

Quaternary Charge-Transfer Complex Enables Photoenzymatic Intermolecular Hydroalkylation of Olefins

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EXPERIMENTAL PROCEDURES

General. Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers and used as received (Sigma-Aldrich, Oakwood Chemical, Combi-Blocks, Chem-Impex, and Acros Chemicals). GDH-105 was purchased from Codexis as cell free lysate and used as received. Silica gel chromatography purifications were carried out using AMD Silica Gel 60. ^1H - and ^{13}C - NMR spectra were recorded on a Bruker UltraShield Plus (500 and 125 MHz, respectively) instrument, and are internally referenced to residual proton signals in CDCl_3 (7.26 ppm). Data for ^1H -NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift relative to CDCl_3 (77 ppm). High resolution mass spectra were obtained on an Agilent 6220 LC/MS with an electrospray ionization time-of-flight (ESI-TOF) detector. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and peaks are reported in terms of frequency of absorption (cm^{-1})

Chromatography. Analytical high-performance liquid chromatography (HPLC) was carried out using an Agilent 1260 Infinity LCMS System. Analytical chiral SFC was done using a JASCO

Cloning. pET22b(+) and pET15a were used as a cloning and expression vector for all enzymes described in this study. Genes for 'ene' reductase enzymes were purchased as gBlocks from IDT and cloned using Gibson Cloning.¹ All genes were cloned between the NdeI and XhoI restriction sites and contained an N-terminal (GluER) or C-terminal (NostocER) 6xHis tag. Cloning for each construct was carried out using BL21 E. coli.

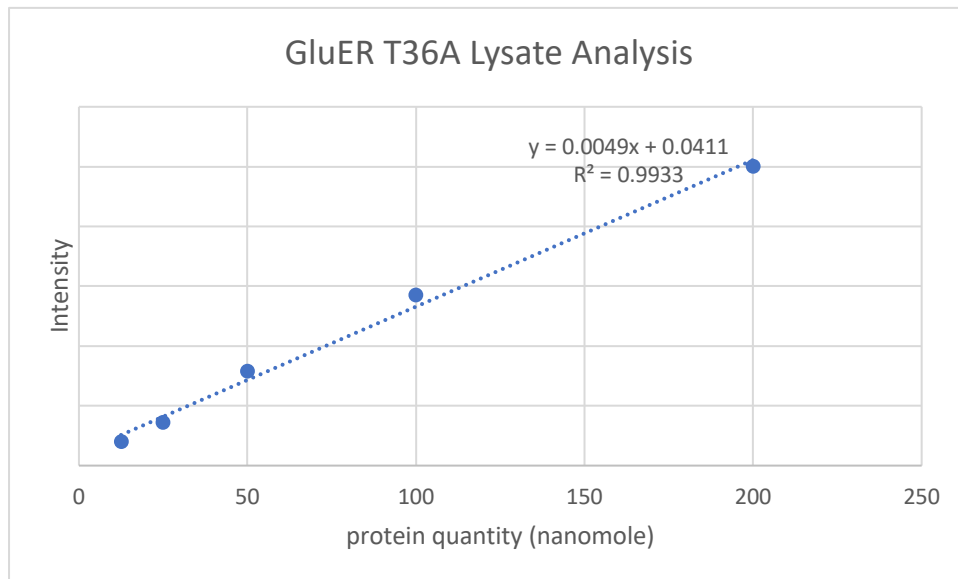
Protein Expression and Purification. Enzymes used in purified protein experiments were expressed in BL21(DE3) E. coli cultures transformed with plasmid encoding ERED variants. Transformed glycerol stocks were used to initiate 10 mL overnight cultures (37 °C, 250 rpm). Expression cultures (500 mL of Turbo Broth with ampicillin (100 $\mu\text{g}/\text{mL}$ final concentration) in a 2L flask) were inoculated with 1-2 ml of the overnight culture and grown to $\text{OD}_{600} = 0.6$ (37 °C, 250 rpm). Once the cell cultures reached an OD of 0.6 they were chilled on ice for 15 minutes prior to the addition of IPTG. For NostocER, expression was induced with 0.1 mM IPTG (24 h 25 °C 250 rpm). Following expression, cells were pelleted and frozen at -80 °C for storage. GluER T36A was expressed using the addition of 4% (v/v) auto inducing mix (sterile filtered mixture of 1.25% glucose, 5% lactose and 15% glycerol). The pellets were kept at -80 °C for at least 24 hrs before thawing for purification. For purification, frozen cells were thawed in ice-cold water and resuspended in buffer A (for GluER: 50 mM TEOA 25 mM imidazole pH 7.0, for all other proteins reported herein: 20 mM KPi, 300 mM NaCl, 30 mM imidazole, pH 7.0). Lysozyme (1 mg/mL), DNase (0.1 mg/mL), FMN (1 mg/mL), and PMSF (1 mg/mL, added as a 35 mg/mL solution in absolute ethanol) were added to the resuspended cells, followed by shaking at room temperature for 30 minutes. The resuspended cells were disrupted by sonication (2 x 4 min, output control 5, 35% duty cycle; Sonicator QSonica Q500 Ultra Sonicator). To pellet insoluble material, lysates were centrifuged at 14,000 x g for 1.5 h at 4

°C. Proteins were purified using a nickel NTA column (5 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTASart purifier FPLC system (GE healthcare). ERED enzymes were eluted with 100 % buffer B (for all proteins reported herein: 20 mM KPi, 300 mM NaCl, 250 mM imidazole pH 7.0) over 5 column volumes. Fractions containing enzyme were pooled, concentrated, and subjected to three exchanges with no-imidazole buffer (for GluER: 50 mM triethanolamine (TEOA), 10% glycerol, pH=7.0, for all other EREDs reported: 20 mM KPi, 300 mM NaCl, pH 7.0) to remove excess salt and imidazole. Concentrated (1.0-1.5 mM) proteins were aliquoted, flash-frozen in liquid N₂, and stored at -80 °C until later use. Protein concentration was determined by A₄₆₅ with calculated extinction coefficients. All proteins other than GluER were used as aliquots pre-expressed and purified according to the procedures detailed in previously published work from the Hyster lab.²

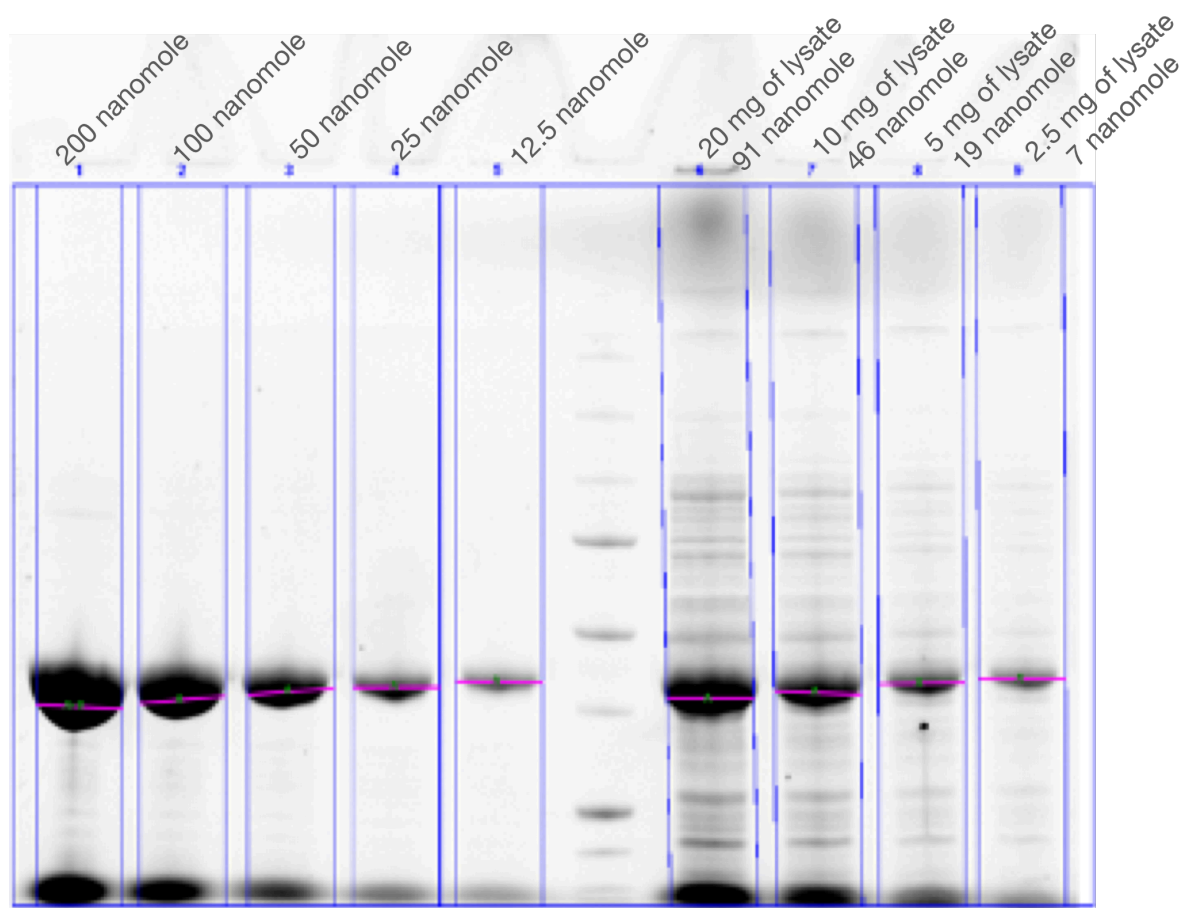
Determination of Extinction Coefficients. Extinction Coefficients for ERED enzymes were calculated based on the extinction coefficient ($12.2 \times 10^{-3} \text{ M}^{-1}\text{cm}^{-1}$ at 464 nm) for free FMN released after protein denaturation (i). Extinction coefficient for NCR: $\epsilon_{464} = 10.5 \times 10^{-3} \text{ M}^{-1}\text{cm}^{-1}$, NostocER: $\epsilon_{461} = 10.6 \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$, OPR: $\epsilon_{464} = 11.1 \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$

Preparation of T36A GluER Lysate. The cells were resuspended in 50 mM TEOA pH = 7 and frozen. They were then thawed and lysozyme (1 mg/mL), DNase (0.1 mg/mL), FMN (1 mg/mL), and PMSF (1 mg/mL, was added as a 35 mg/mL solution in absolute ethanol) were added to the resuspended cells, followed by shaking at room temperature for 30 minutes. The resuspended cells were disrupted by sonication (2 x 4 min, output control 5, 35% duty cycle; Sonicator QSonica Q500 Ultra Sonicator). To pellet insoluble material, lysates were centrifuged at 14,000 x g for 1.5 h at 4 °C. Lysates were aliquoted into 50 mL Falcon tubes (5 mL each) and flash-frozen in liquid N₂. The frozen lysates were lyophilized for 24 h at room temperature. On average, 5 g of lysate was obtained from 4 L Turbo Broth expression media (Induced with autoinducing mix). The lysates were quantified by Stain-Free SDS page gel with a standard curve of purified GluER T36A of a known concentration.

Standard curve:



Protein Gel:



SEQUENCE INFORMATION

Ene-reductase 1 from *Nostoc* sp. PCC7120 (NostocER1)

NostocER1 DNA Sequence

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ATGAGTGATGAAGCGGAGCGCCAACGTGGAAATAATCTTTACAAAAATTCGCCCCCTTCT
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```

NostocER1 amino acid sequence

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SQRVGVRLSPSGTFNDIRDSHPLETFGYVAQALNRFNLSYLHIFEADADIRHGGTVPTSHLRDRF
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TDYPFLAVANKLEHHHHHH
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'Ene'-reductase from *Glucanobacter* Oxydans with mutated T36A (GluER T36A)

GluER T36A DNA sequence

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ATGTCCCCCTGACTCGCGGTGCGGCTGACAAAGAGGCGGTTCCAGCTCCCATATGGC
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CACGCGAAGGCTTAGGTTGGCCGTTTGCGCCGGGAATTTGGTCCGATGCACAGGTTGA
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CATCCGCAACTTCTGGGCCGAACAAT

GluER T36A amino acid sequence

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ERFARGIALQPDDMKTWYSQGPEGYTDYPSATSGPNN

LacER from *Lactobacillus casei* str. Zhang

LacER DNA Sequence

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LacER Protein Sequence

MSGYHFLKPFTFKHQITITLKNRIVIPPMTTRLSFEDGTVTRDEIRYYQQRAGGVGMFITGTANVNA
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Morphinone reductase (MorB)

Genbank Accession Number: AAC43569

DNA Sequence

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Protein Sequence

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Old Yellow Enzyme 1 (OYE1) (GenBank Accession Code: >sp|Q02899|OYE1_SACPS NADPH
 dehydrogenase 1 OS=Saccharomyces pastorianus GN=OYE1)
 Protein Sequence

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DNA Sequence

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Ene-reductase from *Yersenia bercovieri* (YersER)

NCBI Reference Sequence: WP_032896199.1

Protein sequence:

MKTAKLFSPLKVGALTLPNRVFMAPLTRLSIEPGDIPTPLMAEYYRQRASAGLIITEATQISFOAK
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DNA sequence:
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Ene-reductase from *Cupriavidus metallidurans* (RmER)

NCBI Reference Sequence: WP_011519282.1

Protein sequence:

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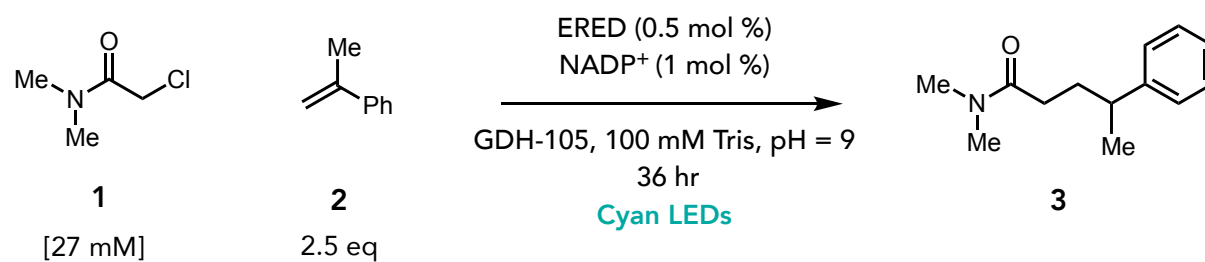
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CGGTCCCAACCCAGAGGGTTAGAGAACTTTTCAAGGACGCGCACTTCGGCCAAAGAC
TCGAGCACCACCATCACCACCACTGA

LacER		-----MSGYHFLKPFTHKHQTITLKNR	22
RmER		-----MPHLFDPYR--IGNLELANR	18
OYE1		-----MS----FVKDFKPQALGDTNLFKPIK--IGNNELHR	31
GluER		-----MPTLFDPID--FGPIHAKNR	18
NostocER	MSDEAERQRGNLYKNSPLLPSISQVSTSOLRETEIMSTNINLFSSYQ--LGELELPNR		58
MorB		-----MPDTSFSNPGLFTPLQ--LGSLSLPNR	25
YersER		-----MKTAKLSPLK--VGALTLPNR	20
		: : *	
LacER	IVIPPMTRLRSFEDGTVTTRDE--IRYYQQRAG-GVGMFITGTANVNALGKGFELEGSVAD		79
RmER	IATAPMCQYSA-QEGN--ATDWHMIHLGQMALSAGALLIIEATAVSPEGRIPTPTDLGLYN		75
OYE1	AVIPPLTRMRALHPGNIPNRDWAVEYYTQRAQRPGMTMITTEGAFISPQAGGYDNAPGVWS		91
GluER	IVMSPLTRGRA-DKEAVPAPIM-AEYYAQRAS--AGLIITEATGISREGLGWPPFAPGWLS		74
NostocER	IVMAPLTRQRA-GEENVPHQLN-AIYYQGRAS--AGLIIEATEATQVTPQGQGYPHTPGIHS		114
MorB	VIMAPLTRSR--TPDSVPGRLO-QIYYQGRAS--AGLIISEATNISPTARGYVYTPGIWT		80
YersER	VFMAPLTRLSIEPGDIPTPLM-AEYYRQGRAS--AGLIITEATQISFQAKGYAGAPGLHT		77
	:	* :	
LacER	DRFIPGLSKLAAAMKTGGT-KAILQIFSAGRMSNSKIILRGE-----QPVSASA		126
RmER	DANEAALGRVLGAVERNHSPIAVTIQLAHAGRKASSEAPWDGGGQIRPDQPRGWTFAPSA		135
OYE1	EEQMVEWTKIFNAIHEKKSFVWVQLWLWGWAFFPDNLARDG-----LRYDS--		137
GluER	DAQVEAWKPIVAGVHAKGG-KIVCQLWHMGRMVHSSVT--GT-----QPVSSSA		120
NostocER	PEQVAGWKLVTDTVHQGG-RIFLQLWHVGRISHPDLPDGG-----LPVAPSA		162
MorB	DAQEAGWKGVVEAVHAKGG-RIALQLWHVGRVSHELVPDGO-----QPVAPSA		128
YersER	QEQLNAWKKITQAVHEEGG-HIAVQLWHVGRISHSSLQPGQQ-----APVAPSA		125
	:	:	*
LacER	VAA-----PRAGYETPRALTSAEIEATHDFGQAVRRAILAGFDGIELHGA		172
RmER	VPH-----AAGEVPPAALDKAGMKKIRDFFVAACKRAARLGIEGIEVHGA		180
OYE1	---ASDNVFMDAEQEAkakannpqhsLTkdeIKQYIKEYVQAakNsIAAGADgVeIHsa		194
GluER	TTAPGEVHTY-----EGKKPFQARAIDAADISRILNDYENAAARNAIRAGFDGVQIHAA		174
NostocER	IAPKGEVLTY-----EGKKPYVTPRALDTSEIPAIVEQYRQGAANALAAGFDGVEIHAA		216
MorB	LKAEGAECFVEFEDGTAGLHPTSTPRALETDEIPGIVEDYRQAAQRAKRAGFDMVEVHAA		188
YersER	IAADTRT-TVRDENGAWVRVPCSTPRALETEEIPGIINDFRQATANAREAGFDYIELHAA		184
	:	:	*
LacER	NTYLIOQQFYSPNSNRRTDEWGGDRDKRMRFPLAVVHEAEKVATIADRPFLLG-YRISPE		231
RmER	HGYLLHQFLSPIANHRTDEYGGSGLENRMRFPLEVDFDAVREAFP--ER---PVWMRVSAT		235
OYE1	NGYLLNQFLDPHSNTRTDEYGGSIENRARFTLEVVDALVEAIGH--EK---VG-LRLSPY		248
GluER	NGYLIDEFLRNGTNHRTDEYGGVPENIRFLKEVTERVIAAIGA--DR---TG-VRLSPN		228
NostocER	NGYLIDQFLRDGTNQRDTDEYGGAIENRARLLLEVTEAITSVWDS--QR---VG-VRLSPS		270
MorB	NACLPNQFLATGTNRRTDQYGGSIENRARFPLEVVDAAEVFGP--ER---VG-IRLTPF		242
YersER	HGYLLHQFMSPASNQRDTQYGGSIENRRTLLEVVDATAAQWSA--ER---IG-IRISPL		238
	:	*	:
LacER	ELEQPGI-----TLDDTLALIDLALKQT-----KIDYLHVSQSDVWRVTSR--NPEDTAI		278
RmER	DWV---PNGWDIE---GTIALSHELKAR-----GSAAVHVSTGGVSPQQAIIKIGPGYQVP		284
OYE1	GVFNMSMGGAETGIVAQYAYVAGELEKRAKAGKRLAFVHLVEPRVTNPFLTETEGETEYEGG		308
GluER	GDTQGCIDSAPET--VFVPAAKLLQDL-----GVAWLELREPGPNGTFGK---TDQPK		276
NostocER	GTFNDIRDSHPLE--TFGYVAQALNRF-----NLSYLHIFEADADIRH-----GDTV		317
MorB	LELFGLTDDEPEA--MAFYLAGELDRR-----GLAYLHFNEPDWIGGD-----TYPEG		289
YersER	GFNFGLDNGEDQEE--AALYLIDELNKR-----HIAYLHISEPDWAGGK-----PYSEA		285
	*	:	
LacER	MNEQIRDHVAGAFFVIIVGGIKTPAD--AEKAAESFDLVAIGHEMIREPHWVQKVLDH--		334
RmER	YAQRVKAIEVGL--PTMAVGLITEAEQAEAIIANNEADIISIARAMLYDPRWPWHAAAKLG		342
OYE1	SNDFFVYSIWKG--PVIRAGNFALHPEVVREEVKDKRTLIGYGRFFISNPDLVDRLEKGLP		366
GluER	LSPQIRKVFLR--PLVLNQDYTFEAAQTA-LAEGKADAIFAGRKFISNPDLPERFARGIA		333
NostocER	PTSHLRDRFTG--TLIVNGGYTREKGDVT-IANKAADLFAVGRKTLFISNPDLPERFVNAP		374
MorB	FREQMRQRFKG--GLIYCGNYDAGRAQAR-LDDNTADAVAFGRPFIANPDLPERFRLGAA		346
YersER	FRDAVRARFKG--VIIGAGAYTAEKAEEL-IEKGFIDAVAFGRSYISNPDLVARLQQHAP		342
	:	:	*
LacER	---DEKAIRYQIAPADLEEELGI---APTFLDFIESISGGAKGVPLTTAQSVTSSNVTDQ		387
RmER	ASVNAPKQYWRSQPRGLEKLFKDAH-----FGQR-----		371
OYE1	LNKYDRDTFYOMSAHGYYIDPTYEEALK-LGWDKK-----		401

GluER	LQPDDMKTWYSQGPEGYTDYPSATSGPNN-----	362
NostocER	LNQADPTTFYGGGEKGYTDYPFLAVANK-----	402
MorB	LNEPDPSTFYGGAEVGYTDYPFLDNHDLRG-----	377
YersER	LNEPDGETFYGGGAKGYTDYPTL-----	365

Table S1. Initial panel of EREDs screened

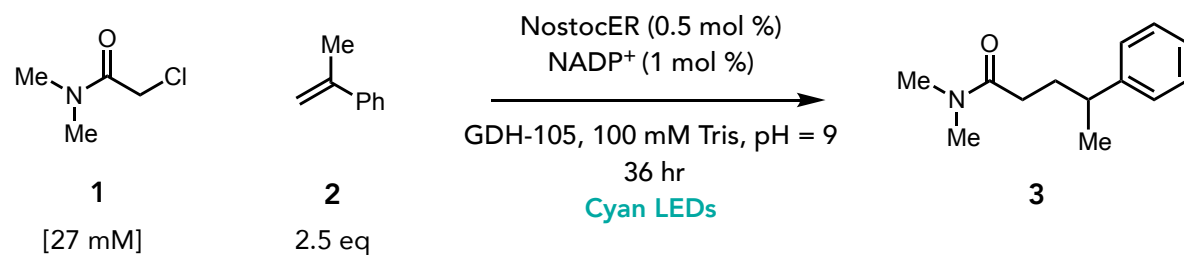


ERED	yield ^a	e.r. ^b
MorB	57%	56:44
OYE1	3%	n.d.
LacER	6%	n.d.
YersER	30%	11:89
RmER	<1%	n.d.
NostocER	37%	10:90
GluER T36A*	80%	97:3

*Reaction run for 24 hrs with 3.5 equivalents of styrene

a) yield determined via LCMS standard curve b) enantiomeric ratio determined via chiral HPLC

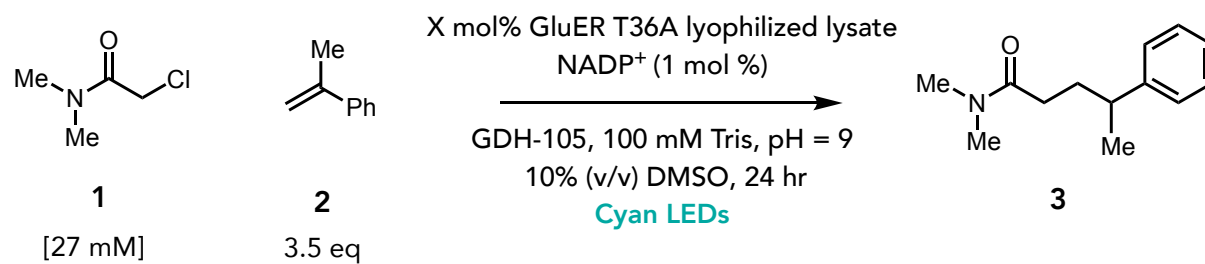
Table S2. Yield optimization studies



optimized condition	yield ^a
standard	37%
with 3.5 eq of α -methylstyrene	50%
with 3.5 eq of α -methylstyrene and 10% (v/v) DMSO	60%
with 3.5 eq of α -methylstyrene and 10% (v/v) DMSO for 24 hours	65%
with 3.5 eq of α -methylstyrene and 10% (v/v) DMSO and 1 mol% NostocER for 24 hours	98%

a) yield determined by LCMS standard curve

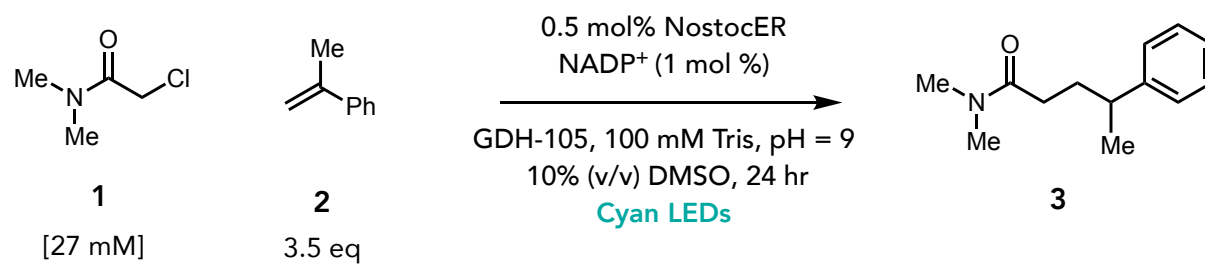
Table S3. Lysate loading screen



X mol%	yield ^a	e.r. ^b
0.25	50%	>99:1
0.5	70%	>99:1
0.75	58%	>99:1
1.0	60%	>99:1

a) yield determined by LCMS standard curve b) enantiomeric ratio determined by chiral HPLC

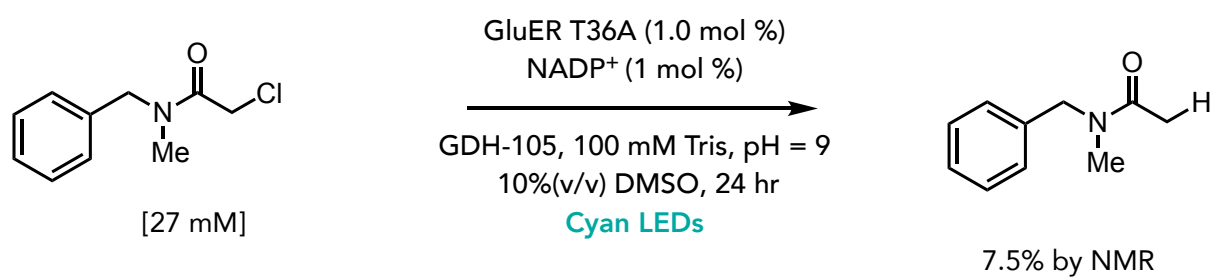
Table S4: Relevant Controls



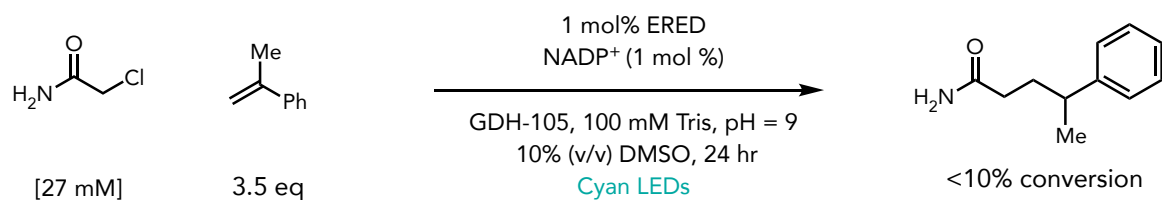
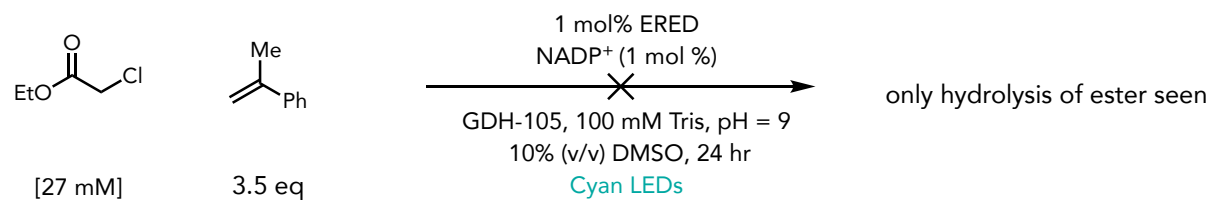
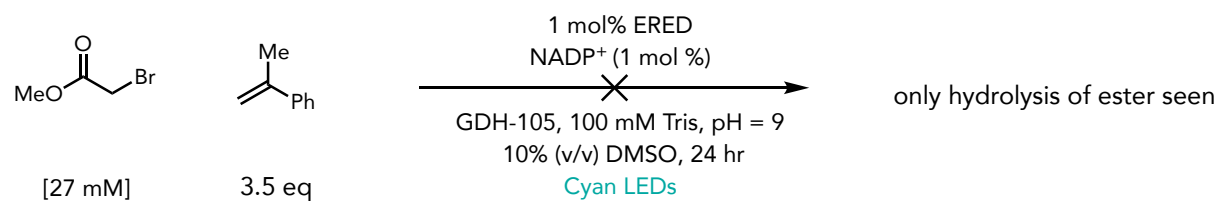
control condition	yield ^a
no enzyme	no reaction
no light	no reaction
no NADP+	no reaction
no GDH	no reaction
no glucose	no reaction
0.5 mol % FMN instead of enzyme	no reaction

a) yield determined via NMR analysis against an internal standard

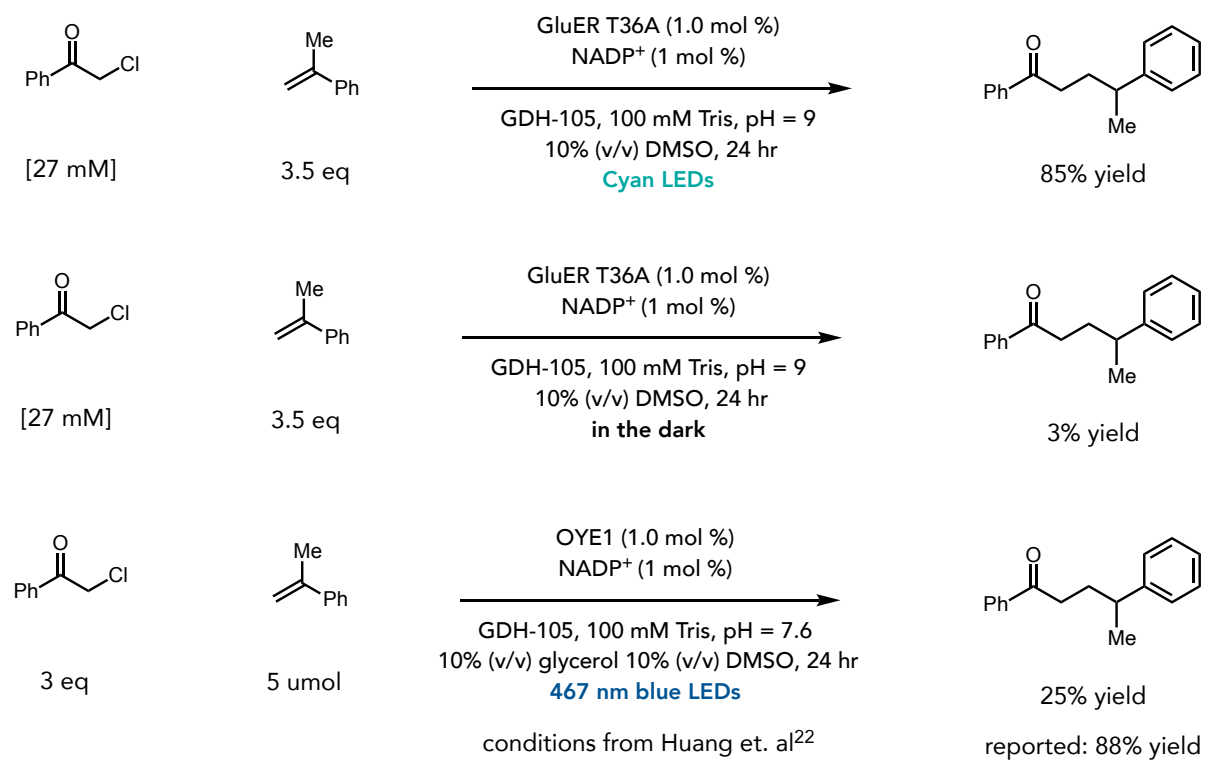
Supplemental Figure 1: Irradiation of chloroamide without alkene



Supplemental Figure 2: Less Reactive Substrates



Supplemental Figure 3: α -chloroacetophenone controls



GENERAL SYNTHESIS PROCEDURES

The α -halocarbonyl substrates were purchased from the commercial sources mentioned before. If not, they were synthesized via these general procedures.

α -chloroamide substrates:

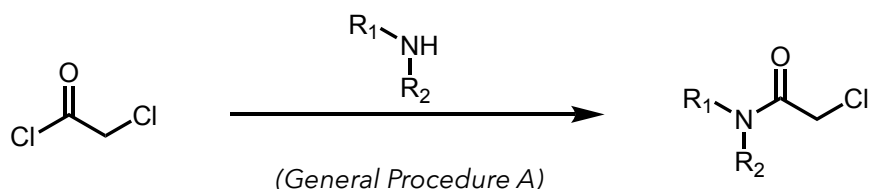


Figure S1. Synthetic scheme for the synthesis of α -chloro amide starting materials

Acylation (General Procedure A):

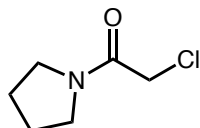
The secondary amine (1 equiv.) is added to a flame dried flask under N_2 pressure containing dry DCM and triethylamine (1.5 equiv.) for a final concentration of 0.25 M for the secondary amine. The chloroacetylchloride purchased from Sigma (1.2 equivalents) is added dropwise to the stirred solution at room temperature. The solution is stirred overnight and then poured into a separatory funnel containing a 1:1 solution of 10% HCl and DCM. The aqueous layer is extracted with DCM. The organic layers are collected, washed with brine, dried with anhydrous Na_2SO_4 and concentrated *in vacuo* to yield a crude chloroamide as an oil. The crude oil is purified via silica gel flash chromatography in a gradient of 12 % EtOAc/Hexanes to 60% EtOAc/Hexanes.

α -substituted styrenes:

If not listed here, the alkenes used in this paper were purchased from the aforementioned sources.

CHARACTERIZATION OF α -CHLORO AMIDES

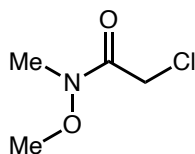
2-chloro-1-(pyrrolidin-1-yl)ethan-1-one (5)



Synthesized using General Procedure A from commercially available pyrrolidine and 2-chloroacetyl chloride. Yield: 67% All spectra match reported values.³

¹H NMR (500 MHz, CDCl₃) δ 4.02 (s, 2H), 3.52 (q, J = 7.2 Hz, 4H), 2.00 (p, J = 6.8 Hz, 2H), 1.89 (p, J = 6.9 Hz, 2H).

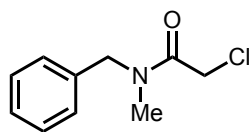
2-chloro-*N*-methoxy-*N*-methylacetamide (6)



Synthesized using General Procedure A from commercially available *N*-methyl,*N*-methoxyamine hydrochloride salt and 2-chloroacetyl chloride. Yield: 61% All spectra match reported values.⁴

¹H NMR (500 MHz, CDCl₃) δ 4.25 (s, 2H), 3.75 (s, 3H), 3.23 (s, 3H).

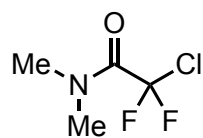
N-benzyl-2-chloro-*N*-methylacetamide (7)



Synthesized using modified General Procedure A (using 3 equivalents of K₂CO₃ instead of triethylamine) from commercially available Benzylmethylamine purchased from Sigma. Yield: 98% All spectra match reported values.⁵

¹H NMR (500 MHz, CDCl₃) δ 7.41 – 7.27 (m, 3H), 7.25 (d, J = 6.9 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 4.61 (s, 2H), 4.15 (s, 1H), 4.11 (s, 1H), 3.00 (s, 2H), 2.98 (s, 1H).

2-chloro-2,2-difluoro-*N,N*-dimethylacetamide (8)



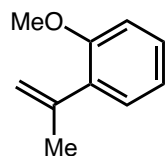
Synthesized through a procedure found in Tang et al using commercially available chlorodifluoroacetic acid and dimethylamine.⁶ Spectra match reported values from Ge et al.⁷
Yield: 55%

¹H NMR (500 MHz, CDCl₃) δ 3.19 (s, 3H), 3.07 (s, 3H).

Characterization of alkene coupling partners

If not listed, the alkenes were purchased from sources listed above and used as purchased.

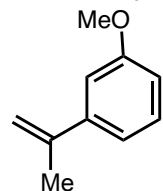
1-methoxy-2-(prop-1-en-2-yl)benzene (9a)



The alkene was synthesized according to a procedure given in Pratsch et al.⁸ All spectra match reported values for this substrate.

¹H NMR (500 MHz, CDCl₃) **δ** 7.21 (ddd, *J* = 24.4, 7.6, 1.8 Hz, 2H), 6.97 – 6.85 (m, 2H), 5.15 (d, *J* = 1.9 Hz, 1H), 5.06 (d, *J* = 1.2 Hz, 1H), 3.84 (s, 3H), 2.12 (s, 3H).

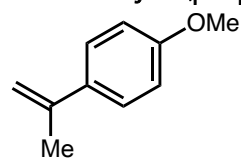
1-methoxy-3-(prop-1-en-2-yl)benzene (9b)



The alkene was synthesized according to a procedure given in Pratsch et al.⁸ All spectra match reported values for this substrate.

¹H NMR (500 MHz, CDCl₃) **δ** 7.24 (d, *J* = 8.0 Hz, 1H), 7.07 (dt, *J* = 7.8, 1.2 Hz, 1H), 7.02 – 6.99 (m, 1H), 6.82 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 5.39 – 5.34 (d, *J* = 1.2 Hz, 1H), 5.09 (d, *J* = 1.5 Hz, 1H), 3.83 (s, 3H), 2.17 (s, 3H).

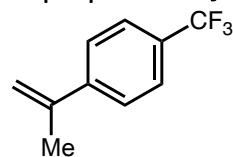
1-methoxy-4-(prop-1-en-2-yl)benzene (9c)



The alkene was synthesized according to a procedure given in Pratsch et al.⁸ All spectra match reported values for this substrate.

¹H NMR (500 MHz, CDCl₃) **δ** 7.42 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 5.29 (s, 1H), 4.99 (s, 1H), 3.82 (s, 3H), 2.17 – 2.10 (m, 3H).

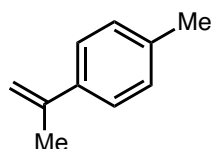
1-(prop-1-en-2-yl)-4-(trifluoromethyl)benzene (9d)



Synthesized according to a procedure detailed by Huang et al.⁹ All spectra match reported values.

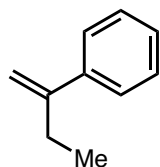
¹H NMR (500 MHz, CDCl₃) δ 7.66 – 7.49 (m, 4H), 5.44 (s, 1H), 5.20 (s, 1H), 2.17 (s, 3H).

1-(prop-1-en-2-yl)-4-(methyl)benzene (9e)



Synthesized according to a procedure detailed by Pratch et al.⁸ All spectra match reported values.

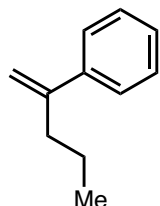
but-1-en-2-ylbenzene (10a)



The alkene was synthesized according to a procedure given in Pratsch et al.⁸ All spectra match reported values for this substrate.

¹H NMR (500 MHz, CDCl₃) δ 7.46 – 7.38 (m, 2H), 7.36 – 7.29 (m, 2H), 7.30 – 7.23 (m, 2H), 5.28 (d, *J* = 1.2 Hz, 1H), 5.06 (s, *J* = 1.5 Hz, 1H), 2.56 – 2.46 (m, 2H), 1.11 (t, *J* = 7.4 Hz, 3H).

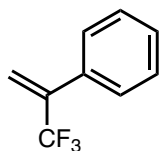
pent-1-en-2-ylbenzene (10b)



The alkene was synthesized according to a procedure in Molloy et al.¹⁰ All spectra match reported values for this substrate.

¹H NMR (500 MHz, CDCl₃) δ 7.45 – 7.38 (m, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.24 (m, 1H), 5.27 (s, 1H), 5.06 (s, 1H), 2.53 – 2.42 (m, 2H), 1.48 (dt, *J* = 14.9, 7.4 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H).

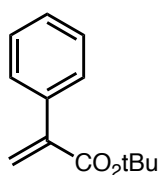
(3,3,3-trifluoroprop-1-en-2-yl)benzene (10c)



The alkene was synthesized according to a procedure in Trost et al.¹¹ All spectra match reported values for this substrate.¹²

¹H NMR (500 MHz, CDCl₃) δ 7.50 – 7.39 (m, 4H), 7.39 (q, J = 2.8 Hz, 5H), 5.96 (d, J = 1.6 Hz, 2H), 5.77 (q, J = 1.8 Hz, 2H).

