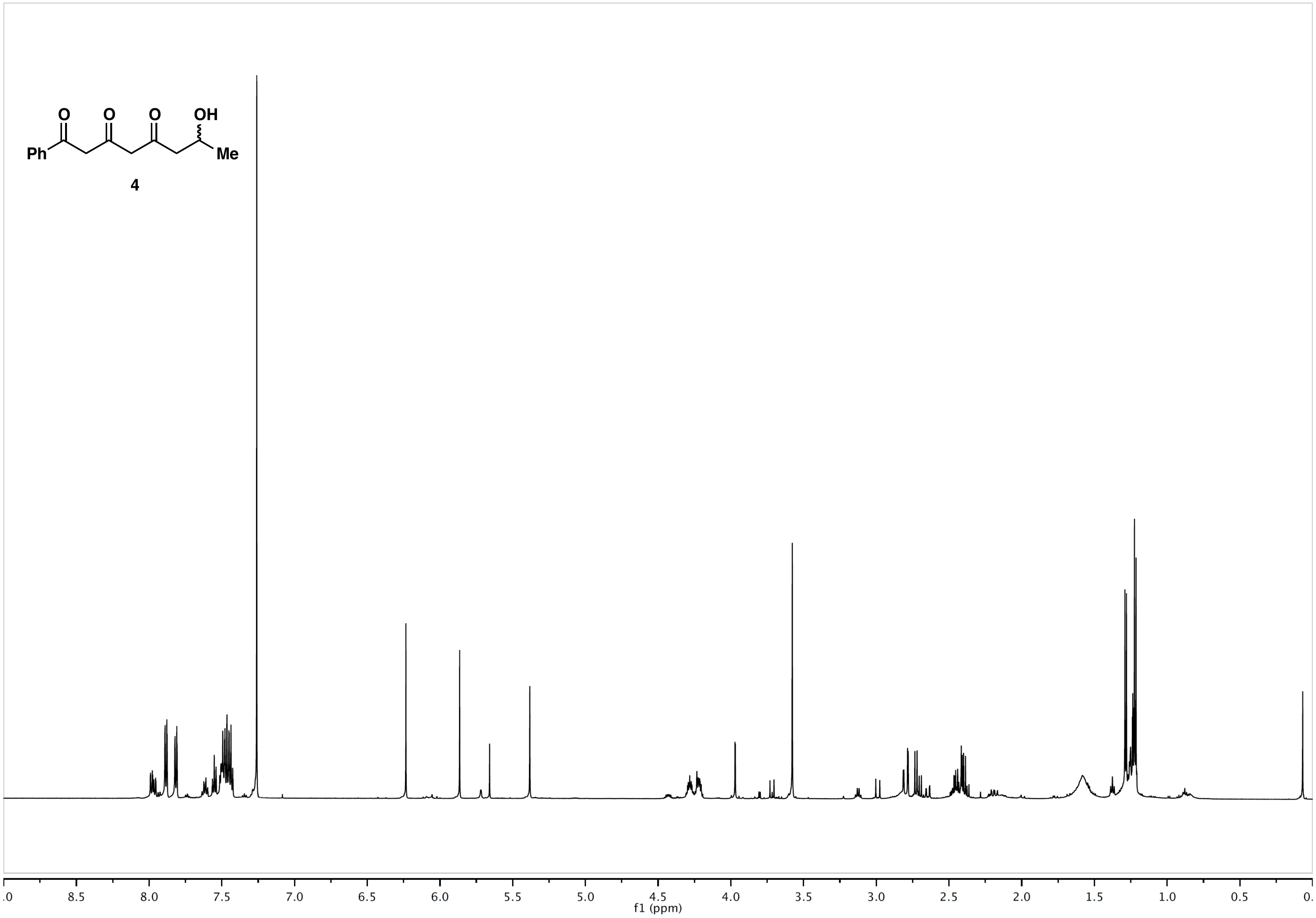
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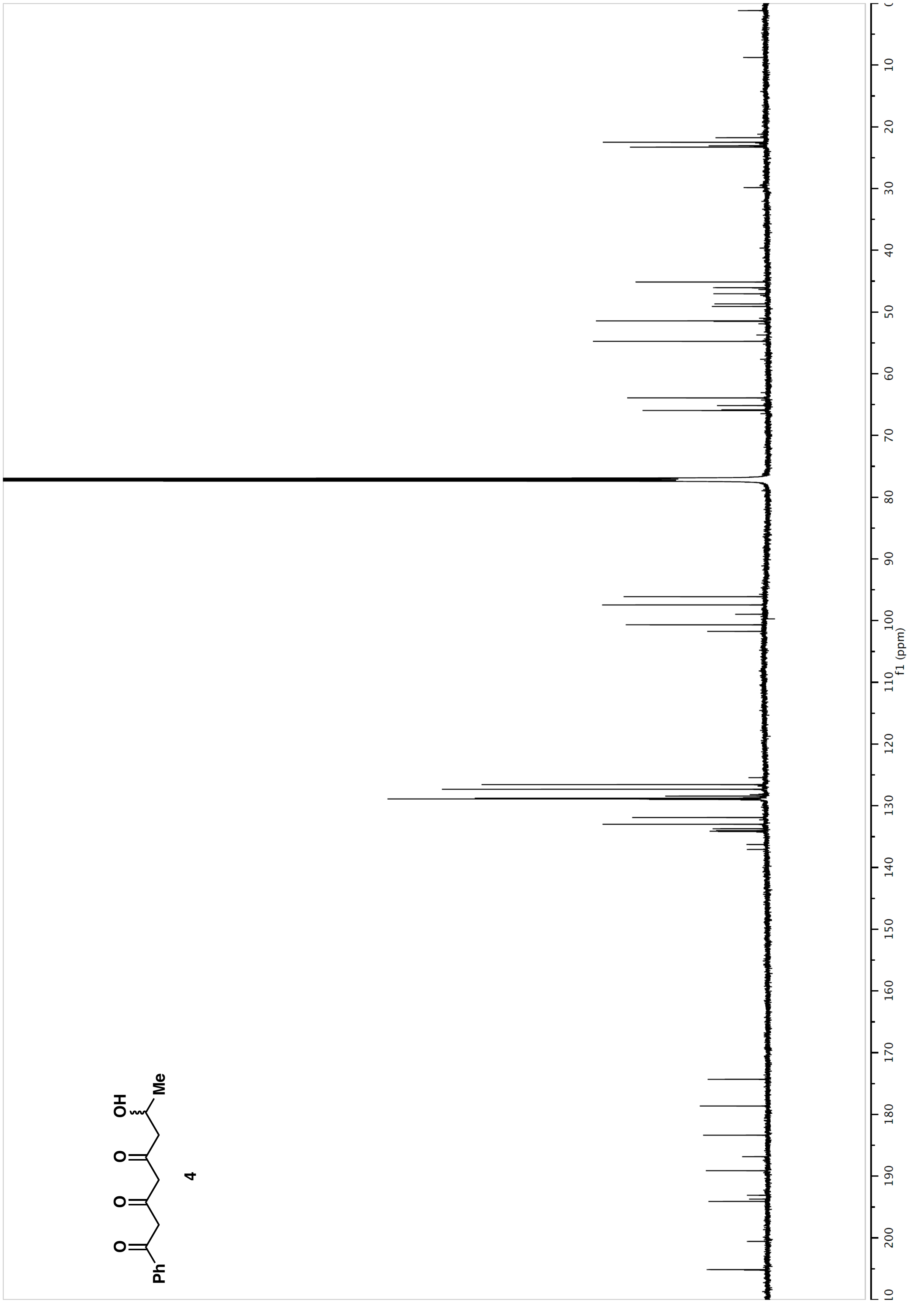
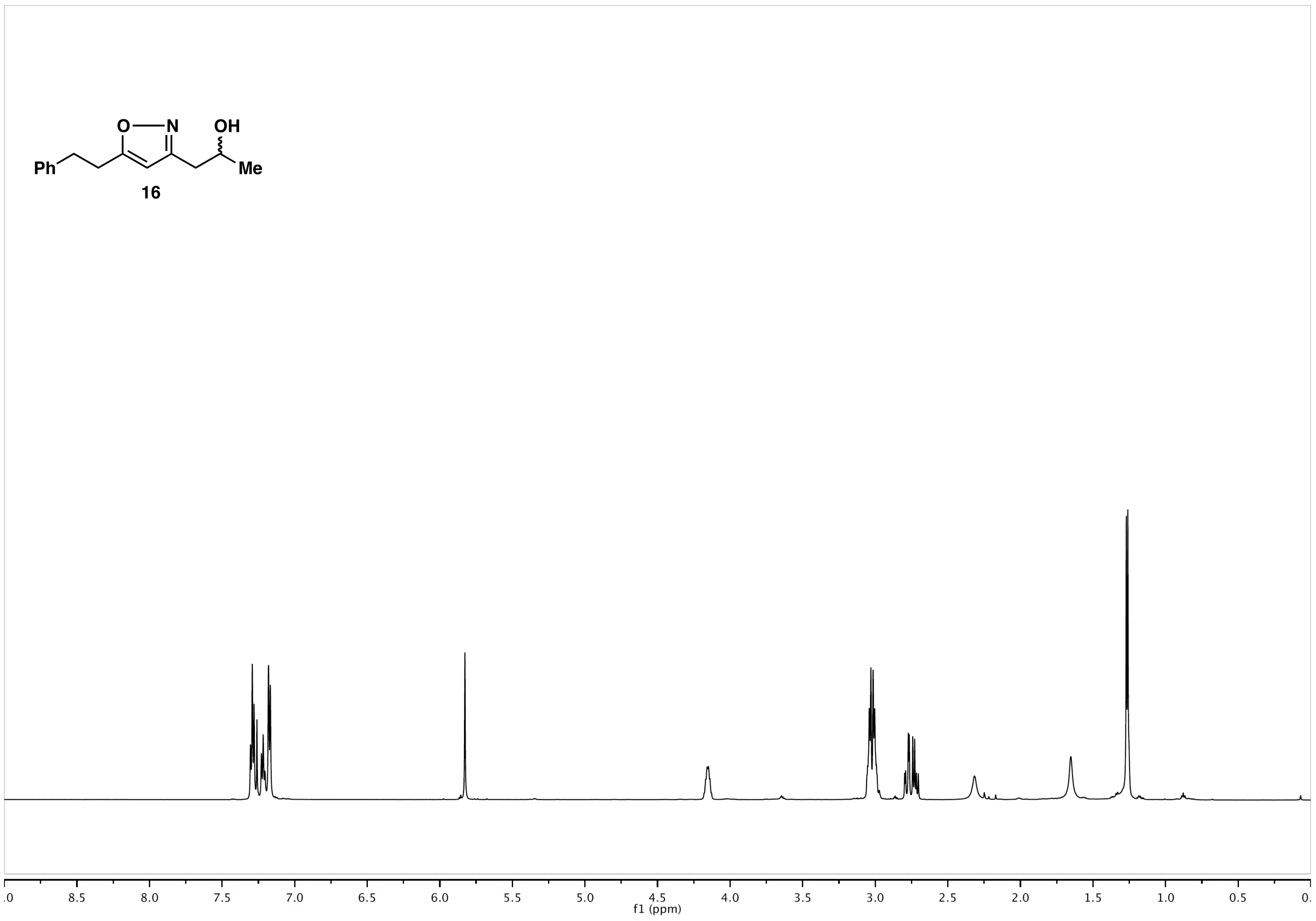
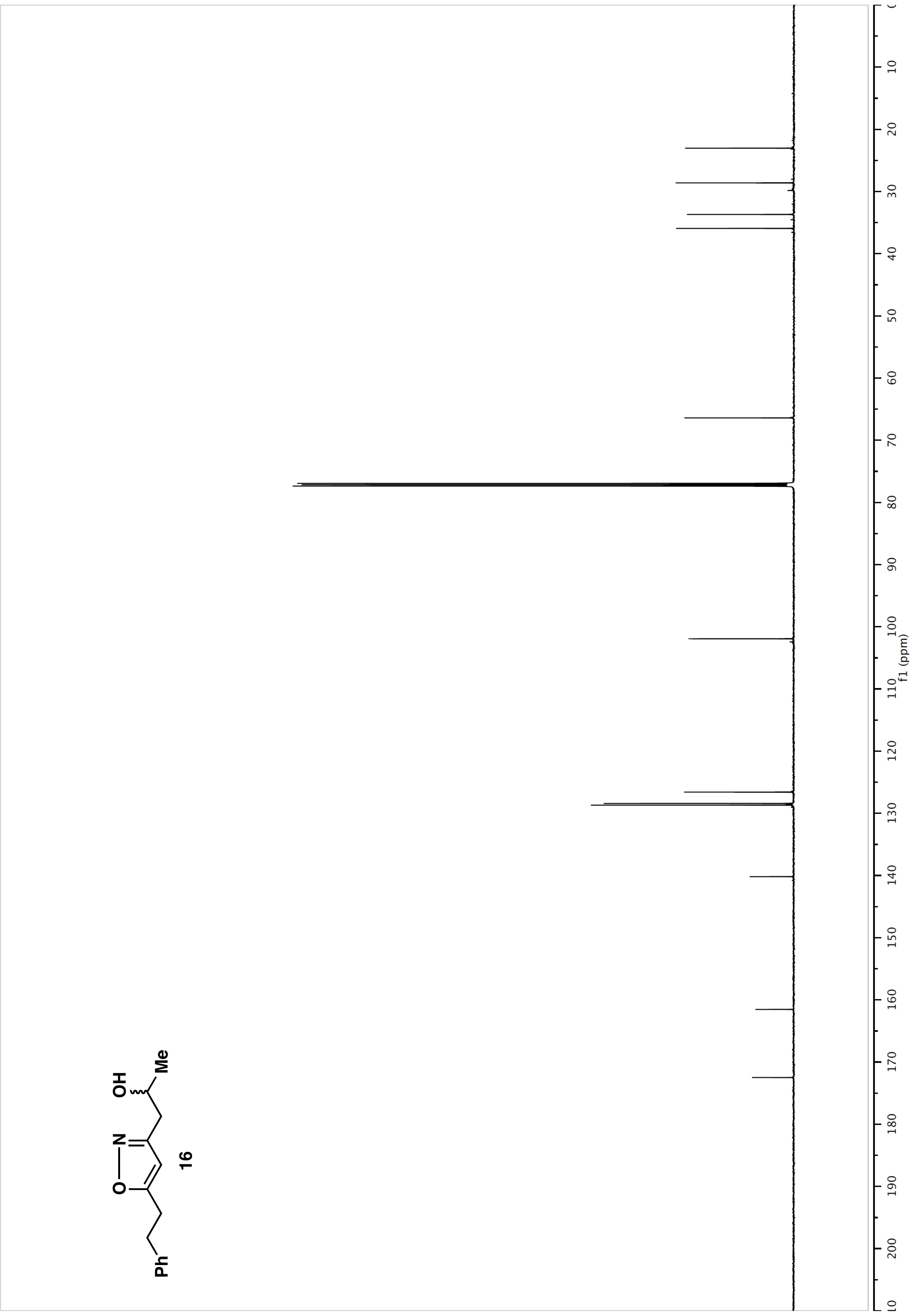
2.0
1.5
1.0
0.5
0.
f1 (ppm)