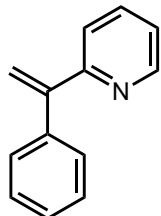
tert-butyl 2-phenylacrylate (11)



The alkene was synthesized according to a procedure in Wang et al.¹³ All spectra match reported values for this substrate.

¹H NMR (500 MHz, CDCl₃) δ 7.44 – 7.38 (m, 2H), 7.38 – 7.28 (m, 3H), 6.25 (d, J = 1.4 Hz, 1H), 5.82 (d, J = 1.4 Hz, 1H), 1.53 (s, 9H), 1.47 (s, 1H).

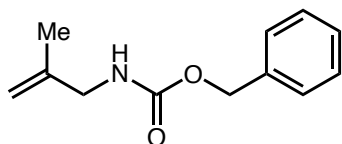
2-(1-phenylvinyl)pyridine (12)



The alkene was synthesized according to a procedure given in Nakano et al.¹⁴ All spectra match reported values.

¹H NMR (500 MHz, CDCl₃) δ 8.67 – 8.62 (m, 1H), 7.64 (td, J = 7.7, 1.9 Hz, 1H), 7.36 (s, 3H), 7.40 – 7.25 (m, 4H), 7.21 (ddd, J = 7.5, 4.9, 1.2 Hz, 1H), 6.00 (d, J = 1.4 Hz, 1H), 5.61 (d, J = 1.5 Hz, 1H)

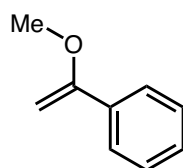
benzyl (2-methylallyl)carbamate (13)



The alkene was synthesized by suspending commercially available 2-methylprop-2-en-amine (1 equiv) in dry DCM with triethylamine (4 equiv). 2 equivalents of benzyl chloroformate was added dropwise at 0°C. The mixture was warmed to room temperature and left to stir overnight. The mixture was diluted with more dichloromethane and washed with saturated sodium bicarbonate and then brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo* to afford a crude oil which was purified via silica gel chromatography (10% to 20% EtoAC in hexanes gradient) to afford the product as a clear oil in 53% yield. The spectra match previously reported values in Sorimachi et al.¹⁵

¹H NMR (500 MHz, CDCl₃) δ 7.40 – 7.29 (m, 5H), 7.25 (d, *J* = 9.9 Hz, 1H), 5.12 (s, 2H), 4.84 (d, *J* = 8.5 Hz, 3H), 3.75 (d, *J* = 6.7 Hz, 2H), 1.74 (s, 3H).

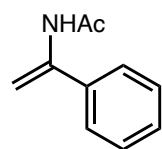
(1-methoxyvinyl)benzene (14)



Synthesized via a procedure found in Willmore et al.¹⁶ The yield was 86%. All spectra match reported values.

¹H NMR (500 MHz, CDCl₃) δ 7.65 – 7.59 (m, 2H), 7.37 – 7.25 (m, 3H), 4.66 (d, *J* = 2.9 Hz, 1H), 4.22 (d, *J* = 3.0 Hz, 1H), 3.75 (s, 3H).

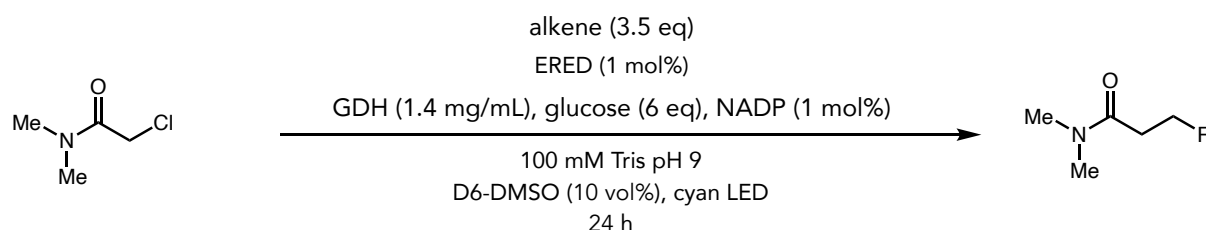
N-(1-phenylvinyl)acetamide (15)



Commercially available 1-phenylethanone oxime (8 mmol, 1 equiv), cuprous iodide (0.1 equiv), sodium bisulfite (3 equiv) and acetic anhydride (2 equiv) was added to dry 1,2-chloroethan under nitrogen atmosphere in a pressure sealed vessel. The reaction was heated at 120°C for 12 hours and monitored by TLC. Once the reaction was completed by TLC, the mixture was diluted with ethyl acetate and then the organic layer was washed with 2N NaOH then brine. The organic layer was dried over sodium sulfate and concentrated. The crude oil was purified by silica flash chromatography to yield a pure colorless oil. The spectra match reported values in Ackermann et al.¹⁷

¹H NMR (500 MHz, CDCl₃) δ 7.43 (d, *J* = 7.2 Hz, 2H), 7.41 – 7.32 (m, 3H), 6.74 (s, 1H), 5.89 (s, 1H), 5.09 (s, 1H), 2.15 (s, 3H).

GENERAL PROCEDURE FOR BIOCATALYTIC RADICAL HYDROALKYLATION



General Procedure for NMR Yield:

All reactions were set up in a Coy® Chamber. Reactions were run with 20 μmol of chloroamide starting material at a final substrate concentration of 27 mM in duplicate. A shell vial with a magnetic cross stir bar was charged with a “master mix” solution of 6 equivalents of (D)-glucose dissolved in 100 mM Tris pH 9 in a volume such that the final concentration of substrate would be 27 mM after addition of all the other reagents. d_6 -DMSO (deuterated DMSO allowed for simplified NMR analysis) was added to the shell vial such that it would be 10% (v/v). To this solution, 200 μL of a 5 mg/mL GDH solution was added. Then 28 μL of a 5 mg/mL solution of NADP⁺ was added. Finally, 1 mol% of ERED was added. Neat chloroamide was then added to the shell vial, followed by alkene. Then the alkene was added. Both reagents were added neat if the density was known. If the density of either of the substrates was not known, they were added as stock solutions in d_6 -DMSO. The vial was sealed with a rubber septa and brought out of the Coy® Chamber where it is placed on a stir plate at 300 rpm under a fan and irradiated with cyan LEDs (50 W Chanzon high power LED chip, $\lambda_{\text{max}} = 490 \text{ nm}$, measured photon flux = 12,000 $\text{mM}/\text{m}^2 \text{ s}$) for 24 hours.

After 24 hours, 6.667 μmol of internal standard (1,3,5-trimethoxybenzene) was added from a stock solution in CDCl_3 or ethyl acetate. Then an equal volume of ether or ethyl acetate was added and the reactions were stirred for 30 minutes. They were centrifuged at 2000 $\times g$ for 2 minutes and organic layer removed. The extraction process was repeated 3-5 times. The organic layers were pooled and concentrated *in vacuo* and then resuspended in CDCl_3 and concentrated *in vacuo* again. The dried crude mixture was resuspended in CDCl_3 and analyzed by ^1H -NMR for yield calculation, where the singlet at $\delta = 6.06 \text{ ppm}$ corresponding to the three aromatic protons of 1,3,5-trimethoxybenzene was set to an integration of 1.00. Integration of varying signals of different product could be used to calculate an effective yield. Following NMR analysis, the CDCl_3 solution was reconcentrated and dissolved in 2% HPLC grade IPA in HPLC hexanes for chiral HPLC analysis.

Photo: NMR yield scale reaction set up



General Procedure for isolated yield:

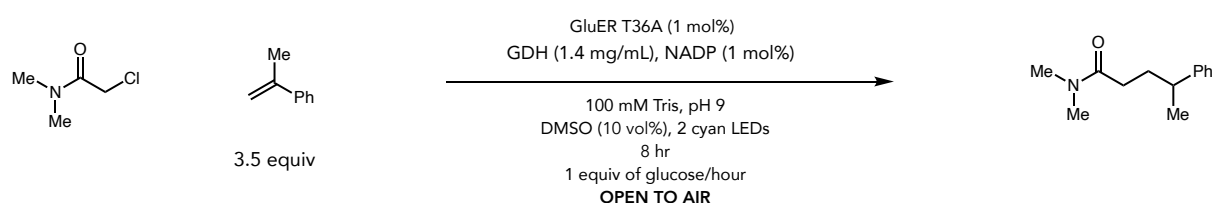
All reactions were set up in an anaerobic Coy® Chamber. They were run with 200 μmol of chloroamide starting material at a final substrate concentration of 27 mM in duplicate. A 25 mL pear-shaped flask with a stir bar was charged with a “master mix” solution of 6 equivalents of (D)-glucose dissolved in 100 mM Tris pH 9 in a volume such that the final concentration of substrate would be 27 mM after addition of all the other reagents. DMSO was added to the flask such that it would be 10% (v/v) of the final volume. To this solution, 2 μL of a 5 mg/mL GDH solution was added. Then 280 μL of a 5 mg/mL solution of NADP⁺ was added. Finally, 1 mol% of ERED was added. The chloroamide was then added to the flask neat. Then the alkene was added. Both reagents were added neat if the density was known. If the density of either of the substrates was not known, it was added as a stock solution in DMSO (70 μmol alkene/73.4 μL of DMSO). The vial was sealed with a rubber septa and brought out of the Coy® Chamber where it is placed on a stir plate at 300 rpm under a fan and immediately irradiated with cyan LEDs (50 W Chanzon high power LED chip, $\lambda_{\text{max}} = 490 \text{ nm}$, measured photon flux = 12,000 mm^2/s) for 24 hours.

Workup was performed as follows: the contents of the reaction flask were poured into a 125 mL Erlenmeyer flask containing 50 mL of 1 M aqueous hydrochloric acid and 50 mL of dichloromethane. This was stirred vigorously for 45 minutes, after which time the biphasic mixture was filtered through a thick pad of Celite® to remove precipitated protein. This pad was washed with dichloromethane (2 x 25 mL). The filtrate was poured into a separatory

funnel and the dichloromethane layer was collected. The aqueous layer was extracted with dichloromethane (2 x 50 mL) (without any emulsion-related difficulties), and the combined organic layers were dried with anhydrous sodium sulfate and concentrated. The crude residue was purified using silica gel chromatography (0 – 60% EtOAc in dichloromethane). Fractions containing product were combined and concentrated to yield the pure coupled product.

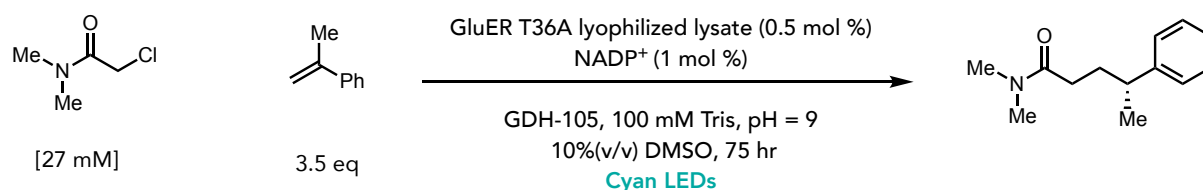
Note: When 2-isopropenylpyridine was used, addition of 1M HCl was avoided. Instead, the reactions were worked up by transferring the contents of the reaction flask to a 40 mL vial. In order to quantitatively transfer, the mostly empty reaction flask was washed with diethyl ether and water, and the contents were also transferred to the 40 mL vial. The biphasic mixture was shaken vigorously, centrifuged at 2000 xg for 2 minutes, and the organic layer was collected. This was repeated 5 times. The combined organic layers were dried with sodium sulfate, filtered, and concentrated. The crude residue was purified using silica gel chromatography (0 – 10% MeOH in dichloromethane). Fractions containing product were combined and concentrated to yield the pure coupled product.

General Procedure for experiment run open to air:



On the benchtop, a shell vial with a magnetic cross stir bar was charged a volume of degassed 100 mM Tris pH 9 such that the final concentration of substrate would be 27 mM after addition of all the other reagents. Degassed D6-DMSO (deuterated DMSO allowed for simplified NMR analysis) was added to the shell vial such that it would be 10% (v/v). To this solution, 200 μ L of a 5 mg/mL GDH solution was added. Then 28 μ L of a 5 mg/mL solution of NADP⁺ was added. Finally, 1 mol% of ERED was added. Neat chloroamide was then added to the shell vial, followed by alkene. Glucose was added one equivalent at a time until the solution turned clear and a rubber septa was placed on the vial with a needle outlet. The vial was placed on a stir plate at 300 rpm under a fan and irradiated with cyan LEDs (50 W Chanzon high power LED chip, λ_{max} = 490 nm, measured photon flux = 12,000 mW/m² s) for 8 hours. Every hour, one equivalent of glucose in 10 μ L of degassed buffer was added via Hamilton microsyringe. After 8 hours, the reactions were worked up according to the procedure for NMR analysis.

REPRESENTATIVE PROCEDURE ON GRAM-SCALE (MODEL SUBSTRATE RUN)



To a 1L round bottom flask was added a football shaped stir bar, (D)-glucose (8.9 g, 6 equiv.), GDH lyophilized lysate (411.3 mg), NADP⁺ (57.6 mg, approx. 1 mol%) and GluER-T36A lyophilized lysate (8.4 g, lysate concentration 4.9 nmol/mg, 0.5 mol%, 8.4 mg/mg chloroamide). The resulting flask and its contents were then taken into a Coy® anaerobic chamber. The contents of the flask were dissolved by the addition of reaction buffer (300 mL, 100 mM Tris HCl Buffer, 10% v/v DMSO, pH = 9). To the resulting yellow solution was added 2-chloro-N,N-dimethylacetamide (1.0 g, 0.846 mL, 1 equiv.) followed by α -methylstyrene (3.4 g, 3.74 mL, 3.5 equiv.). Once all the contents were added, the reaction vessel was capped with a rubber septum, and the seal was wrapped with electrical tape (see photos below). The reaction vessel was then removed from the anaerobic chamber and placed in a water dish connected to a chiller (set to 12 °C) with four surrounding 497 nm LEDs and stirred at 600 rpm. Irradiation was initiated and the reaction was allowed to stir under exposure to light for 75 hours, when LCMS showed mostly complete consumption of amide. The white cloudy mixture was then poured into a 4L flask, washed several times with 1M HCl (1L) and DCM (1L). Approximately 8 g of celite was added and the biphasic mixture was stirred for 1.5 h, then filtered through a pad of celite into a 4L vacuum flask. The filtrate was poured into a 5L separatory funnel and the layers were separated. The aqueous layer was further extracted with DCM (3 x 1L). The organic layers were pooled and dried with sodium sulfate, filtered, and concentrated in vacuo to afford a crude material as a brown oil. The oil was purified by flash chromatography on a Biotage (SNAP KP-Sil 340g column) with a gradient of 0 - 50% EtOAc in DCM to give pure (R)-N,N-dimethyl-4-phenylpentanamide as a yellow oil (1.0243 g, 4.99 mmol, 61% yield, >99:1 er).

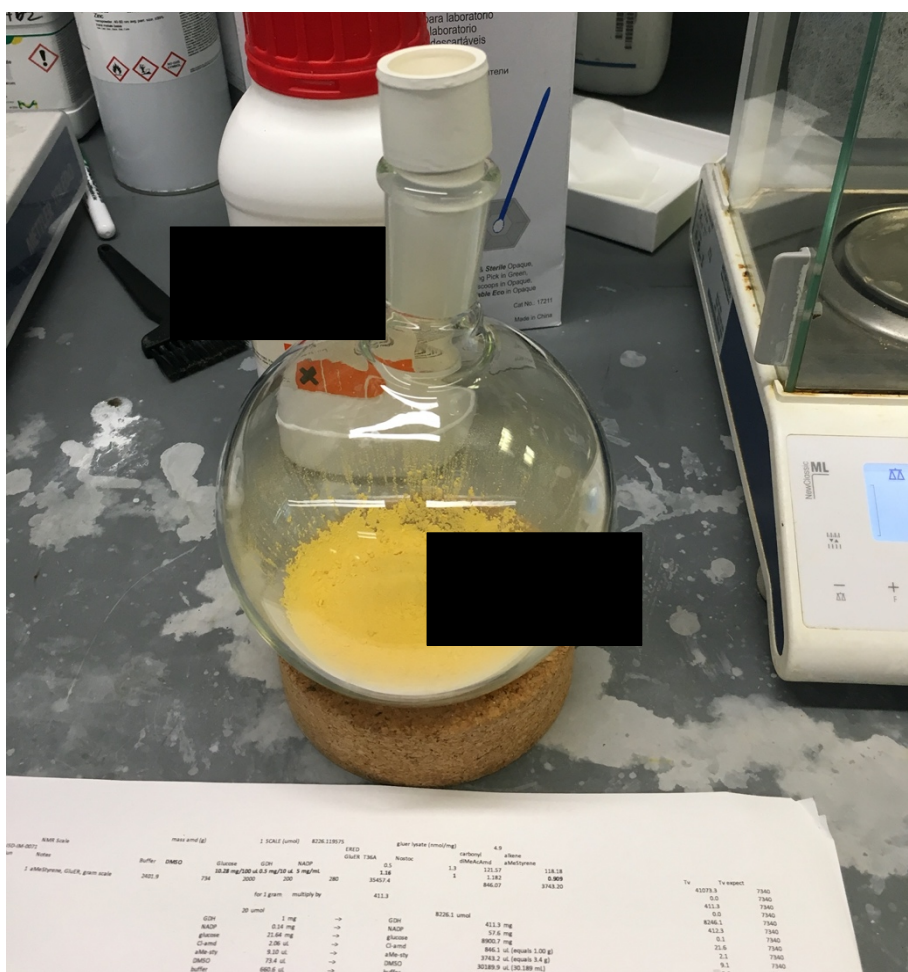


Photo: Addition of dry reagents (lysate, glucose, NADP+, GDH).

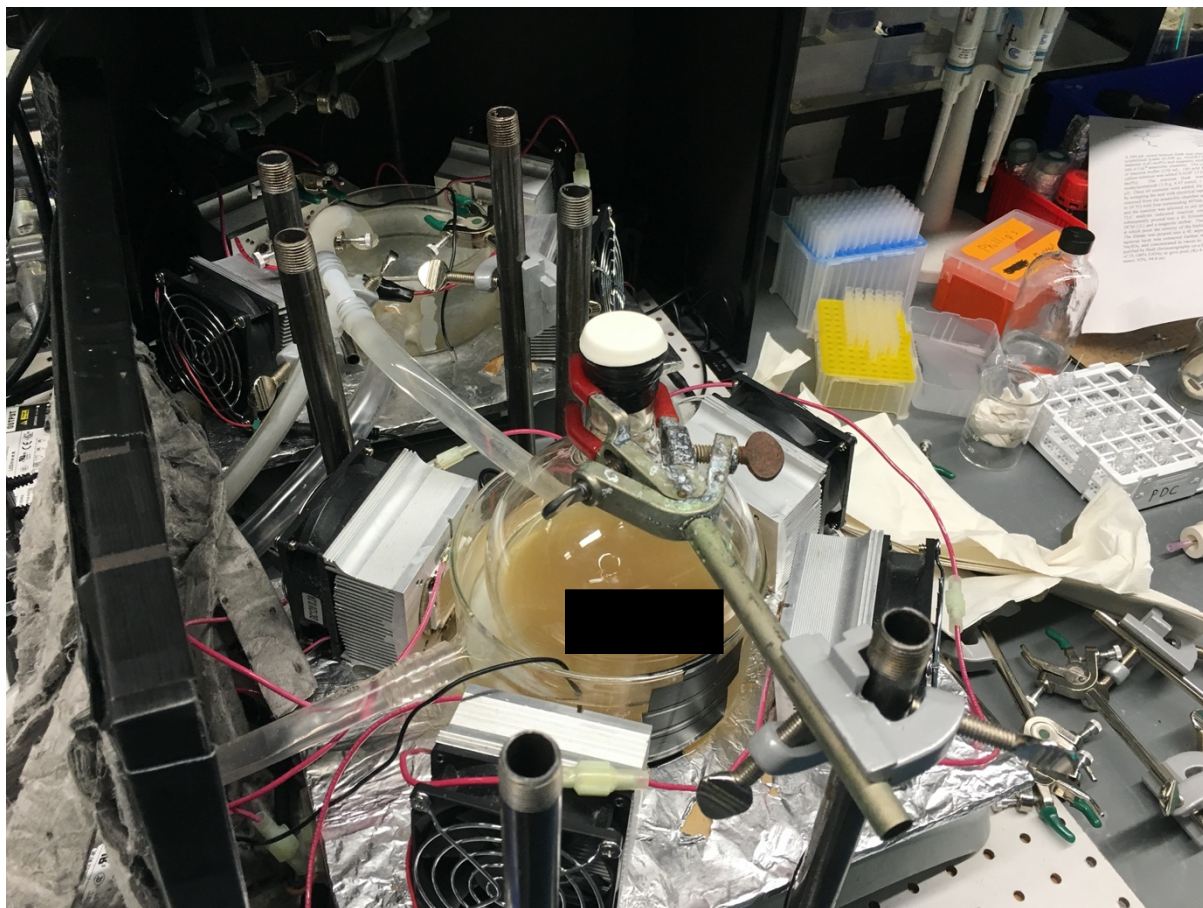


Photo: After reaction setup, picture of reactor just before turning on the cyan LEDs.

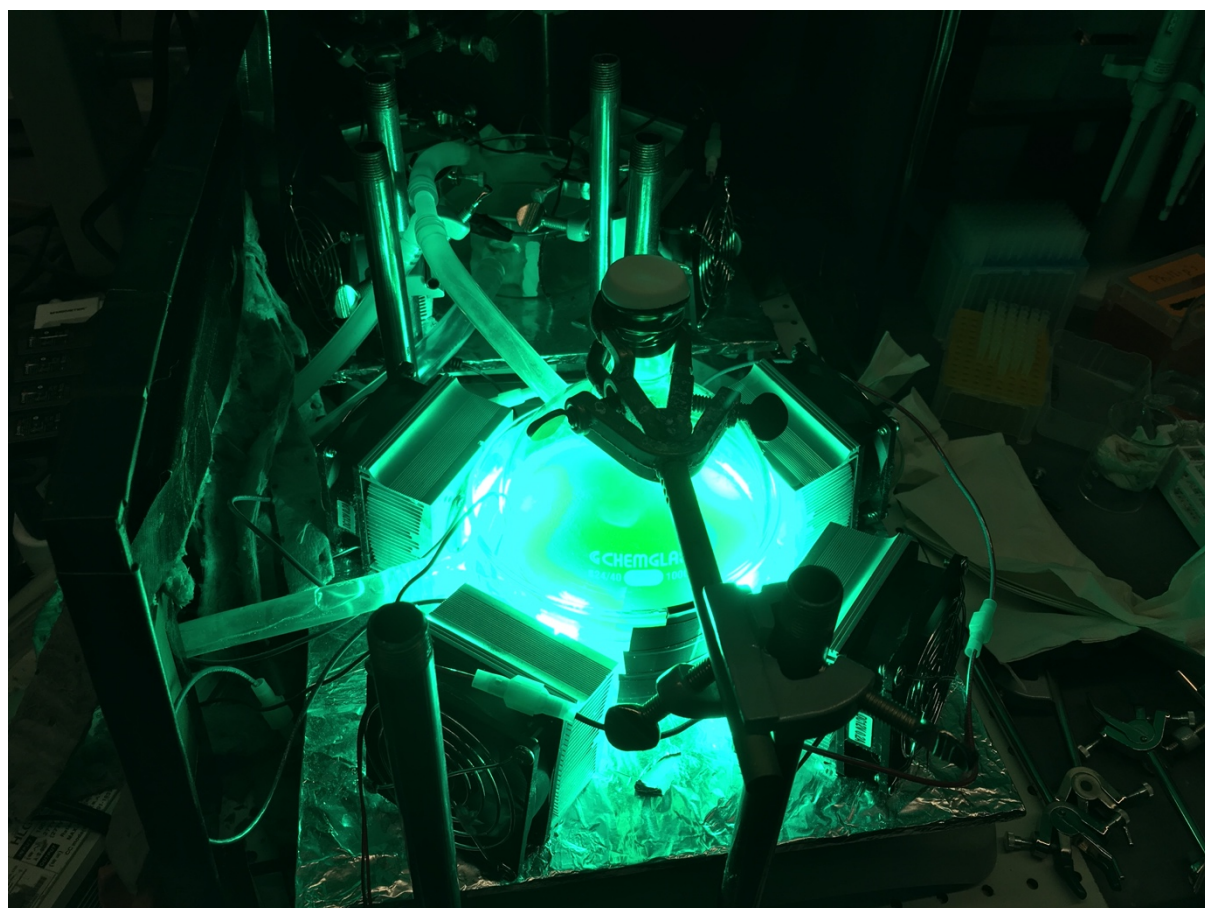


Photo: During reaction while illuminated with cyan LEDs and water chiller.

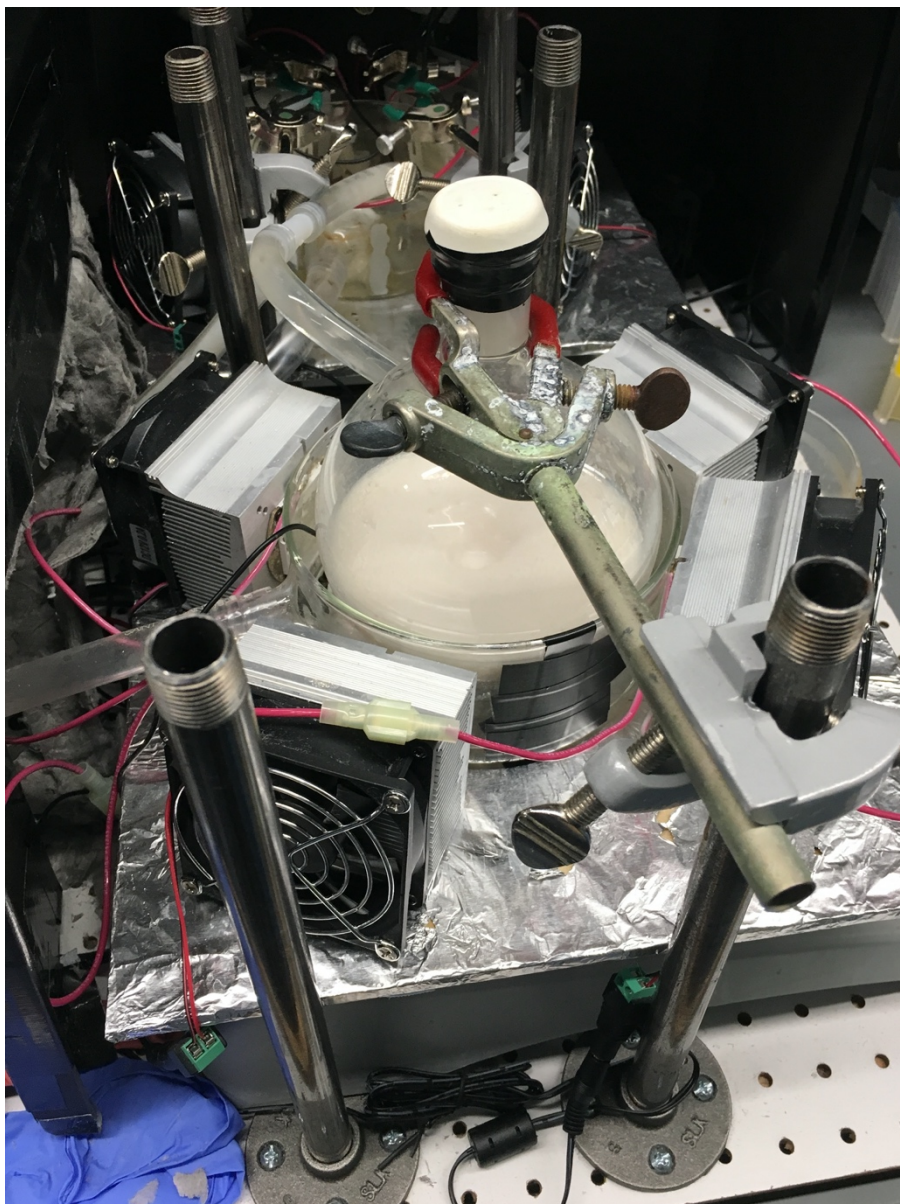


Photo: Appearance of reaction after completion.

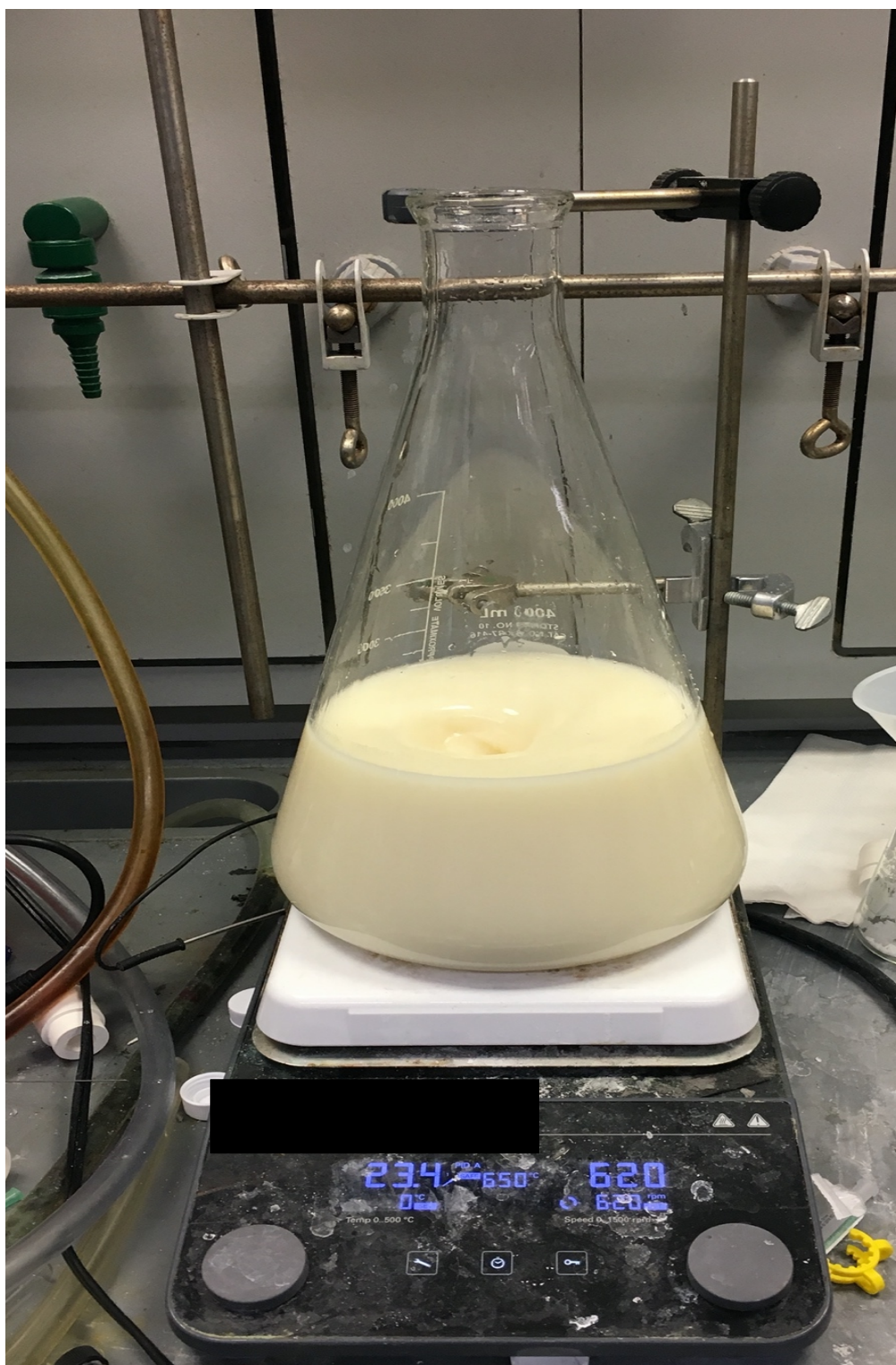


Photo: After addition of 1M HCl and dichloromethane, stirring.

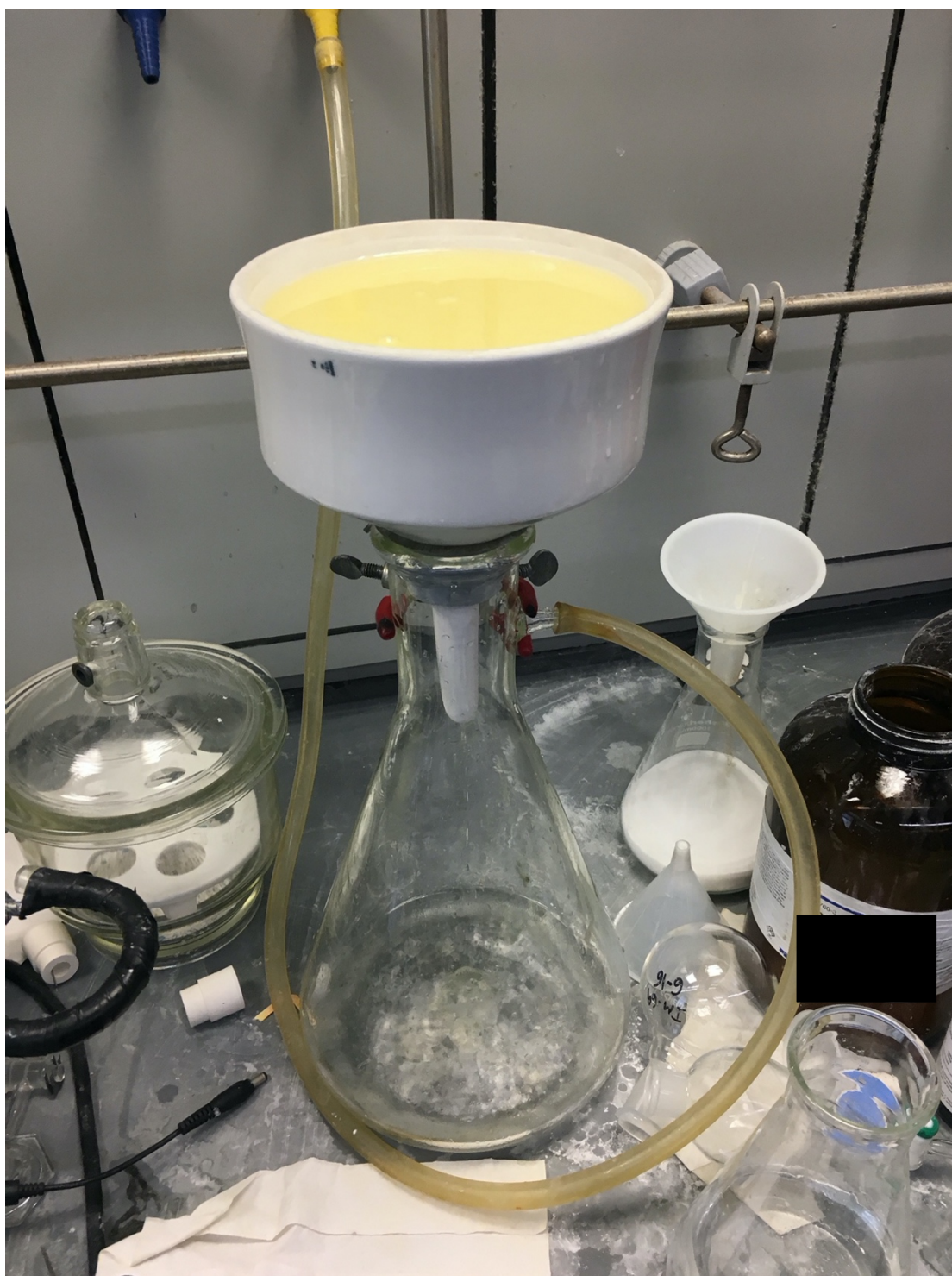


Photo: Filtering through celite.

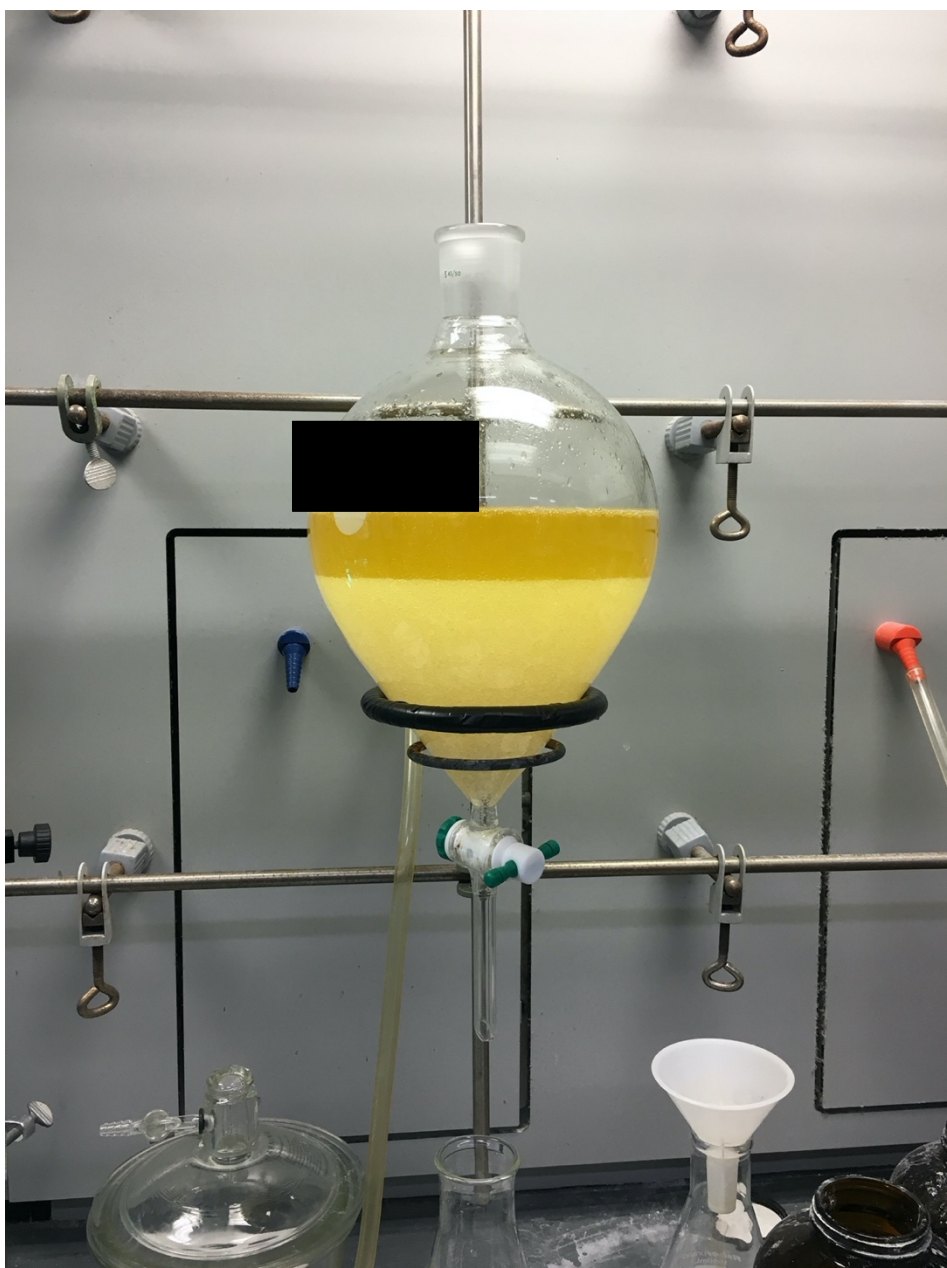
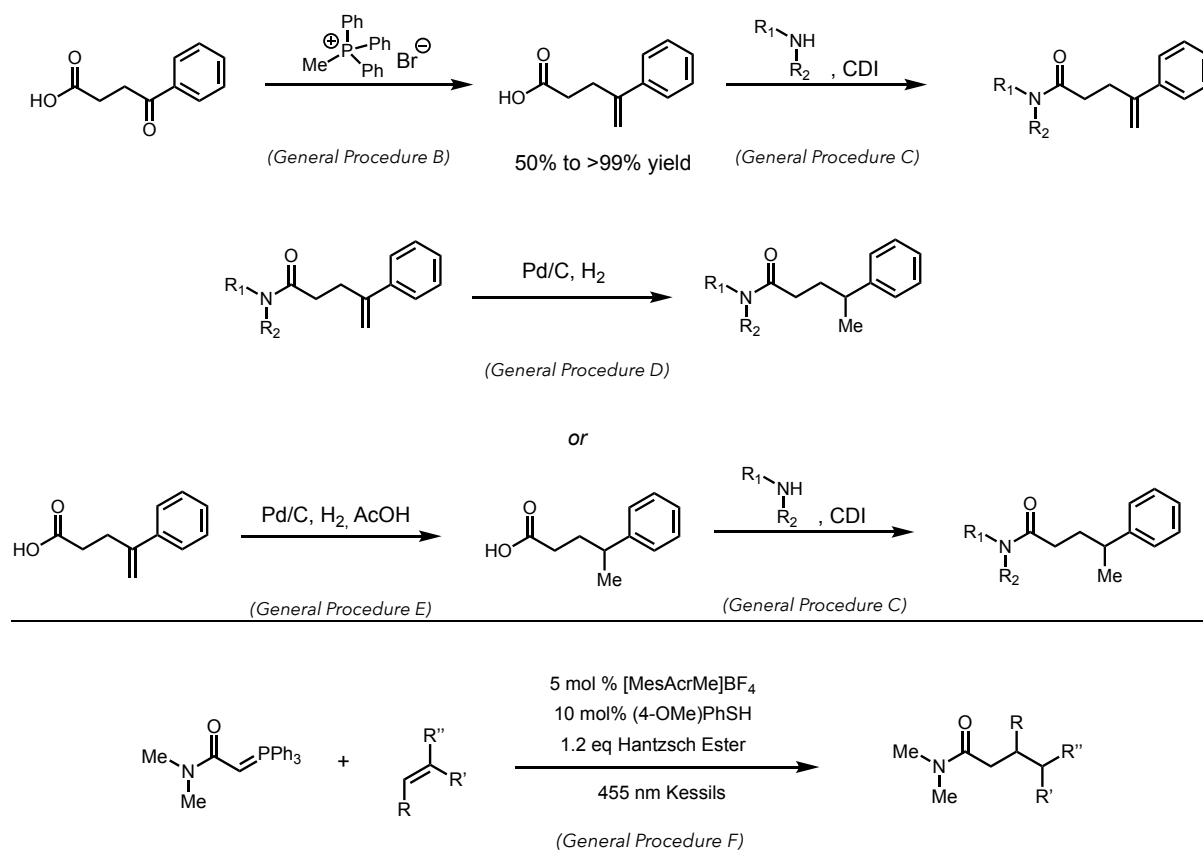


Photo: Separation of filtrate with separatory funnel.



Photo: Filtering organic layer (dried with sodium sulfate).

GENERAL PROCEDURE FOR SYNTHESIS OF RACEMIC STANDARDS



General Procedure B (Wittig):

The Wittig reagent (1.3 equivalents) and potassium tert-butoxide (2.6 equivalents) were added to a flame dried flask containing a magnetic stir bar and dry THF. The flask was placed under N₂ atmosphere. The reactants were allowed to stir for one hour before the 3-benzoylpropionic acid was added. The reaction was stirred for overnight and partitioned into a separatory funnel containing DCM and 10% HCl and extracted with DCM. The organic layers were collected, washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo* to furnish pure product. For the reactions run with methyl triphenylphosphonium bromide and ethyl triphenylphosphonium iodide, the reaction yielded pure product. For the reaction run with propyltriphenylphosphonium bromide, the product was purified using a Biotage KP-SIL 50g column with TLC conditions of 75:25 ethyl acetate:hexanes. Yields for this step ranged from 50% to >99%.

General Procedure C:

The product from General Procedure B or E was added to a flame dried flask containing a magnetic stir bar and dry DCM under N₂ atmosphere. 1.3 equivalents of 1,1'-carbonyldiimidazole (CDI) was added slowly and the mixture was stirred for 1 hour. Then 2 equivalents of the amine were added dropwise and the reaction was stirred overnight. The

reaction mixture was poured into a separatory funnel containing DCM and 10% HCl. The organic layers were collected and extracted again with saturated sodium bicarbonate. The organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo* to furnish pure product.

General Procedure D

The product from General Procedure C was added to a flask containing methanol and sparged with N₂. 5 weight % of Pd/C was added and the flask was sparged with H₂ for one hour. After one hour, the flask was kept under H₂ atmosphere overnight. The reaction mixture was filtered through a celite pad to remove residual Pd/C and the filtrate was concentrated *in vacuo* to furnish the racemic product in pure.

General Procedure E

The product from General Procedure A was added to a flame-dried flask containing methanol and 8 equivalents of acetic acid. The flask was sparged with N₂. Then 5 weight % of Pd/C was added and the flask was sparged with H₂ for one hour. After one hour, the reaction flask was left under H₂ overnight. The reaction mixture was filtered through a celite pad to remove residual Pd/C and the filtrate was concentrated *in vacuo* to furnish the racemic product.

General Procedure F

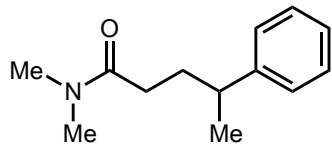
The ylide was prepared from commercially available N,N-dimethylchloroacetamide and triphenylphosphine using a modified procedure from Vicente et al.¹⁸ The acetamide was refluxed in dry ether with the triphenylphosphine for 26 hours and then cooled to room temperature. The solid is filtered and 5% NaOH is added dropwise to the filtrate and the mixture is stirred for 20 minutes then DCM is added (3 times the amount of water). The mixture is separated and washed with DCM. The organic layers are combined, dried over sodium sulfate and concentrated *in vacuo* to yield yellow solid (84% yield) that was used in the next step without further purification.

Reactions were run using a modified procedure from Das et al.¹⁹

9-mesityl-10-methylacridinium tetrafluoroborate (5 mol %), Hantzsch ester (1.2 equiv) and ylide (1 equiv) were added to an 8 mL vial and dissolved in dry DCM (4 mL). 4-methoxythiophenol (10 mol%) and 3 equivalents of alkene substrate were added and the vial was freeze-pumped-thawed three times to degas the mixture. It was then irradiated with 467 nm Kessil Lamps (10 cm from light source) with a cooling fan for 12 hours. The reaction mixtures were concentrated *in vacuo* and the pure product was isolated via prep TLC (3:1 dichloromethane:ethyl acetate for most products, 9:1 dichloromethane:methanol for more products containing basic residues).

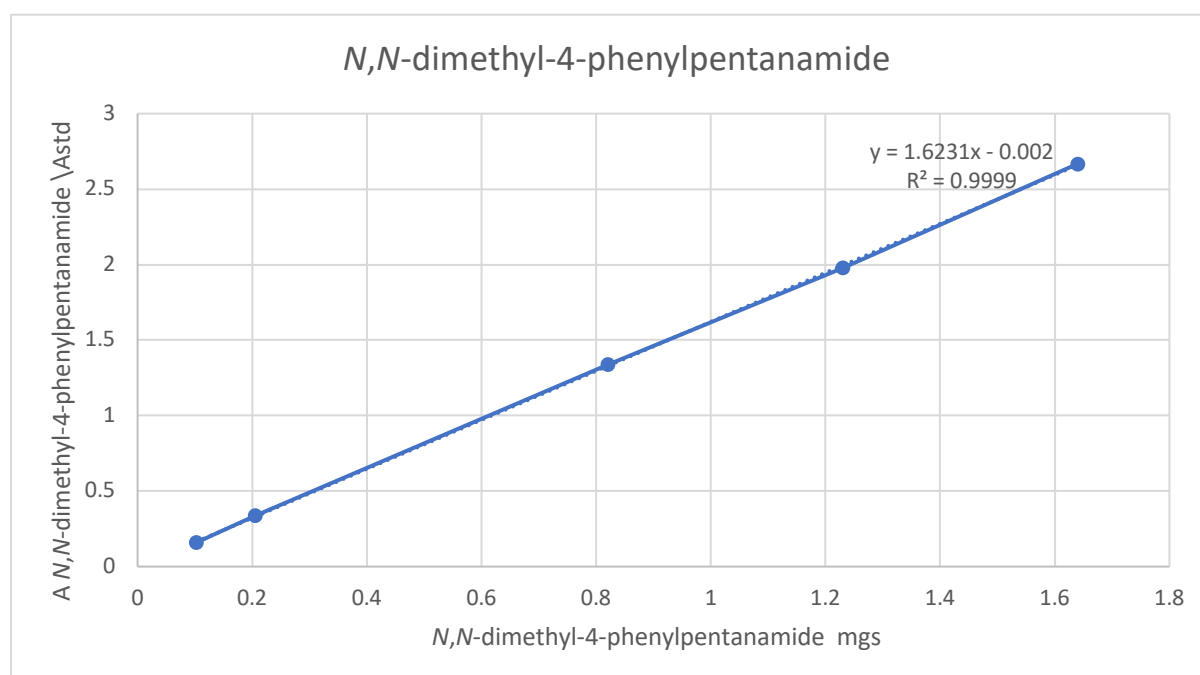
PRODUCT CHARACTERIZATION

N,N-dimethyl-4-phenylpentanamide (3)



The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-benzoylpropionic acid, methyltriphenylphosphonium bromide and dimethylamine (2.0M solution in THF). Yield over General Procedure C and D was 90%.

Standard Curve: Measured with product generated from the rac standard synthesis. All sample vials contain a final volume of 2.2 mL MeCN and include 100 μ L of a 2 mg/mL solution of 1,3,5-tribromobenzene standard in MeCN. The y-axis measurement is the ratio of the LCMS peak areas of the compound of interest to the standard.



Yield with NostocER

Trial 1: >99%

Trial 2: 92%

Average: 96%

Enantioselectivity: 10:90 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: >99%

Trial 2: >99%

Average: >99%

Enantioselectivity: >99:1 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

Isolated yield: 90%, >99:1 e.r.

Yield when reaction was run open to air: 15%

Gram scale with 0.5 mol % GluER T36A lyophilized lysate: 61%, >99:1 e.r.

From the gram scale, absolute configuration was determined. The literature value for (-)-(R)-*N,N*-dimethyl-4-phenylpentanamide is $[\alpha]_D^{22} = [-26.32]$.²⁰ Recorded value was $[\alpha]_D^{22} = -24.86$.

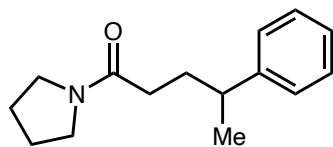
¹H NMR (400 MHz, CDCl₃) **δ** 7.33 – 7.26 (m, 2H), 7.19 (dd, *J* = 5.3, 2.5 Hz, 3H), 2.87 (s, 6H), 2.80 – 2.68 (m, 1H), 2.28 – 2.08 (m, 2H), 2.06 – 1.82 (m, 2H), 1.28 (d, *J* = 6.9 Hz, 3H).

¹³C NMR (126 MHz, CDCl₃) **δ** 173.05, 146.63, 128.41, 127.02, 126.07, 39.57, 33.29, 31.28, 22.59.

IR: 2956 cm⁻¹, 2928 cm⁻¹, 2870 cm⁻¹, 1642 cm⁻¹, 1602 cm⁻¹, 1493 cm⁻¹, 1451 cm⁻¹, 1397 cm⁻¹, 1265 cm⁻¹, 1145 cm⁻¹

HRMS: calc'd [M+H]⁺ 206.153936, found 206.15418

4-phenyl-1-(pyrrolidin-1-yl)pentan-1-one (16)



The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-benzoylpropionic acid, methyltriphenylphosphonium bromide and pyrrolidine. Yield over General Procedure C and D was >99%.

Yield with NostocER

Trial 1: 89%

Trial 2: 87%

Average: 88%

Enantioselectivity: 67:33 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

Yield with GluER T36A

Trial 1: 89%

Trial 2: 91%

Average: 90%

Isolated Yield: 90%

Enantioselectivity: 4:96 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

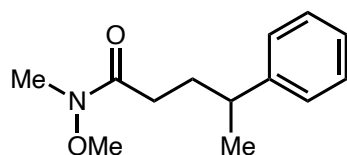
^1H NMR (500 MHz, CDCl_3) δ 7.31 – 7.26 (m, 2H), 7.21 – 7.15 (m, 3H), 3.42 (q, J = 6.3 Hz, 2H), 3.19 (q, J = 6.5 Hz, 2H), 2.75 (dq, J = 9.3, 6.9, 5.2 Hz, 1H), 2.20 – 1.95 (m, 3H), 1.95 – 1.74 (m, 5H), 1.27 (d, J = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 171.68, 146.87, 128.54, 127.22, 126.20, 46.55, 45.71, 39.74, 33.14, 32.89, 26.17, 24.51, 22.82.

IR: 2960 cm^{-1} , 2871 cm^{-1} , 1636 cm^{-1} , 1449 cm^{-1} , 1342 cm^{-1} , 763 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 232.169586, found 232.17070

N-methoxy-*N*-methyl-4-phenylpentanamide (17)



The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-benzoylpropionic acid, methyl triphenylphosphonium bromide and *N*-methoxy-*N*-methylamine hydrochloride. Yield over General Procedure C and D was >99%.

For the isolated yield, general isolated yield procedure was performed except using diethyl ether as the organic solvent, not dichloromethane.

Yield with NostocER

Trial 1: 51%

Trial 2: 53%

Average: 52%

Enantioselectivity: 29:71 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: 71%

Trial 2: 75%

Average: 73%

Isolated yield: 47%

Enantioselectivity: >99:1 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

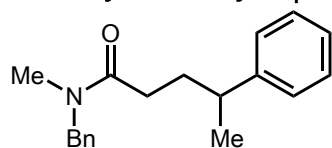
^1H NMR (500 MHz, CDCl_3) δ 7.29 (dd, J = 8.2, 7.0 Hz, 2H), 7.21 – 7.15 (m, 3H), 3.67 (s, 2H), 3.52 (s, 3H), 3.13 (s, 3H), 3.08 (s, 2H), 2.75 (dt, J = 9.3, 6.7 Hz, 1H), 2.28 (dq, J = 18.4, 11.0, 9.0 Hz, 2H), 2.01 – 1.82 (m, 2H), 1.28 (d, J = 6.9 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 174.74, 163.57, 146.77, 128.52, 127.23, 126.20, 61.19, 60.70, 39.66, 36.30, 32.84, 32.26, 30.21, 22.66.

IR: 2959 cm^{-1} , 2931 cm^{-1} , 1649 cm^{-1} , 1493 cm^{-1} , 1451 cm^{-1} , 1416 cm^{-1} , 1384 cm^{-1} , 1177 cm^{-1} , 998 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$: 222.14883 found 222.14898

***N*-benzyl-*N*-methyl-4-phenylpentanamide (18)**



The racemic standard was synthesized via General Procedures B, C, and E from commercially available 4-benzoylpropionic acid, methyl triphenylphosphonium bromide and benzylmethylamine. Yield over General Procedure E and C was 45%.

Yield with NostocER

Trial 1: 54%

Trial 2: 56%

Average: 55%

Enantioselectivity: 76:24 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: 74%

Trial 2: 75%

Average: 75%

Enantioselectivity: >99:1 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

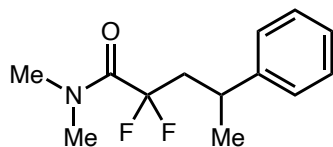
¹H NMR (500 MHz, CDCl₃) **δ** 7.48 – 7.29 (m, 6H), 7.28 (s, 1H), 7.25 – 7.17 (m, 2H), 7.09 (d, J = 7.4 Hz, 1H), 4.62 (q, J = 14.3 Hz, 1H), 4.47 – 4.34 (m, 1H), 2.97 (d, J = 2.1 Hz, 2H), 2.90 – 2.73 (m, 3H), 2.28 (tdd, J = 21.0, 10.6, 5.3 Hz, 2H), 2.18 – 1.91 (m, 2H), 1.33 (ddd, J = 25.8, 6.7, 2.0 Hz, 3H).

¹³C NMR (126 MHz, CDCl₃) **δ** 173.51, 173.14, 146.74, 146.69, 137.65, 136.84, 128.96, 128.67, 128.58, 128.56, 128.14, 127.63, 127.40, 127.20, 127.16, 126.46, 126.25, 126.21, 53.40, 50.86, 39.71, 39.62, 34.81, 34.06, 33.62, 33.45, 31.67, 31.26, 22.76.

IR: 3026 cm⁻¹, 2959 cm⁻¹, 2927 cm⁻¹, 1643 cm⁻¹, 1493 cm⁻¹, 1451 cm⁻¹, 1400 cm⁻¹

HRMS: calc'd [M+H]⁺: 282.18523, found 282.18536

2,2-difluoro-*N,N*-dimethyl-4-phenylpentanamide (19)



The racemic standard was synthesized using the following procedure: **3** was suspended in dry THF in a flame dried flask under nitrogen. LDA (1.1 eq) was then added dropwise at -78°C. Then mixture was stirred for an hour at this temperature, then *N*-fluorobenzenesulfonimide (1.1 eq) was added dropwise. The mixture was warmed to room temperature and stirred for 6 hours. Then it was recooled to -78°C and another 1.1 eq of LDA was added at -78°C and the mixture left to stir for 1 hour. Then another 1.1 eq of *N*-fluorobenzenesulfonimide was added dropwise and the mixture was warmed to room temperature and left to stir overnight. The reaction was quenched with saturated ammonium chloride and extracted with ether. The organic extracts were combined, washed with brine and dried over sodium sulfate and then concentrated in vacuo. The product was purified by column chromatography (hexanes/ethyl acetate) to afford pure product (50% yield).

Yield with NostocER

Trial 1: 92%,

Trial 2: >99%

Average: 96%

Enantioselectivity: 2:98 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: 38%,

Trial 2: 36%

Average: 37%

Enantioselectivity: 97:3 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

¹H NMR (500 MHz, CDCl₃) **δ** 7.34 – 7.27 (m, 2H), 7.25 – 7.16 (m, 3H), 3.19 (p, *J* = 6.9 Hz, 1H), 3.10 (t, *J* = 2.0 Hz, 3H), 2.93 (s, 3H), 2.49 (ddtd, *J* = 63.0, 21.7, 15.3, 6.7 Hz, 2H), 1.35 (d, *J* = 7.0 Hz, 3H), 1.20 (d, *J* = 6.9 Hz, 3H).

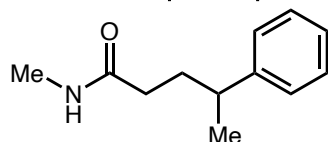
¹³C NMR (126 MHz, CDCl₃) **δ** 163.61, 146.91, 129.29, 128.57, 126.97, 126.36, 124.66, 120.51, 49.12, 42.56, 42.39, 42.21, 37.09, 33.98, 23.52, 21.83.

¹⁹F NMR (376 MHz, CDCl₃) **δ** -97.66 (dd, *J* = 21.9, 15.4 Hz)

IR: 2966 cm⁻¹, 1664 cm⁻¹, 1494 cm⁻¹, 1403 cm⁻¹, 1342 cm⁻¹, 1186 cm⁻¹, 1139 cm⁻¹, 1058 cm⁻¹, 699 cm⁻¹

HRMS: calc'd [M+H]⁺ 242.135096, found 242.13883

N-methyl-4-phenylpentanamide (20)



The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-benzoylpropionic acid, methyltriphenylphosphonium bromide and methylamine . Yield over General Procedure C and D was 91%

Yield with NostocER

Trial 1: 59%

Trial 2: 62%

Average: 61%

Enantioselectivity: 19:81 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

Yield with GluER T36A

Trial 1: 17%

Trial 2: 29%

Average: 23%

Enantioselectivity: >99:1 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

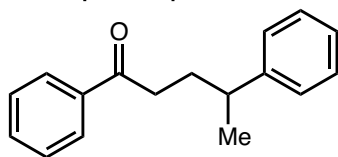
^1H NMR (500 MHz, CDCl_3) δ 7.29 (dd, $J = 8.3, 6.9$ Hz, 2H), 7.22 – 7.14 (m, 3H), 5.33 (s, 1H), 2.74 (d, $J = 4.8$ Hz, 3H), 2.69 (td, $J = 5.9, 4.7, 2.2$ Hz, 1H), 2.09 – 1.94 (m, 3H), 1.93 – 1.79 (m, 1H), 1.26 (d, $J = 6.9$ Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 173.64, 146.55, 128.60, 127.17, 126.30, 39.70, 34.83, 33.88, 26.36, 22.67.

IR : 3289.1 cm^{-1} , 2957 cm^{-1} , 1644 cm^{-1} , 1558.6 cm^{-1} , 1493 cm^{-1} , 1451 cm^{-1} , 1409 cm^{-1} , 1375 cm^{-1} , 1265 cm^{-1} , 1158 cm^{-1} , 1026 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 192.138286, found: 192.13859

1,4-diphenylpentan-1-one (21)



The racemic standard was synthesized using a modified procedure from Leonori et al from commercially available ethyl-3-oxo-3-phenyl-propanoate and 1-(bromomethyl)vinylbenzene and then hydrogenation of the alkene using General Procedure D.²¹ All spectra match reported values.²²

Yield with NostocER

Trial 1: 44%

Trial 2: 45%

Average: 45%

Enantioselectivity: 70:30 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OJ-H

Yield with GluER T36A

Trial 1: 87%

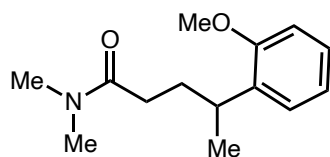
Trial 2: 76%

Average: 82%

Enantioselectivity: >99:1 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OJ-H

¹H NMR (400 MHz, CDCl₃) **δ** 7.90 – 7.80 (m, 2H), 7.58 – 7.46 (m, 1H), 7.41 (ddd, *J* = 8.1, 6.6, 1.3 Hz, 2H), 7.37 – 7.27 (m, 2H), 7.24 – 7.11 (m, 3H), 2.95 – 2.70 (m, 3H), 2.14 – 1.92 (m, 2H), 1.32 (d, *J* = 6.9 Hz, 3H).

4-(2-methoxyphenyl)-*N,N*-dimethylpentanamide (22a)



The racemic standard was synthesized using General Procedure F with alkene **9a**. Yield was 15%.

^1H NMR (500 MHz, CDCl_3) δ 7.21 – 7.13 (m, 2H), 6.92 (td, J = 7.5, 1.2 Hz, 1H), 6.85 (dd, J = 8.1, 1.2 Hz, 1H), 3.80 (s, 3H), 3.22 (q, J = 7.1 Hz, 1H), 2.90 (s, 3H), 2.86 (s, 3H), 2.30 – 2.07 (m, 3H), 1.93 (tdd, J = 8.0, 6.2, 3.0 Hz, 2H), 1.23 (d, J = 6.9 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 173.48, 157.21, 134.88, 126.98, 126.93, 120.82, 110.52, 55.44, 37.29, 35.46, 32.33, 31.91, 31.74, 21.22.

Yield with NostocER

Trial 1: 97%,

Trial 2: 98%

Average: 98%

Enantioselectivity: 12:88 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: 91%,

Trial 2: 95%,

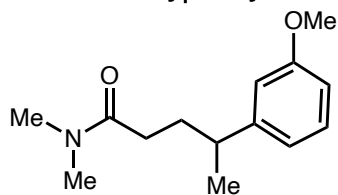
Average: 93%

Enantioselectivity: >99:1 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

IR: 2928 cm^{-1} , 1646 cm^{-1} , 1492 cm^{-1} , 1463 cm^{-1} , 1398 cm^{-1} , 1239 cm^{-1} , 774 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$: 236.16451, found 236.16475

4-(3-methoxyphenyl)-*N,N*-dimethylpentanamide (22b)



The racemic standard was synthesized using General Procedure F from alkene **9b**. Yield was 5%.

Yield with NostocER

Trial 1: >99%

Trial 2: >99%

Average: >99%

Enantioselectivity: 3:97 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: >99%

Trial 2: >99%

Average: >99%

Enantioselectivity: 91:9 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

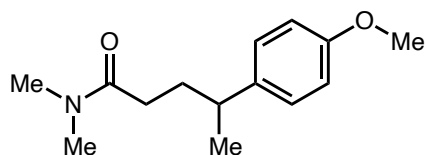
^1H NMR (500 MHz, CDCl_3) **5** 7.72 – 7.62 (m, 2H), 7.60 – 7.37 (m, 3H), 7.21 (dd, J = 8.9, 7.6 Hz, 1H), 6.81 – 6.70 (m, 3H), 3.80 (s, 3H), 2.88 (d, J = 28.8 Hz, 6H), 2.72 (dt, J = 9.5, 6.6 Hz, 1H), 2.27 – 2.08 (m, 2H), 2.07 – 1.78 (m, 2H), 1.27 (d, J = 7.0 Hz, 6H).

^{13}C NMR (126 MHz, CDCl_3) **5** 192.53, 132.30, 132.10, 128.70, 128.61, 113.07, 111.27, 55.29, 39.81, 37.30, 33.34, 31.49, 29.85.

IR: 2929 cm^{-1} , 1642 cm^{-1} , 1493 cm^{-1} , 1453 cm^{-1} , 1398 cm^{-1} , 1362 cm^{-1} , 1265 cm^{-1} , 1142 cm^{-1} , 1102 cm^{-1} , 762 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 236.16451, found 236.16473

4-(4-methoxyphenyl)-*N,N*-dimethylpentanamide (22c)



The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-(4-methoxy)benzoylpropionic acid, methyltriphenylphosphonium bromide and dimethylamine (2.0M solution in THF). Yield for General Procedure B was 60%. Yield over General Procedure C and D was >99%.

Yield with NostocER

Trial 1: 92%

Trial 2: 90%

Average: 91%

Enantioselectivity: 8:92 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

Yield with NostocER Y219F

Trial 1: 98%

Trial 2: >99%

Average: >99%

Enantioselectivity: 8:92 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: 71%

Trial 2: 75%

Average: 73%

Enantioselectivity: >99:1 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

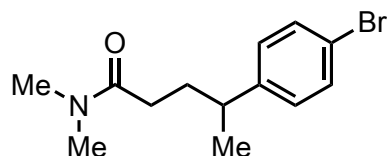
^1H NMR (500 MHz, CDCl_3) δ 7.12 – 7.08 (m, 2H), 6.86 – 6.81 (m, 2H), 3.79 (s, 3H), 2.90 (s, 3H), 2.84 (s, 3H), 2.69 (ddd, J = 9.6, 7.2, 5.4 Hz, 1H), 2.21 – 2.07 (m, 2H), 2.01 – 1.75 (m, 2H), 1.24 (d, J = 6.9 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 173.20, 157.97, 138.89, 128.03, 113.91, 55.37, 38.89, 37.30, 35.48, 33.62, 31.53, 22.96.

IR: 2954 cm^{-1} , 2835 cm^{-1} , 1641 cm^{-1} , 1558 cm^{-1} , 1540 cm^{-1} , 1512 cm^{-1} , 1456 cm^{-1} , 1398 cm^{-1} , 1234 cm^{-1} , 1178 cm^{-1} , 1035 cm^{-1} , 831 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 236.16451 found 236.16739

4-(4-bromophenyl)-*N,N*-dimethylpentanamide (22d)



The racemic standard was synthesized via General Procedure F from commercially available 4-bromo- α -methylstyrene. Yield was 25%.

Yield with NostocER

Trial 1: 98%,

Trial 2: 96%

Average: 97%

Enantioselectivity: 90:10 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

Yield with GluER T36A

Trial 1: 77%,

Trial 2: 65%

Average: 71%

Enantioselectivity: 78:26 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

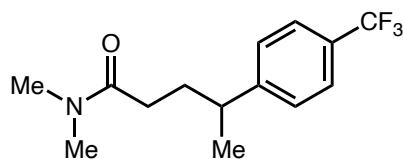
^1H NMR (500 MHz, CDCl_3) δ 7.41 (d, J = 8.1 Hz, 2H), 7.07 (d, J = 8.1 Hz, 2H), 2.91 (s, 3H), 2.86 (s, 3H), 2.77 – 2.67 (m, 1H), 2.19 – 2.04 (m, 2H), 1.97 (ddt, J = 15.1, 9.0, 6.1 Hz, 1H), 1.91 – 1.73 (m, 1H), 1.25 (d, J = 6.8 Hz, 4H).

^{13}C NMR (126 MHz, CDCl_3) δ 172.79, 145.87, 131.64, 128.99, 119.83, 39.22, 37.28, 35.51, 33.25, 31.30, 22.65.

IR: 2926 cm^{-1} , 1663 cm^{-1} , 1647 cm^{-1} , 1488 cm^{-1} , 1397 cm^{-1} , 1147 cm^{-1} , 1008 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 284.06445, found 284.06483

***N,N*-dimethyl-4-(4-(trifluoromethyl)phenyl)pentanamide (22e)**



The racemic standard was synthesized via general procedure F from alkene **9d**. Yield was 11%.

Yield with NostocER

Trial 1: 80%,

Trial 2: 93%

Average: 87%

Enantioselectivity: 93:7 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, AS-H

Yield with GluER T36A

Trial 1: 36%,

Trial 2: 29%

Average: 33%

Enantioselectivity: 88:12 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, AS-H

^1H NMR (500 MHz, CDCl_3) δ 7.55 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 2.97 – 2.77 (m, 5H), 2.24 – 2.07 (m, 2H), 2.04 – 1.83 (m, 1H), 1.29 (d, J = 6.9 Hz, 3H).

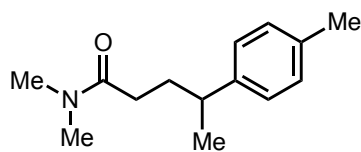
^{13}C NMR (126 MHz, CDCl_3) δ 151.04, 127.55, 125.56, 125.53, 125.50, 39.60, 37.27, 35.54, 33.15, 31.24, 22.53.

^{19}F NMR (376 MHz, CDCl_3) δ -62.33 (s).

IR: 2960 cm^{-1} , 1642 cm^{-1} , 1326 cm^{-1} , 1162 cm^{-1} , 1119 cm^{-1} , 1068 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 274.14133 found 274.14362

N,N-dimethyl-4-(4-(methyl)phenyl)pentanamide (22f)



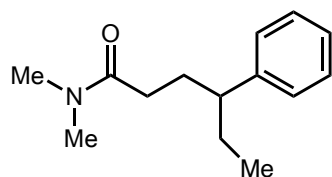
The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-(4-methyl)benzoylpropionic acid, methyltriphenylphosphonium bromide and dimethylamine (2.0M solution in THF). Yield for General Procedure B was 52%. Yield over General Procedure C and D was >99%.

^1H NMR (500 MHz, CDCl_3) δ 7.17 – 7.00 (m, 4H), 2.87 (d, J = 28.4 Hz, 6H), 2.76 – 2.61 (m, 1H), 2.32 (s, 3H), 2.23 – 2.08 (m, 2H), 2.02 – 1.79 (m, 2H), 1.25 (d, J = 6.9 Hz, 3H).

IR: 2922 cm^{-1} , 2867 cm^{-1} , 1641 cm^{-1} , 1513.4 cm^{-1} , 1454 cm^{-1} , 1394 cm^{-1} , 1142 cm^{-1} , 1054 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$: 220.16958, found: 220.16950

N,N-dimethyl-4-phenylhexanamide (23a)



The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-benzoylpropionic acid, ethyl triphenylphosphonium iodide and dimethylamine (2.0M solution in THF) bromide. Yield for General Procedure B was 70% and yield over General Procedure C and D was >99%.

Yield with NostocER

Trial 1: 78%

Trial 2: 77%

Average: 78%

Enantioselectivity: 11:89 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

Yield with NostocER Y219F

Trial 1: 87%

Trial 2: 82%

Average: 85%

Enantioselectivity: 4:96 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: 91%

Trial 2: 99%

Average: 95%

Enantioselectivity: 97:3 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

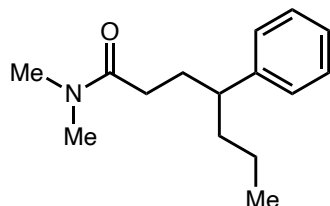
^1H NMR (500 MHz, CDCl_3) δ 7.28 (t, J = 7.5 Hz, 2H), 7.21 – 7.10 (m, 3H), 2.84 (d, J = 36.3 Hz, 6H), 2.45 (tdd, J = 10.2, 5.3, 2.9 Hz, 1H), 2.22 – 1.99 (m, 3H), 1.89 – 1.76 (m, 2H), 1.75 – 1.64 (m, 1H), 1.64 – 1.52 (m, 1H), 0.78 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 173.30, 145.08, 128.47, 127.90, 126.24, 47.56, 37.25, 31.71, 31.40, 30.10, 12.28.

IR: 2958 cm^{-1} , 2927 cm^{-1} , 2872 cm^{-1} , 1642 cm^{-1} , 1493 cm^{-1} , 1451 cm^{-1} , 1396 cm^{-1} , 1265 cm^{-1} , 1143 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 220.16959, found 220.17005

N,N-dimethyl-4-phenylheptanamide (23b)



The racemic standard was synthesized via General Procedures B, C, and D from commercially available 4-benzoylpropionic acid, propyl triphenylphosphonium bromide and dimethylamine (2.0M solution in THF). After General Procedure B, the crude material was purified using flash column chromatography (TLC conditions 75:25 EtOAc/Hex) to give pure product. Yield over General Procedure B, C and D was 71%.

Yield with NostocER

Trial 1: 41%

Trial 2: 42%

Average: 42%

Enantioselectivity: 86:14 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

Yield with GluER T36A

Trial 1: 43%

Trial 2: 37%

Average: 40%

Enantioselectivity: 85:15 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD

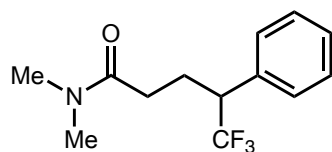
^1H NMR (500 MHz, CDCl_3) δ 7.28 (t, J = 7.6 Hz, 2H), 7.21 – 7.15 (m, 1H), 7.15 – 7.10 (m, 2H), 2.89 (s, 3H), 2.79 (s, 3H), 2.60 – 2.46 (m, 1H), 2.17 – 1.98 (m, 3H), 1.89 – 1.73 (m, 1H), 1.69 – 1.51 (m, 2H), 1.30 – 1.06 (m, 2H), 0.83 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 173.08, 145.24, 128.35, 127.71, 126.07, 45.38, 39.41, 37.12, 35.34, 31.92, 31.31, 20.68, 14.13.

IR: 2954 cm^{-1} , 2928 cm^{-1} , 1647 cm^{-1} , 1493 cm^{-1} , 1452 cm^{-1} , 1395 cm^{-1} , 1265 cm^{-1} , 1142 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 234.18507, found 234.18564

5,5,5-trifluoro-*N,N*-dimethyl-4-phenylpentanamide (23c)



The racemic standard was synthesized and purified using the general biocatalytic procedure for isolated yield and MorB. The yield was 15%, 69:31 er. Note: The product decomposes under exposure to UV light, potentially to the corresponding styrene with geminal vinylic fluorides (a strong chromophore).

Yield with NostocER

Trial 1: 41%,

Trial 2: 37%

Average: 39%

Enantioselectivity: 15:85 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD-H

Yield with GluER T36A

Trial 1: 22%,

Trial 2: 22%,

Average: 22%

Enantioselectivity: 88:12 e.r., 2% IPA 98% hexanes, 45 min, OD-H

^1H NMR (500 MHz, CDCl_3) δ 7.42 – 7.27 (m, 6H), 3.51 – 3.38 (m, 1H), 2.91 (s, 3H), 2.81 (s, 3H), 2.48 – 2.38 (m, 1H), 2.25 – 2.10 (m, 3H).

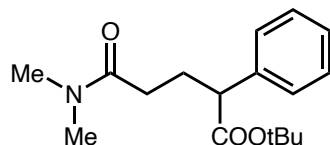
^{13}C NMR (126 MHz, CDCl_3) δ 171.62, 134.56, 129.22, 128.90, 128.68, 128.40, 128.37, 128.35, 128.32, 128.14, 127.56, 125.92, 49.27, 49.05, 37.10, 35.53, 29.86, 24.38, 24.36.

^{19}F NMR (376 MHz, CDCl_3) δ -69.60

IR: 2921 cm^{-1} , 2030 cm^{-1} , 1733.5 cm^{-1} , 1641 cm^{-1} , 1257 cm^{-1} , 1145 cm^{-1} , 1108 cm^{-1} , 703

HRMS: calc'd $[\text{M}+\text{H}]^+$ 260.12568, found 260.12577

tert-butyl 5-(dimethylamino)-5-oxo-2-phenylpentanoate (23d)



The racemic standard was synthesized via General Procedure F. Yield was 27%.

Yield with NostocER

Trial 1: 90%,

Trial 2: 93%

Average: 92%

Enantioselectivity: 11:89 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD-H

Yield with GluER T36A

Trial 1: 88%,

Trial 2: 75%

Average: 82%

Enantioselectivity: 62:38 e.r., HPLC method: 2% IPA 98% hexanes, 45 min, OD-H

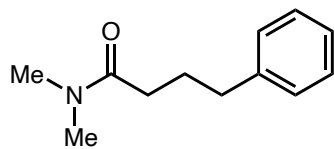
^1H NMR (500 MHz, CDCl_3) δ 7.30 (dd, J = 6.7, 1.4 Hz, 5H), 7.25 – 7.21 (m, 1H), 3.55 (t, J = 7.6 Hz, 1H), 2.91 (d, J = 11.3 Hz, 6H), 2.42 – 2.18 (m, 3H), 2.14 – 2.02 (m, 1H), 1.39 (s, 9H).

^{13}C NMR (126 MHz, CDCl_3) δ 173.14, 172.35, 139.35, 128.66, 128.05, 127.20, 51.98, 37.30, 35.52, 31.08, 28.86, 28.09.

IR: 2977 cm^{-1} , 2932 cm^{-1} , 2030 cm^{-1} , 1722 cm^{-1} , 1642 cm^{-1} , 1493 cm^{-1} , 1453 cm^{-1} , 1396 cm^{-1} , 1367 cm^{-1} , 1257 cm^{-1} , 1145 cm^{-1} , 750 cm^{-1} , 700 cm^{-1}

HRMS: calc'd $[\text{M}+\text{Na}]^+$ 314.17266, found 314.17281

***N,N*-dimethyl-4-phenylbutanamide (24)**



Synthesized using general procedure C from commercially available 4-phenylbutanoic acid and dimethylamine (2.0 M in THF) 90% yield. Spectra matches previously reported.²³

Yield with NostocER:

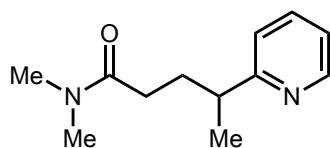
Trial 1: 42%

Trial 2: 53%

Average: 47%

¹H NMR (500 MHz, CDCl₃) **δ** 7.29 (dd, *J* = 7.7, 1.2 Hz, 2H), 7.21 – 7.15 (m, 3H), 2.68 (t, *J* = 7.5 Hz, 2H), 2.34 – 2.26 (m, 2H), 2.02 – 1.92 (m, 2H).

***N,N*-dimethyl-4-(pyridin-2-yl)pentanamide (25)**



The racemic standard was synthesized using General Procedure F using commercially available 2-isopropenylpyridine. The yield was 20%.

Yield with NostocER

Trial 1: 72%,

Trial 2: 74%

Average: 73%

Enantioselectivity: 13:87 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ-H

Yield with GluER T36A

Trial 1: 52%,

Trial 2: 65%,

Average: 59%

Enantioselectivity: 90:10 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ-H

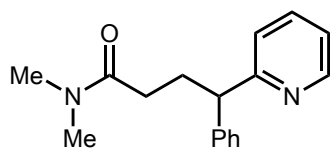
^1H NMR (500 MHz, CDCl_3) **δ** 8.63 – 8.46 (m, 1H), 7.62 (td, J = 7.7, 1.8 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 7.15 – 7.09 (m, 1H), 2.89 (d, J = 7.2 Hz, 7H), 2.35 – 2.13 (m, 2H), 2.13 – 1.93 (m, 2H), 1.32 (d, J = 6.9 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) **δ** 173.04, 165.82, 149.15, 136.74, 122.00, 121.46, 41.65, 37.36, 35.50, 32.34, 31.51, 21.27.

IR: 3365 cm^{-1} , 2958 cm^{-1} , 2924 cm^{-1} , 2853 cm^{-1} , 1595 cm^{-1} , 1478 cm^{-1} , 1435 cm^{-1} , 1404 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 207.14919, found 207.14923

***N,N*-dimethyl-4-phenyl-4-(pyridin-2-yl)butanamide (26)**



The racemic standard was synthesized using General Procedure F using alkene **12**. The yield was 29%.

Yield with NostocER

Trial 1: 48%,

Trial 2: 50%

Average: 49%

Enantioselectivity: 81:19 e.r., HPLC method: 20% IPA 80% hexanes, 45 min, OJ

Yield with GluER T36A

Trial 1: 77%,

Trial 2: 92%,

Average: 85%

Enantioselectivity: 68:32 e.r., HPLC method: 20% IPA 80% hexanes, 45 min, OJ

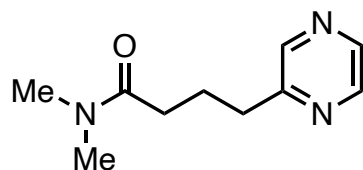
^1H NMR (500 MHz, CDCl_3) **5** 8.56 (ddd, $J = 4.9, 1.9, 0.9$ Hz, 1H), 7.56 (td, $J = 7.7, 1.9$ Hz, 1H), 7.37 – 7.31 (m, 2H), 7.29 (d, $J = 2.0$ Hz, 2H), 7.21 – 7.14 (m, 2H), 7.09 (ddd, $J = 7.5, 4.9, 1.2$ Hz, 1H), 4.15 (t, $J = 7.8$ Hz, 1H), 2.89 (d, $J = 21.9$ Hz, 6H), 2.58 (dq, $J = 13.5, 7.8$ Hz, 1H), 2.49 – 2.35 (m, 1H), 2.27 (t, $J = 7.6$ Hz, 2H).

^{13}C NMR (126 MHz, CDCl_3) **5** 172.85, 163.53, 149.28, 143.52, 136.66, 132.29, 128.68, 128.17, 126.67, 123.09, 121.54, 52.98, 37.34, 35.51, 31.70, 30.46.

IR: 3361 cm^{-1} , 1620 cm^{-1} , 1494 cm^{-1} , 1434 cm^{-1} , 1403 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 269.16484, found 269.16493

N,N-dimethyl-4-(pyrazin-2-yl)butanamide (27)



The product standard was made using commercially available vinyl pyrazine and model substrate (1) using the general procedure for NMR analysis of the biocatalytic reaction and NostocER (4 reactions were run at 20 μ mol each and pulled together). After NMR analysis all of the crude material was pooled, evaporated, resuspended in 1:1 Pentane:Et₂O and ~3 eq of HCl (from 4N HCl in dioxane) was added to precipitate the pyrazinium salts. The suspension was then filtered, and the filter was washed several times with 1:1 Pentane:Et₂O. Then, water was added to redissolve the salts, followed by enough of sat. Na₂CO₃ to until pH ~ 12 was reached. Then this aqueous layer was extracted several times with EtOAc, dried with Na₂SO₄, filtered and concentrated. An NMR showed essentially pure pyrazine product, with an isolated yield at 35% averaged over the four runs.