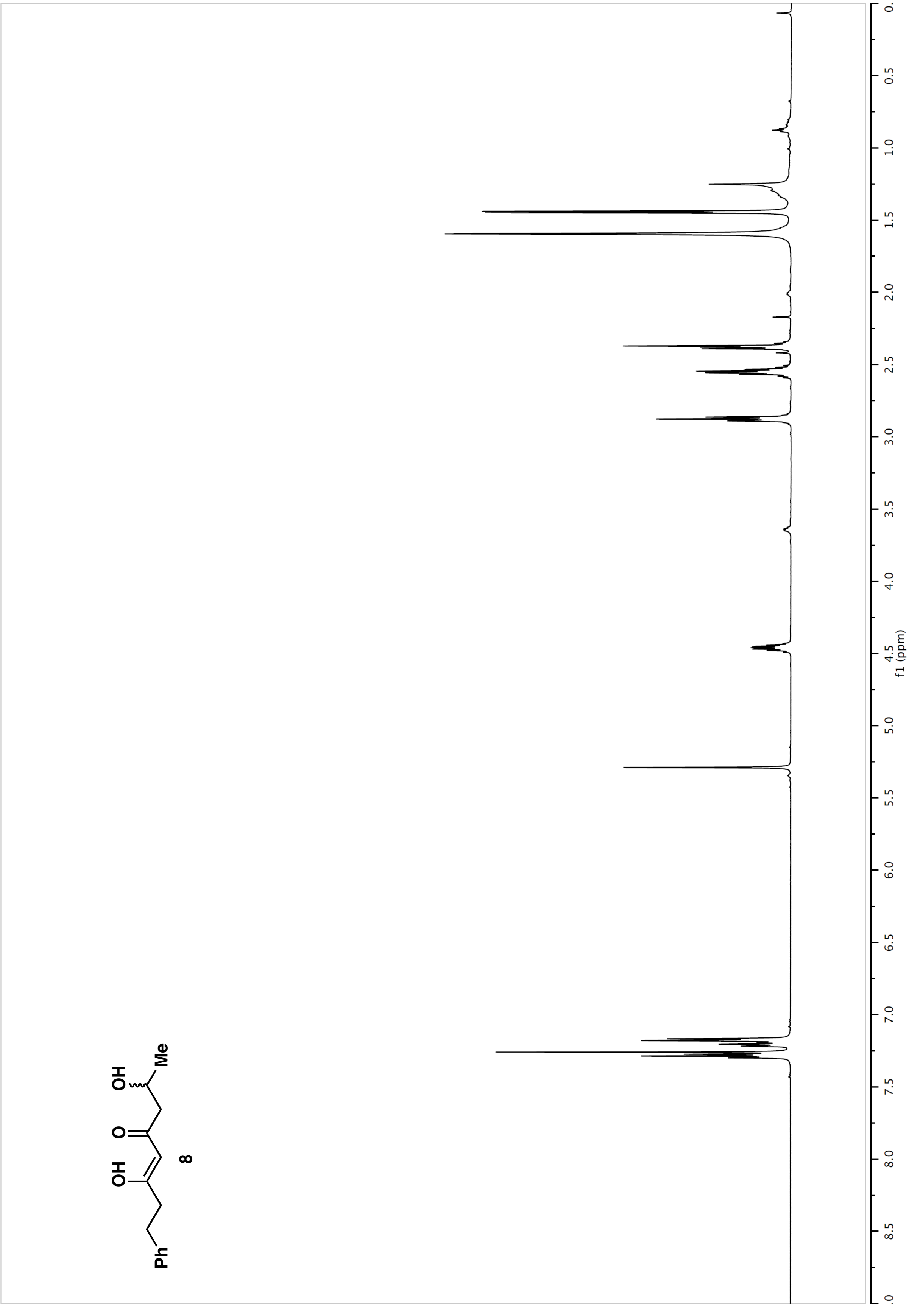

4
Ph
O
O
O
OH
Me
f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210



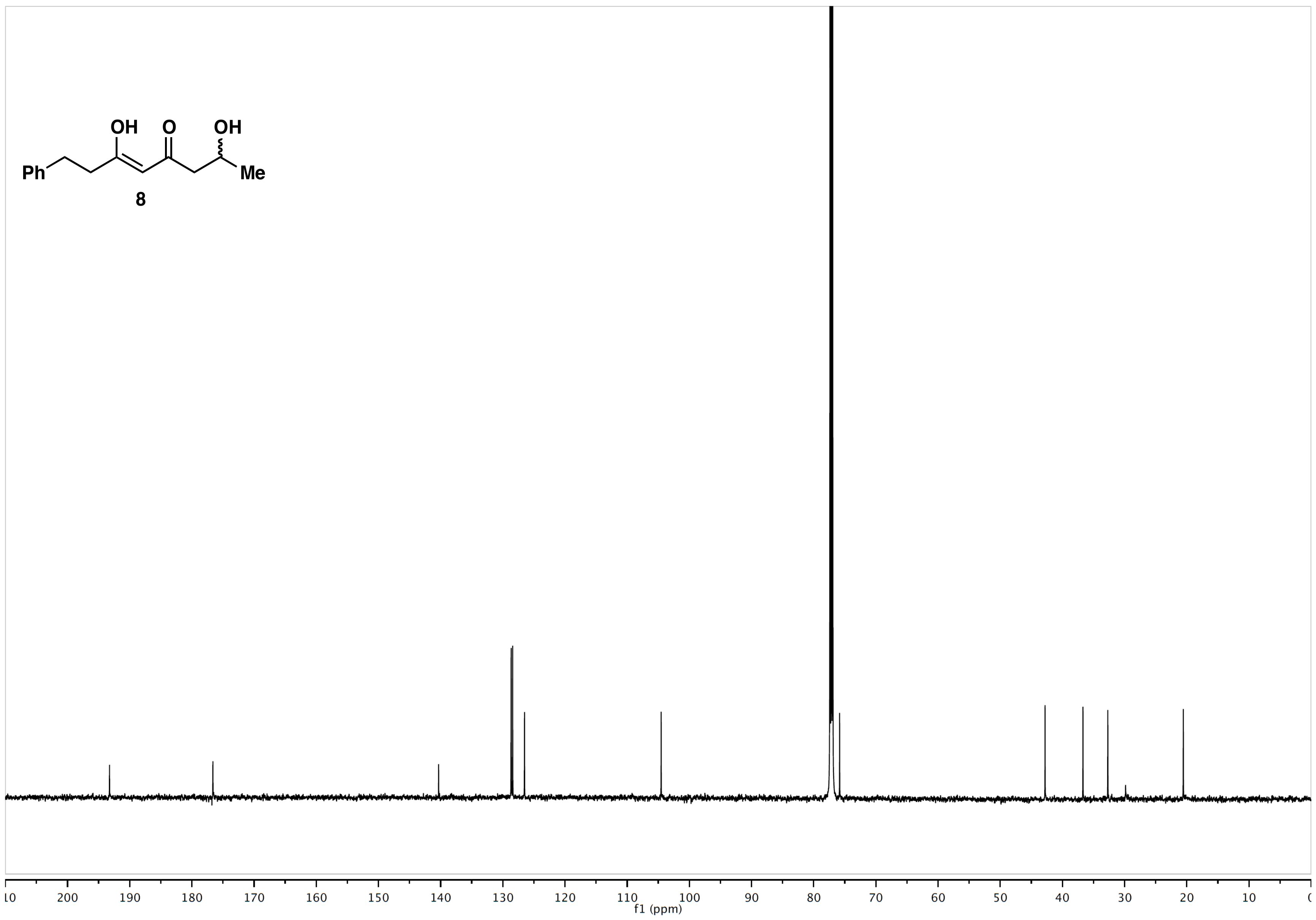
16

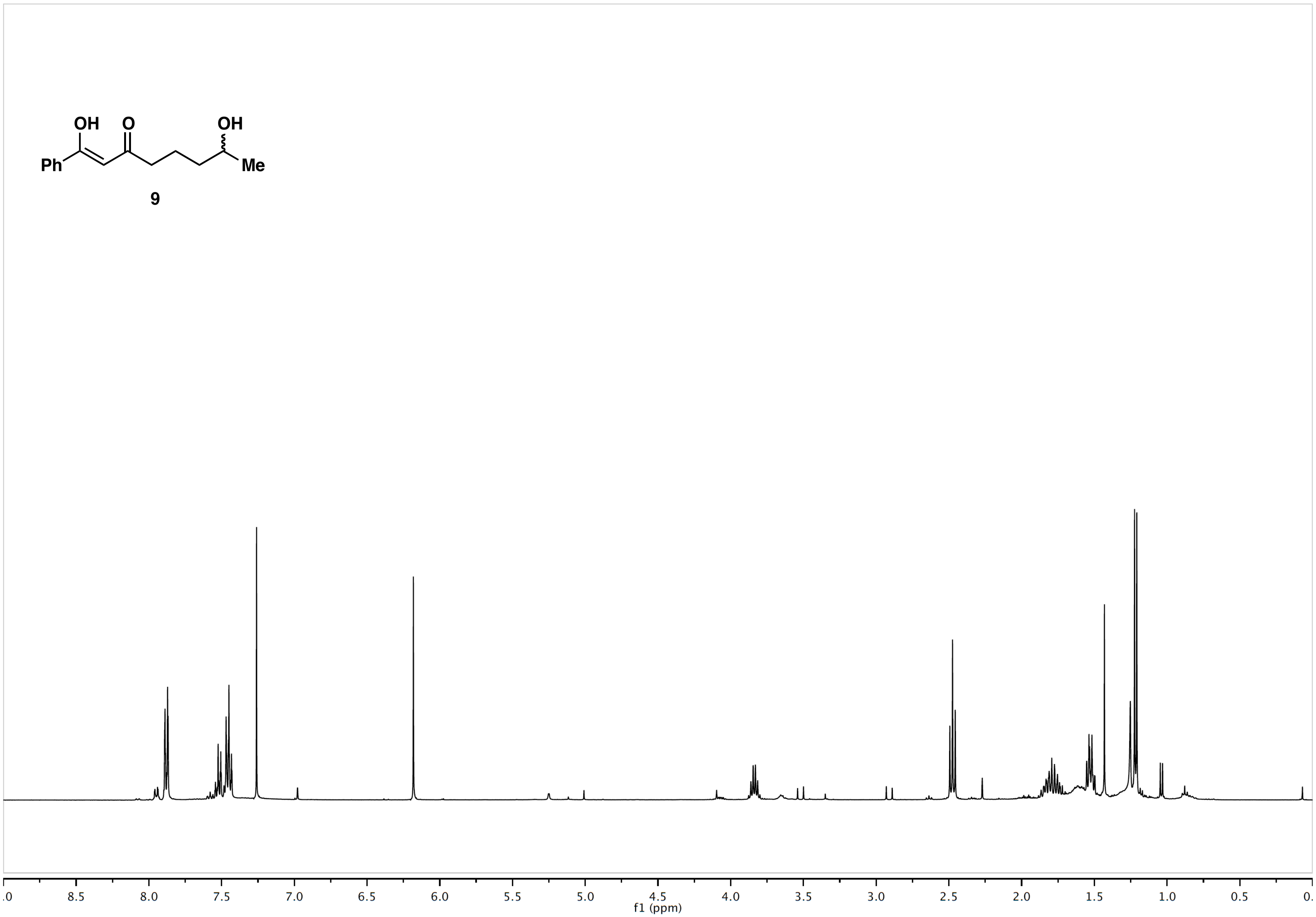

Ph
O
N
OH
Me
f1 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

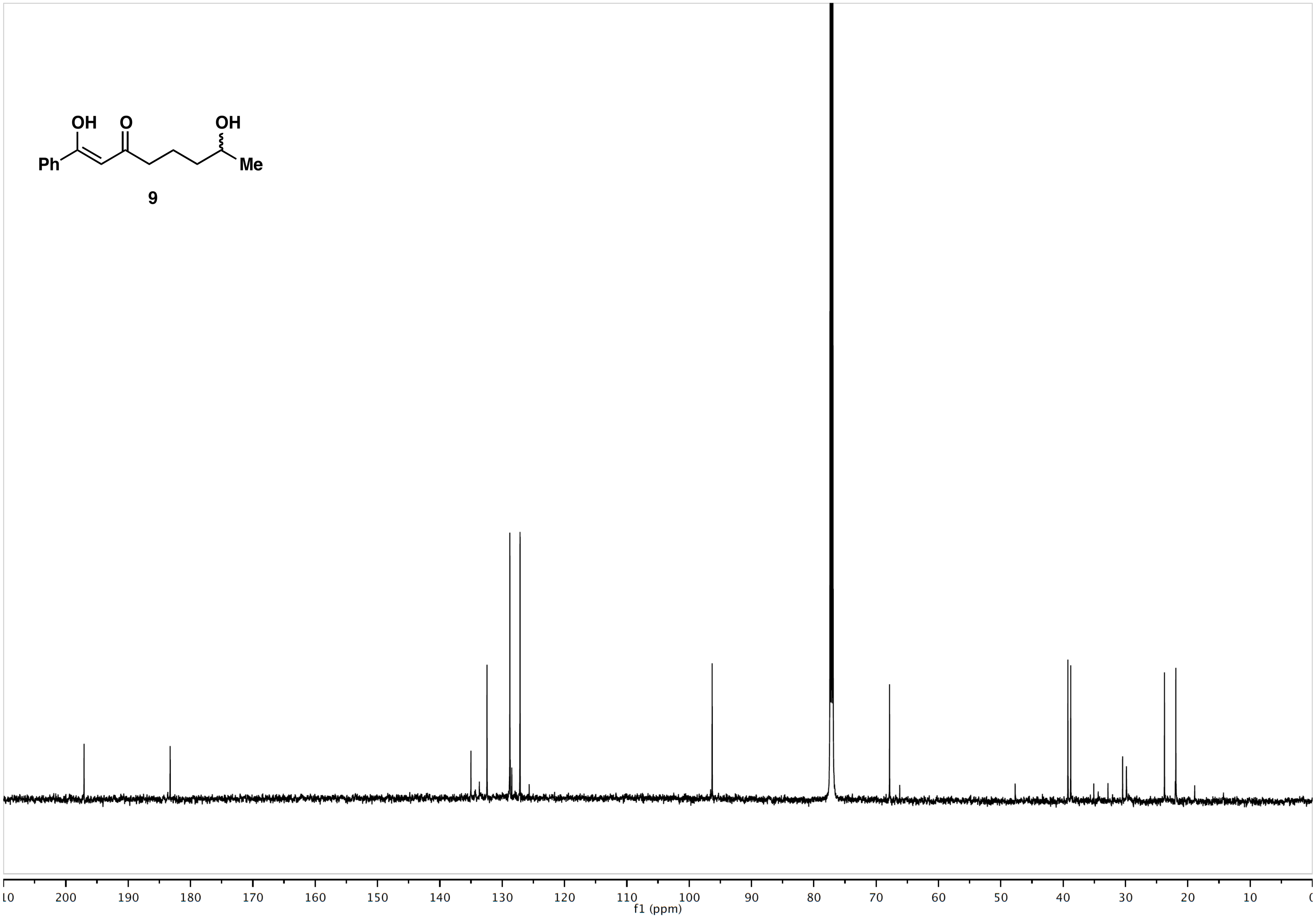
16



8

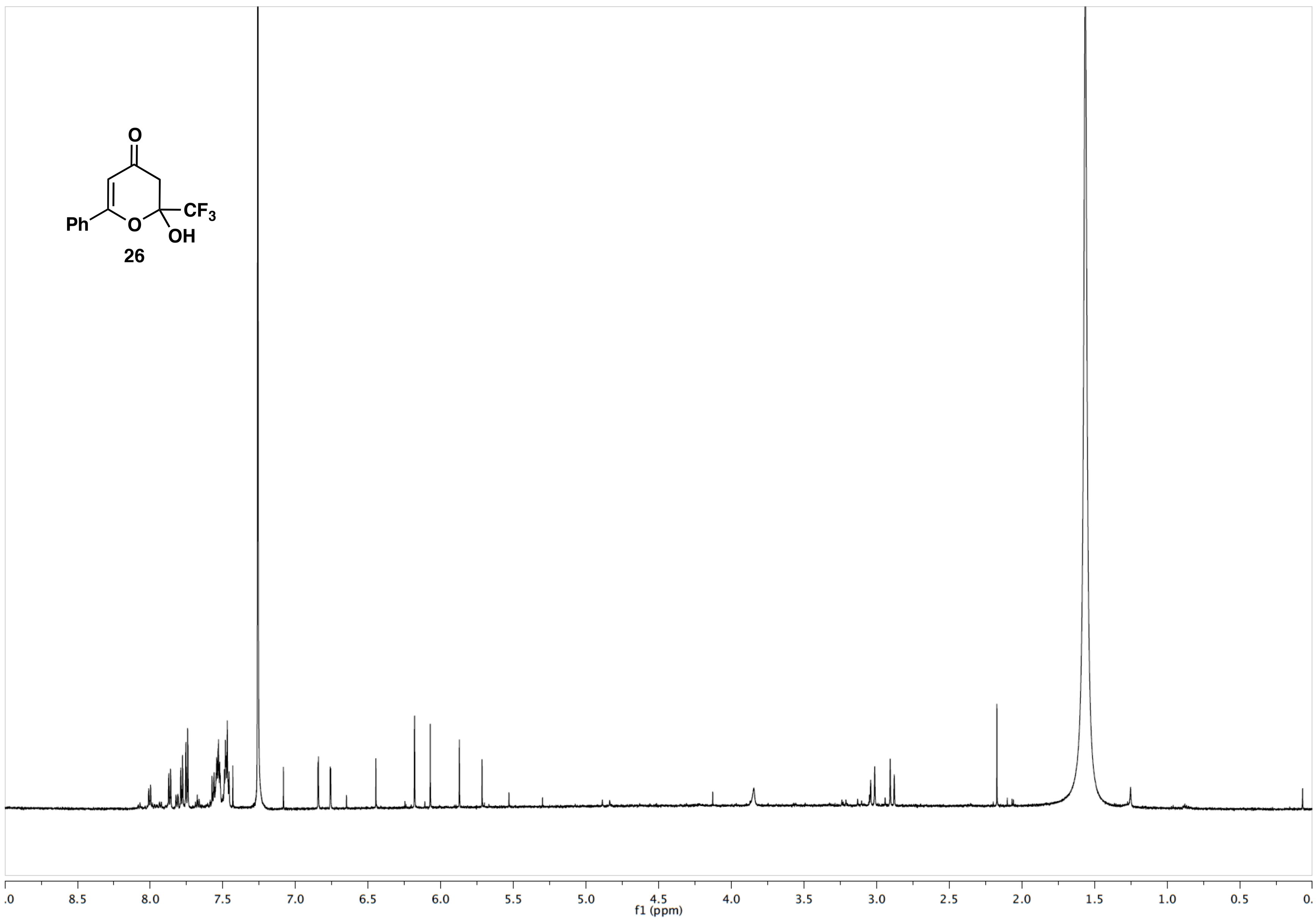

OH
O
OH
Ph
Me
8
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

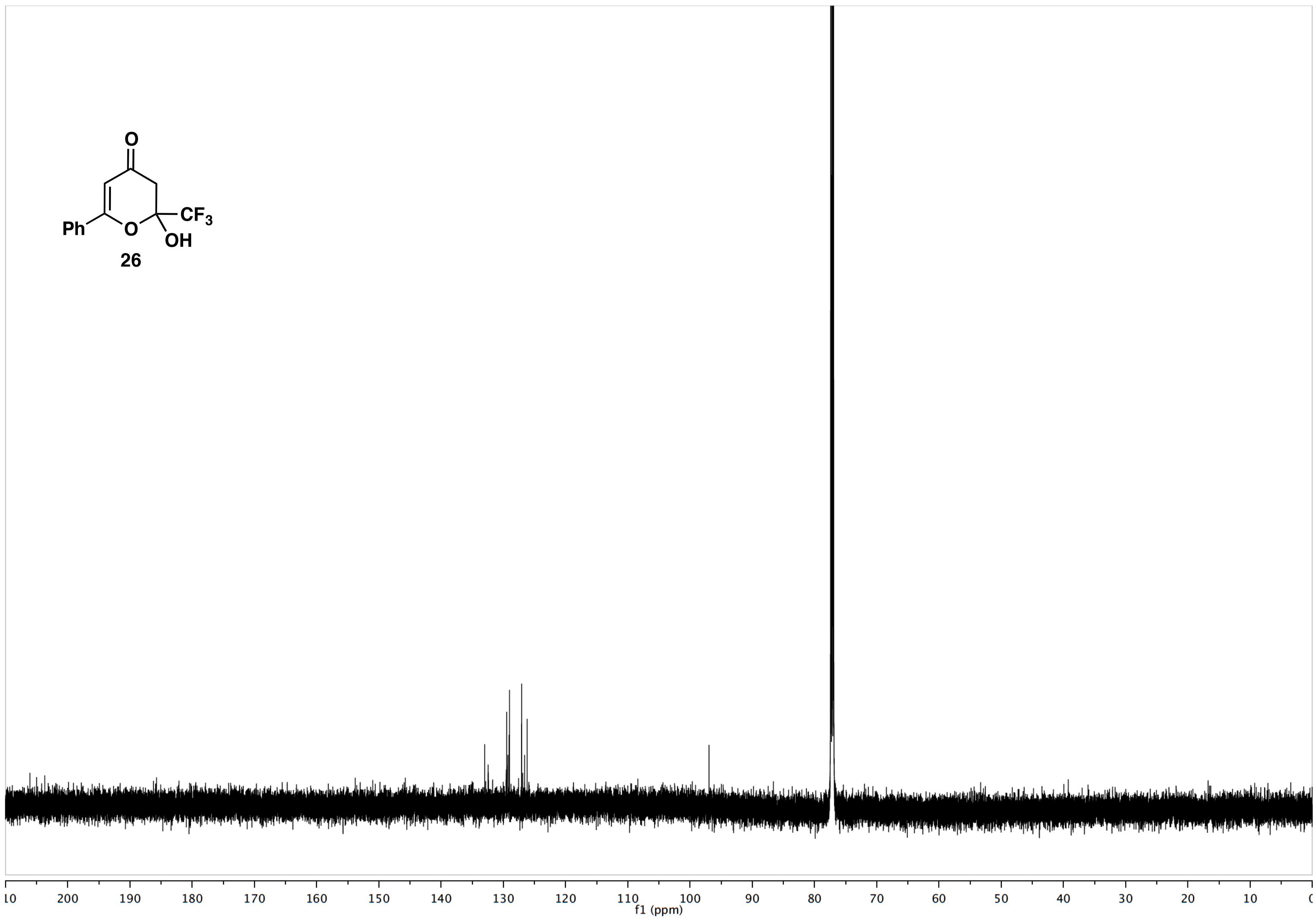

OH
O
OH
Ph
Me
9
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



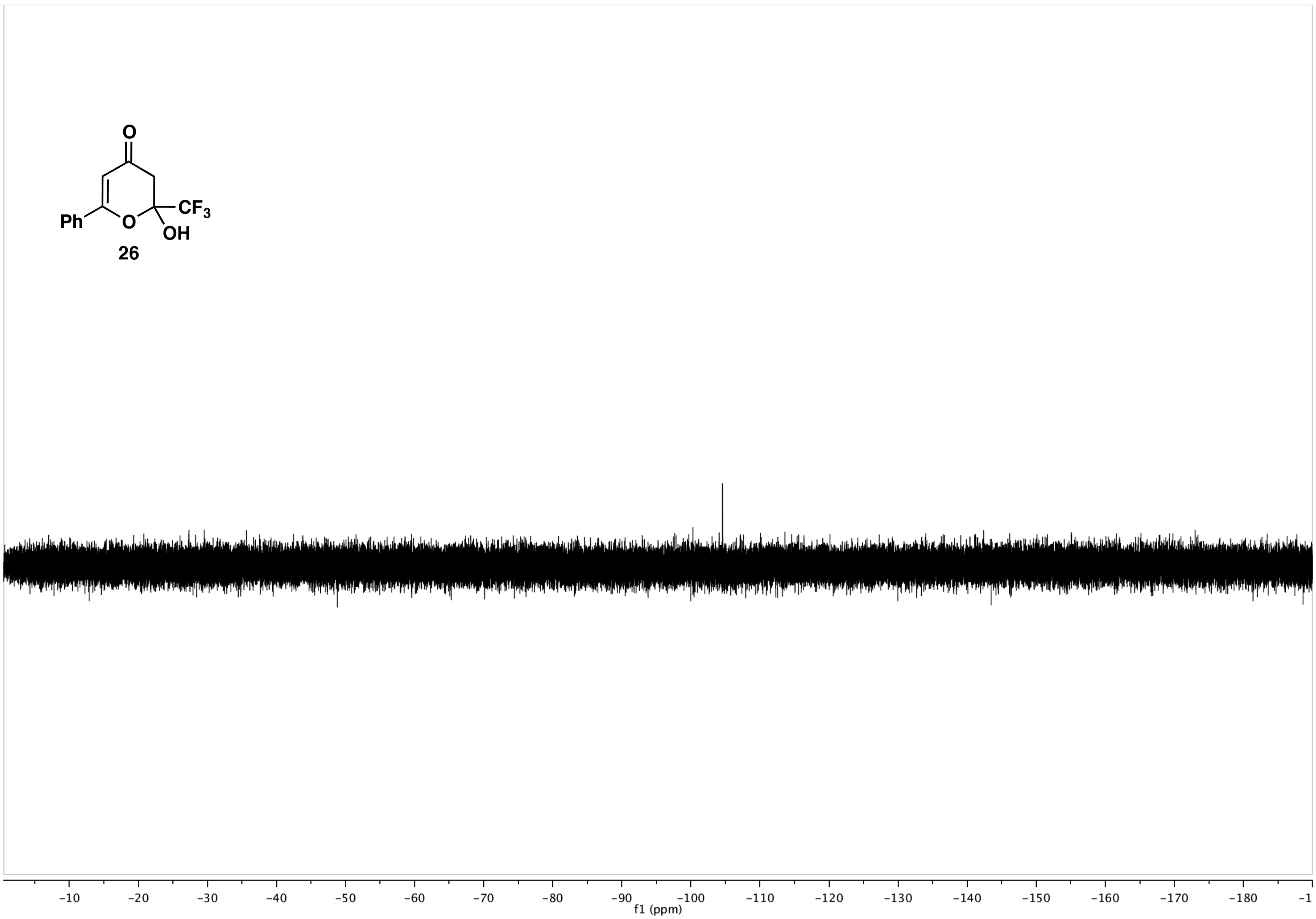