Yield with NostocER

Average: 35%

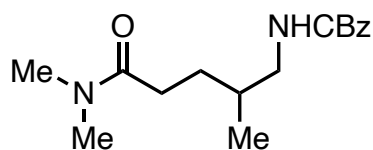
¹H NMR (500 MHz, CDCl₃) δ 8.63 – 8.31 (m, 5H), 2.98 (s, 3H), 2.95 (s, 3H), 2.93 – 2.88 (m, 3H), 2.39 (t, *J* = 7.3 Hz, 2H), 2.17 – 2.08 (m, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 171.71, 145.98, 144.28, 142.87, 142.43, 35.38, 34.85, 32.49, 23.93

IR: 3335 cm⁻¹, 1631 cm⁻¹, 1404 cm⁻¹

HRMS: calc'd [M+H]⁺ 194.12879, found 194.12876

benzyl (5-(dimethylamino)-2-methyl-5-oxopentyl)carbamate (28)



The racemic standard was synthesized via General Procedure F (36% yield) using alkene 13.

Yield with NostocER

Trial 1: 46%,

Trial 2: 44%

Average: 45%

Enantioselectivity: 20:80 e.r., HPLC method: 20% IPA 80% hexanes, 30 min, OJ

Yield with NostocER Y219F

Trial 1: 41%,

Trial 2: 43%

Average: 42%

Enantioselectivity: 24:76 e.r., HPLC method: 20% IPA 80 % hexanes, 30 min, OJ

Yield with GluER T36A

Trial 1: 63%,

Trial 2: 70%,

Average: 67%

Enantioselectivity: 53:47 e.r., HPLC method: 20% IPA 80 % hexanes, 30 min, OJ

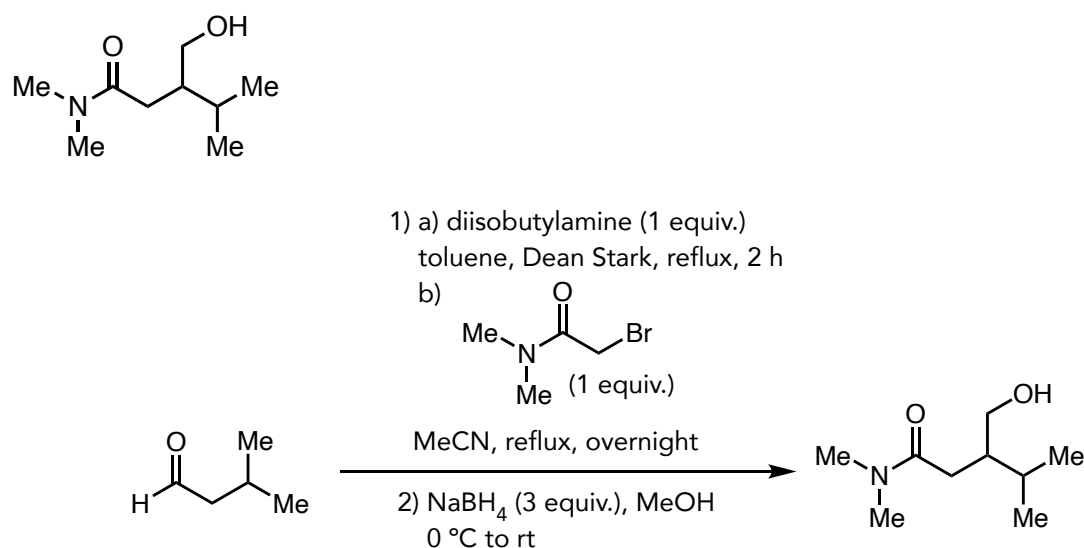
^1H NMR (500 MHz, CDCl_3) **δ** 7.35 (d, J = 4.3 Hz, 4H), 7.34 – 7.28 (m, 1H), 5.09 (s, 3H), 3.09 (q, J = 6.4 Hz, 2H), 2.99 (s, 3H), 2.93 (s, 3H), 2.42 – 2.24 (m, 2H), 1.77 – 1.63 (m, 2H), 1.53 – 1.40 (m, 1H), 0.92 (d, J = 6.5 Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) **δ** 173.03, 156.76, 136.85, 128.63, 128.22, 128.17, 66.70, 46.60, 37.35, 35.61, 33.46, 30.63, 29.04, 17.77.

IR: 3306 cm^{-1} , 2954 cm^{-1} , 2928 cm^{-1} , 1711 cm^{-1} , 1631 cm^{-1} , 1575 cm^{-1} , 1536 cm^{-1} , 1453 cm^{-1} , 1250 cm^{-1} , 1140 cm^{-1} , 1107 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 293.185966 found 293.18660

3-(hydroxymethyl)-*N,N*,4-trimethylpentanamide (29)



Step 1) Enamine-assisted alkylation

To a 100 mL flame-dried round bottom flask under nitrogen was added isovaleraldehyde (1.07 mL, 10 mmol, 1 equiv.), toluene (50 mL), and diisobutylamine (1.75 mL, 10 mmol, 1 equiv.). A Dean-Stark apparatus and reflux condenser were attached to the flask, and the mixture was heated at reflux for 2 hours. At this point, TLC showed little to no aldehyde remaining, and the flask was allowed to cool to room temperature. The toluene was then evaporated in vacuo, and the reaction mixture was redissolved in acetonitrile (50 mL). 2-bromo-*N,N*-dimethylacetamide (1.08 mL, 10 mmol, 1 equiv.) was then added and the solution was heated to reflux overnight. After allowing the reaction mixture to cool to room temperature, acetic acid (4.7 mL) and water (14 mL) were added and the mixture was stirred for 30 minutes at room temperature and for 1 hour at 45 °C. Acetonitrile was then removed under reduced pressure, and the mixture was extracted with dichloromethane. The organic phase was washed with a saturated solution of sodium bicarbonate, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was then taken forward to the next step without any further purification.

Step 2) NaBH₄ reduction

The crude mixture was dissolved in MeOH (0.1 M), cooled to 0 °C and NaBH₄ (3 equiv.) was carefully added. The reaction was allowed to stir to room temperature over 3 hours, at which point the reaction mixture was concentrated in vacuo. To the crude residue was added sat. NH₄Cl and water. The aqueous mixture was extracted with EtOAc (3x). The organic layers were pooled, dried with sodium sulfate, filtered, and concentrated. The crude product was purified by column chromatography (5-18% EtOAc in dichloromethane, then 1-10% MeOH in dichloromethane). The pure product was isolated as a yellow oil (13% yield over two steps.)

Yield with NostocER

Trial 1: 62%,

Trial 2: 58%

Average: 60%

e.r. 50:50, HPLC method: 5% IPA 95% Hexanes 30 min OJ-H

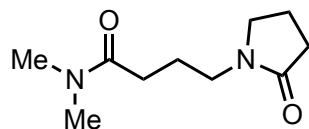
^1H NMR (500 MHz, CDCl_3) δ 3.69 (dd, $J = 10.9, 3.7$ Hz, 1H), 3.54 (dd, $J = 10.9, 7.8$ Hz, 1H), 3.05 (s, 3H), 2.96 (s, 3H), 2.50 (dd, $J = 15.9, 3.0$ Hz, 1H), 2.35 (dd, $J = 15.9, 9.8$ Hz, 1H), 2.02 – 1.89 (m, 1H), 1.84 – 1.72 (m, 1H), 0.91 (dd, $J = 11.1, 6.9$ Hz, 6H).

^{13}C NMR (126 MHz, CDCl_3) δ 174.42, 65.65, 43.16, 37.77, 35.95, 34.59, 29.94, 20.13, 19.65.

IR: 3406 cm^{-1} , 2956 cm^{-1} , 2872 cm^{-1} , 1620 cm^{-1} , 1540 cm^{-1} , 1503 cm^{-1} , 1485 cm^{-1} , 1400 cm^{-1} , 1368 cm^{-1} , 1264 cm^{-1} , 1155 cm^{-1} , 1049 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 174.14886, found 174.14912

***N,N*-dimethyl-4-(2-oxopyrrolidin-1-yl)butanamide (30)**



To pyrrolidine-one (42.5 mmol, 1 equivalent) was added metallic sodium (6 equivalents) and the mixture was heated to 90 °C. Then tetrahydrofuran-2-one (1 equiv) was added dropwise at 120 °C. The reaction was run at this temperature for 24 hours. The reaction was quenched with 5% aq NaOH and washed with DCM. The extracts were pooled, washed with brine and dried with magnesium sulfate and concentrated. The crude was semi purified by column (0 to 10% methanol to DCM) and the acid was carried through General Procedure C with commercially available dimethylamine, which gave pure product after silica purification. Yield was 90% over two steps.

Yield with NostocER

Trial 1: 42%,

Trial 2: 30%

Average: 36%

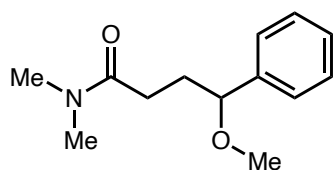
^1H NMR (500 MHz, CDCl_3) δ 3.41 (t, J = 7.0 Hz, 2H), 3.33 (t, J = 7.1 Hz, 2H), 2.99 (s, 3H), 2.94 (d, J = 4.7 Hz, 3H), 2.34 (dt, J = 22.9, 7.7 Hz, 4H), 2.04 – 1.96 (m, 2H), 1.88 (p, J = 7.3 Hz, 2H).

^{13}C NMR (126 MHz, CDCl_3) δ 175.28, 172.28, 47.18, 42.29, 37.37, 35.59, 31.25, 30.48, 22.77.

IR: 3362 cm^{-1} , 2922 cm^{-1} , 2852 cm^{-1} , 1635 cm^{-1} , 1604 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 199.14410, found 199.14410

4-methoxy-*N,N*-dimethyl-4-phenylbutanamide (31)



The racemic standard was synthesized by stirring γ -phenyl- γ -butyrolactone in 200 mL of a 2M solution of dimethylamine in THF under nitrogen at room temperature (29.8 mmol scale). The mixture was concentrated *in vacuo* to yield crude material that was taken to the next step.

The crude product was resuspended in dry THF and put under N₂ and sodium hydride was added (0.98 equiv) dropwise at 0°C. After 30 minutes iodomethane was added dropwise (1 equiv) and the mixture was warmed to room temperature and left to stir overnight. The reaction was quenched with water and the extracted with ether. The organic layers were pulled, dried over sodium sulfate and concentrated. The crude mixture was purified via silica chromatography to yield pure product (40% yield).

Yield with NostocER

Trial 1: 54%

Trial 2: 56%

Average: 55%

Enantioselectivity: 76:24 e.r., HPLC method: 2% IPA 98% hexanes, 40 min, OJ

Yield with GluER T36A

Trial 1: 33%

Trial 2: 33%

Average: 33%

Enantioselectivity: 27:73 e.r., HPLC method: 2% IPA 98% hexanes, 40 min, OJ

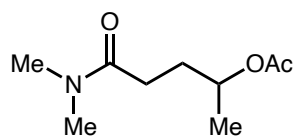
¹H NMR (500 MHz, CDCl₃) δ 7.38 – 7.24 (m, 5H), 4.22 (dd, *J* = 7.3, 5.8 Hz, 1H), 3.22 (s, 3H), 2.94 (d, *J* = 10.5 Hz, 6H), 2.38 (td, *J* = 7.0, 6.5, 4.6 Hz, 2H), 2.12 – 1.96 (m, 2H).

¹³C NMR (126 MHz, CDCl₃) δ 172.80, 142.08, 128.53, 127.68, 126.72, 83.01, 56.85, 37.31, 35.52, 33.43, 29.32.

IR: 2930 cm⁻¹, 2820 cm⁻¹, 1647 cm⁻¹, 1493 cm⁻¹, 1453 cm⁻¹, 1398 cm⁻¹, 1102 cm⁻¹

HRMS: calc'd [M+H]⁺ 222.148856, [M-OMe] 190.12319, found 190.12354 (-OMe)

5-(dimethylamino)-5-oxopentan-2-yl acetate (32)



γ -valerolactone was suspended in 200 mL of 2M dimethylamine in THF was stirred under a nitrogen atmosphere at room temperature for 2 days. The solvent was removed under reduced pressure to give crude product which was carried onto the next step without further purification.

The crude product was resuspended in dry DCM with DMAP (0.2 equiv.), acetic anhydride (2.5 equiv.) and triethylamine (4 equiv.). The mixture was resuspended in ether, washed with 10% HCl and water. The organic layer was dried in sodium sulfate and concentrated, and the residue was purified by silica flash chromatography (10-100% EtOAc in DCM). The overall yield was 2.5% over two steps (low product yield due to high solubility in aqueous media and due to volatility).

Yield with NostocER

Trial 1: 54%,

Trial 2: 54%

Average: 54%

Enantioselectivity: 26:74 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

Yield with GluER T36A

Trial 1: 77%,

Trial 2: 82%,

Average: 80%

Enantioselectivity: 68:32 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

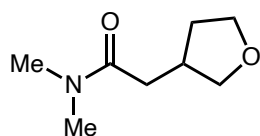
^1H NMR (500 MHz, CDCl_3) **δ** 4.94 (ddd, $J = 7.9, 6.3, 4.7$ Hz, 1H), 2.97 (d, $J = 21.9$ Hz, 6H), 2.44 – 2.27 (m, 2H), 2.03 (s, 3H), 1.98 – 1.82 (m, 2H), 1.25 (d, $J = 6.2$ Hz, 4H).

^{13}C NMR (126 MHz, CDCl_3) **δ** 172.27, 170.94, 70.78, 37.31, 35.60, 31.42, 29.42, 21.51, 20.28.

IR: 3369 cm^{-1} , 2924 cm^{-1} , 2853 cm^{-1} , 1711 cm^{-1} , 1605 cm^{-1} , 1376 cm^{-1} , 1258 cm^{-1} , 1065 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 210.11006, found 210.1101

***N,N*-dimethyl-2-(tetrahydrofuran-3-yl)acetamide (33)**



The racemic standard was synthesized via General Procedure C from commercially available 2-(tetrahydrofuran-3-yl)acetic acid and dimethylamine (2.0M in THF) . Yield over General Procedure C and D was 67%.

Yield with NostocER

Trial 1: 60%

Trial 2: 51%

Average: 57% yield

Enantioselectivity: 50:50 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

Yield with GluER T36A

Trial 1: 17%, e.r.: 49:51

Trial 2: 13%, e.r.: 50:50

Average: 15%

Enantioselectivity: 50:50 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

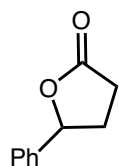
^1H NMR (500 MHz, CDCl_3) δ 3.98 (dd, J = 8.6, 7.0 Hz, 1H), 3.86 (td, J = 8.2, 5.1 Hz, 1H), 3.74 (q, J = 7.7 Hz, 1H), 3.42 (dd, J = 8.6, 6.0 Hz, 1H), 2.97 (d, J = 24.1 Hz, 6H), 2.71 (p, J = 7.1 Hz, 1H), 2.48 – 2.33 (m, 2H), 2.15 (dtd, J = 12.7, 7.7, 5.1 Hz, 1H), 1.55 (dq, J = 12.3, 7.4 Hz, 1H).

^{13}C NMR (126 MHz, CDCl_3) δ 171.83, 73.52, 67.80, 37.33, 35.62, 35.48, 32.40.

IR: 2932 cm^{-1} , 2896 cm^{-1} , 1639 cm^{-1} , 1502 cm^{-1} , 1401 cm^{-1} , 1154 cm^{-1} , 1108 cm^{-1} , 1077 cm^{-1} , 1043 cm^{-1}

HRMS: calc'd $[\text{M}+\text{H}]^+$ 158.117556, found 158.11743

γ -phenyl- γ -butyrolactone (34)



This standard is commercially available.

Yield with NostocER

Trial 1: 89%,

Trial 2: 86%

Average: 88%

Enantioselectivity: 15:84 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

Yield with GluER T36A

Trial 1: 56%,

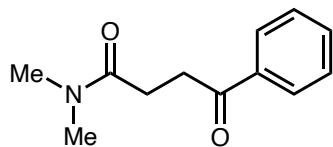
Trial 2: 64%,

Average: 60%

Enantioselectivity: 95:5 e.r., HPLC method: 5% IPA 95% hexanes, 45 min, OJ

^1H NMR (500 MHz, CDCl_3) δ 7.40 (dd, $J = 8.3, 6.3$ Hz, 2H), 7.34 (td, $J = 7.2, 1.3$ Hz, 3H), 5.52 (dd, $J = 8.0, 6.1$ Hz, 1H), 2.74 – 2.56 (m, 3H), 2.28 – 2.09 (m, 1H).

N,N-dimethyl-4-oxo-4-phenylbutanamide (35)



Synthesized via general procedure C using commercially available 4-phenyl-4-oxo-butyric acid and dimethyl amine (yield was 90%). All spectra match reported values in Tanaka et al.²⁴

Yield with NostocER:

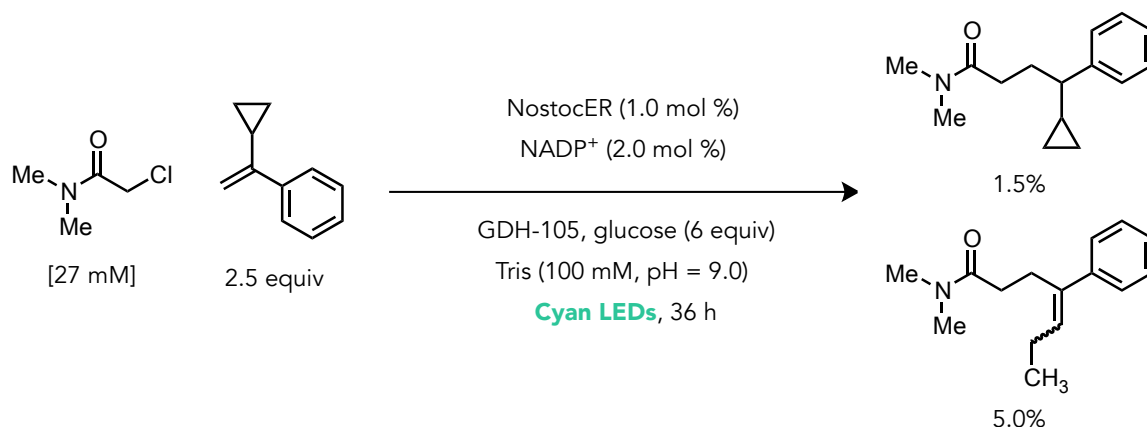
Trial 1: 91%

Trial 2: 89%

Average: 90%

¹H NMR (500 MHz, CDCl₃) **δ** 8.05 – 7.96 (m, 2H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 3.36 (t, *J* = 6.6 Hz, 2H), 3.03 (d, *J* = 63.6 Hz, 6H), 2.79 (t, *J* = 6.6 Hz, 2H).

CYCLOPROPANE RING OPENING EXPERIMENT



The coupling reaction of *N,N*-dimethyl chloroacetamide and α -cyclopropyl styrene is run to test for the existence of radical intermediates along the reaction pathway. Due to the general lower reactivity of α -substituted styrenes with substituents larger than ethyl, the loadings of NostocER and NADP⁺ are doubled and the scale of the chloroamide is increased to 0.29 mmol (35.25 mg). 6 equivalents of glucose and 0.1 mg GDH-105 lysate per mg of substrate (3.525 mg) are weighed out and added to a 25 mL round-bottom flask. Then, inside a Coy® anaerobic chamber 7.42 mL of thoroughly degassed 100 mM Tris buffer, pH 9 and NADP⁺ (863 μ L of a 5 mg/mL stock solution, 5.8 μ mol, 2.0 mol%) and purified WT NostocER (3.05 mL of a 0.95 mM solution, 2.9 μ mol, 1.0 mol%) are added. The yellow color of the oxidized FMN quickly disappears when added to the reducing solution. Once the solution is completely clear, 30 μ L of *N,N*-dimethyl chloroacetamide are dissolved into the mixture, followed by 116 μ L of α -cyclopropyl styrene. The flask is capped with a rubber septum, taken to the bench, and allowed to stir for 5 min under nitrogen atmosphere before turning on the 50W cyan LED. After 36 h of irradiation the reaction mixture is poured into an Erlenmeyer flask containing a biphasic mixture of 50 mL DCM and 50 mL 1 M HCl and stirred vigorously for 45 min-1h. The resulting biphasic mixture is vacuum filtered through a pad of Celite®. The clarified biphasic filtrate is transferred to a separatory funnel and the DCM layer collected. The aqueous portion is extracted with additional DCM (2x 50 mL) and the combined organic layer is dried with sodium sulfate, filtered, and evaporated. The resultant crude residue is purified with an Isolera™ One flash chromatography system using a C18 stationary phase, and an acetonitrile/water gradient as the mobile phase. Two products are isolated, the ring-closed 4-cyclopropyl-*N,N*-dimethyl-4-phenylbutanamide (1.0 mg, 1.5% yield) and the ring-opened *N,N*-dimethyl-4-phenylhept-4-enamide (3.3 mg, 83:17 olefin isomer ratio, 5.0% yield), providing strong evidence for the involvement of radical species along the reaction coordinate.

IDENTIFICATION OF THE CHARGE TRANSFER COMPLEX (EDA COMPLEX)

All samples were prepared in an MBraun glovebox with O₂ level less than 0.1 ppm. Cuvettes were specially designed with a GL18 cap to maintain oxygen free conditions for the duration of the experiment.

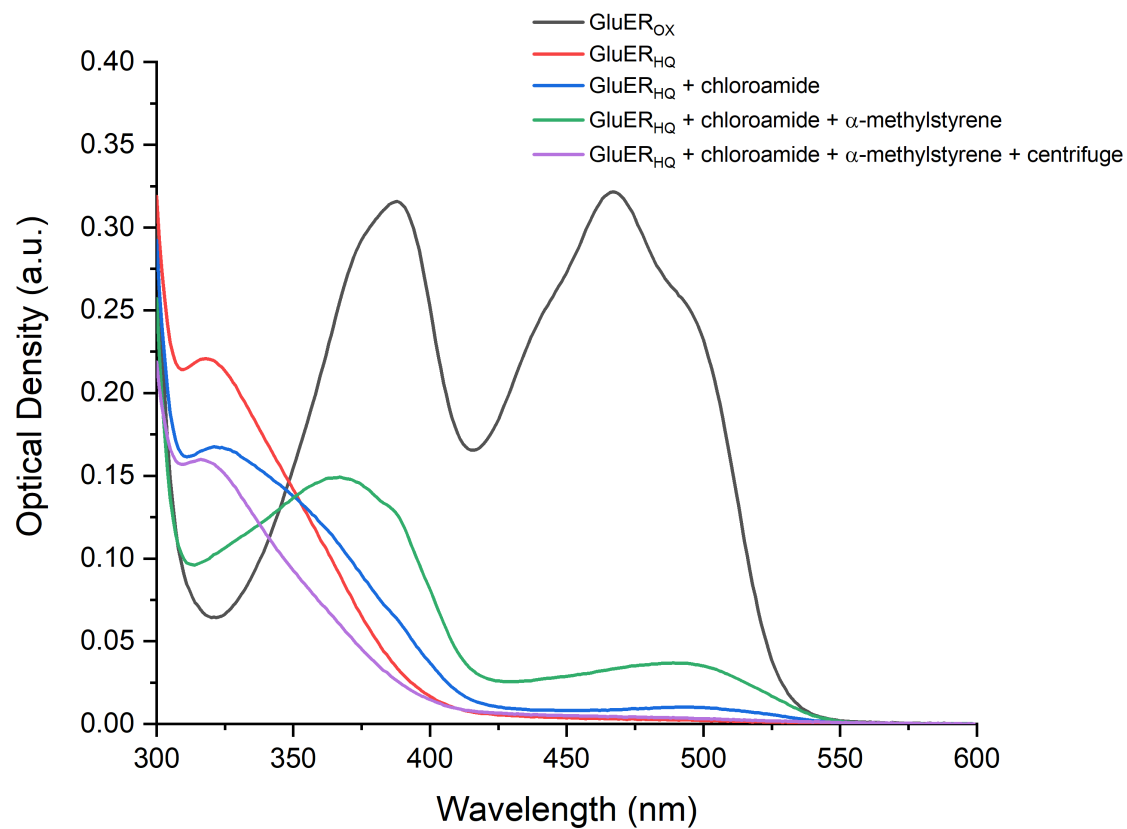
To generate the charge-transfer state in solution 20 nmol of ERED is diluted to a final volume of 1.0 mL in Tris buffer (pH = 9.0) and absorption spectra is taken. The cuvette is taken back into the MBraun and 120 nmol (1.2 equiv) sodium dithionite is added to generate FMN_{hq} and an absorption spectrum is taken (figure S4, red trace). 9 μ L of substrate **1** is added (3800:1 mol ratio substrate:enzyme) to the solution and an absorption spectrum is taken, showing a weak new absorptive feature at ~500 nm (figure S4, blue trace). Then 18 μ L of alpha-methylstyrene is added to 50 μ L of degassed THF and the total volume of the mixture (68 μ L) is transferred to the cuvette and an absorption spectrum is taken. A strong new absorptive feature at 360 nm and 500 nm appears (Supplemental Figure 4, green trace). The mixture is then removed from the cuvette and placed into Amicon Ultra Centrifugal Filters (0.5 mL, 10 kDa cutoff) and centrifuged in the MBraun at 12500 rpm for 3 minutes to remove small molecules. The protein is resuspended in Tris Buffer and the centrifugal process is repeated 3 times. Then the resuspended protein is transferred back to the cuvette and a spectrum is taken to show recovered FMN_{hq} (Supplemental Figure 4, purple trace).

Interestingly, when the model substrate (**1**) is added to a solution of reduced GluER_{hq}, only a small spectral growth is seen, again peaking around 490 nm (Supplemental Figure 4 blue trace). When alkene is added to the N,N-dimethylchloroamide and GluER_{hq} solution, a large charge transfer state can be observed in Supplemental Figure 4 (green trace) which is similar to the ones observed by Biegasiewicz et.al. Centrifugation of the solution in 10 kDa filters to remove small molecules shows complete recovery of the GluER_{hq}, which is consistent with this being a noncovalent interaction. Alkene (**2**) plus GluER_{hq} yields no noticeable spectral changes (Supplemental Figure 5), suggesting the alkene can only take place in charge transfer complex formation if **1** is present in the active site. This feature is not reproduced when the flavin is not protein bound (Supplemental Figure 8). This provides strong evidence for enzyme-templated ternary charge transfer complex where the enzyme organizes the chloroamide and alkene near the flavin in such a way that both the alkene and the FMN can donate into the chloroamide C-Cl σ^* , which allows for facile homolytic cleavage upon excitation at 490 nm and almost instantaneous coupling of the chloroamide with the alkene. Peaks around 320 nm can be contributed to excess dithionite, not the charge transfer state.

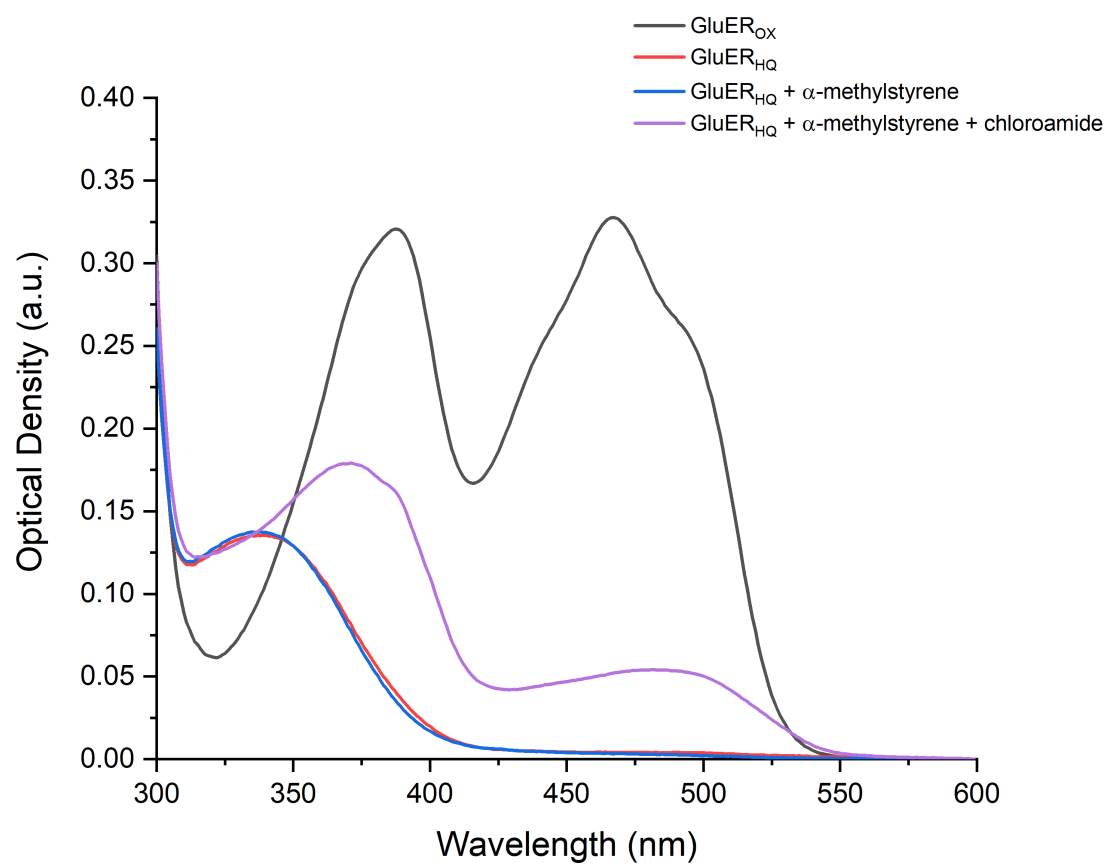
This charge transfer state is consistent across both enzymes used in this study, as NostocER with the model chloroamide and styrene exhibits the same behaviour as seen in Supplemental Figure 6. NostocER is a worse binder of alpha-methylstyrene, as twice the amount of alkene needs to be added to achieve the same relative absorbance of the charge transfer state then the charge transfer state achieved with GluER. However, it is not consistent once we moved to the more easily reduced 2-chloroacetophenone substrate class (Supplemental Figure 6). As can be seen in the blue trace, addition of substrate to GluER_{hq} gives recovery of oxidized flavin which suggests GluER_{hq} reduces the substrate without charge transfer state excitation. Efforts to keep the solution in the dark and then take the absorbance spectrum only returned traces similar to the blue trace. Running this reaction

under normal LCMS analysis reaction conditions in the dark yielded 3.5% of coupled product and about 10-15% of the hydrodehalogenated product. Due to the increased yield with light, it is likely a charge transfer mechanism is operative but cannot be seen due to background ground state reduction of the starting material. This suggests the charge transfer state reported in Huang et al is likely due to the presence of an equilibrium mixture of reduced and oxidized flavin and not the real charge transfer state of the protein and substrate.²² Since they did not conduct their charge transfer studies using stoichiometric to protein reductant, instead an excess of D-glucose and a catalytic turnover system, they were able to reach an equilibrium absorbance of oxidized flavin and reduced flavin which would give the trace they reported as a charge transfer state in their paper.

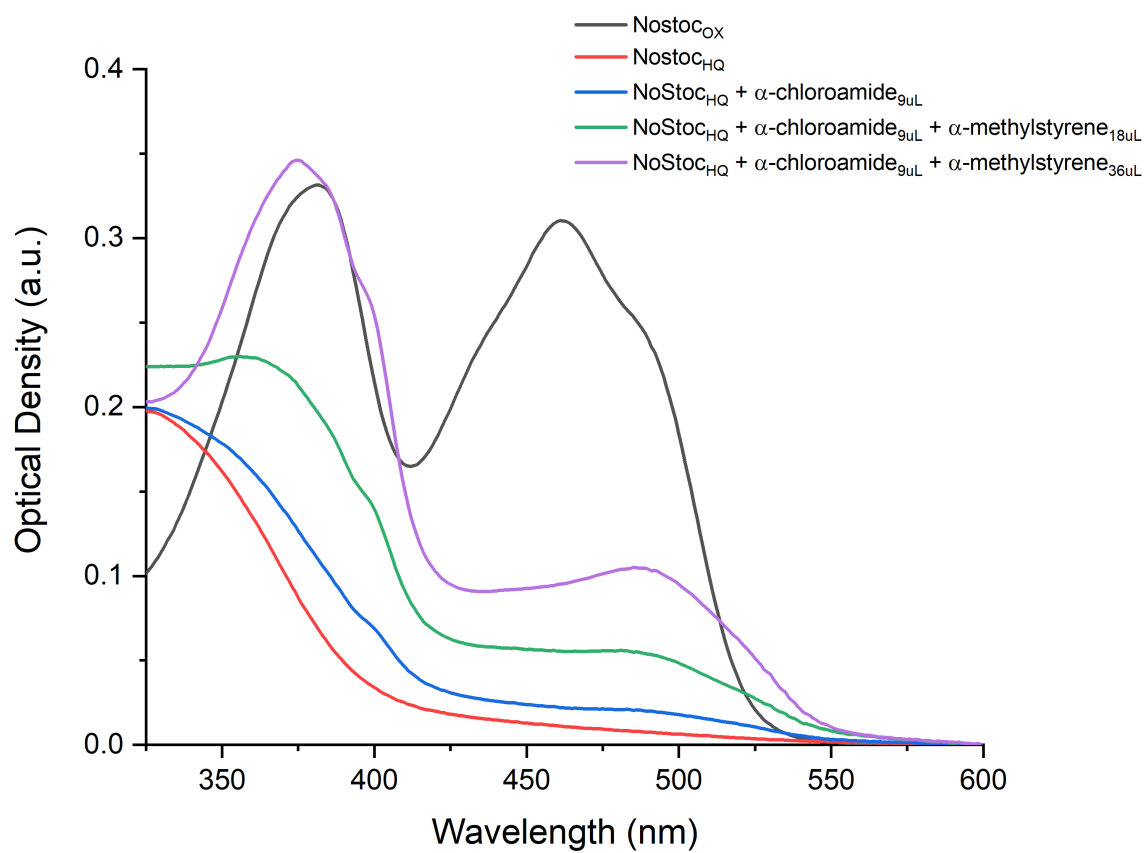
Supplemental Figure 4: GluER T36A mediated Charge Transfer State



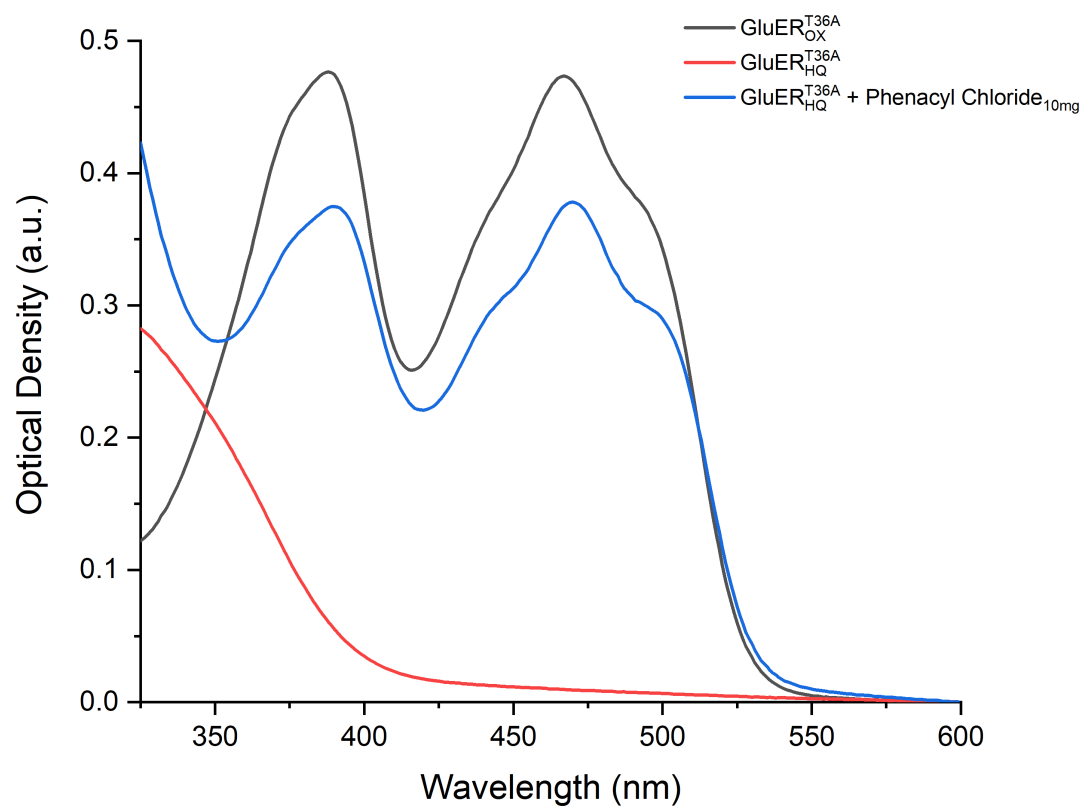
Supplemental Figure 5: GluER T36A mediated Charge Transfer State with α -methyl styrene added first



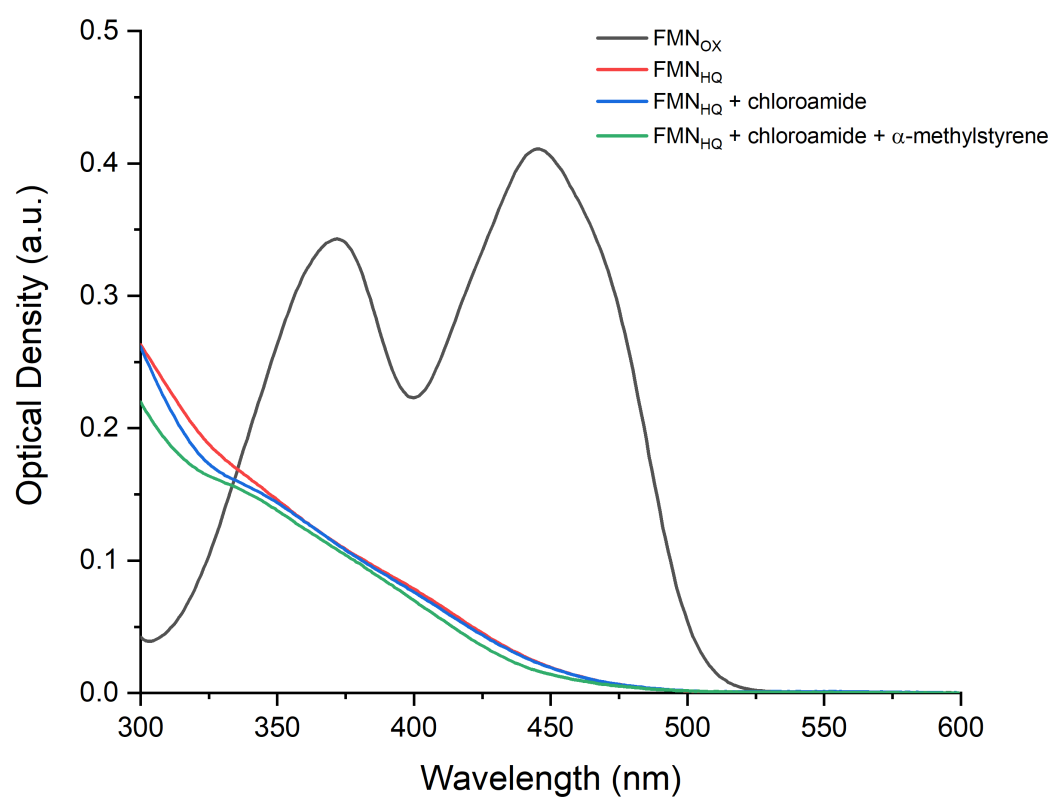
Supplemental Figure 6: NostocER mediated Charge Transfer State



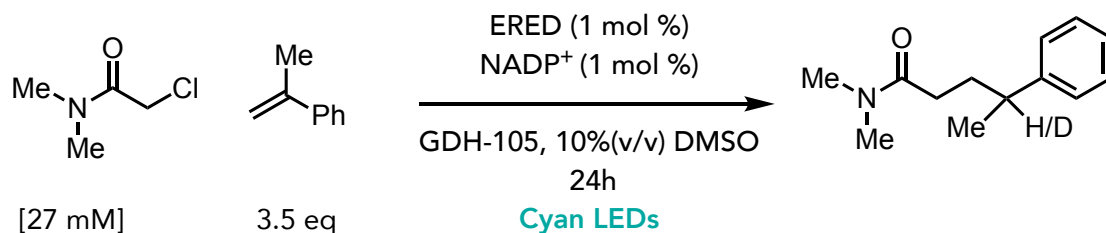
Supplemental Figure 7: Return of Oxidized Flavin when chloroacetophenone is added to reduced GluER T36A



Supplemental Figure 8: Free FMN does not form a charge transfer complex



Deuterium Incorporation Experiments



A series of isotopic labeling experiments were conducted with the aim of understanding the source of hydrogen incorporated into the γ -position of the product. A pair of isotopic labeling experiments are run at 1 mol% ERED catalyst loading at 20 μ mol scale using the model substrate, 2-chloro-N,N-dimethylacetamide ([S]=25 mM) and α -methylstyrene, in order to assess the source of the hydrogen atom incorporated into the product. Two reactions are run side by side for 24 hours, one containing 6 equivalents of (D)-1-²H-glucose in 100 mM Tris, pH=9 (reaction buffer) made in H₂O, the other containing (D)-glucose at the same loading run in reaction buffer made in D₂O. Upon completion of the reaction, the work up for NMR analysis is performed.