9

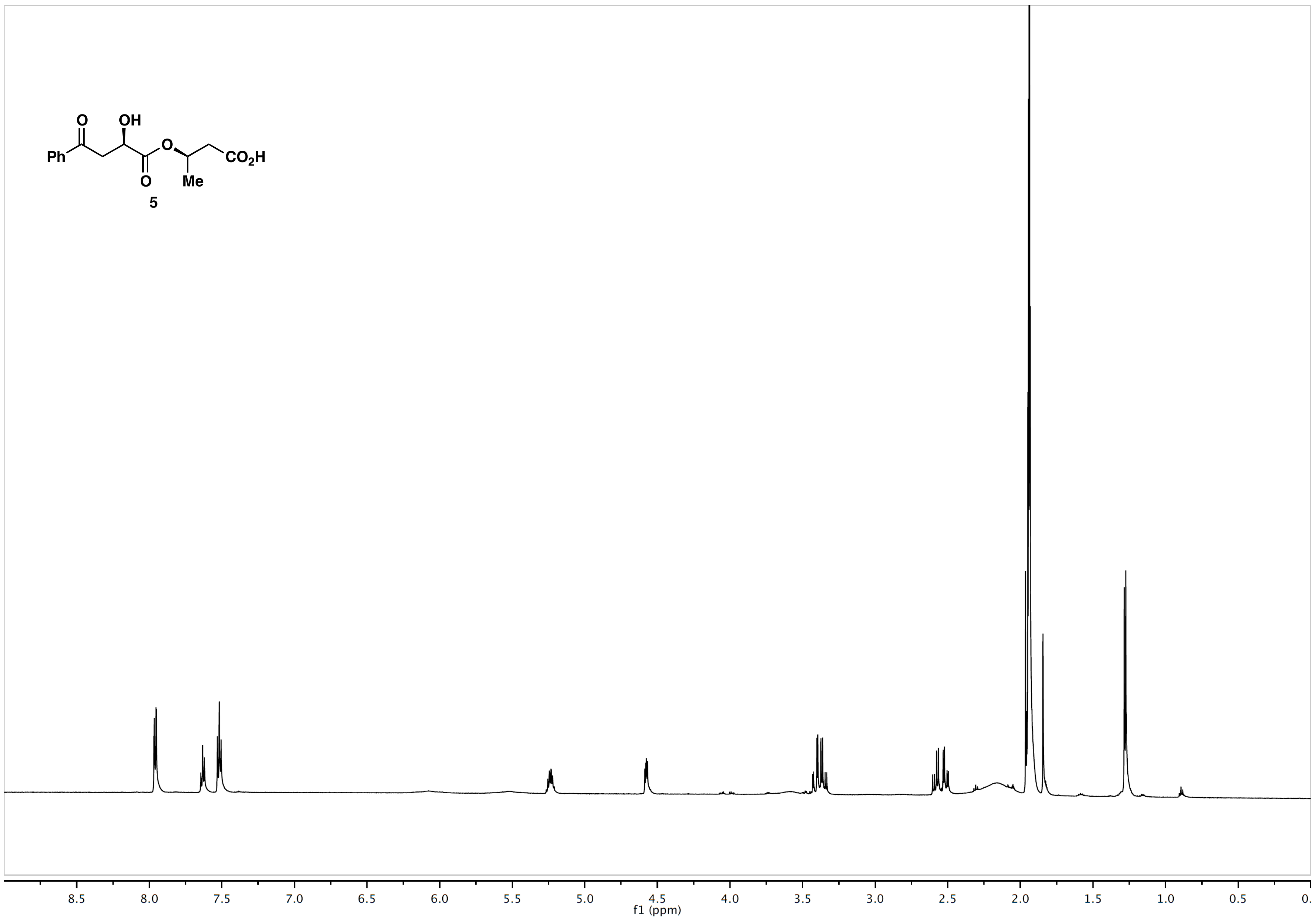
OH
O
OH
Ph
Me
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

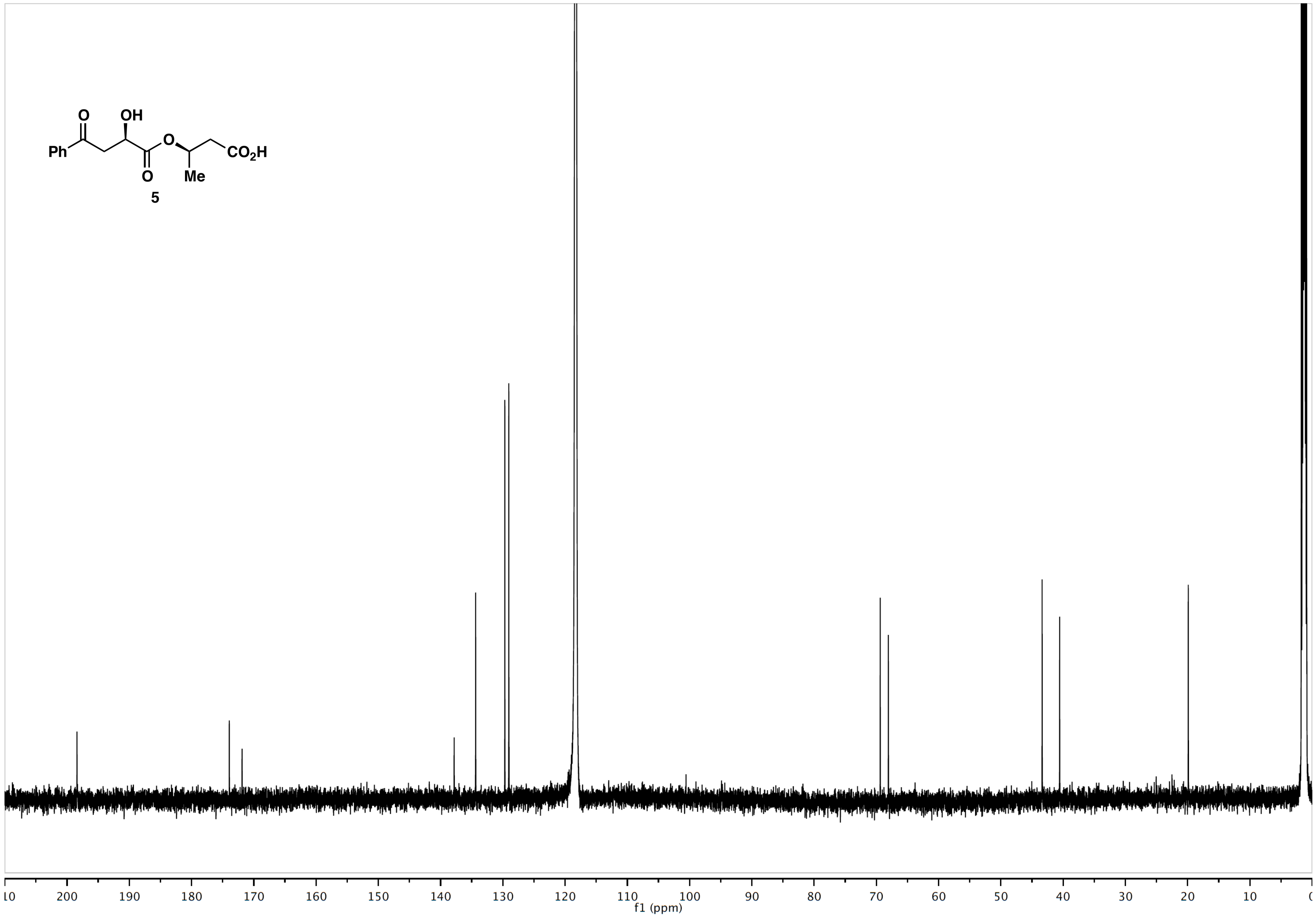

O
Ph
O
CF3
OH
26
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
f1 (ppm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.

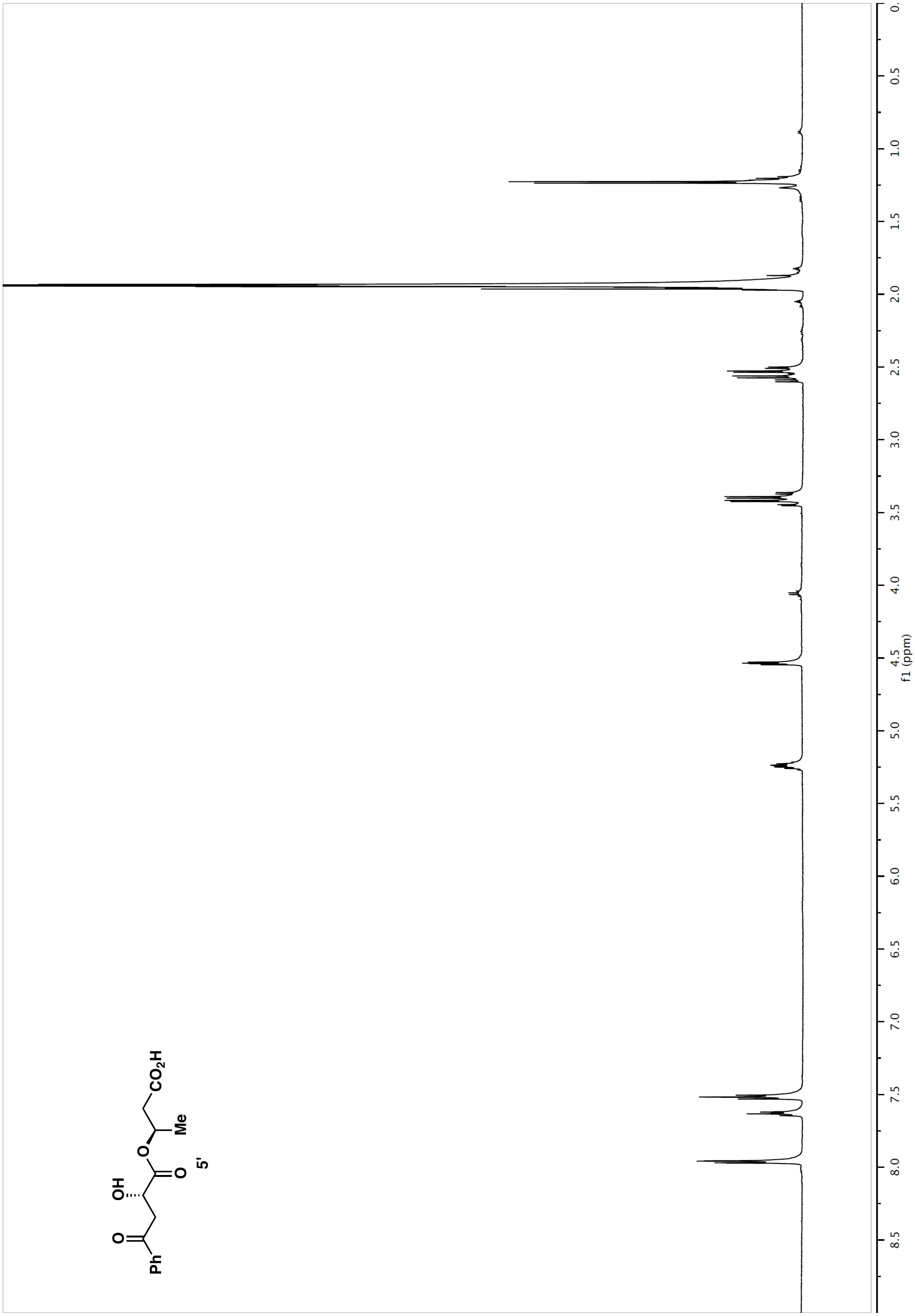

O
Ph
O
CF3
OH
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

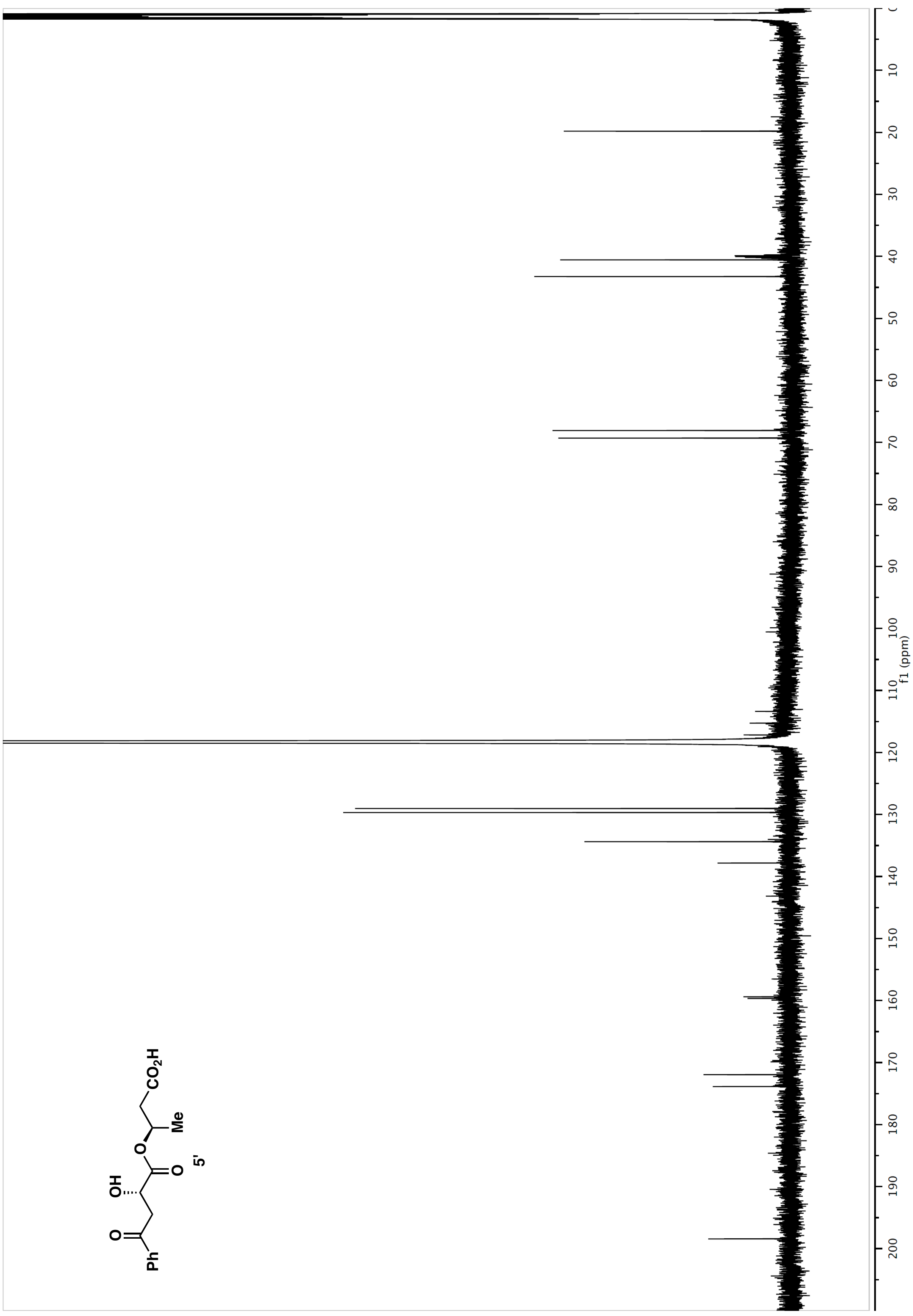
26


O
Ph
O
CF3
OH
26
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-1
f1 (ppm)

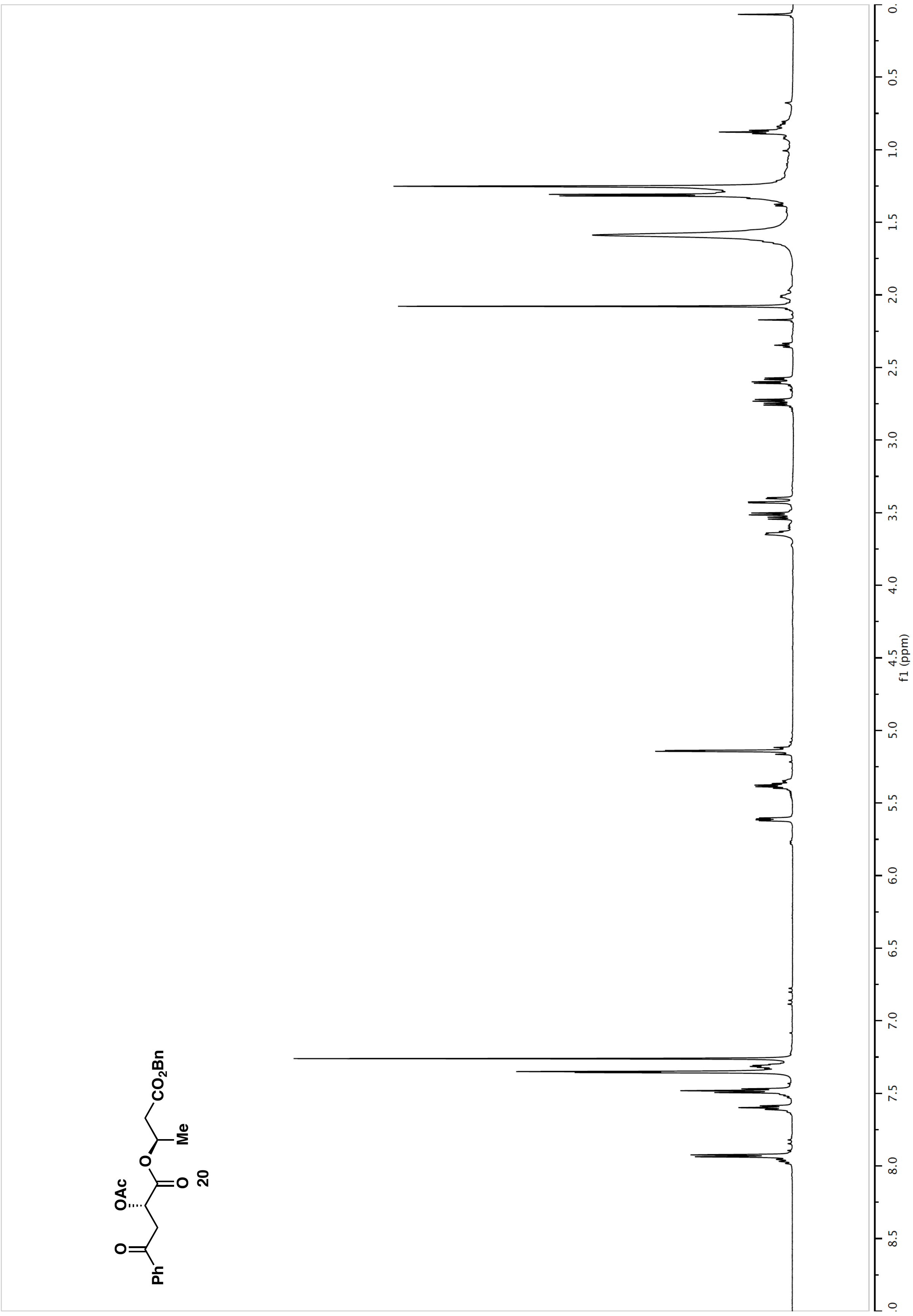

O
OH
Ph
O
O
Me
CO2H
5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)