The extent of deuterium incorporation in the ²H-glucose reaction and D₂O reaction can be calculated from Eqn. 1 using integrations of the corresponding C-H and C-D peaks in the quantitative ¹³C-NMR spectrum for either the methyl carbon adjacent to the deuterium bearing carbon or the methylene carbon adjacent to the deuterium bearing carbon or the deuterium bearing carbon itself (Figure S7-Figure S13). Due to the increased sensitivity of integrating the carbon peaks of the carbons adjacent to the deuterated carbon, the methyl carbon and the beta carbon were used to determine deuterium incorporation. MestReNova's (version 10.0.2) "fit region" tool was used to get accurate peak integrations, with the standard settings (lower and upper boundaries set to 0.1 and 100 Hz, respectively, with simulated annealing enabled, coarse iterations-500, fine iterations-200). The calculated deuterium incorporation for each ERED tested is presented in Figure S7 to Figure S13. The tabulated results for deuterium incorporation are presented in Table S5.

The two EREDs that offer the highest levels of γ -enantioselectivity for the model reaction; NostocER and GluER T36A were tested with either 1-²H-glucose as the deuterium source in protic buffer or glucose in D₂O as the deuterium source. GluER T36A, which produces the product enantiomer opposite to that formed with NostocER at 2:98 er displays the highest fidelity of deuterium incorporation with 1-²H-glucose at 93%, with <2% incorporation observed with D₂O buffer. NostocER and 1-²H-glucose leading to only 75% incorporation of deuterium, while in D₂O giving 21% incorporation along with a decrease in enantioselectivity.

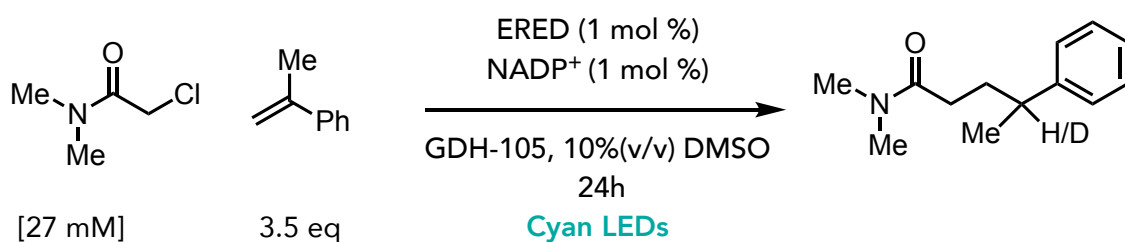
Unlike previously reported chemistry from our group, significant levels of deuterium are noted under both sets of conditions for NostocER. Previous studies by Ghisla et al.²⁵ have shown that for free FMN_{hq} the exchange rates of the key N₅ hydrogen with the surrounding solution exhibits a strong pH dependence, with the slowest exchange rates occurring at pH

= 9, the same pH at which the reactions are run. This suggests the diminished deuterium incorporation in NostocER is not due to N₅ hydrogen exchange. The fact that high product er is observable despite low levels of deuterium incorporation with 1-²H-glucose suggests that in certain cases it may be possible for active site tyrosines to cooperatively act as HAT catalysts that react with the same face of the prochiral radical as FMN_{sq}, while others can erode it by reacting with the opposite face, assuming that the mutation's effect on the relative orientation between flavin and the prochiral radical is negligible. Mutagenesis studies in NostocER reported previously in our lab¹⁴ replacing two active site tyrosines conserved among many members of the OYE family^{26, 27} with phenylalanine lead to the result that the Y106F variant decreased product enantioselectivity significantly from 90:10 er to 67:33 whereas the Y219F variant increased it to 95:5. We hypothesized that this meant Y106 was a hydrogen atom source that gave the same facial selectivity as the FMN HAT in NostocER while Y219 was a hydrogen atom source that gave the opposite enantiomer.

To examine this hypothesis, the same deuterium incorporation studies were conducted with NostocER Y106F and NostocER Y219F. As expected, Nostoc Y219F had slightly increased deuterium incorporation from ²H-glucose (78% vs. 75% in wild type) and significantly decreased deuterium incorporation in D₂O. The lack of complete suppression of deuterium incorporation when the reaction is run in D₂O and the minimal increase of deuterium incorporation when the reaction is run with deuteroglucose indicates other active site tyrosines perhaps also play a significant role. Nostoc Y106F had significantly decreased deuterium incorporation in the reaction run from ²H-glucose and comparable levels of deuterium incorporation from the reaction run in D₂O. We attribute the reduced deuterium levels in the D₂O reaction to the presence of protons in the reaction from the buffer the enzyme is stored in (this makes up roughly 1/7 of the total reaction volume). Kinetically, the proton is expected to undergo HAT transfer faster than deuterium, which would lead to a suppression of deuterium incorporation from a tyrosine HAT source and preferential incorporation of a proton instead due to the significant presence of protons. This would decrease the amount of deuterium incorporation from tyrosine observed, despite the significant amount of radical termination from tyrosine in the reaction. Since the N₅ hydrogen does not easily undergo exchange with solvent at the pH this reaction is run at, it suggests the lack of deuterium incorporation when the reaction is run with deuteroglucose is a better indicator of Nostoc Y106F having more significant radical termination from an alternative HAT source.

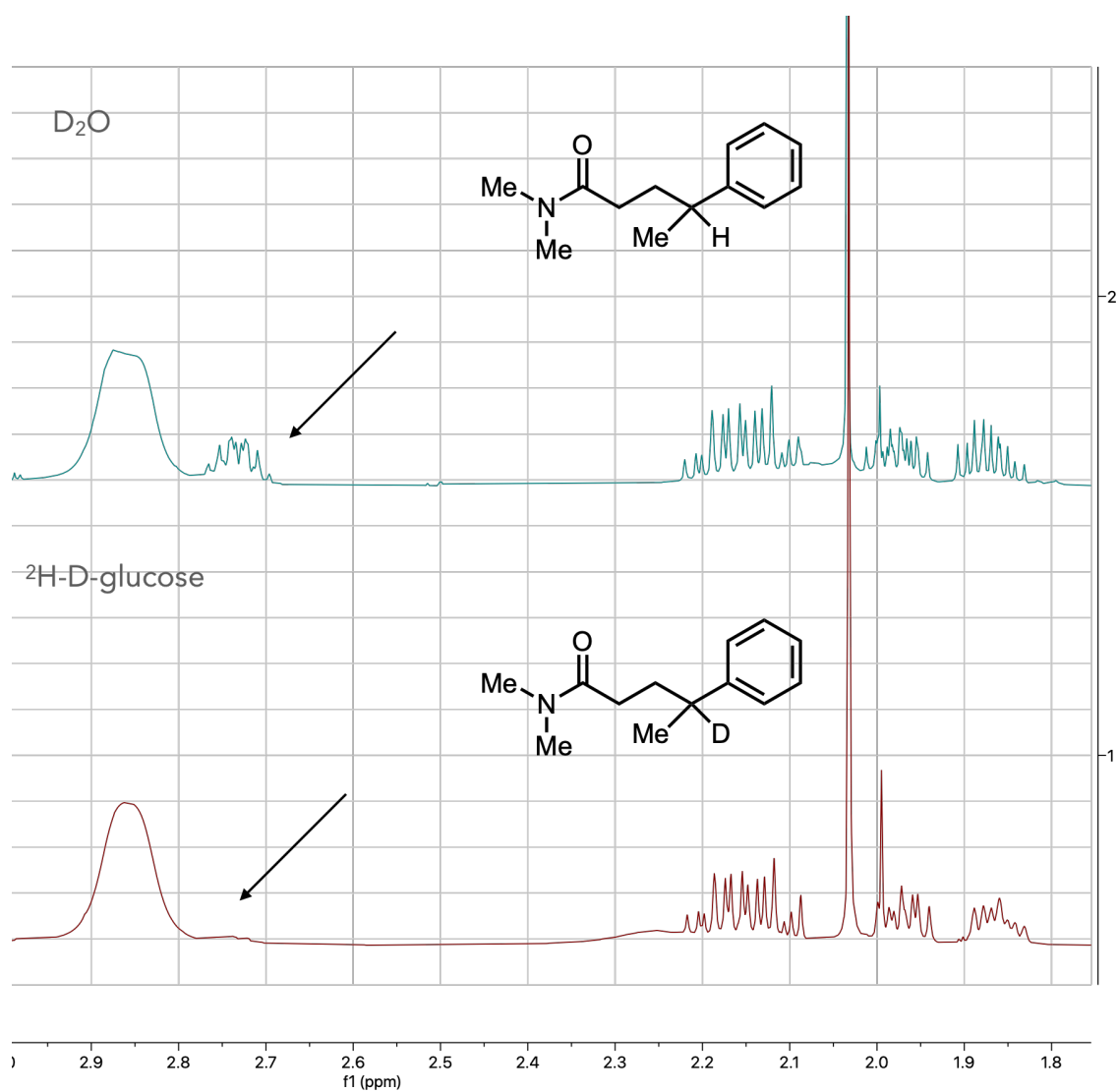
$$\%D_{\text{incorp, methyl/beta C}} = \left[1 - \left(\frac{A_1}{A_1 + A_2} \right) \right] \times 100 \quad [\text{Eqn. 1}]$$

Table S5: Deuterium Incorporation results

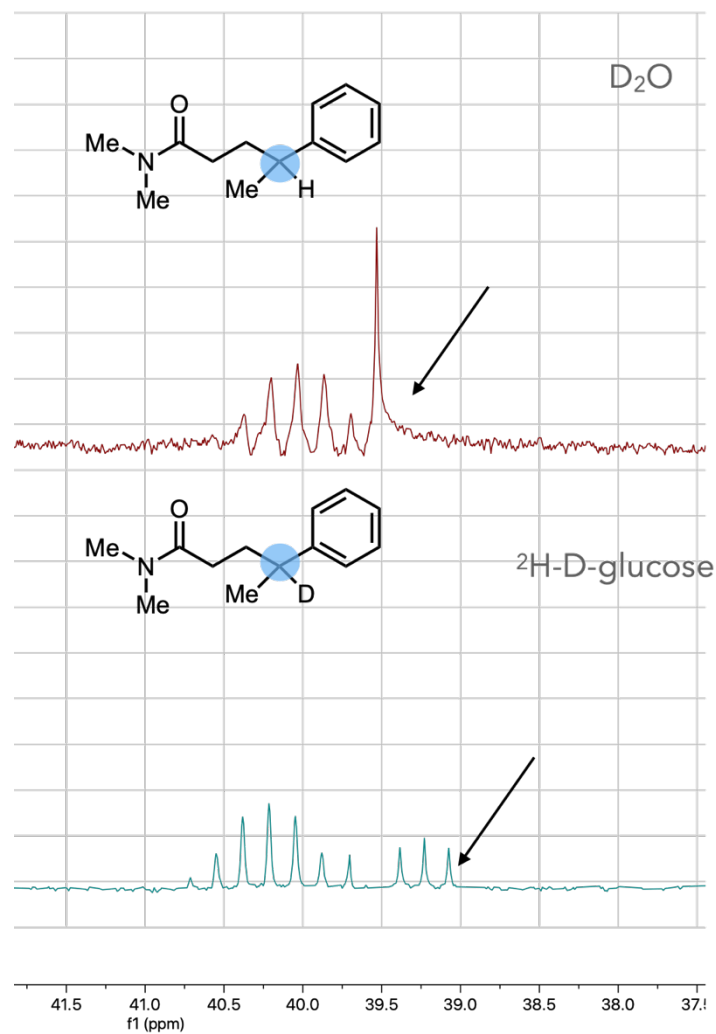


Deuterium source	ERED	yield	e.r.	% D inc
Isotopically Labelled Flavin <i>d</i> ₁ -glucose, H ₂ O Buffer	GluER T36A	97%	2:98	93%
	NostocER	94%	92:8	75%
	NostocER Y219F	>99%	95:5	78%
	NostocER Y106F	76%	67:33	17%
Isotopically Labelled Buffer <i>H</i> ₁ -glucose, D ₂ O Buffer	GluER T36A	>99%	2:98	<2%
	NostocER	96%	88:11	21%
	NostocER Y219F	>99%	94:6	12%
	NostocER Y106F	80%	64:36	23%

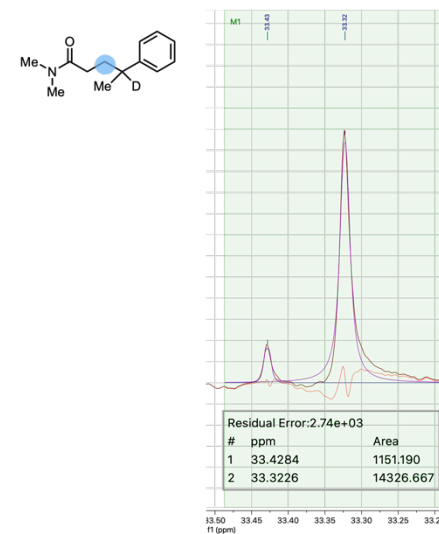
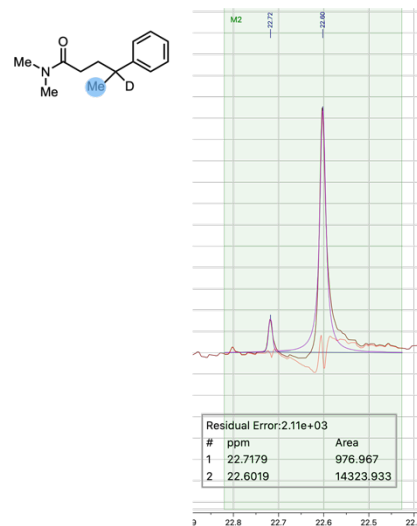
Supplemental Figure 9: GluER T36A ^1H NMR



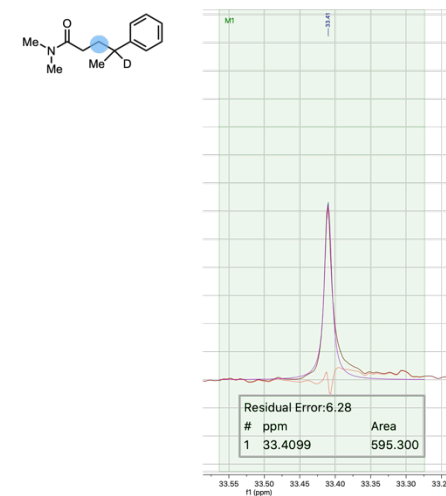
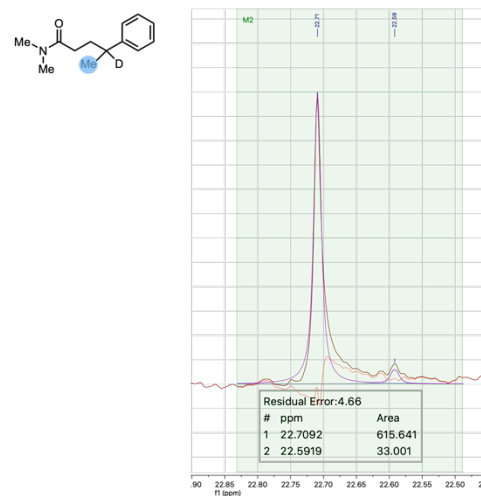
Supplemental Figure 10: GluER T36A ^{13}C NMR and Integrated Carbon Data



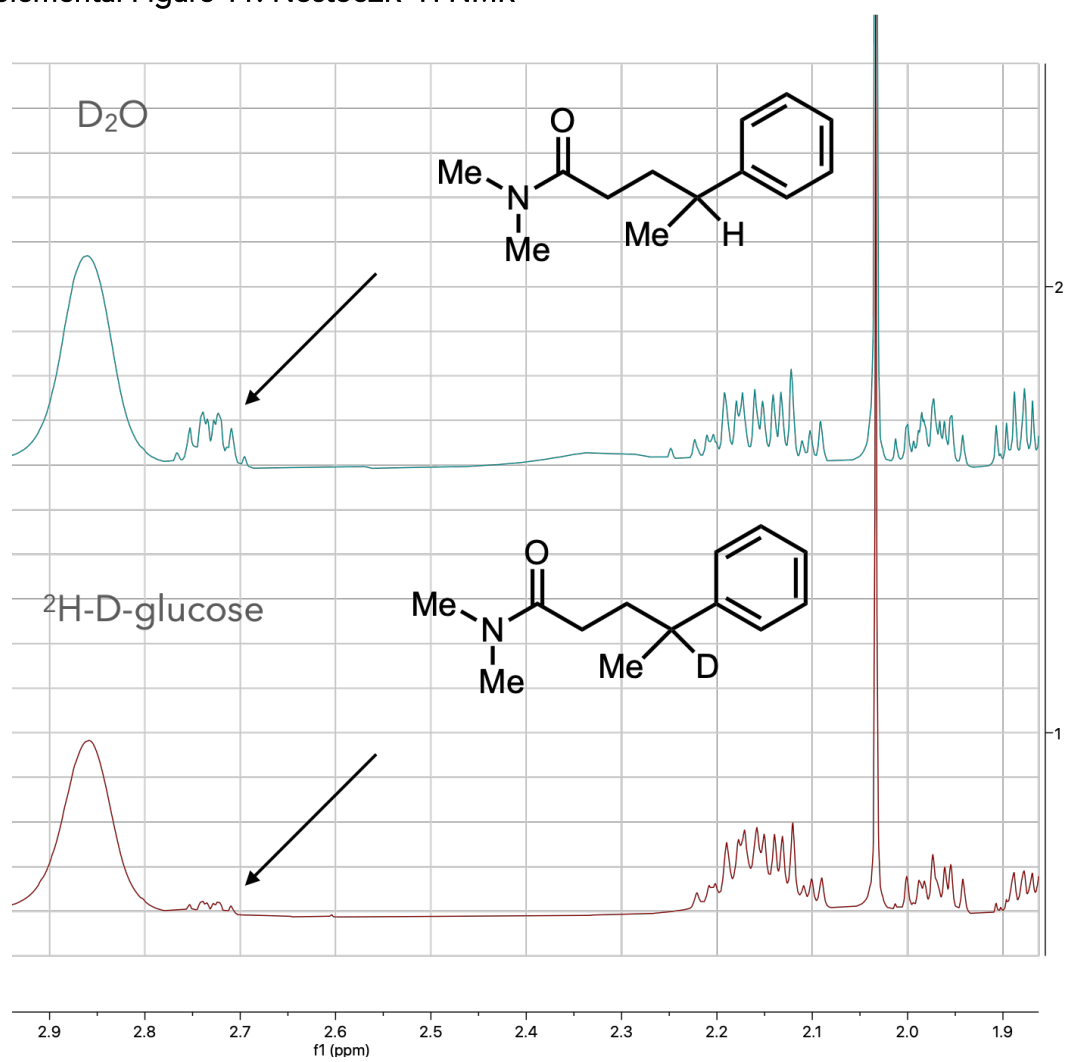
GluER T36A ^{13}C NMR $^2\text{H-glucose}$



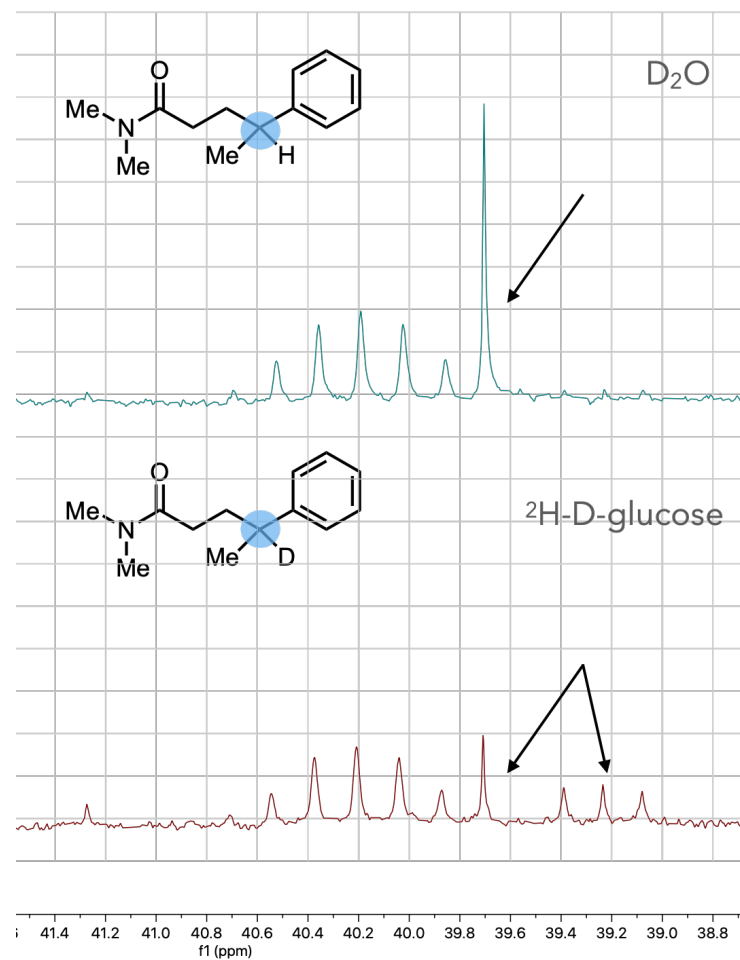
GluER T36A ^{13}C NMR D_2O



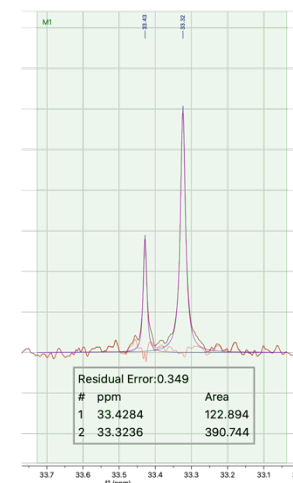
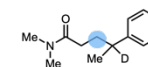
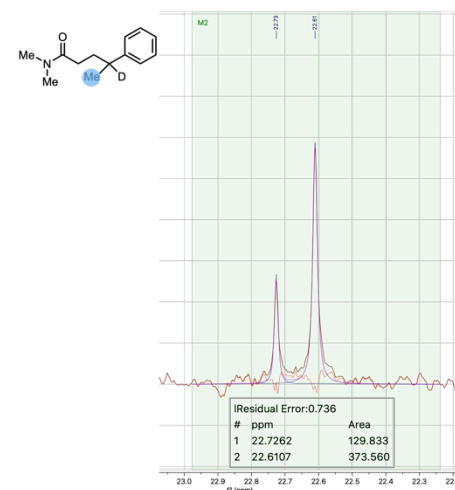
Supplemental Figure 11: NostocER ^1H NMR



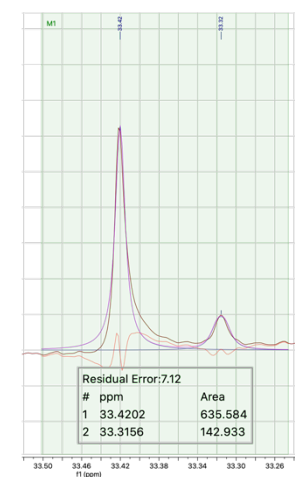
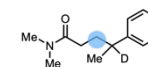
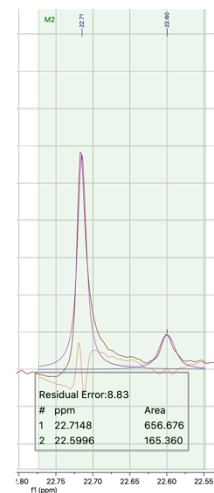
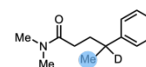
Supplemental Figure 12: Nostoc ER ^{13}C NMR and Carbon integrations



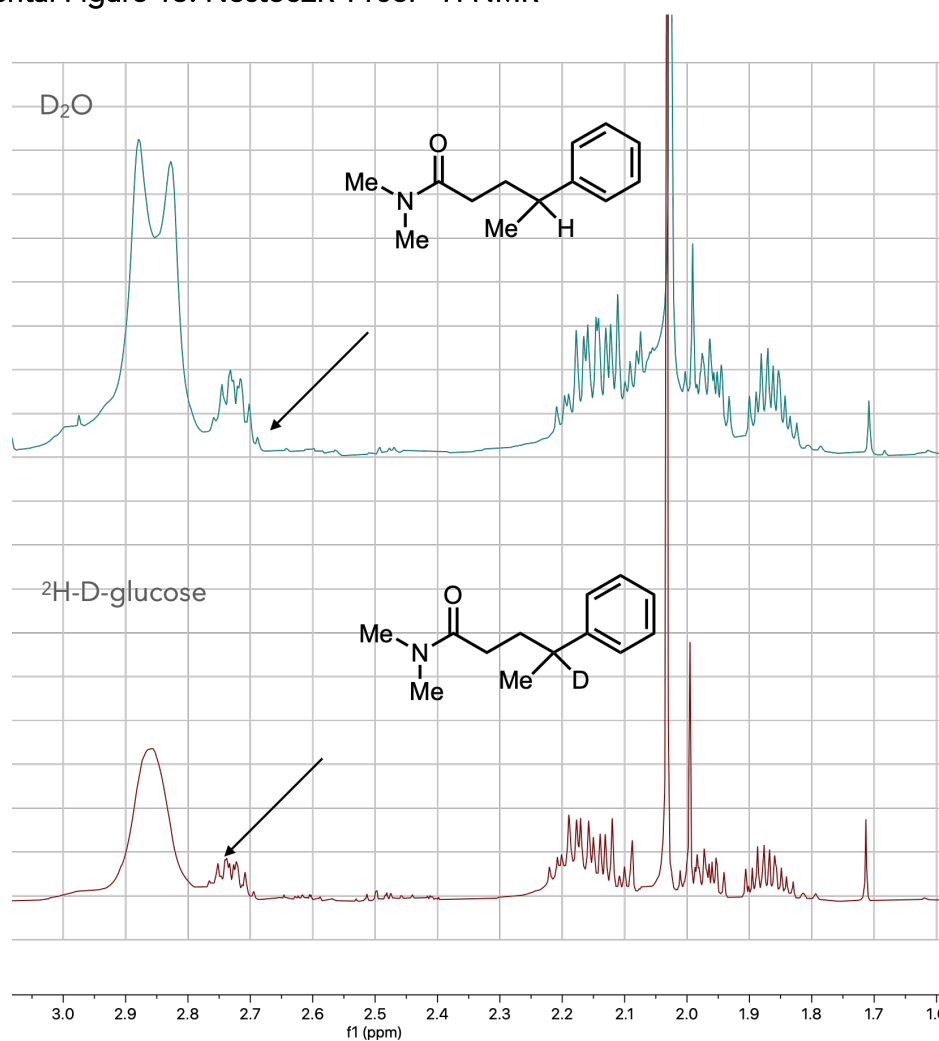
NostocER ^{13}C NMR $^2\text{H-glucose}$



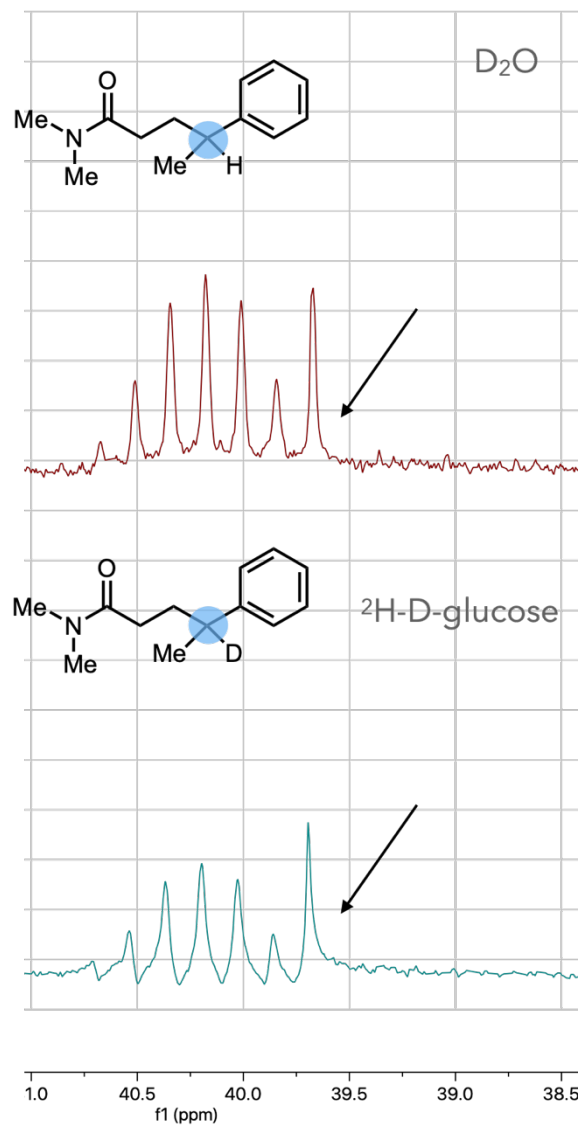
NostocER ^{13}C NMR D_2O



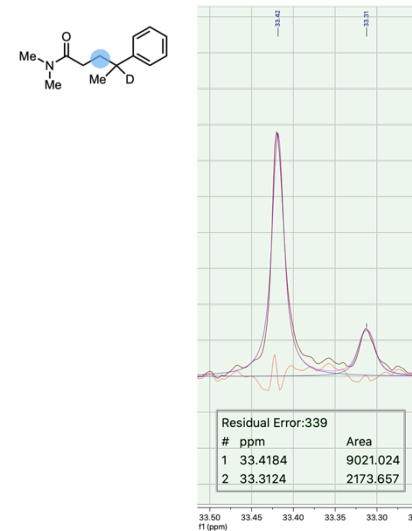
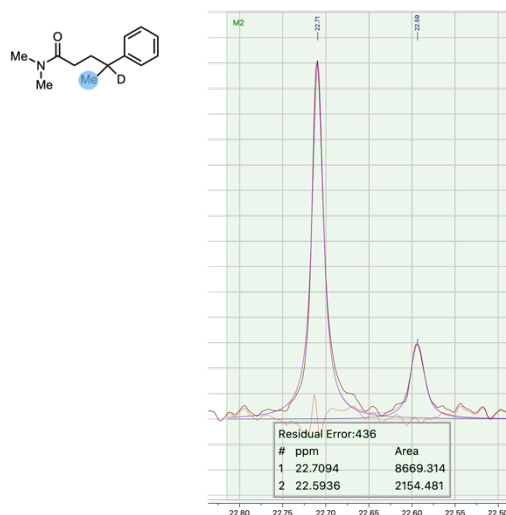
Supplemental Figure 13: NostocER Y106F ^1H NMR



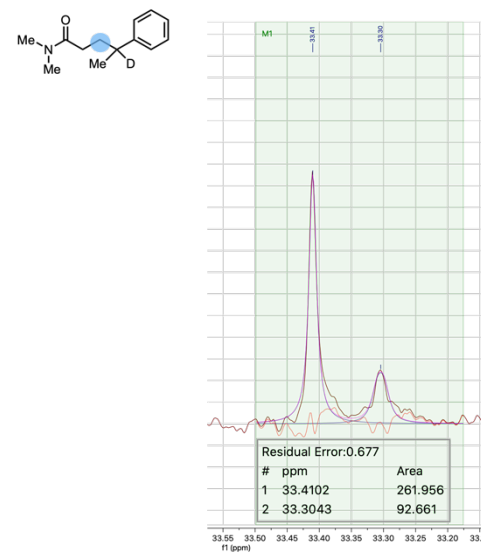
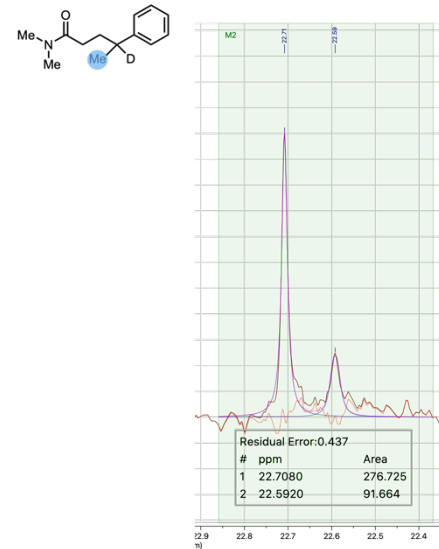
Supplemental Figure 14: NostocER Y106F ^{13}C NMR and Carbon Integrations



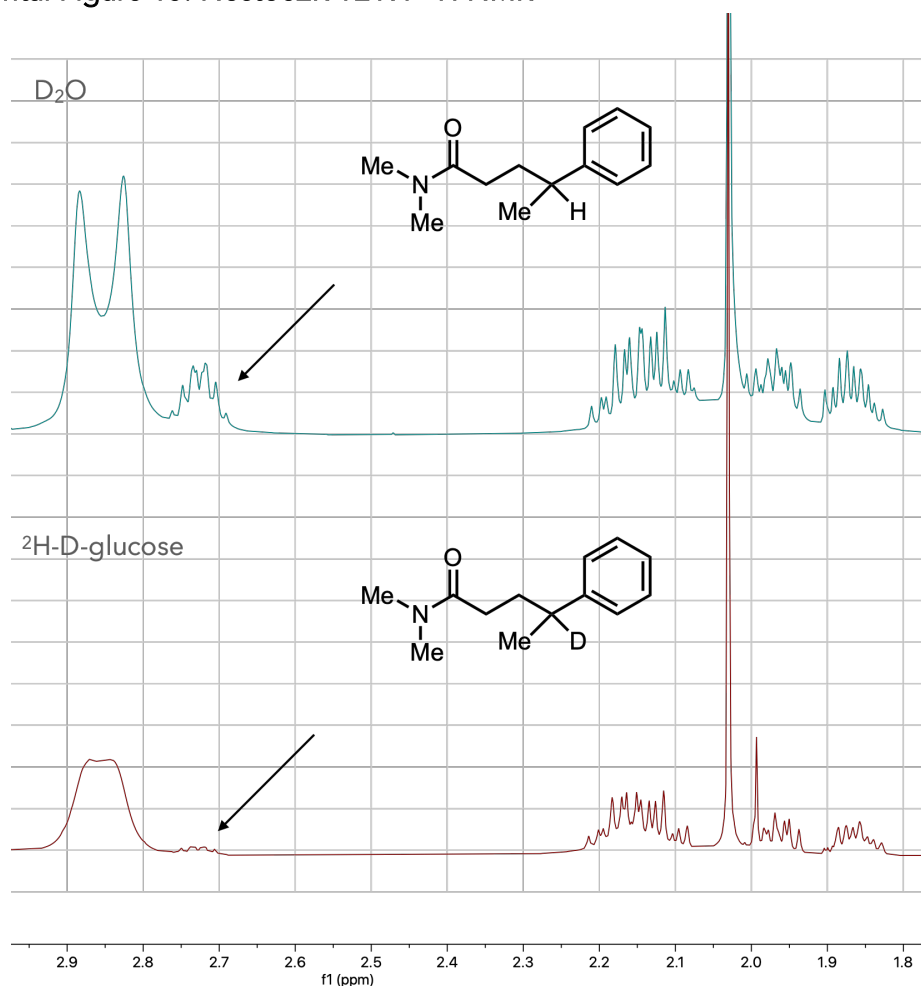
NostocER Y106F ^{13}C NMR $^2\text{H-glucose}$



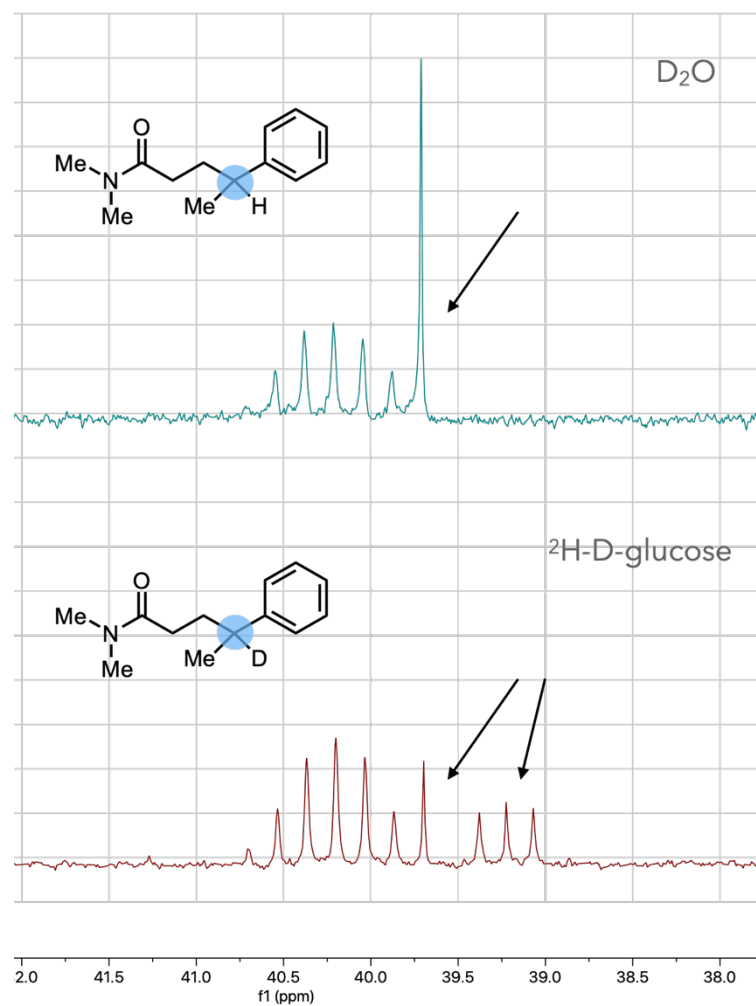
NostocER Y106F ^{13}C NMR D_2O



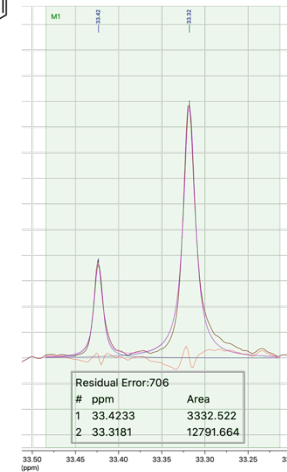
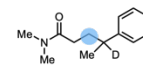
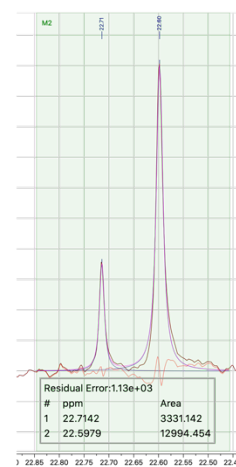
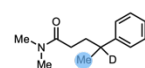
Supplemental Figure 15: NostocER Y219F ^1H NMR



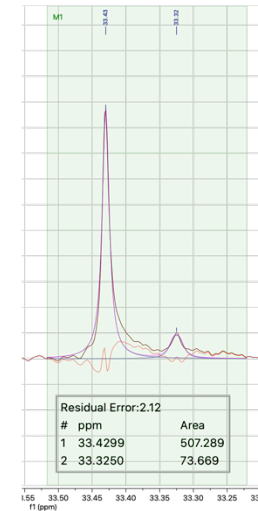
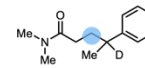
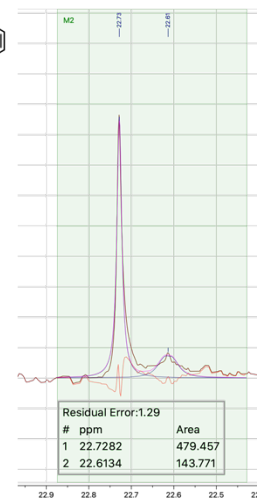
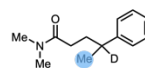
Supplemental Figure 16: NostocER Y219F ^{13}C NMR and Carbon Integrations



NostocER Y219F ^{13}C NMR $^2\text{H-glucose}$



NostocER Y219F ^{13}C NMR D_2O



Photophysics Studies

Transition absorption spectroscopy was conducted in a sealed 2mm quartz cuvette which was prepared in a MBraun oxygen free glove box. The total volume of the solution was 1mL and each sample and contents are listed below.

Sample 1: 950uL 100mM Tris pH 9, 200nmoles GluER T36A, 6000nmoles of sodium dithionite, 50uL of tetrahydrofuran

Sample 2: Sample 1 + 138umoles α -methyl styrene

Sample 3: Sample 1 + 88umoles *N,N*-dimethyl α -chloroacetamide

Sample 4: Sample 1 + 138umoles α -methyl styrene + 88umoles *N,N*-dimethyl α -chloroacetamide

The commercial experimental setup from Ultrafast Systems (Florida, USA) was used to conduct the short-time transient absorption spectroscopic studies. The pump beam was generated by seeding a Coherent optical parametric amplifier with 2mJ of 800nm light, 45fs in duration. The pulse was centered at 370nm with an energy of 300nJ. The probe pulse is generated by focusing 800nm into a vertically translated CaF₂ crystal. A filter is used to remove light red of 700nm. The experiments were performed at magic angle by rotating the pump polarization 55° relative to the probe.

The global analysis of GluER_{hq} (Supplemental Figure 17) is shown below and shows a biexponential decay of 60 ps and 220ps and are spectrally very similar and possibly related to a concerted protein motion. Upon addition of α -methyl styrene the overall excited state lifetime of the hydroquinone increases but shows no new intermediate states and resembles without substrate except for the increases in lifetime from 220ps to 245ps and a reduction in the first lifetime from 60ps to 17ps.

Addition of *N,N*-dimethyl α -chloroacetamide causes the formation of a long lived species that is similar to oxidized flavin and possible covalent adduct in a similar manner to a covalent adduct formed upon irradiation of reduced lactose oxidase and α -bromopropionic acid.²⁸ We see the formation of an initial state of the homolytic cleavage of the chloride, which decays in 9ps to the neutral semiquinone, following that a time constant of 77ps is observed for the decay of the neutral semiquinone to the oxidized flavin in green. Also, a large shoulder to the red trace appears which could be attributed to substrate that did not undergo HAT in the active site. This shoulder appeared to decay on a 785ps time scale leaving a long lived species in blue that could be a combination of both oxidized flavin and a covalent adduct.

Addition of both the *N,N*-dimethyl α -chloroacetamide and α -methyl styrene substrate causes the formation of a long lived species that is just the protein bound form of oxidized flavin. We see the formation of an initial state of the homolytic cleavage of the chloride, which decays in 5ps to the neutral semiquinone in red. This state persists for 45ps before decaying into the blue state which appears to be oxidized flavin and neutral semiquinone, and then decaying to just oxidized flavin on a 300ps timescale.

The same set of experiments were performed with NostocER.

Sample 5: 950uL 100mM Tris pH 9, 200nmoles NostocER, 6000nmoles of sodium dithionite, 50uL of tetrahydrofuran

Sample 6: Sample 1 + 138umoles α -methyl styrene

Sample 7: Sample 1 + 88umoles *N,N*-dimethyl α -chloroacetamide

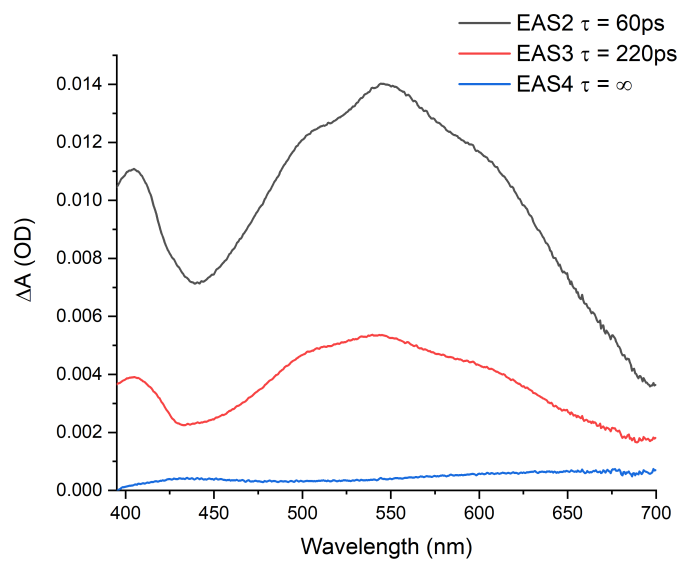
Sample 8: Sample 1 + 138umoles α -methyl styrene + 88umoles *N,N*-dimethyl α -chloroacetamide

The global analysis of NostocER_{hq} (Supplemental Figure 25) is shown below shows a biexponential decay of 1 ps and 123ps and are spectrally very similar and possibly related a concerted protein motion. Upon addition of α -methyl styrene the overall excited state lifetime of the hydroquinone increases but shows no new intermediate states and resembles without substrate except for the increases in lifetime from 123ps to 164ps.

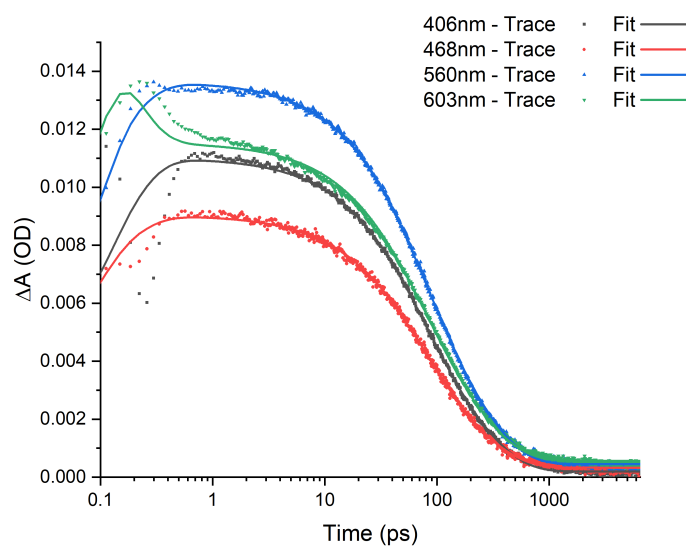
When *N,N*-dimethyl α -chloroacetamide is added to the solution a long lived species is formed which is not oxidized flavin and is a molecular intermediate. The analysis shows the formation of an excited state which decays in 3ps to a state resembling the neutral semiquinone which again decays in 81 ps to a long lived species that has a lifetime of 2200ps and decays to a final curve in green which has an infinite lifetime, the molecular adduct. This is the same adduct formed in the GluER T36A case, however NostocER forms with it much higher efficiencies. GluER T36A appears to favour the formation of hydrodehalogenated product in the absence of the coupling partner, α -methylstyrene, whereas NostocER favours the formation of a covalent intermediate over the hydrodehalogenated product under the same conditions.

When *N,N*-dimethyl α -chloroacetamide is added to the solution with α -methyl styrene a long lived species is formed which is oxidized flavin. The analysis shows the formation of an excited state which decays in 5ps to a state resembling the neutral semiquinone which again decays in 131 ps to a long lived species what has a lifetime of 2000ps and decays to a final curve in green which has an infinite lifetime, oxidized flavin. This shows that both substrates need to be in the active site upon photo-excitation for the radical coupling to occur.

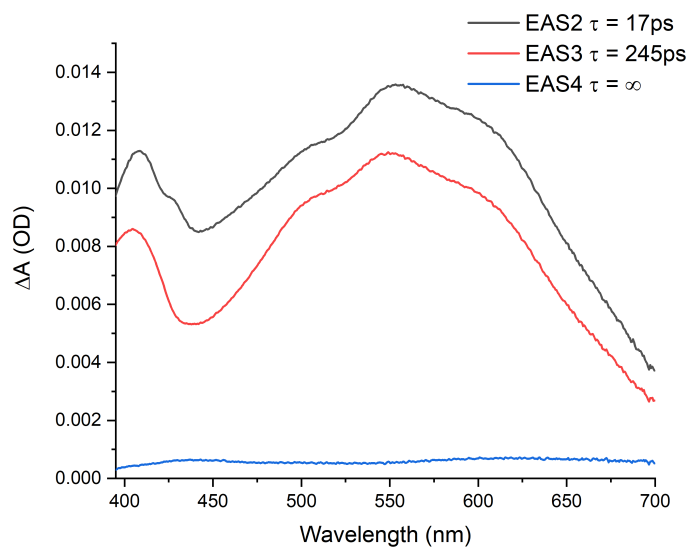
Supplemental Figure 17: Evolutionary associated difference spectra of sample 1



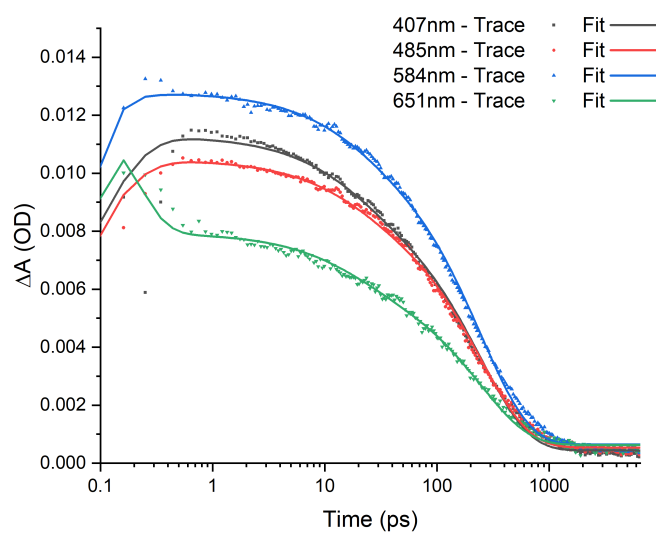
Supplemental Figure 18: Evolutionary associated difference spectra fits at different wavelengths of sample 1



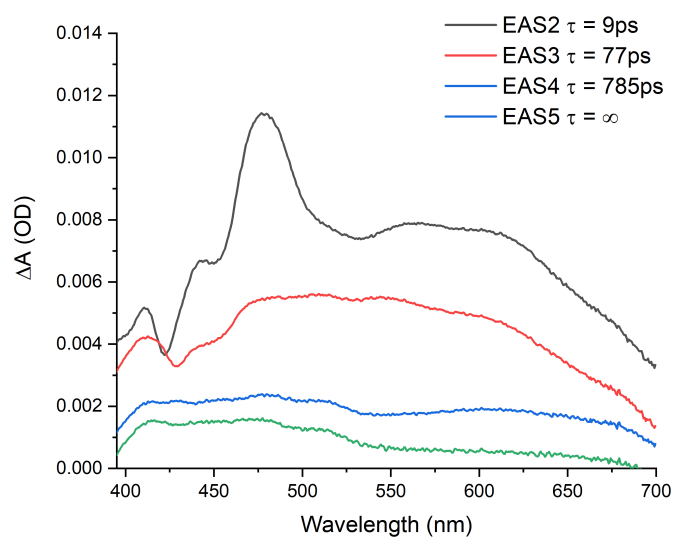
Supplemental Figure 19: Evolutionary associated difference spectra of sample 2



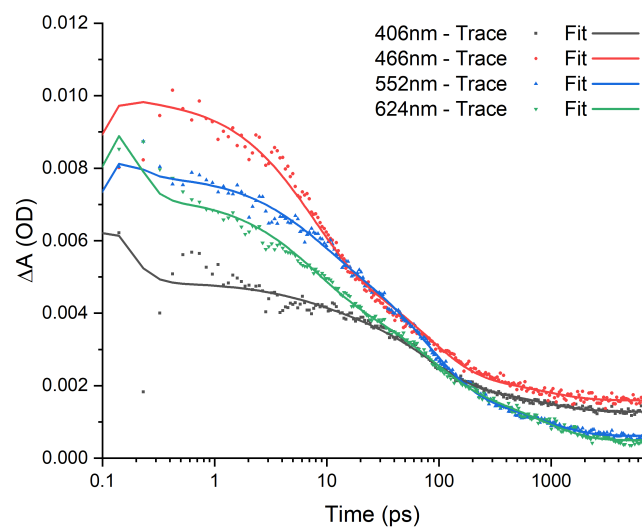
Supplemental Figure 20: Evolutionary associated difference spectra fits at different wavelengths of sample 2



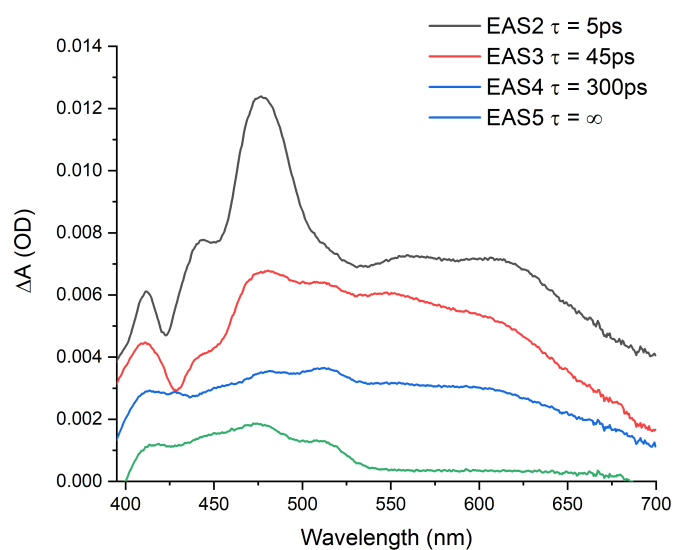
Supplemental Figure 21: Evolutionary associated difference spectra of sample 3



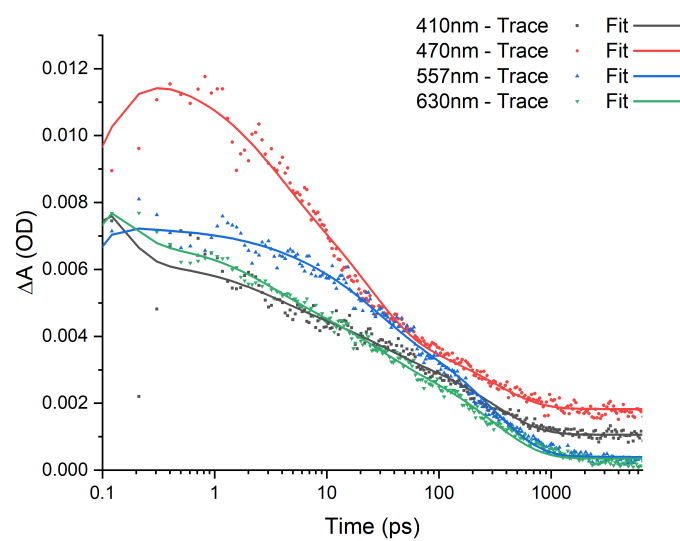
Supplemental Figure 22: Evolutionary associated difference spectra fits at different wavelengths of sample 3



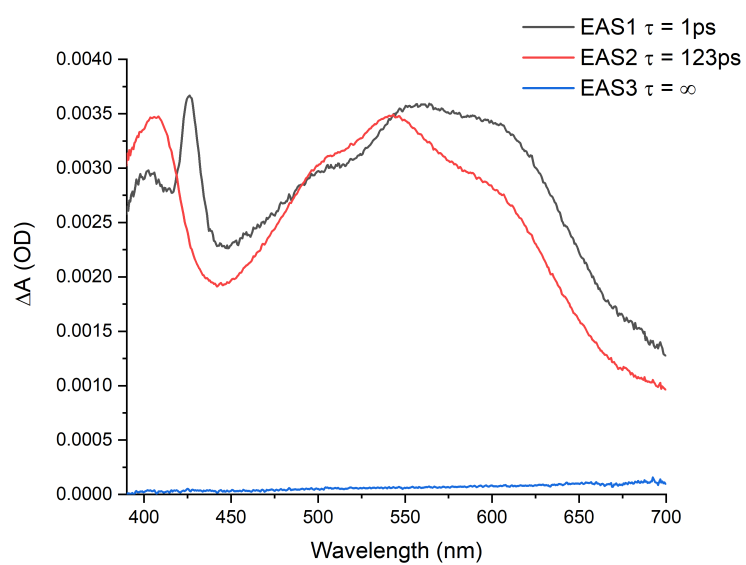
Supplemental Figure 23: Evolutionary associated difference spectra of sample 4



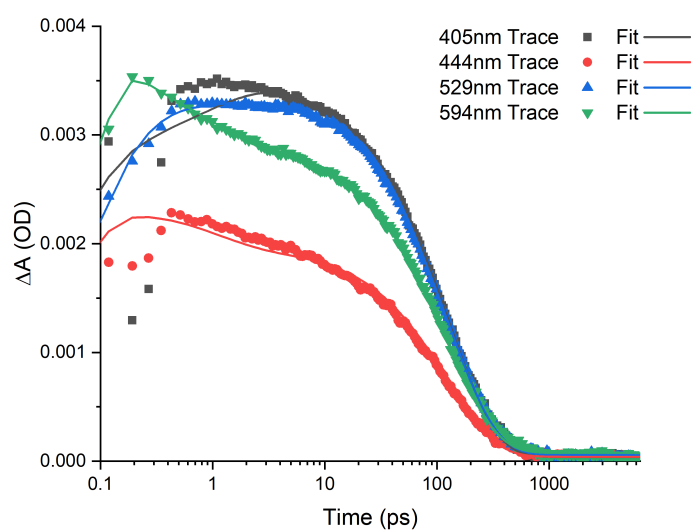
Supplemental Figure 24: Evolutionary associated difference spectra fits at different wavelengths of sample 4



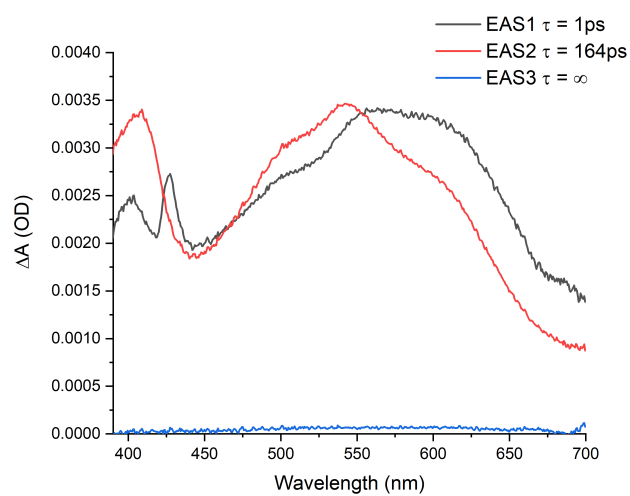
Supplemental Figure 25: Evolutionary associated difference spectra of sample 5



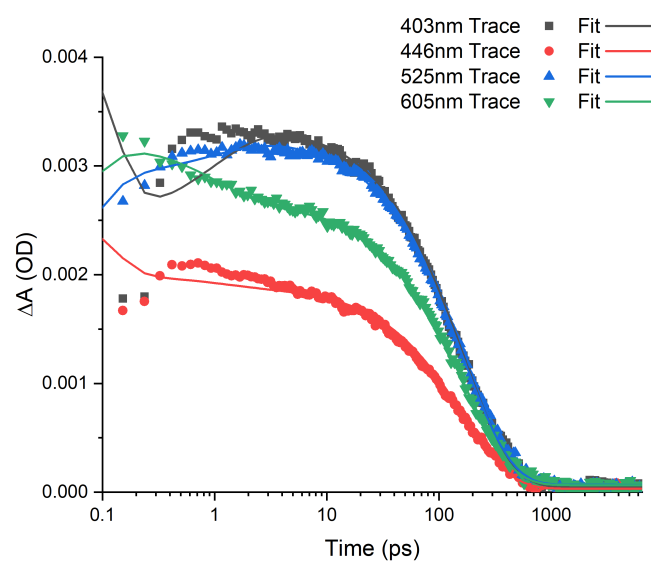
Supplemental Figure 26: Evolutionary associated difference spectra fits at different wavelengths of sample 5



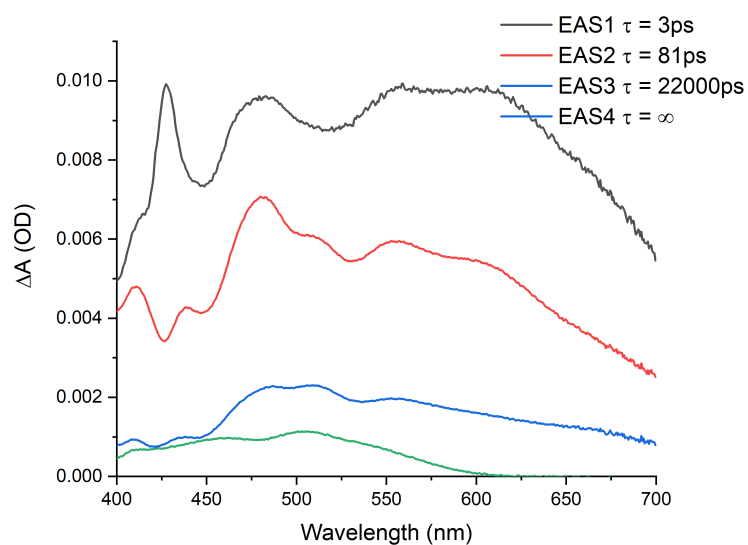
Supplemental Figure 27: Evolutionary associated difference spectra of sample 6



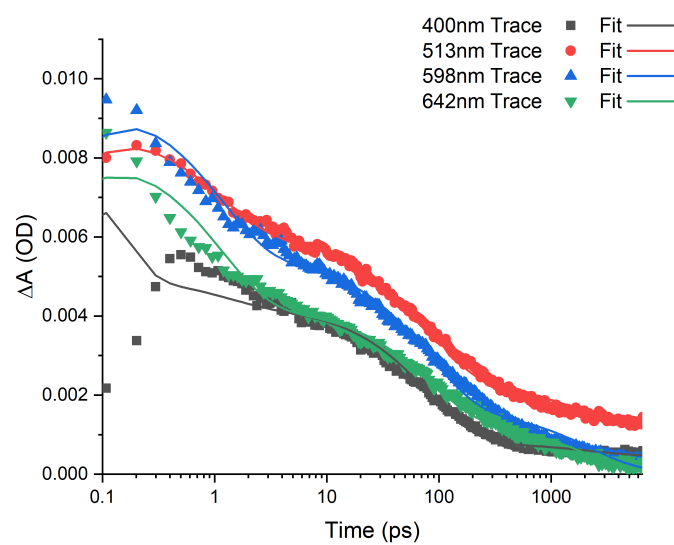
Supplemental Figure 28: Evolutionary associated difference spectra fits at different wavelengths of sample 6



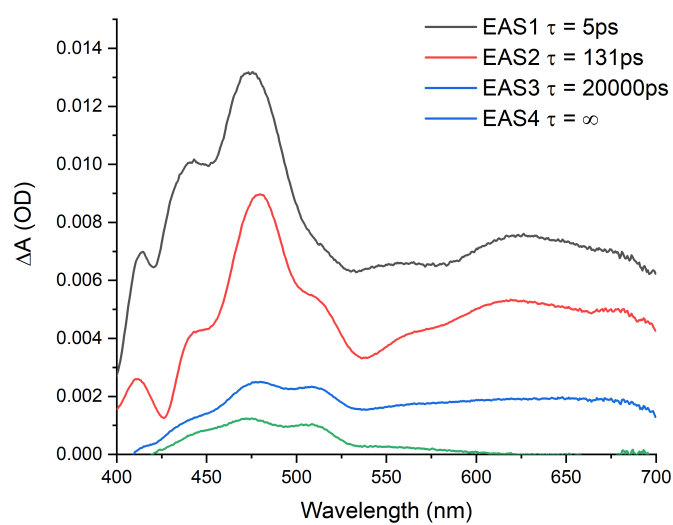
Supplemental Figure 29: Evolutionary associated difference spectra of sample 7



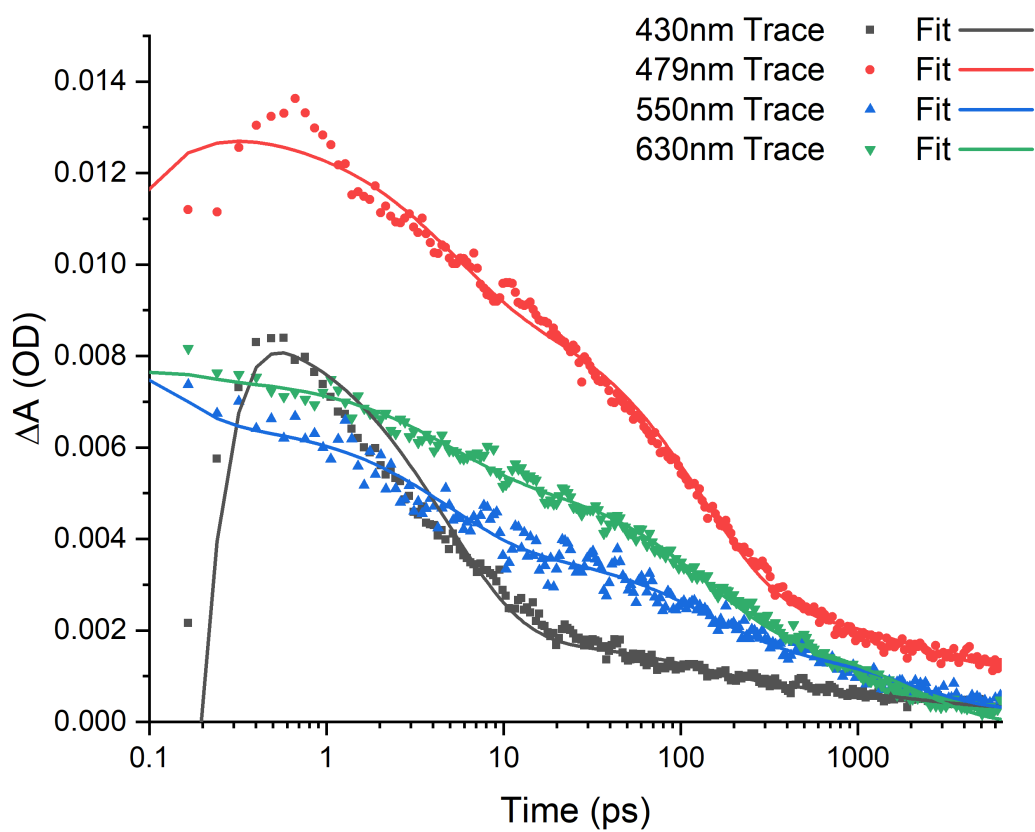
Supplemental Figure 30: Evolutionary associated difference spectra fits at different wavelengths of sample 7



Supplemental Figure 31: Evolutionary associated difference spectra of sample 8

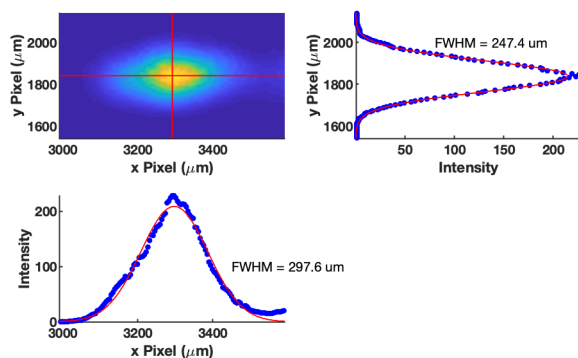


Supplemental Figure 32: Evolutionary associated difference spectra fits at different wavelengths of sample 8



Quantum Yield

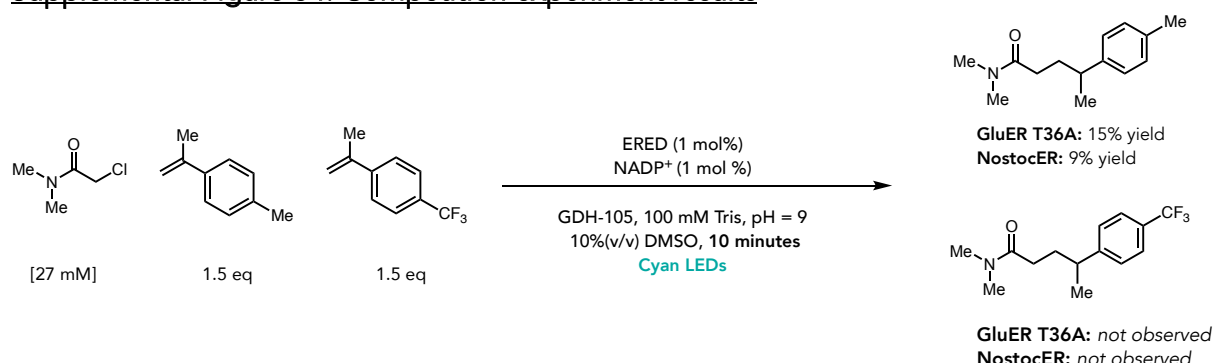
Supplemental Figure 33: Representative Pump cross section for GluER T36A



To determine quantum yield we go back to previous studies and utilized the same methodology as in Biegasiewicz et al.²⁹ A quantum yield of 12% is determined when both the α -chloro substrate and α -methyl styrene are in the binding pocket of GluER T36A. When only α -chloro substrate is in the binding pocket we determine a quantum yield to be 8% for GluER T36A. Quantum yield of 10% for NostocER is observed when both substrates are in solution.

Competition experiment to assess the importance of the alkene in the charge transfer excitation

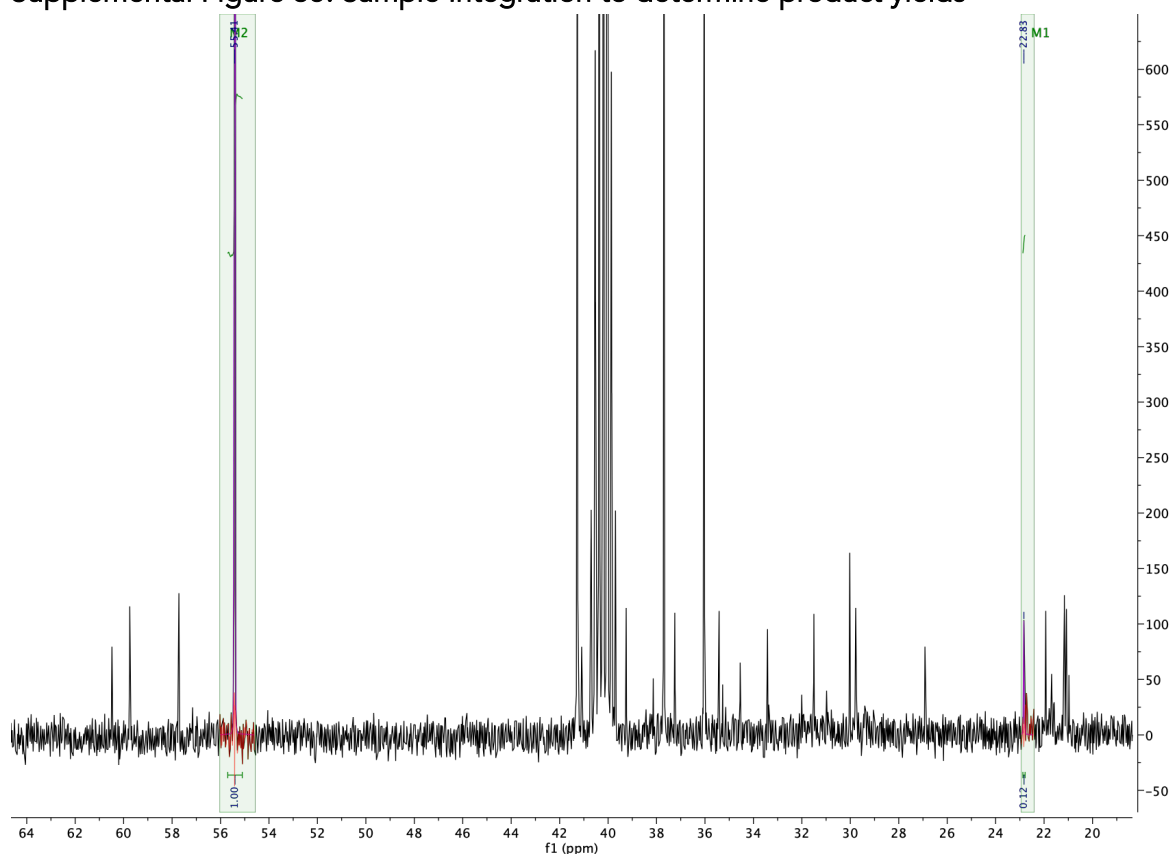
Supplemental Figure 34: Competition experiment results



To further establish the need for donation of the alkene π bond density into the charge transfer complex for mesolytic cleavage of the C-Cl σ bond to occur, a competition experiment was conducted between two electronically different alkenes with similar steric properties. In duplicate, a standard biocatalytic reaction was run using 20 μ mol of N,N-dimethylchloroacetamide and a total of 3.5 equivalents of alkene was added as a 1.5 to 1 mixture of 4-methyl- α -methylstyrene and 4-trifluoromethyl- α -methylstyrene. The reaction was irradiated for 10 minutes with cyan LEDs and then worked up according the standard NMR workup procedure.

Analysis of the product yield and the product ratios was performed using quantitative ^{13}C NMR against the internal standard trimethoxybenzene. MestReNova's (version 10.0.2) "fit region" tool was used to get accurate peak integrations, with the standard settings (lower and upper boundaries set to 0.1 and 100 Hz, respectively, with simulated annealing enabled, coarse iterations-500, fine iterations-200). A sample integration is shown in Supplemental Figure 34. Yield was determined using Eqn. 2

Supplemental Figure 35: Sample Integration to determine product yields



#	Name	From	To	Error	Peak #	ppm	Height	Width(Hz)	L/G	Area
1	M2	54.569	56.028	160	1	55.4148	1298.63	1.4	0.22	11226.045
2	M1	22.418	22.939	97.7	1	22.8338	102.89	2.1	0.79	1262.326

$$\% \text{ yield} = A_{\text{pdt}} / A_{\text{trimethoxybenzene}}$$

Discussion: We reasoned the electronically deficient *p*-trifluoromethyl α -methylstyrene would serve as a worse donor in the charge transfer complex compared to the *p*-methyl α -methylstyrene. This would severely diminish the quantum efficiency of the coupling reaction with *p*-trifluoromethyl α -methylstyrene, so in the initial rate regime (low conversions) we should see selective incorporation of the *p*-methyl α -methylstyrene. Pleasingly, we see exactly that with no discernable formation of the coupling product with *p*-trifluoromethyl α -methylstyrene and most of the conversion going to forming the coupling product with *p*-methyl α -methylstyrene. While not definitive on its own, these results coupled with evidence from the transient absorbance spectroscopy and the charge transfer studies suggests that the alkene electron density is involved in the excitation of the charge transfer state and the degree of donation and therefore the quantum efficiency of the reaction is dependent on the alkene's electronics.

HRMS Experiment to elucidate the presence of alkylated Flavins

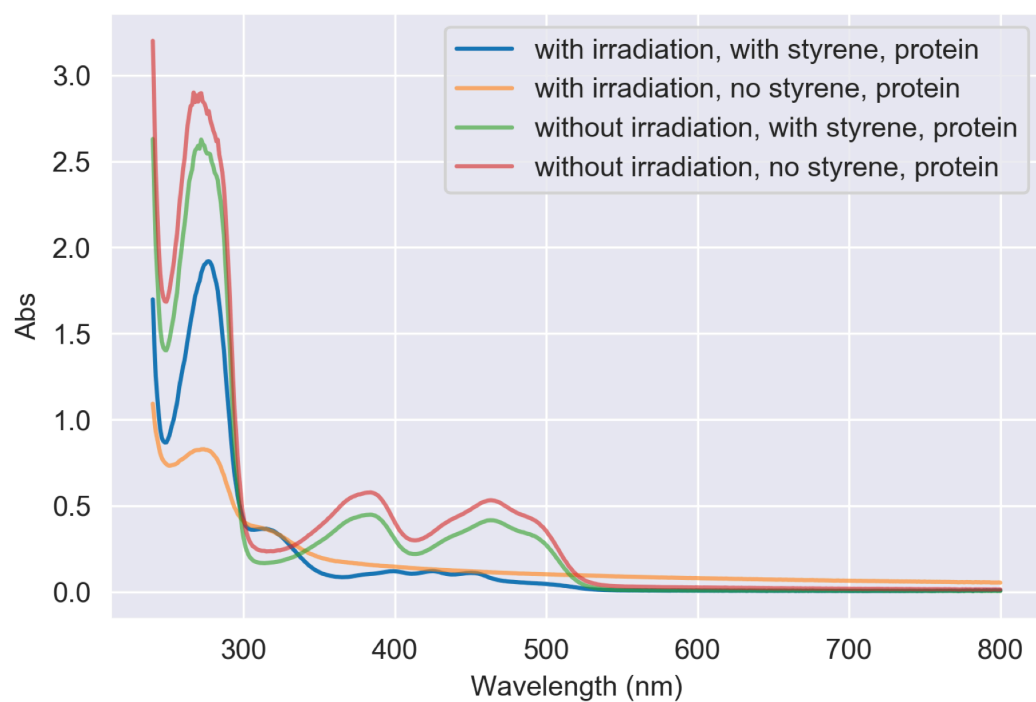
All reactions were performed in a Coy Chamber. They were run with 10 μ mol of chloroamide starting material at a final substrate concentration of 27 mM in duplicate. A shell vial with a magnetic cross stir bar was charged with a "master mix" solution of 6 equivalents of (D)-glucose dissolved in 100 mM Tris pH 9 in a volume such that the final concentration of substrate would be 27 mM after addition of all the other reagents. DMSO was added to the shell vial such that it would be 10% (v/v). To this solution, 100 μ L of a 5 mg/mL GDH solution was added. Then 14 μ L of a 5 mg/mL solution of NADP⁺ was added. Finally, 1 mol% of ERED was added. The chloroamide was then added to the shell vial neat and 3.5 equivalents of alkene was added, if applicable. The vial was sealed with a rubber septa and brought out of the Coy Chamber where it is placed on a stir plate at 300 rpm under a fan and immediately irradiated with cyan LEDs (50 W Chanzon high power LED chip, λ_{max} = 490 nm, measured photon flux = 12,000 mW/m² s) for 24 hours.

After 24 hours, the reactions were removed from the light and put into Amicon centrifugal filters (5 mL reservoir, 10 kDa cutoff) and resuspended in HPLC H₂O. They were centrifuged at 2400 x g for 10 minutes and the protein was resuspended in HPLC H₂O and centrifuged again. The protein was resuspended and centrifuged four times to ensure removal of all small molecules. Then the protein solution was placed in a 2 mL quartz cuvette and an absorption spectrum was taken (Supplemental Figure 36). After that, the protein solution was transferred into an eppendorf tube and the protein was heat denatured and centrifuged at 14500 rpm for 5 minutes to release the free flavin. The free flavin solution was placed into a 2 mL quartz cuvette and another absorption spectrum was taken (Supplemental Figure 37). The free flavin solution was diluted into a HPLC acetonitrile + 0.1% FA (1:100 dilution) and a negative mode HRMS was obtained (Supplemental Figure 39 and 40).

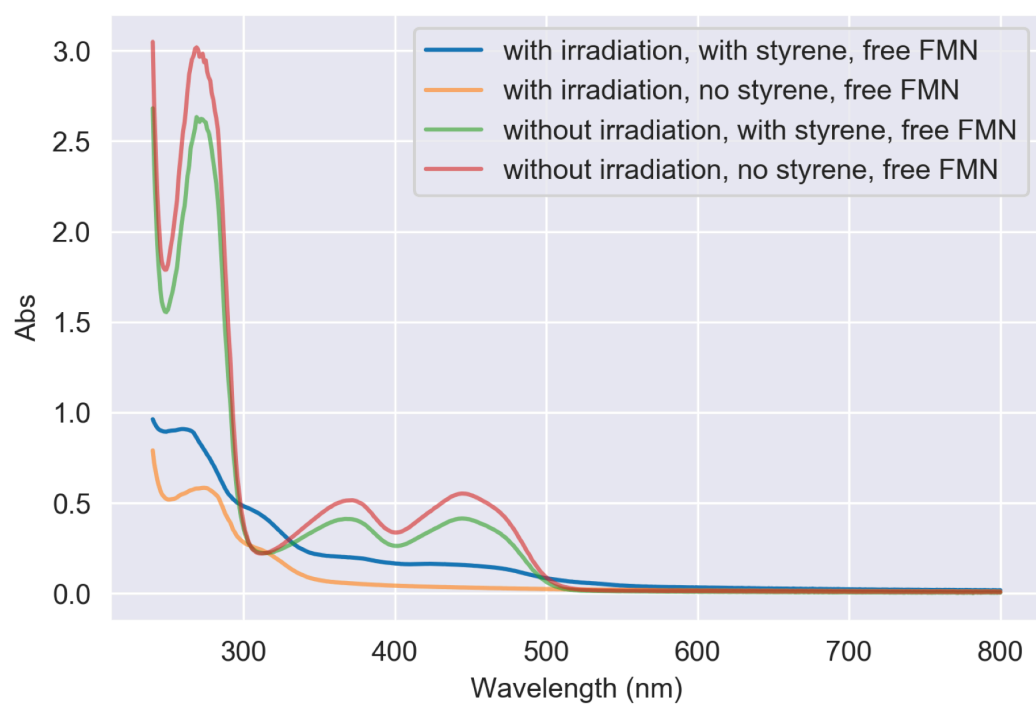
NostocER was chosen as the model enzyme because transient absorption spectroscopy saw formation of stable intermediate upon irradiation of the ERED with N,N-dimethylchloroacetamide. Massey et al have shown that alkylation of protein bound flavins can occur upon irradiation of the flavoenzyme with alpha-halocarbonyls and these covalent flavin adducts are stable.^{28, 30} It is therefore feasible the nature of this intermediate is an alkylated flavin species. As can be seen in the HRMS traces irradiation is necessary to see any mass corresponding to an alkylated flavin adduct. If just chloroamide (1) is added with irradiation for 24 hours, then a mass corresponding to two alkylations of FMN by the chloroamide substrate is seen (Supplemental Figure 39) and the UV/Vis trace looks remarkably like the flavin hydroquinone (Supplemental Figure 36 orange trace and Supplemental Figure 37 red trace). If styrene is present, then a mixture of masses is seen, corresponding to singly alkylated flavin, doubly alkylated flavin and oxidized flavin (Supplemental Figure 40) Additionally, a new spectral peak can be seen between the oxidized flavin peaks (Supplemental Figure 36 blue trace). This suggests flavin alkylation is a side pathway from the concerted coupling of chloroamide with styrene. It is likely that one alkylation occurs at the C_{4a} position upon irradiation of the chloroamide and FMN complex, the adduct isomerizes to the N₅ adduct where it can be re-reduced and undergo another SET event with another molecule of chloroamide and get alkylated again. When styrene is present, coupling of the chloroamide with styrene competes with the first alkylation. Then the singly alkylated flavin is a competent catalyst for the coupling of chloroamide with

styrene, so the second alkylation event is inhibited, which explains the observation of masses corresponding to both singly and doubly alkylated flavin when styrene is present. Further investigation is required to determine the exact catalytic relevance of these species, as well as their structures. However, due to the nearly instantaneous nature of the coupling of alkene to chloroamide upon charge transfer complex excitation and increased quantum yield when both are in the active site, it appears unlikely this is a main catalytic route to product. Instead, this is likely a catalyst degradation pathway, as the doubly alkylated flavin cannot be reduced and serve as an electron donor in a charge transfer complex.