O
OH
Ph
O
CO2H
O
Me
5
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

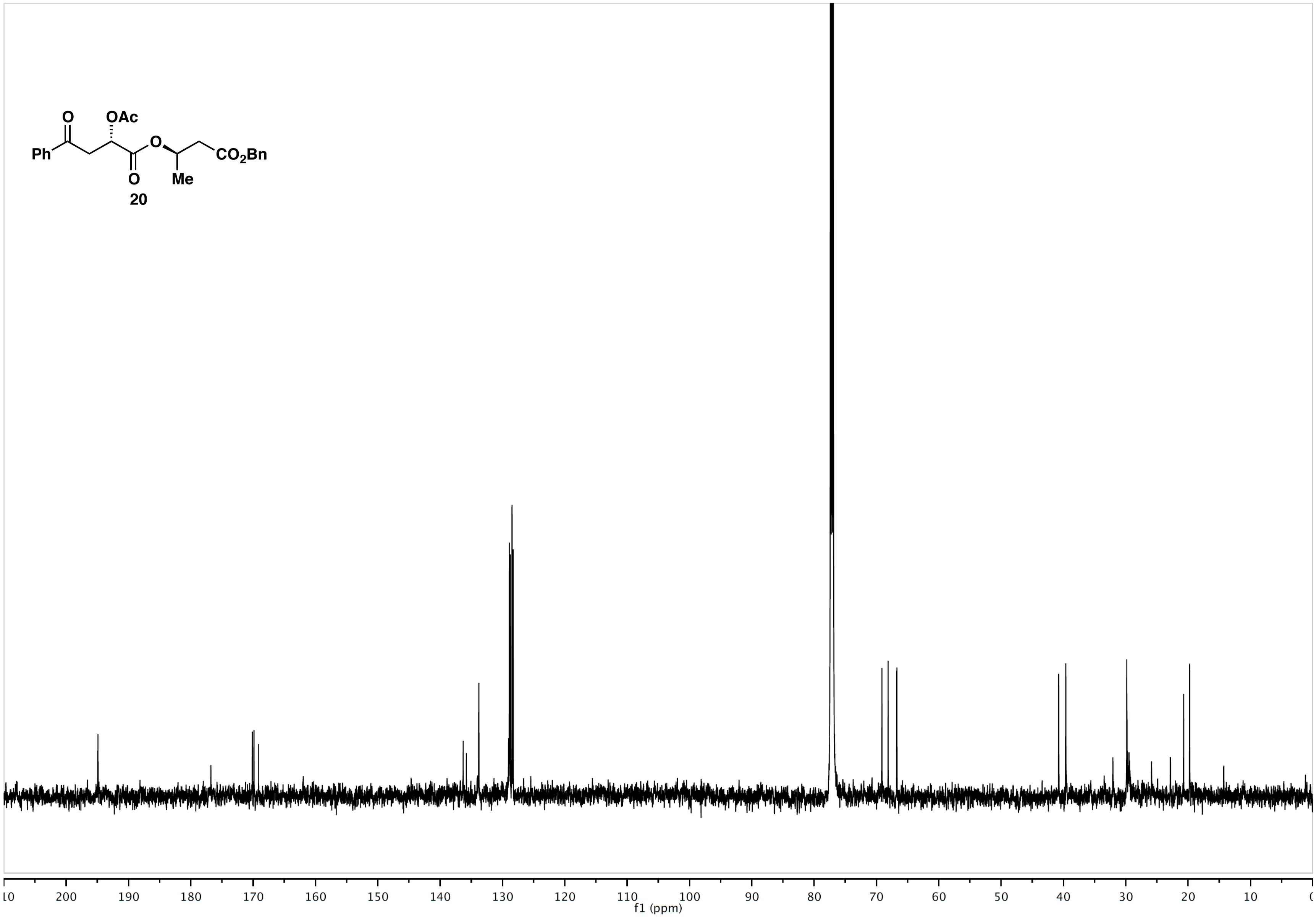

5'
Ph
O
OH
O
O
Me
CO2H
f1 (ppm)
0.
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5

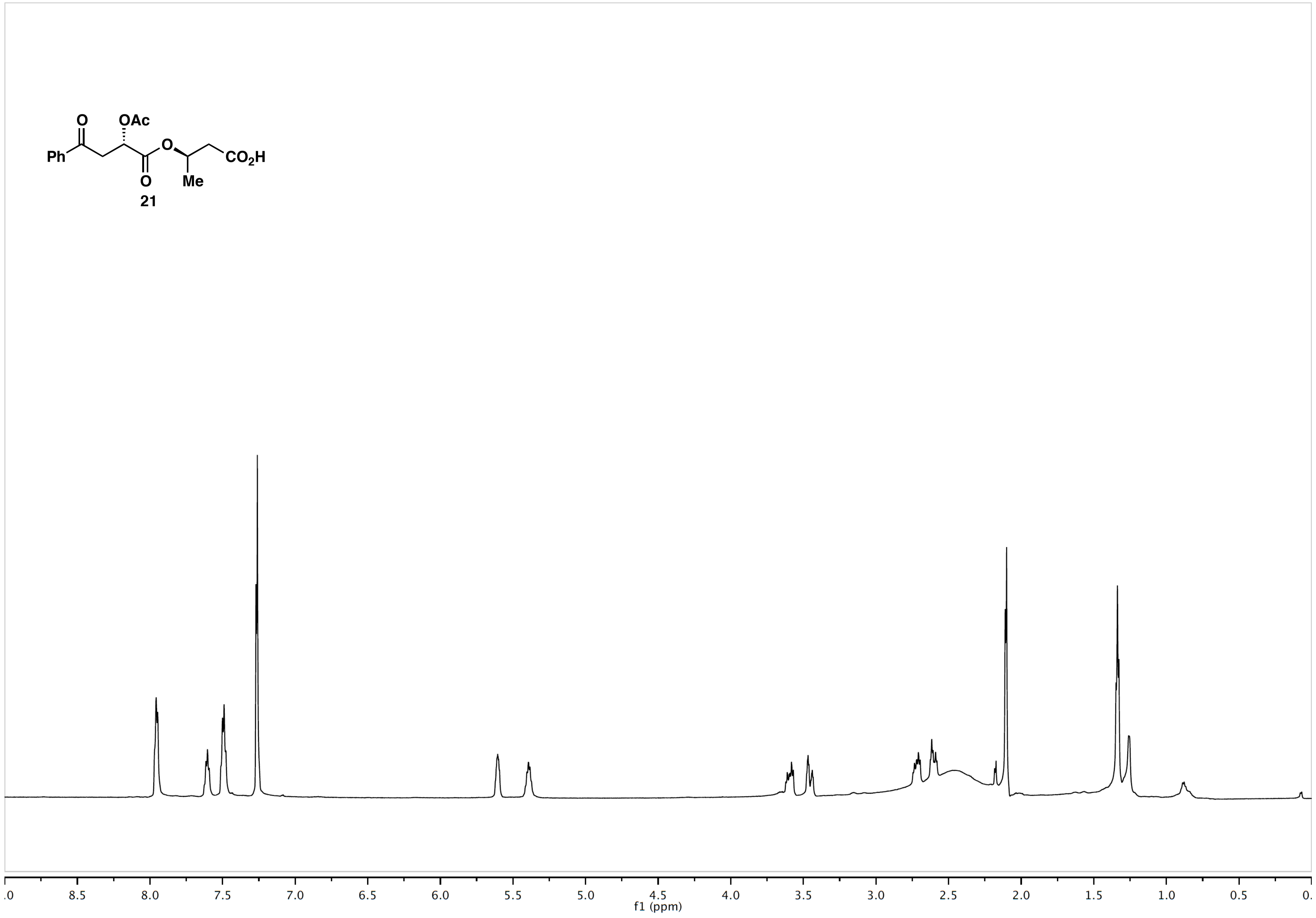

5'