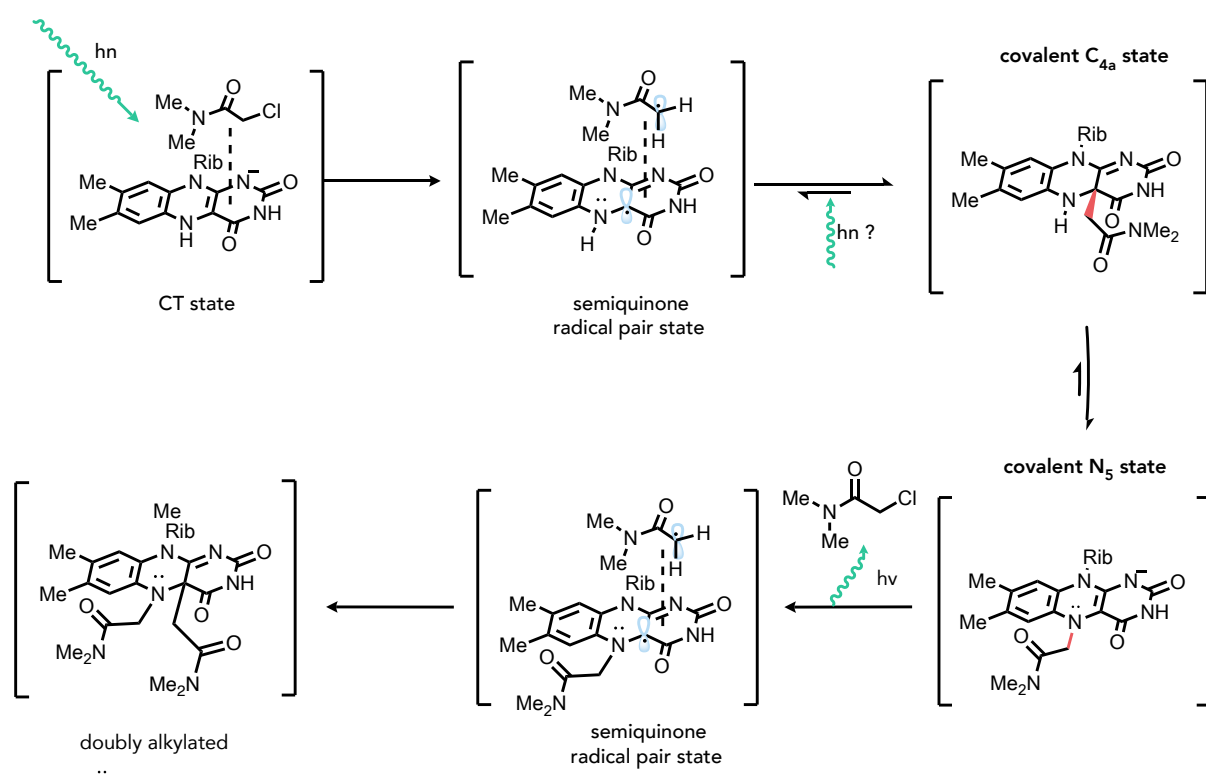
Supplemental Figure 36: Traces of protein bound FMN



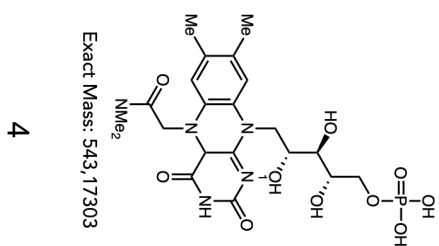
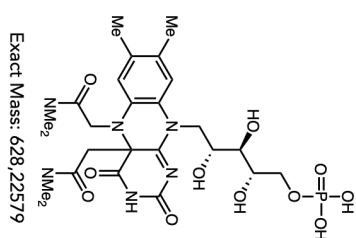
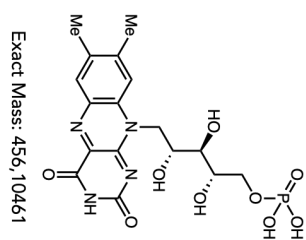
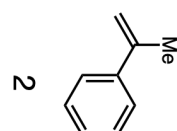
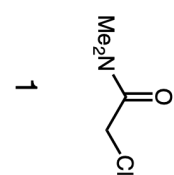
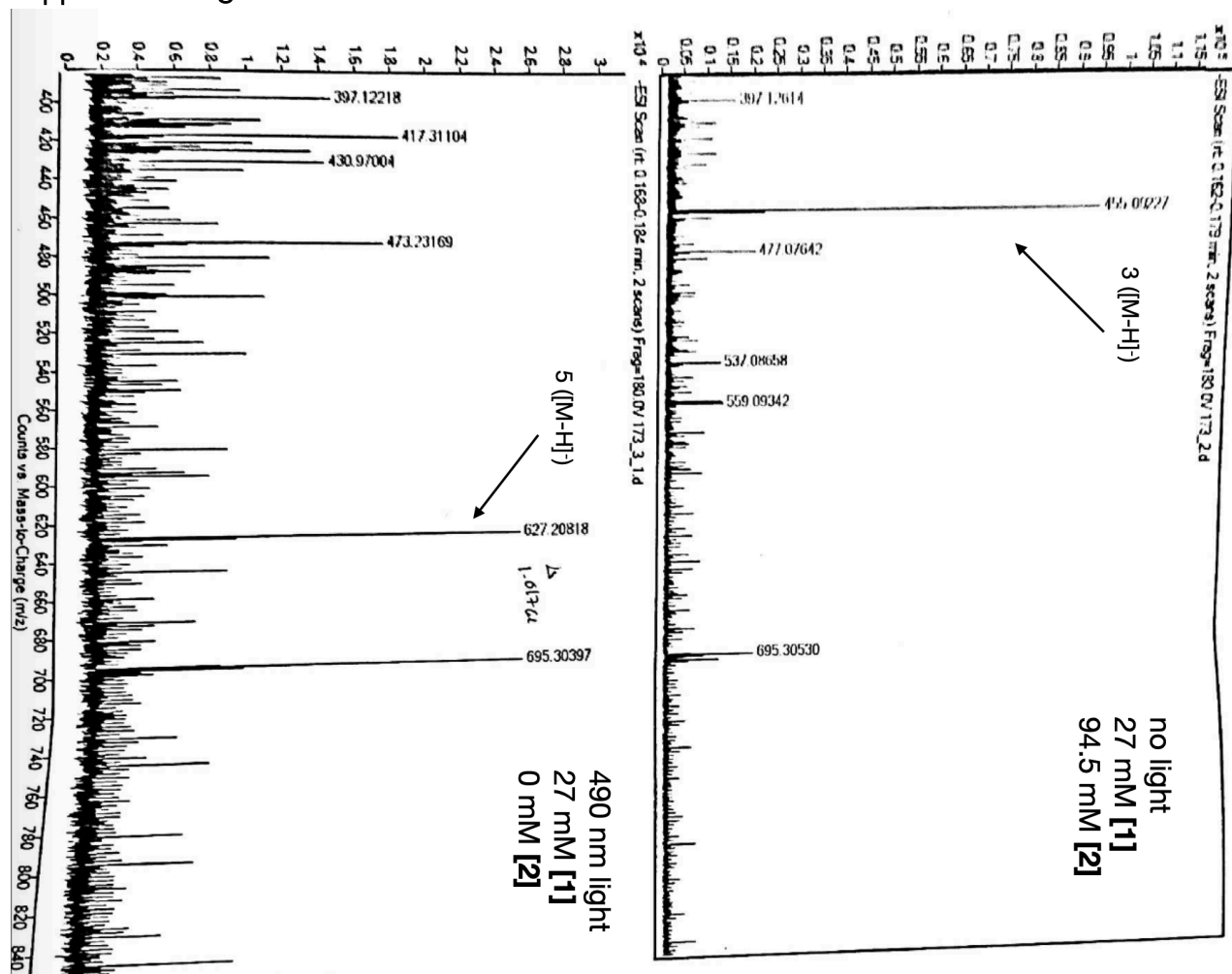
Supplemental Figure 37: UV/Vis traces of free FMN



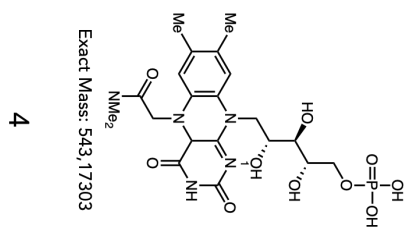
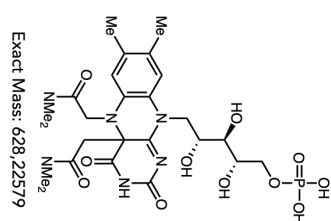
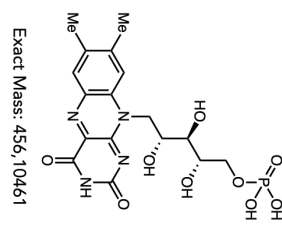
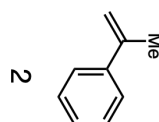
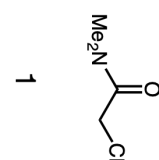
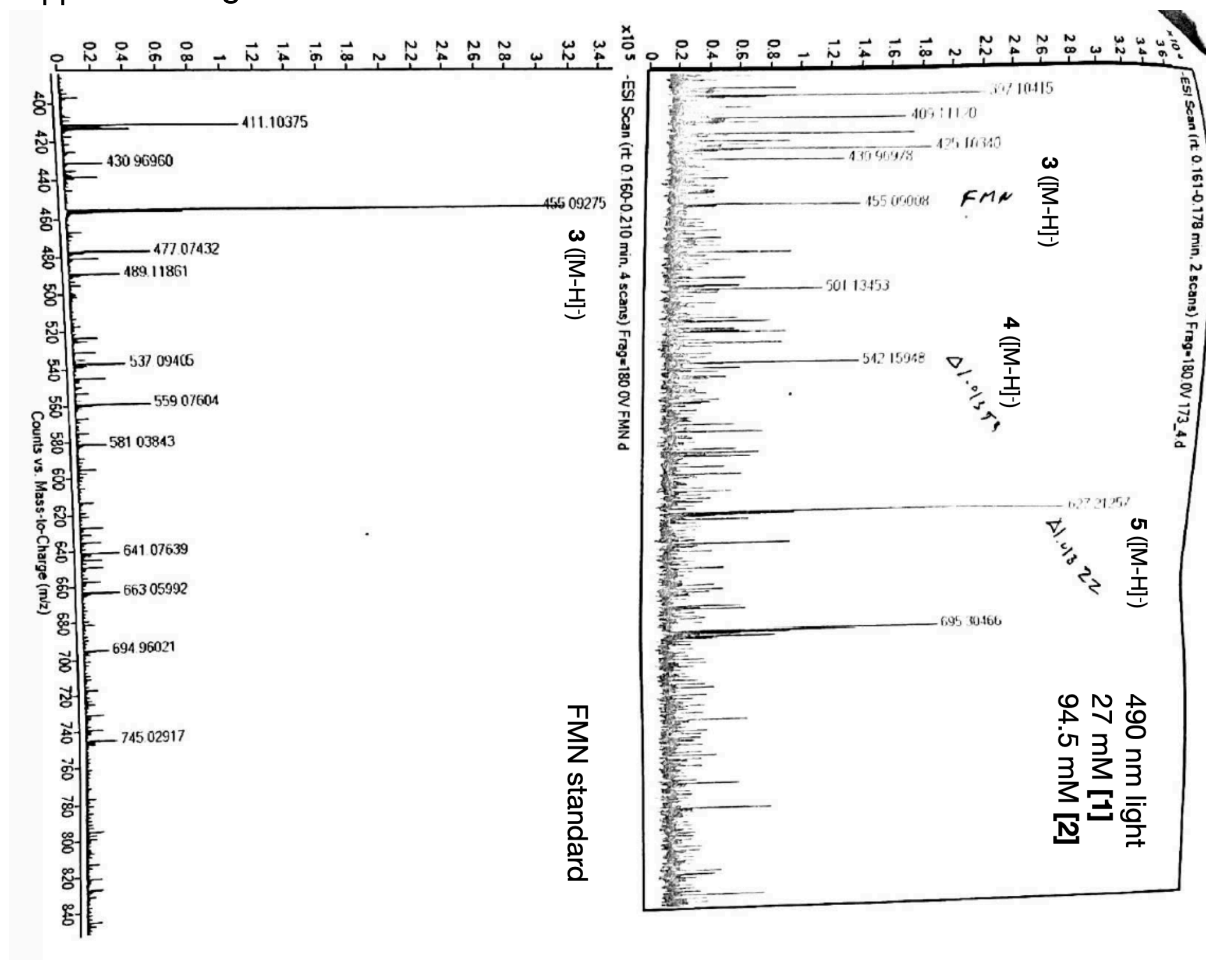
Supplemental Figure 38: Proposed mechanism for flavin alkylation



Supplemental Figure 39: HRMS traces of reaction mixtures



Supplemental Figure 40: HRMS of FMN from reaction and free FMN standard

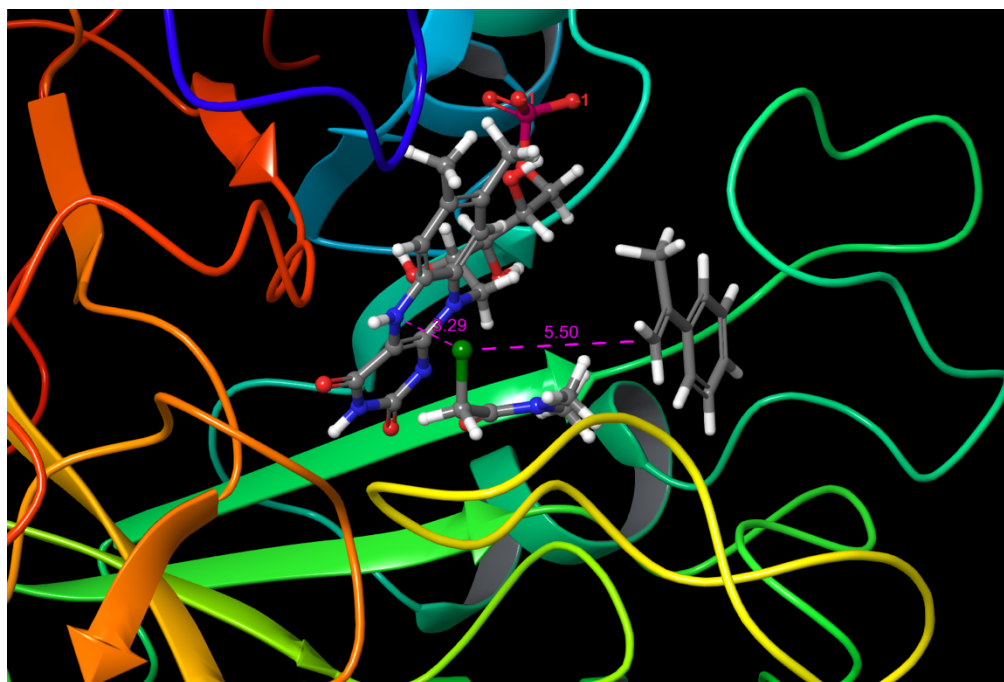


Docking Studies and Electron Donor Acceptor Calculations

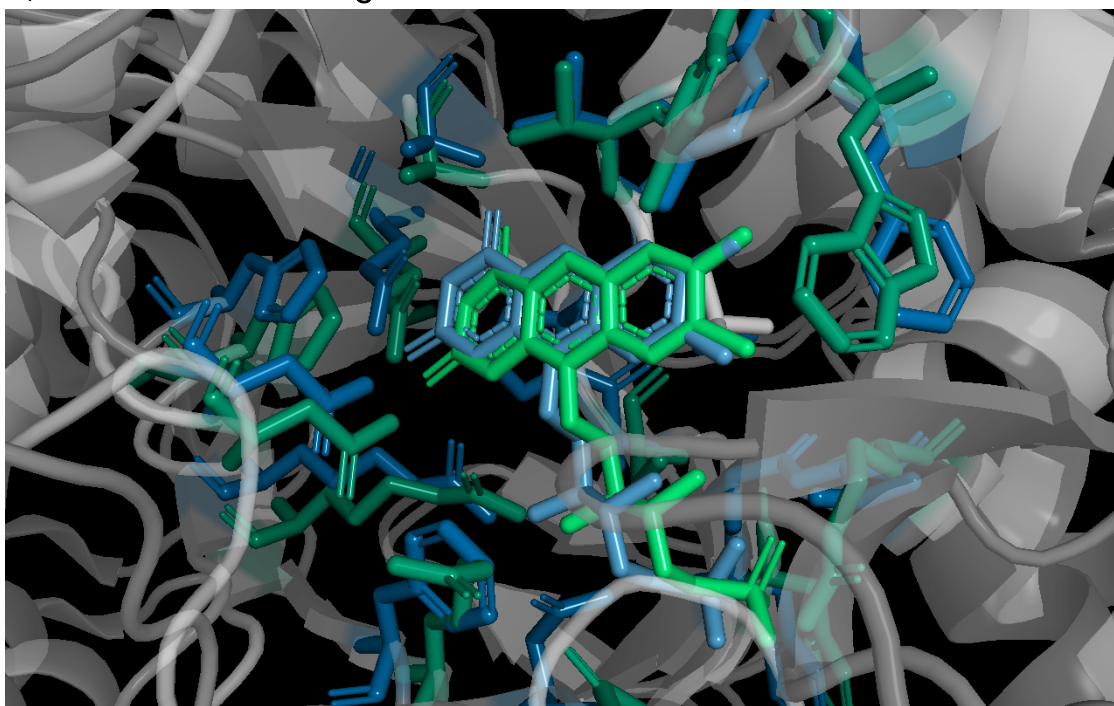
As a starting point the initial structure of oxidized flavin from GluER T36A crystal structure was used (PDB ID:6MYW). FMN_{ox} was then reduced to the anionic hydroquinone form where the tail was frozen and the remaining atoms were optimized in Gaussian 16 software package using CAM-B3LYP and 6-311+G** basis set.³¹ The optimized flavin hydroquinone was then inserted into the protein scaffold using a least squares minimization to the original oxidized flavin.

The point charges of both ligands and the hydroquinone were calculated with the Jaguar software using a DFT with B3LYP functional and a 6-311+G** basis set.³² Substrate **1** was docked into the active site using Glide program and the top twenty lowest energy conformers were selected and subjected to TD-DFT using CAM-B3LYP in the Gaussian 16 software package.^{33,34,35} The conformer which showed a transition near 500nm was then used for docking of substrate **2**. This pose positioned the chlorine 3.29Å from the hetero-nitrogen in the flavin molecule as shown in Supplemental Figure 41. The ten lowest energy poses with both substrates were visually examined, and one pose aligned the alkene on substrate **2** with the chlorine on substrate **1**. This positioned substrate **2** 5.50Å away from the chlorine atom as shown in Supplemental Figure 41. From these docking studies and an active site overlay made in pymol (Supplemental Figure 42), a rationalization for enantioselectivities could not be made due to the similarity in active site structure, Further docking studies are needed to determine the origin of enantioselectivity for the hydrogen atom transfer step

Supplemental Figure 41: Docked pose of GluER T36A with substrate 1 and substrate 2



Supplemental Figure 42: Overlay of the active sites of GluER T36A (green) and NostocER (blue) curtained on the FMN ligand



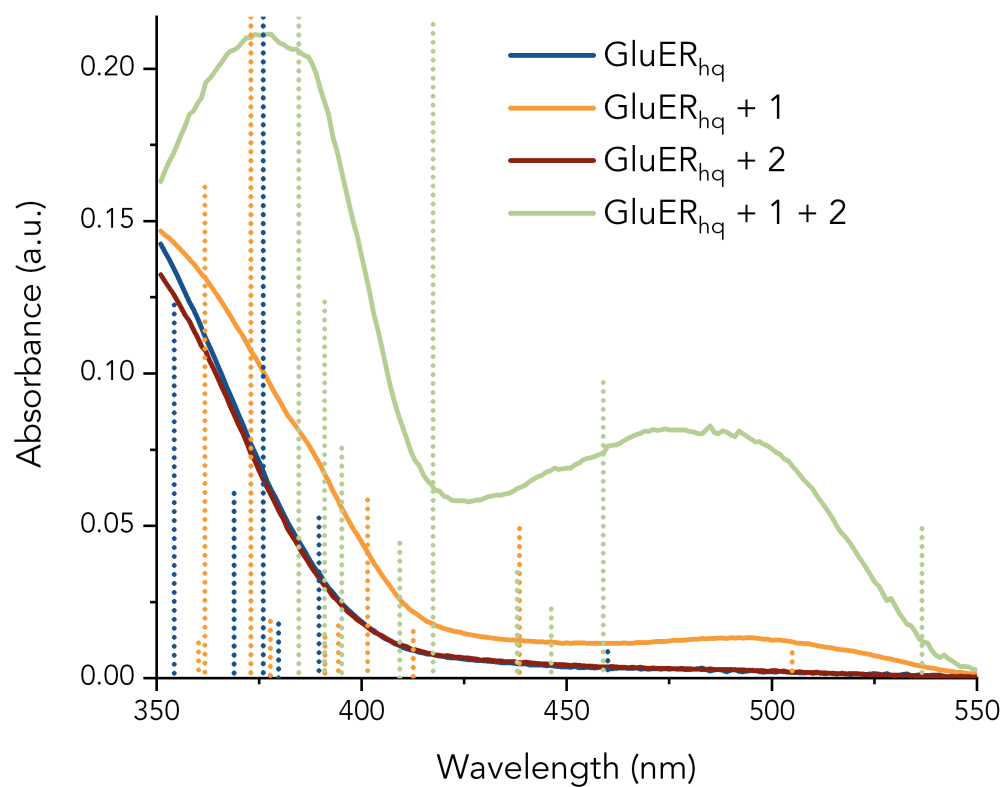
To examine whether the change in the absorption spectrum was the result of substrate **2** interacting to form a quaternary complex TD-DFT studies were employed. Using Gaussian 16 the first ten excited states were calculated with TD-DFT employing CAM-B3LYP and 6-311+G** basis set. Three excited state calculations were performed for: anionic hydroquinone, anionic hydroquinone + *a*-chloroamide, and the anionic hydroquinone + *a*-chloroamide + *a*-methyl-styrene. The vertical transition energy and oscillator strength are listed in **Table S6**. As shown in Supplemental Figure 43, the normalized vertical transition energy for the first 10 excited states of the GluER FMN_{hq} + substrates **1** + **2** corresponds well with the charge transfer complex absorbance observed.

Table S6: Vertical transition energies and associated oscillator strength

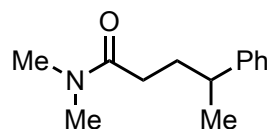
FMN _{hq}		FMN _{hq} + 1		FMN _{hq} + 1 + 2	
Wavelength (nm)	Oscillator Strength (a.u.)	Wavelength (nm)	Oscillator Strength (a.u.)	Wavelength (nm)	Oscillator Strength (a.u.)
459.98	0.001	505.01	0.0012	659.51	0
389.63	0.0135	438.5	0.0099	536.63	0.0017
379.71	0.0036	412.63	0.0027	458.88	0.0037
376.06	0.0603	401.42	0.0119	446.25	6E-4
368.9	0.0158	394.35	0.0031	437.9	0.0011
354.29	0.0334	391.09	0.0023	417.45	0.0086
345.99	0.001	377.78	0.0034	409.27	0.0015
340.05	9E-4	373.05	0.0457	395.19	0.0028
313.59	0.0065	361.76	0.0338	390.96	0.0048

304.82	0.0057	360.25	0.0019	384.7	0.0087
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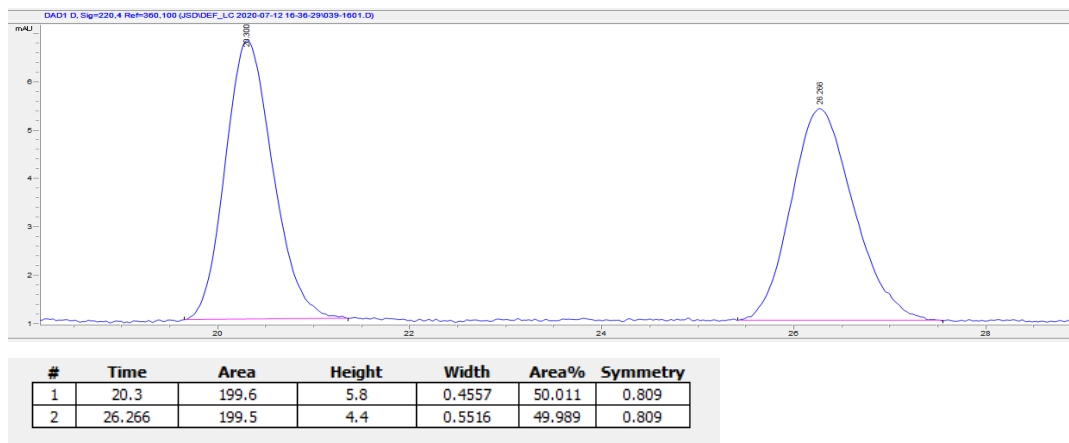
Supplemental Figure 43: Charge Transfer Complex absorbance spectra overlaid with the normalized vertical electronic excitation energies for the first 10 excited states of GluER FMN_{hq}, GluER FMN_{hq} + 1 and GluER FMN_{hq} + 1 + 2.



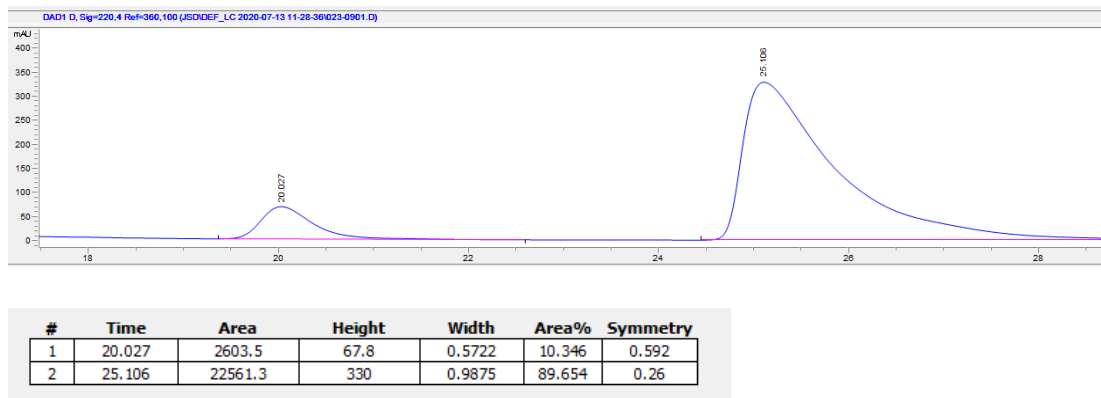
HPLC Traces of Products



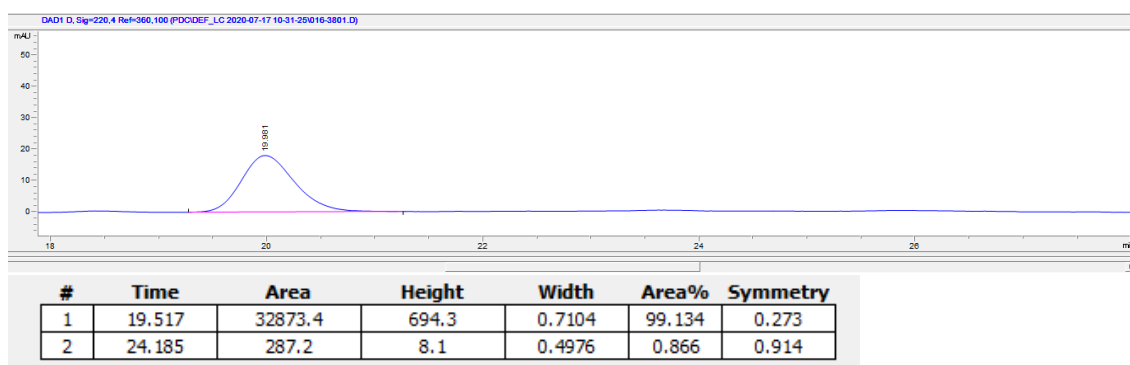
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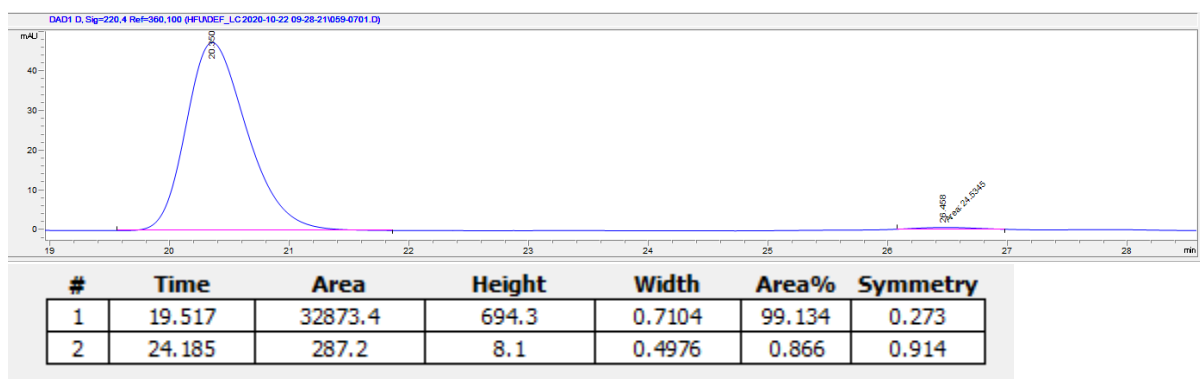
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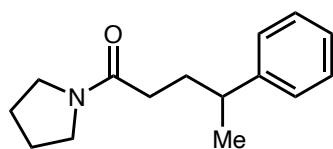


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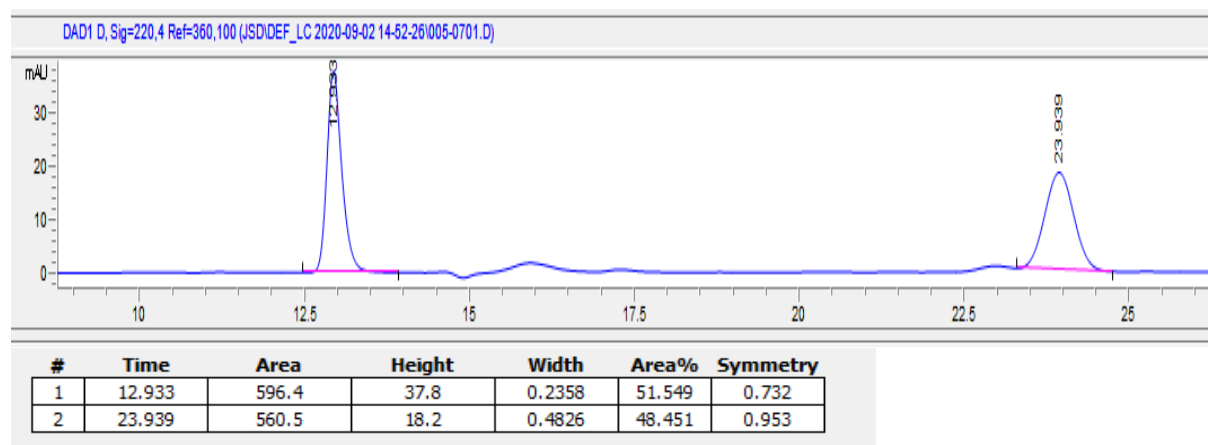


GluER T36A lyophilized lysate (gram scale):

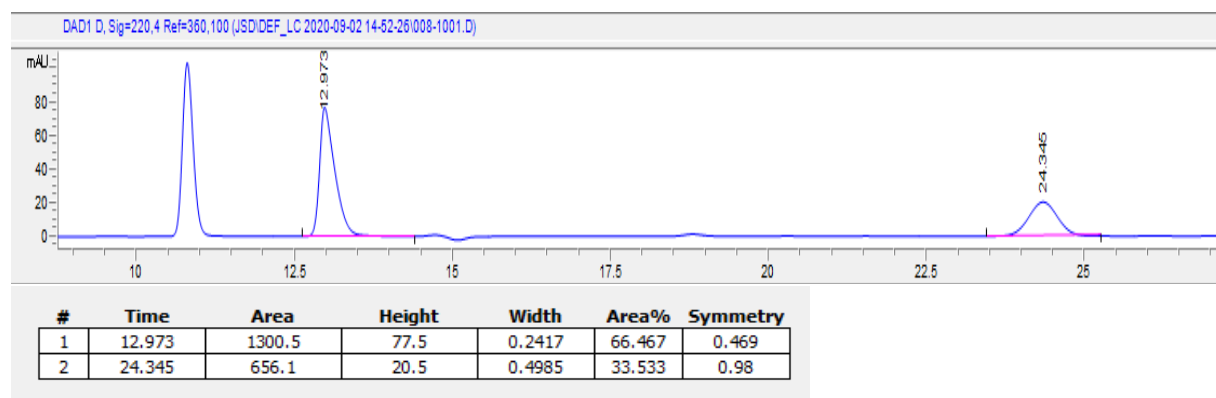




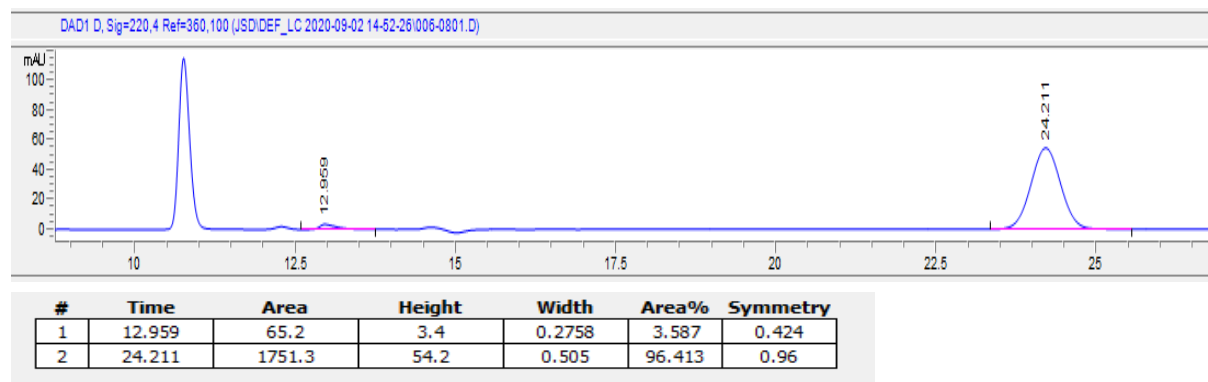
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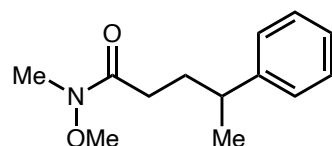


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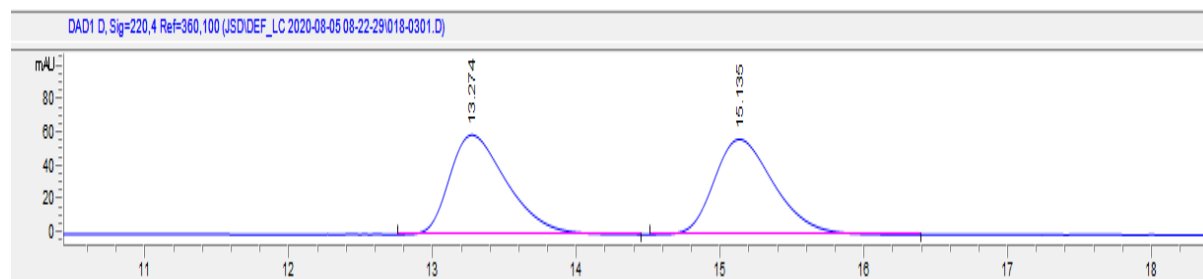


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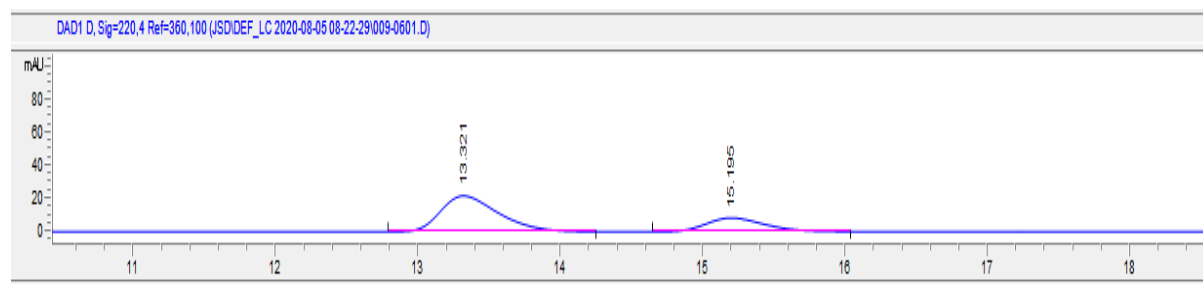


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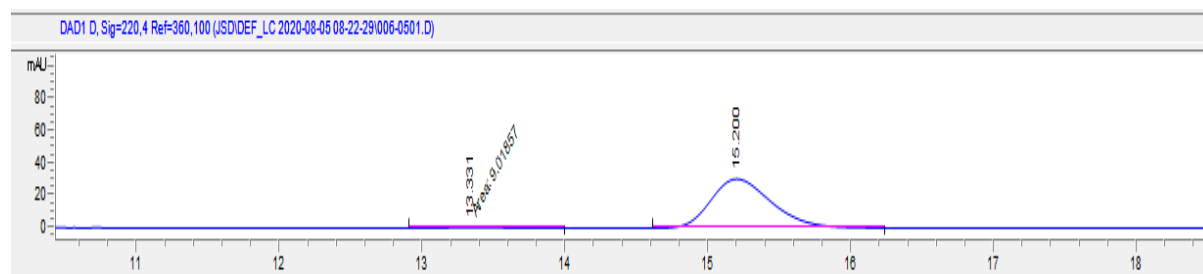
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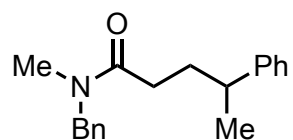


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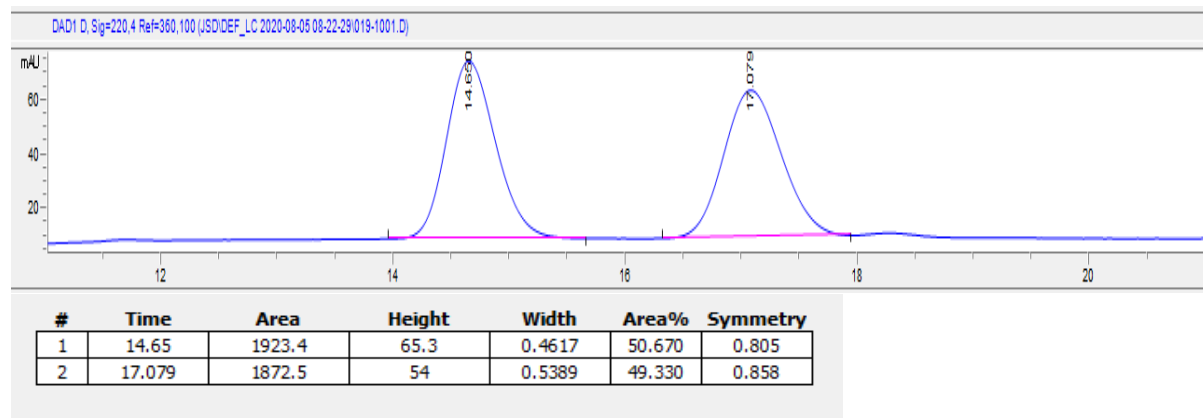
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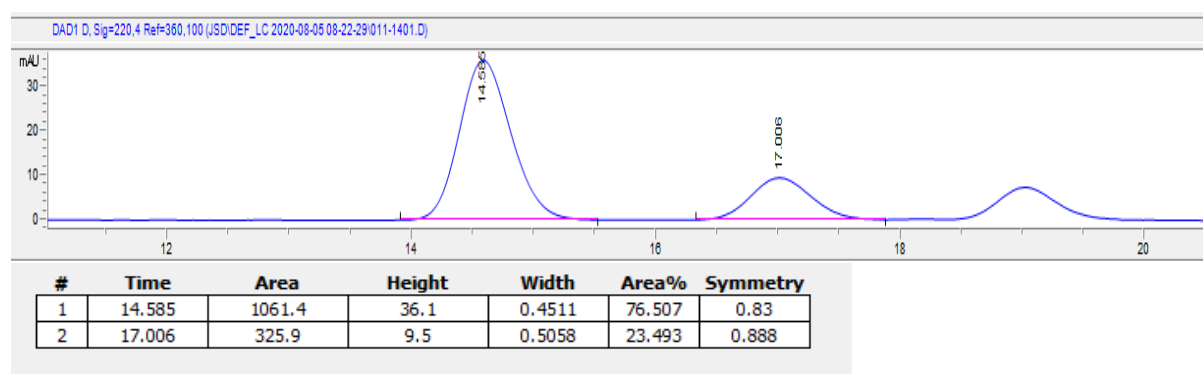
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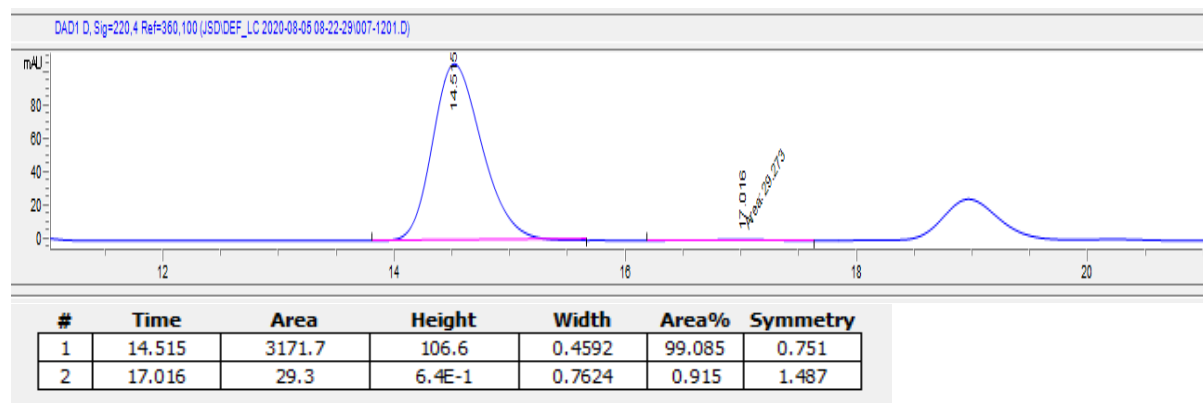
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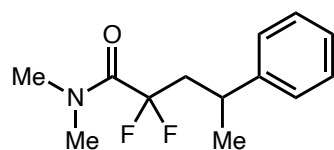