Ph
O
OH
O
O
Me
CO_2H
f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

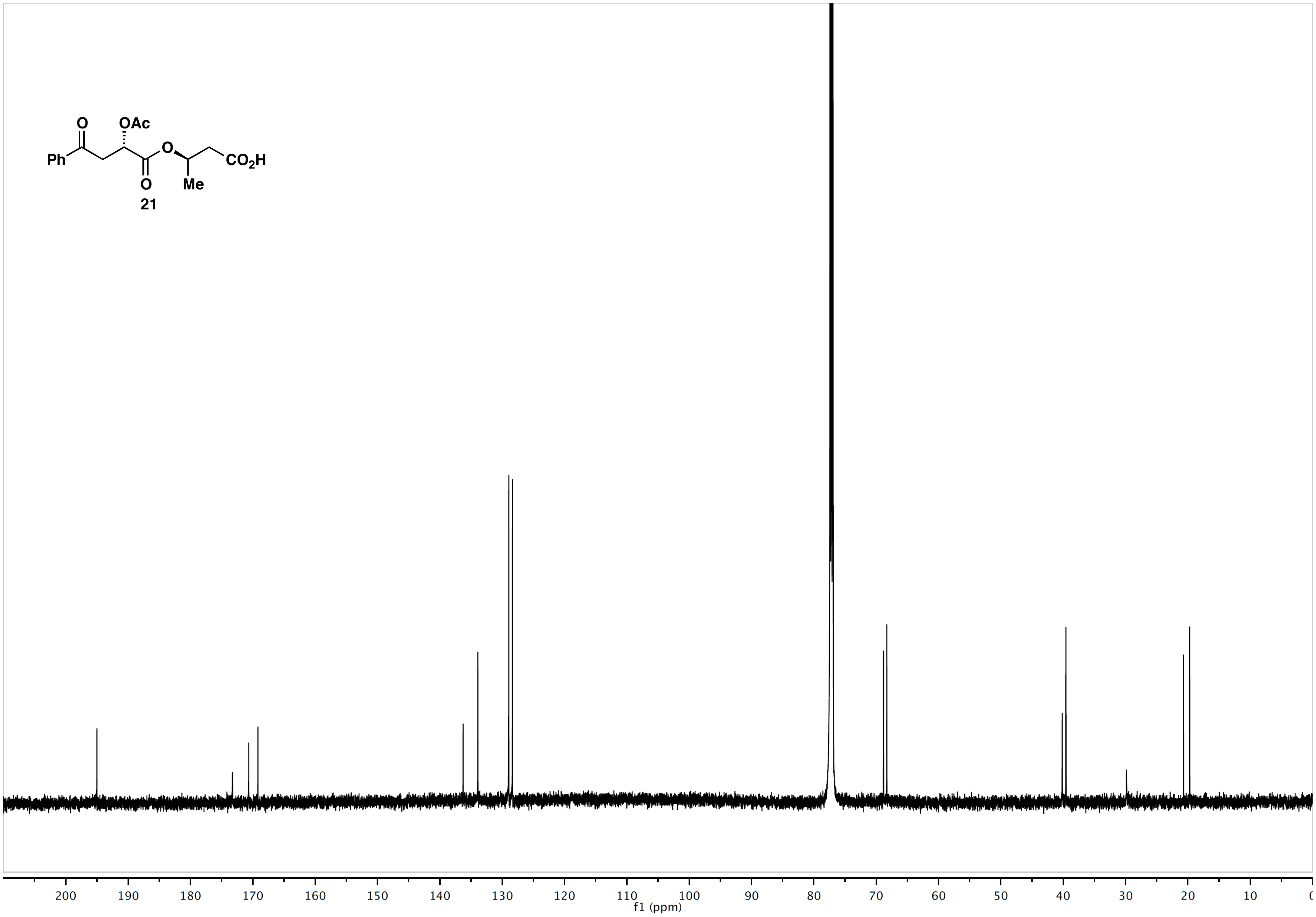

20

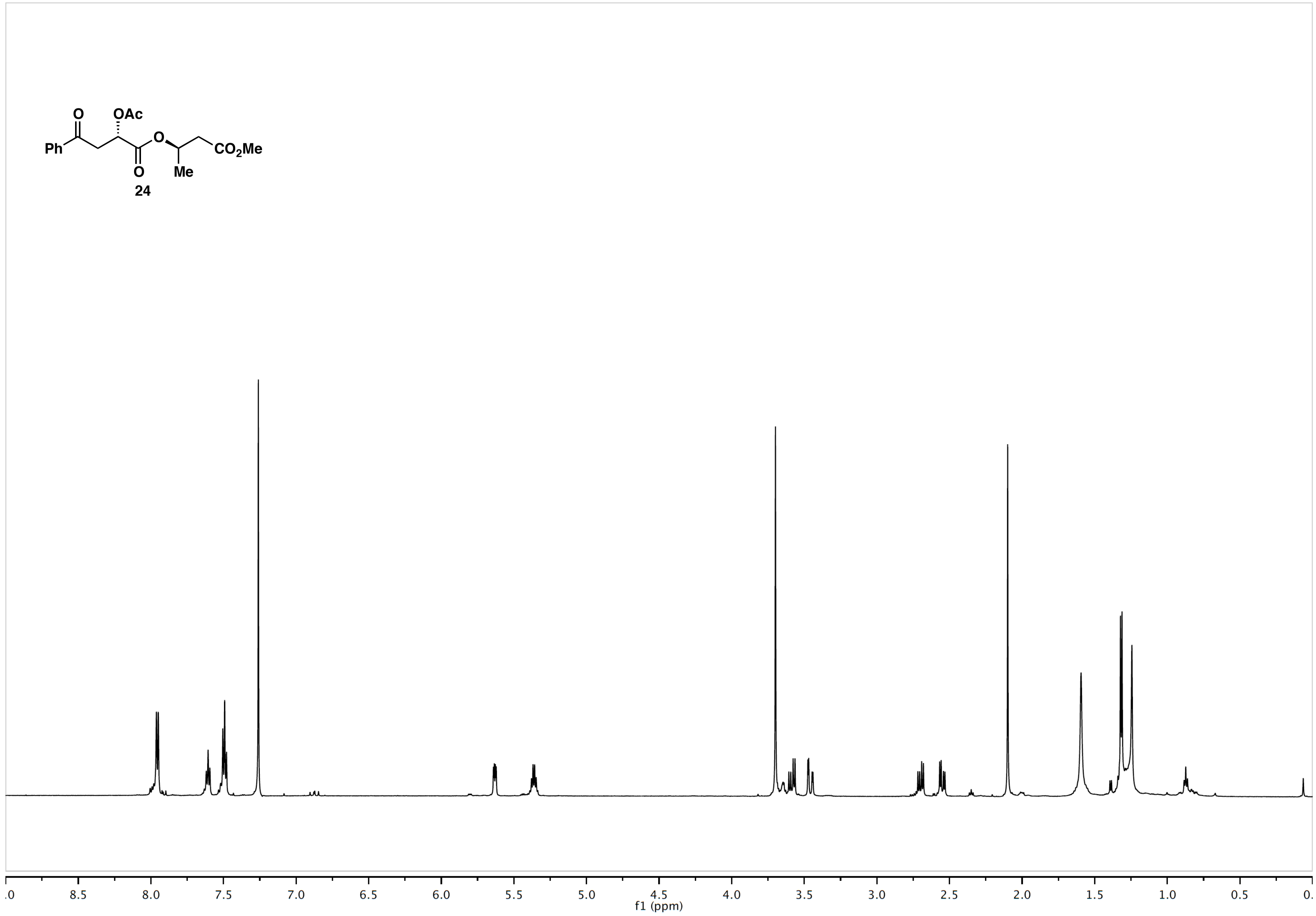
Ph
O
OAc
O
O
Me
CO2Bn
0.
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
f1 (ppm)
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
.0

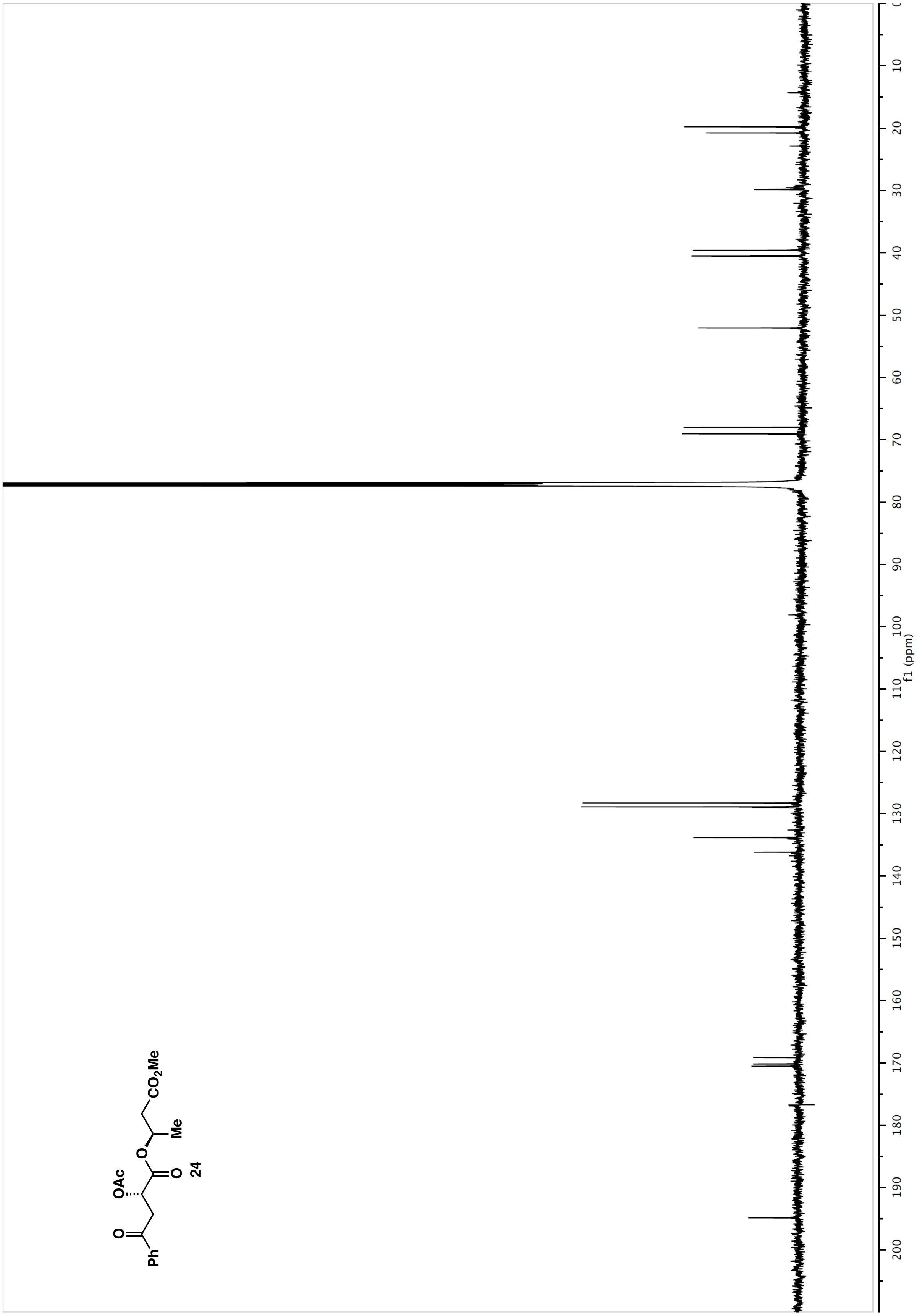

O
OAc
Ph
O
O
CO2Bn
Me
20
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


21

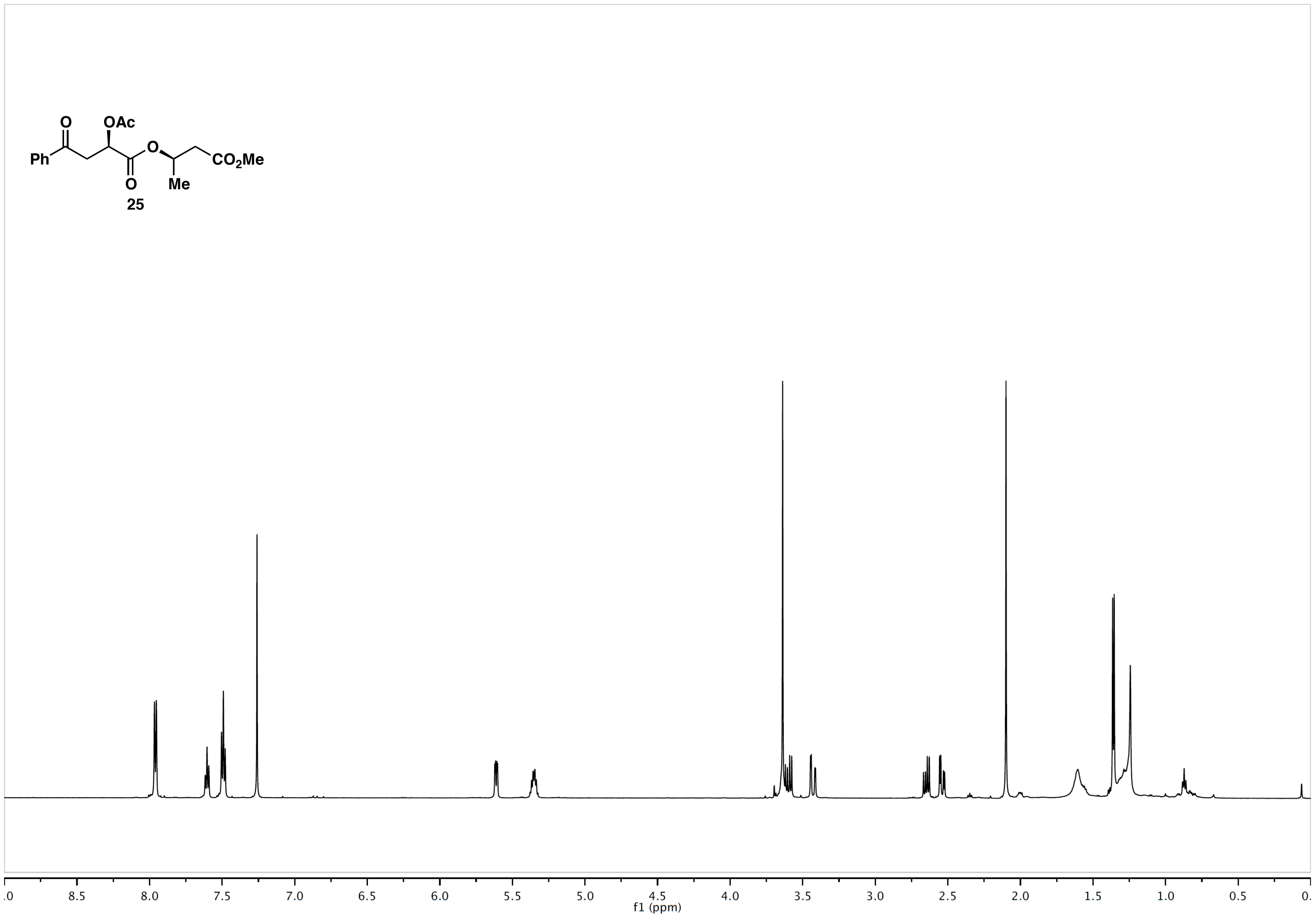
Ph
O
OAc
O
O
Me
CO2H
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)


O
OAc
Ph
O
CO$_2$H
O
Me
21
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
OAc
Ph
O
CO$_2$Me
O
Me
24
.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.
f1 (ppm)

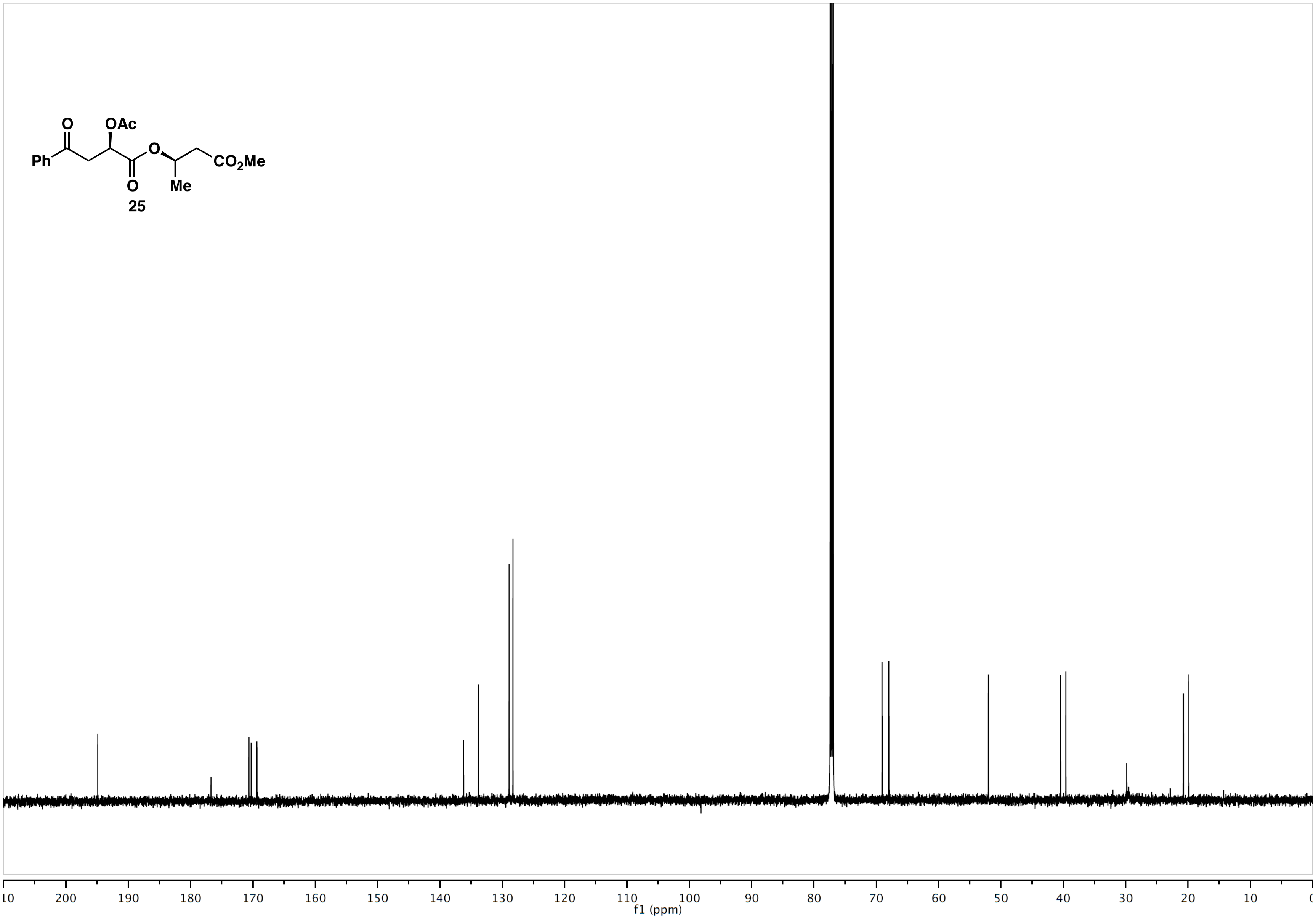

O
OAc
O
O
Me
CO2Me
Ph
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)

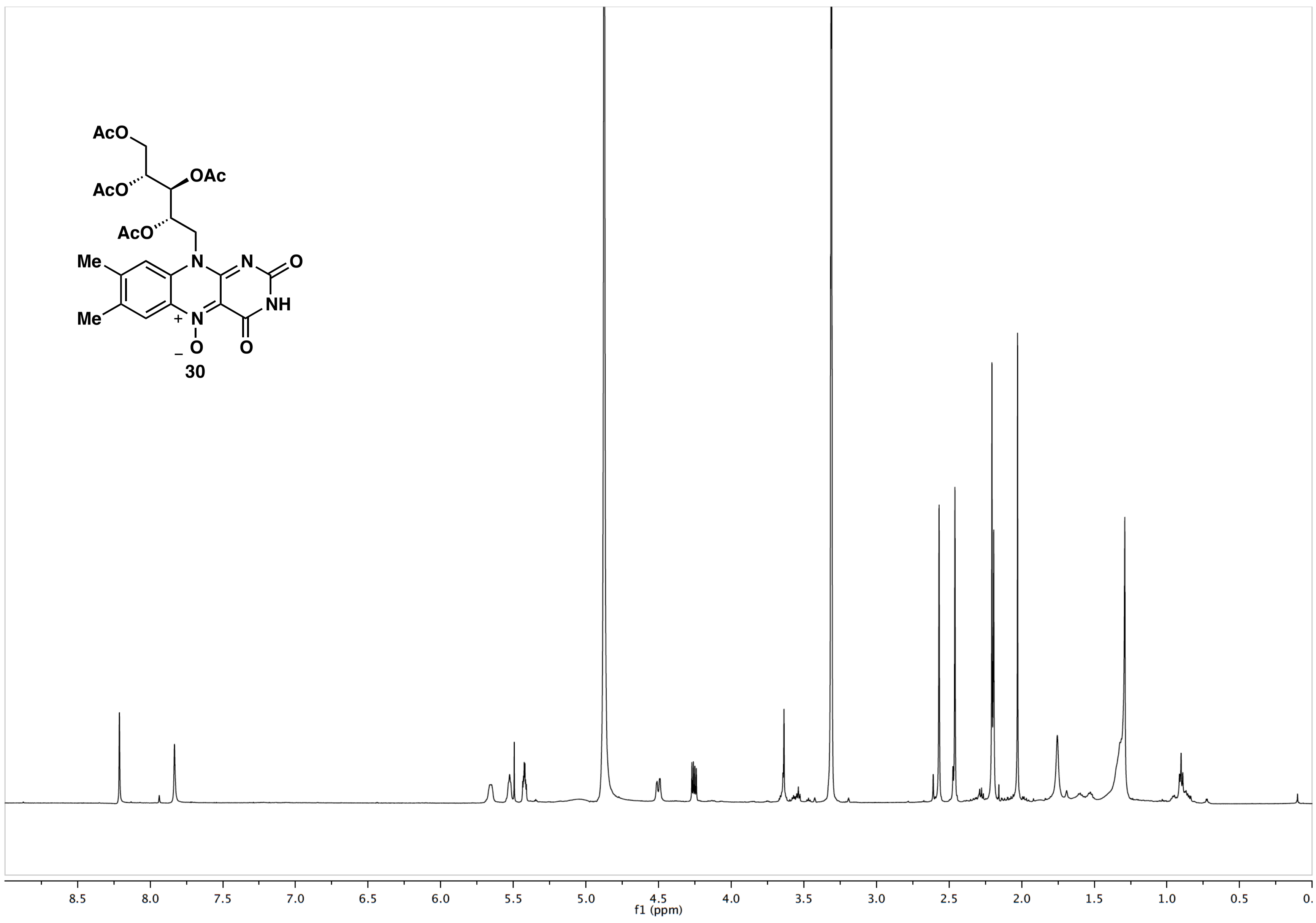
24



25

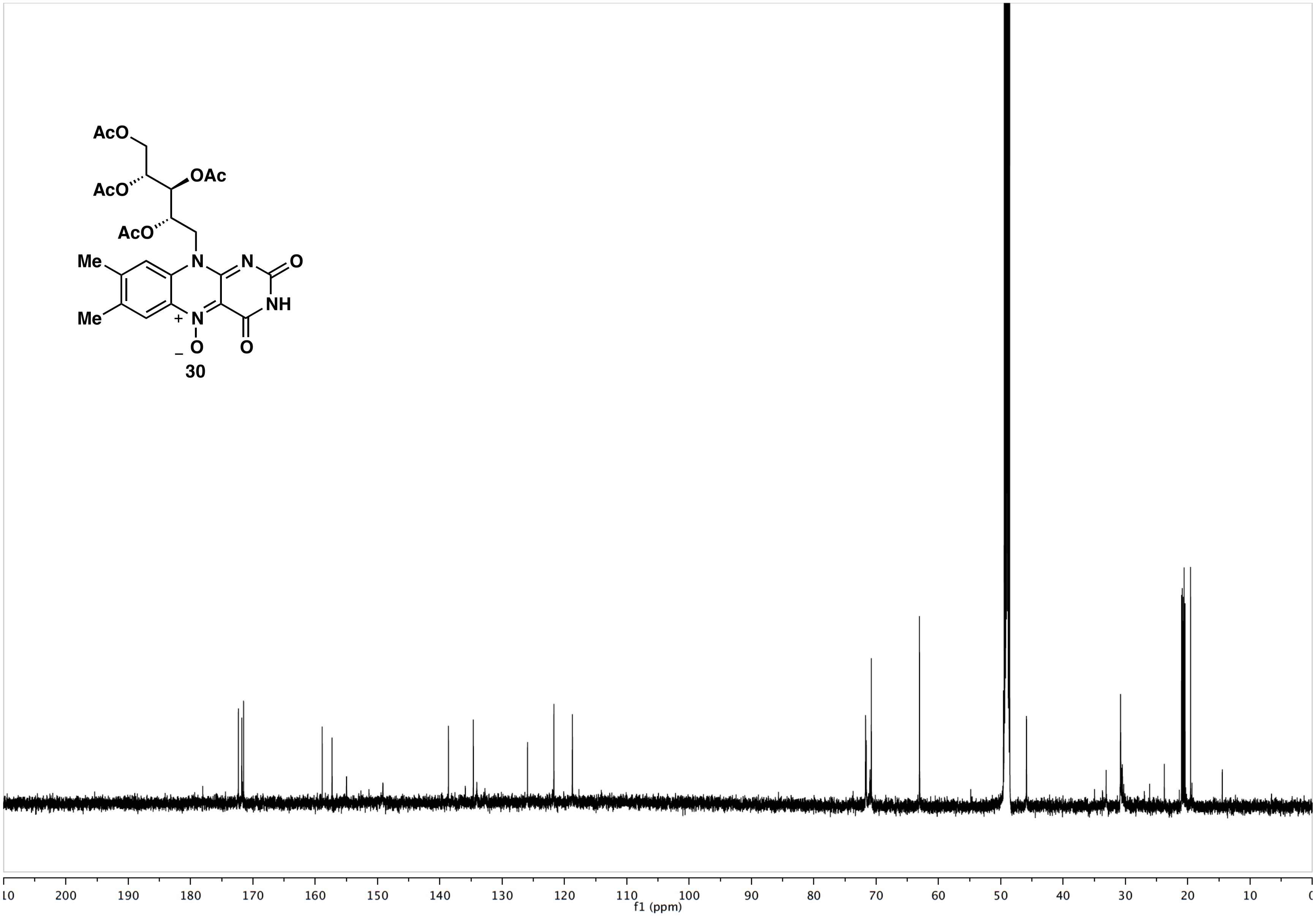
O
OAc
Ph
O
O
CO2Me
Me
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)


O
OAc
Ph
O
CO2Me
O
Me
25
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



30

AcO
AcO
OAc
AcO
Me
Me
N
N
O
NH
N
O
O
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.
f1 (ppm)



30

AcO
AcO
OAc
AcO
Me
Me
N
N
O
NH
N
+
–
O
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)