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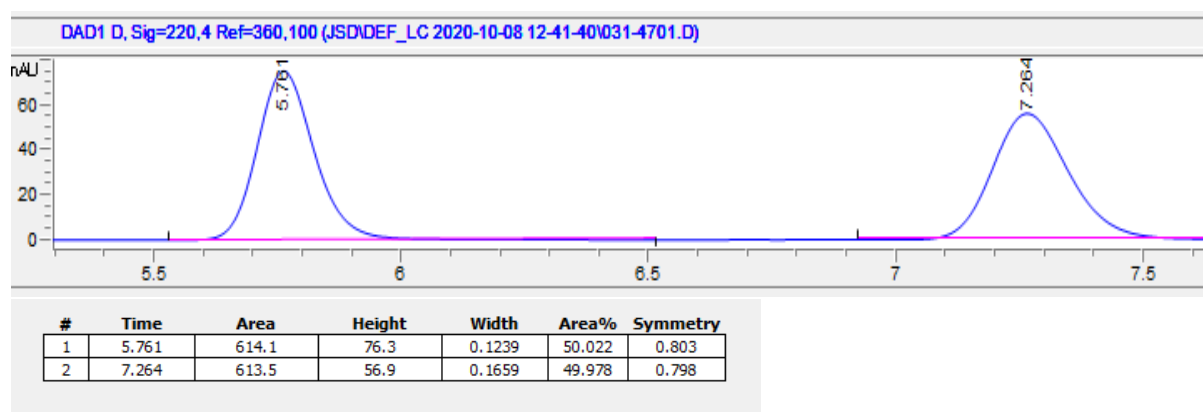


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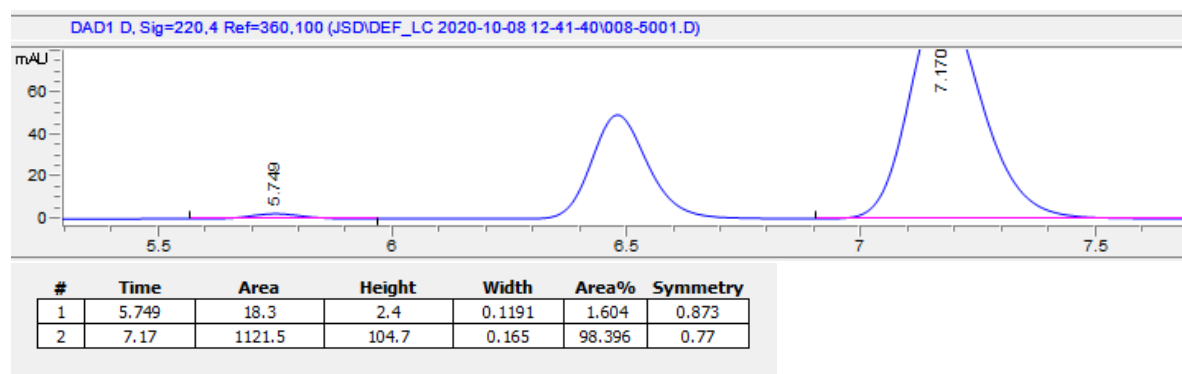




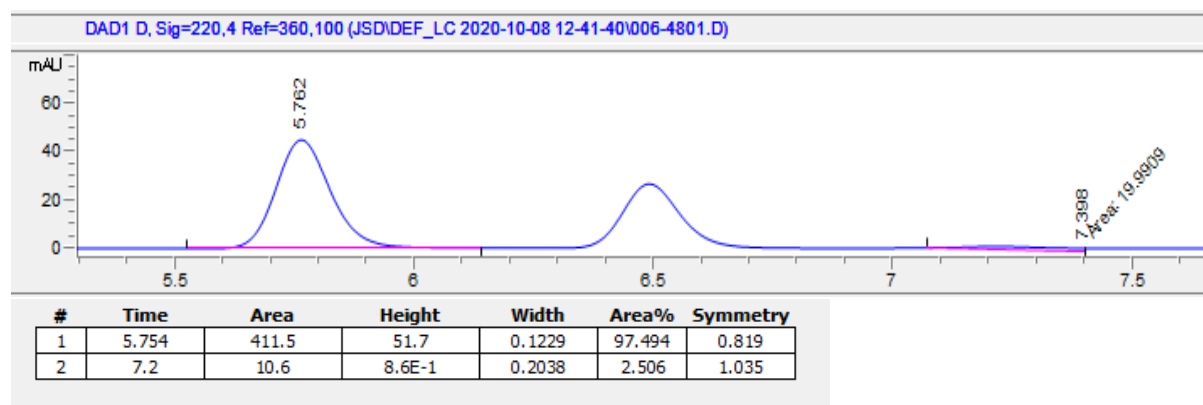
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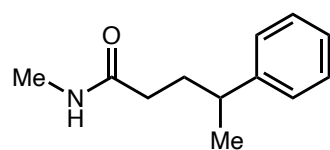


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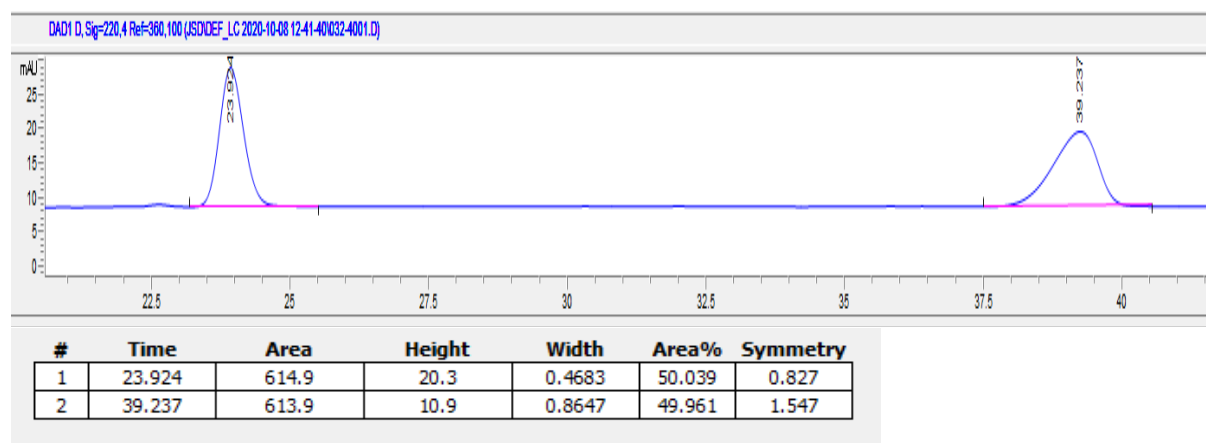


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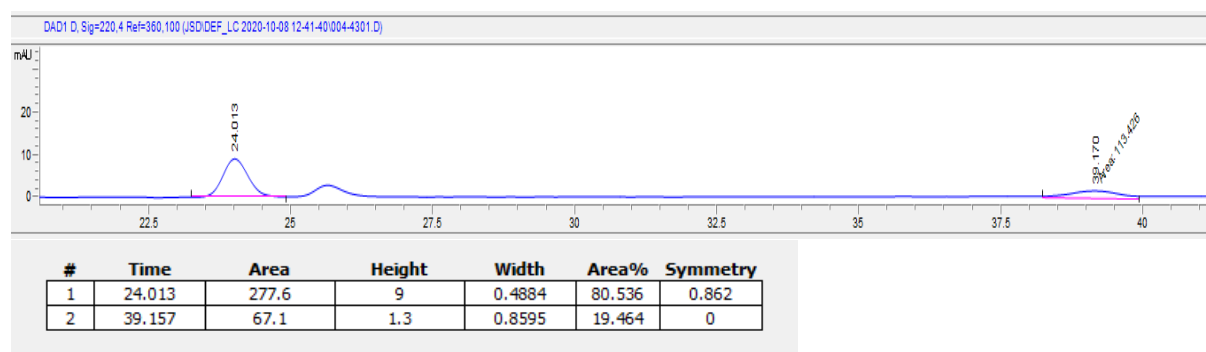




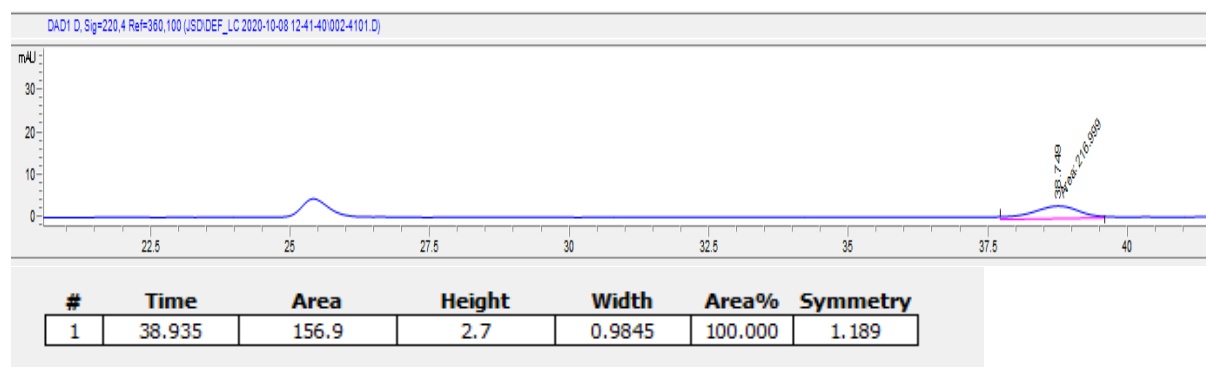
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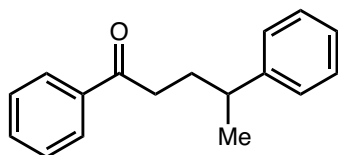


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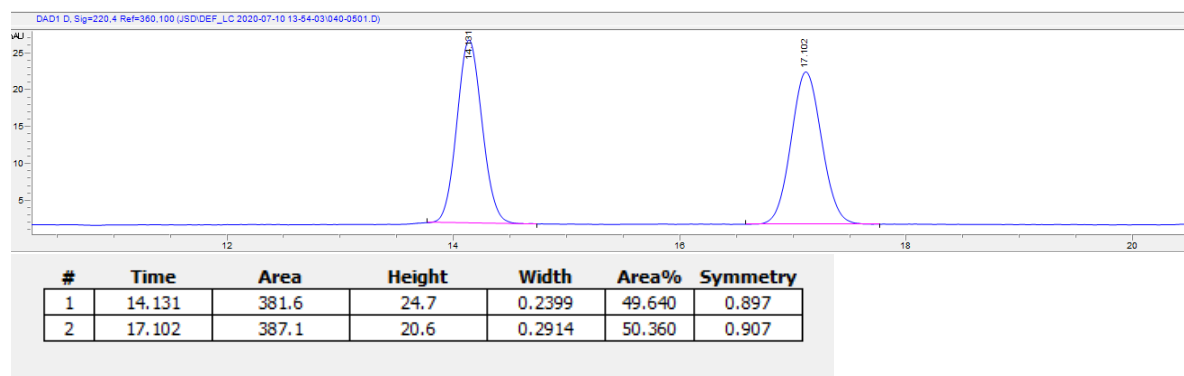


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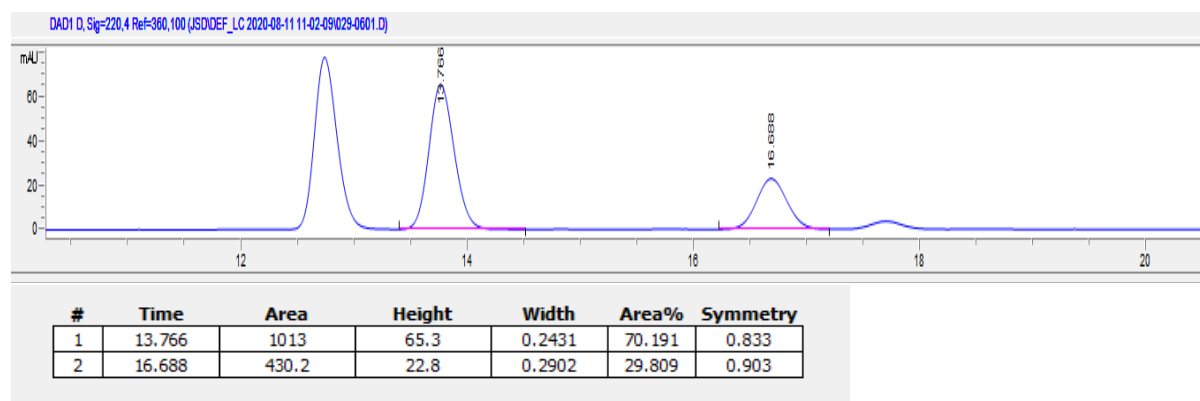




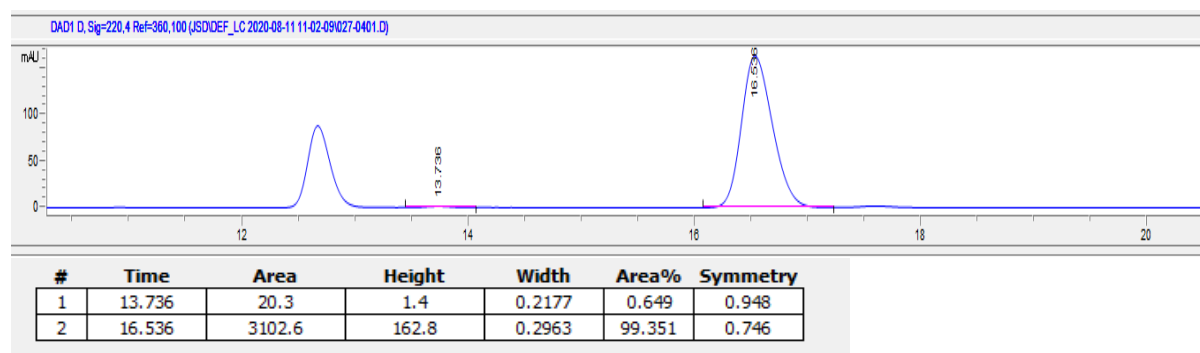
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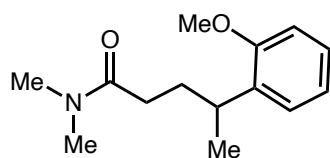


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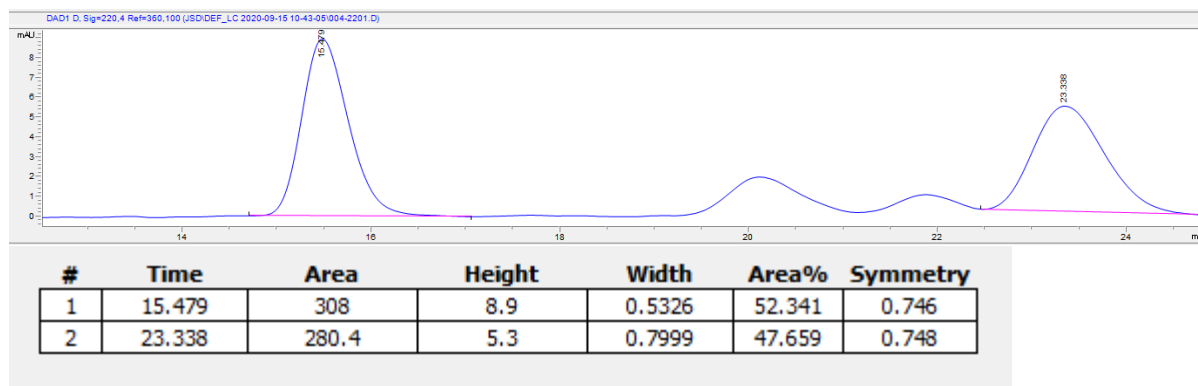


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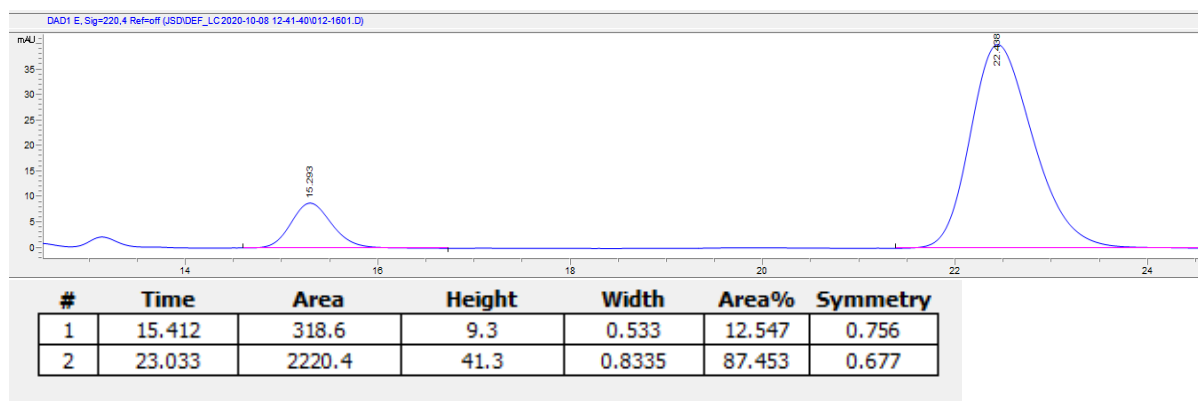




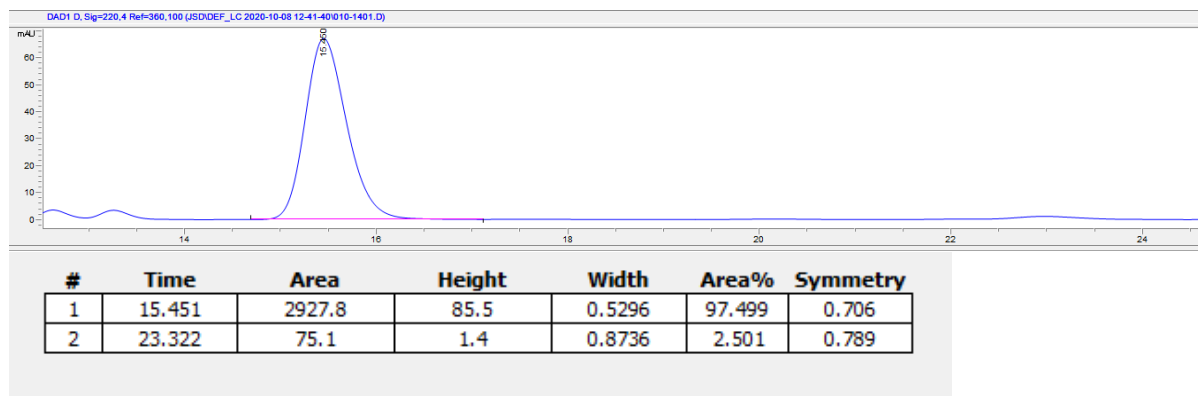
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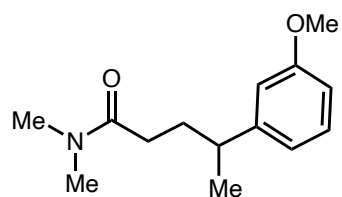


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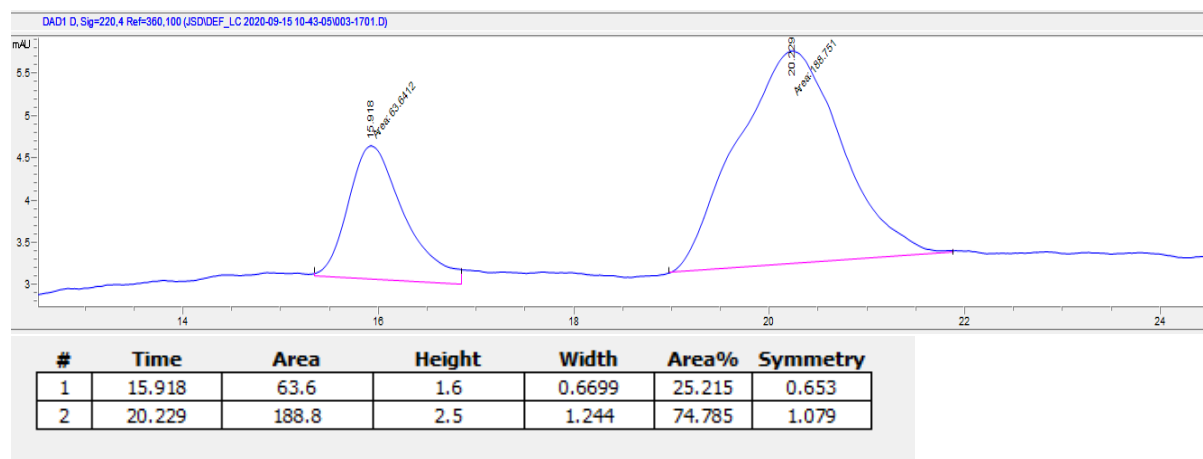


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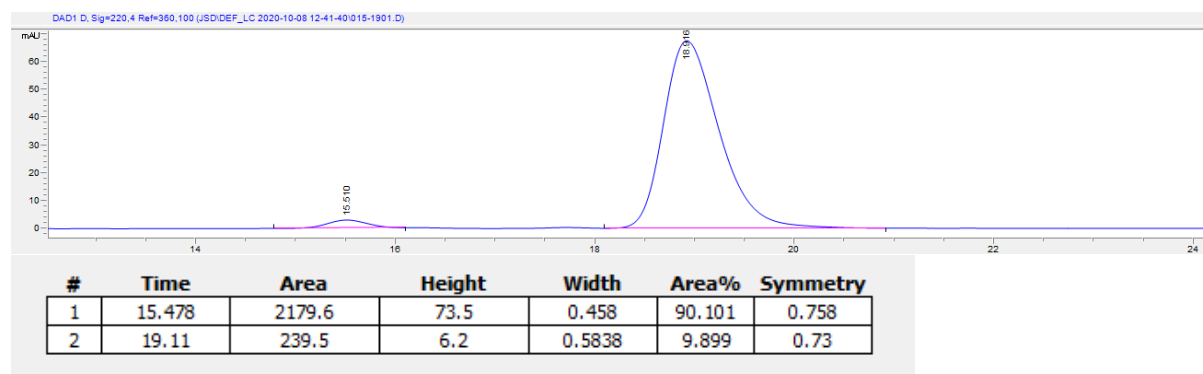




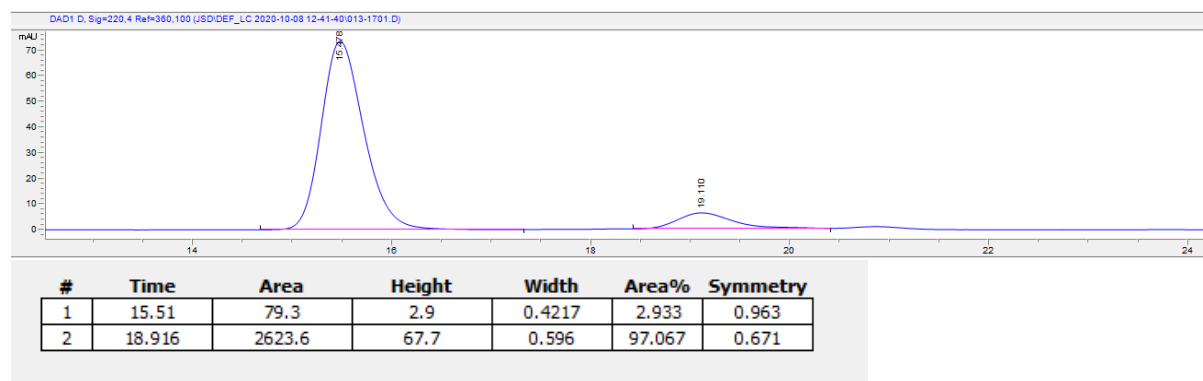
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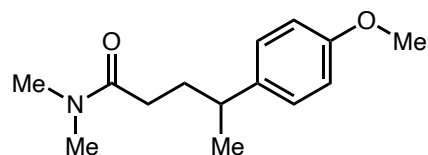


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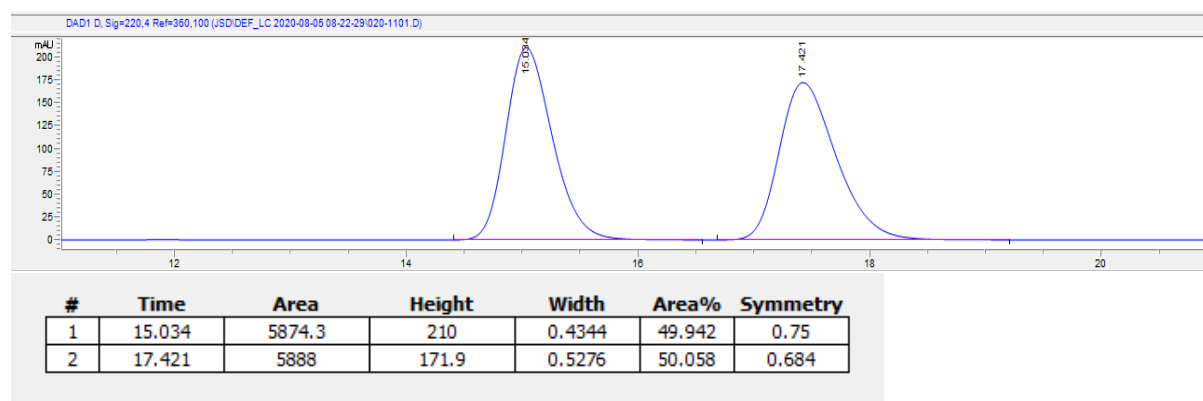


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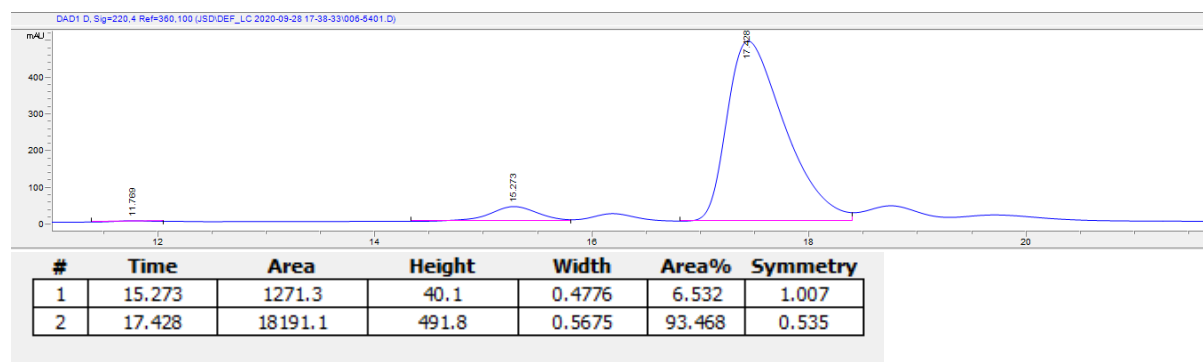




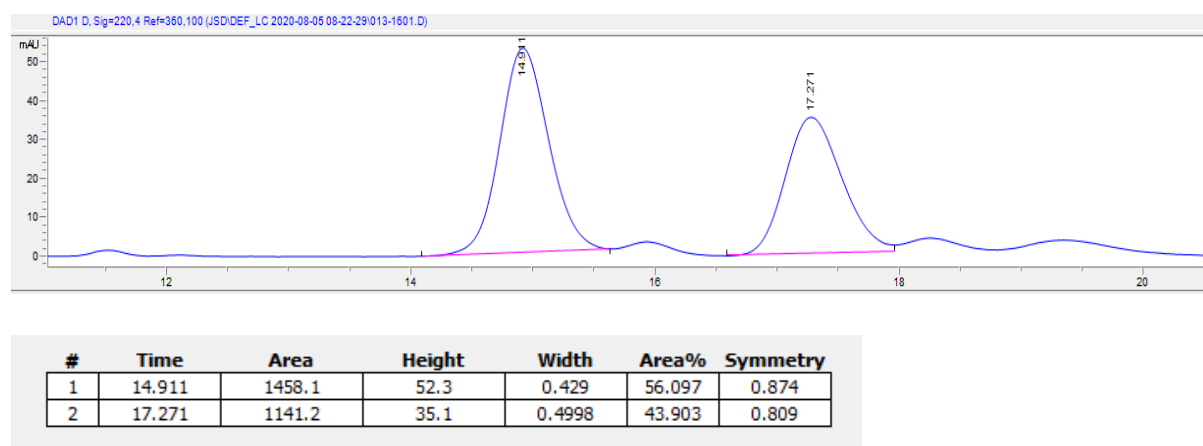
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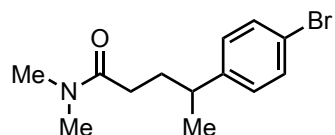


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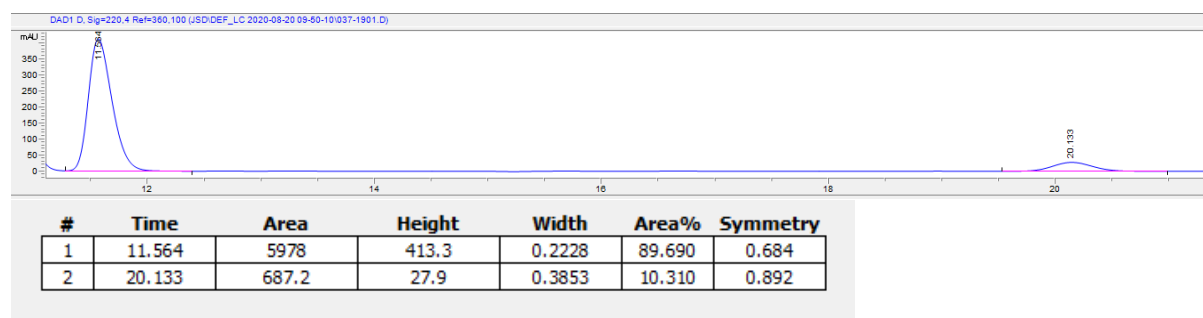




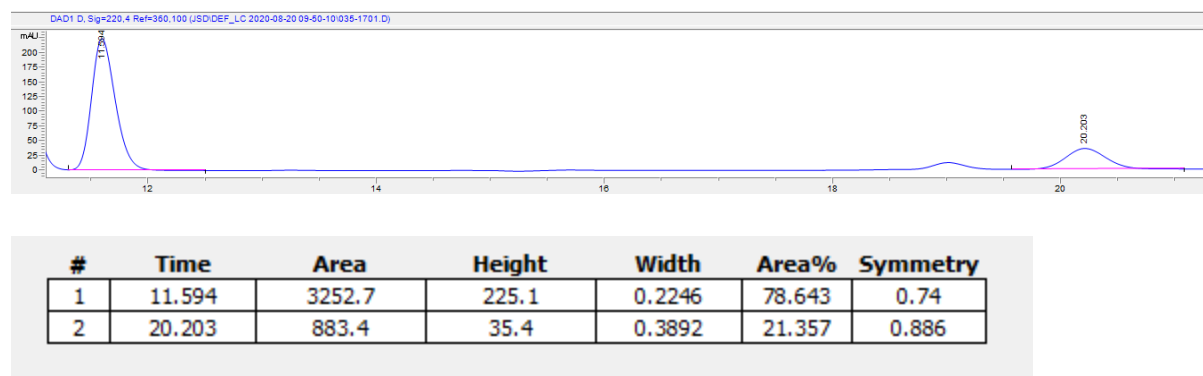
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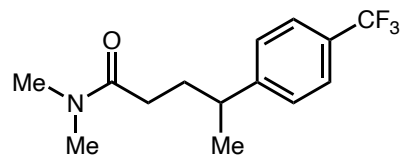


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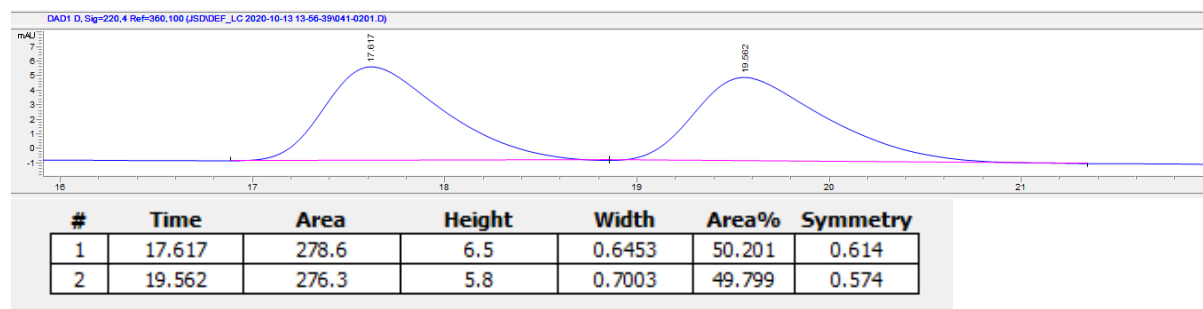


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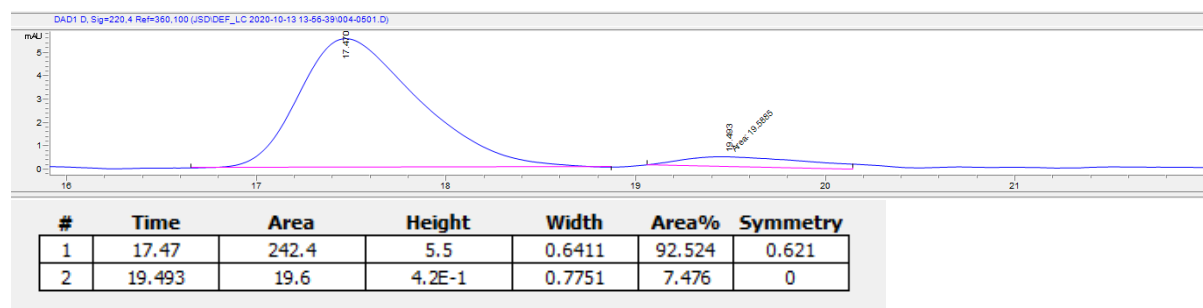




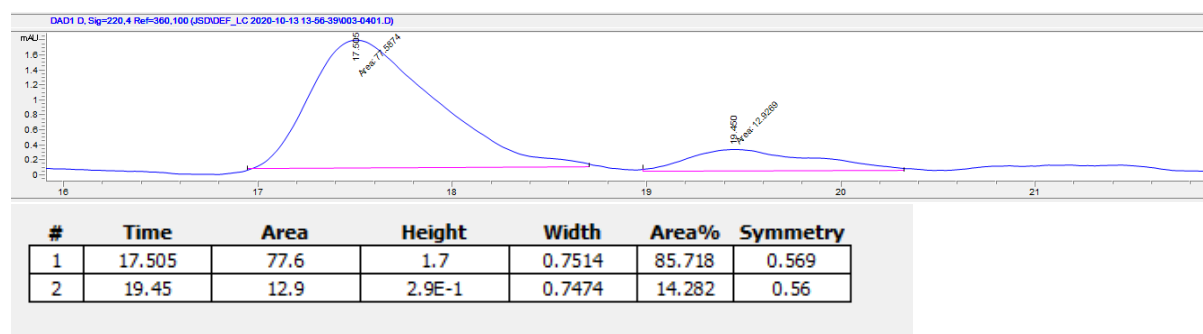
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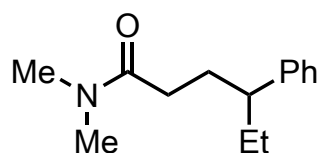


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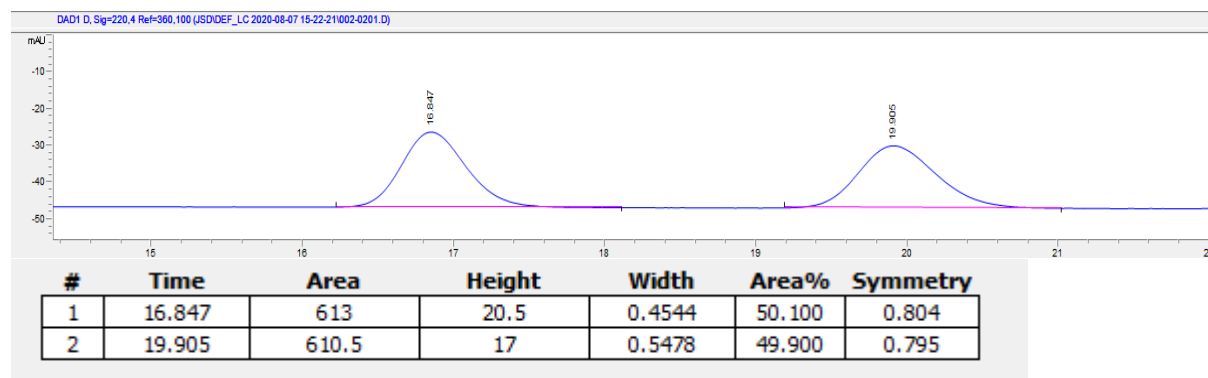


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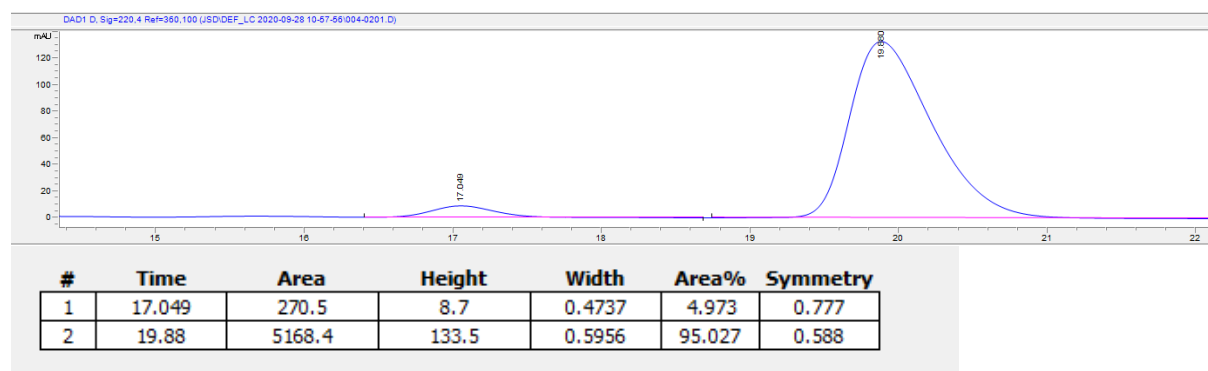




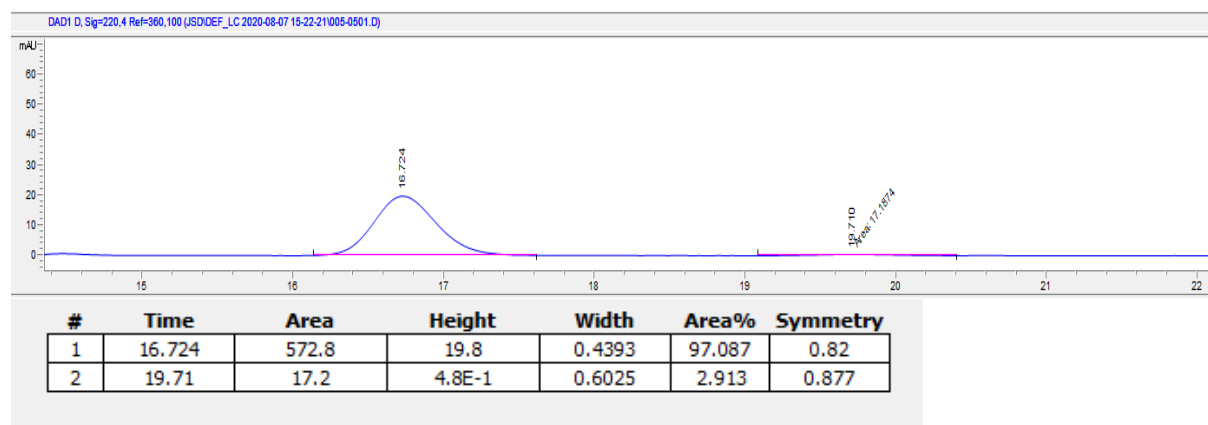
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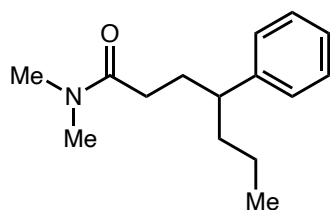


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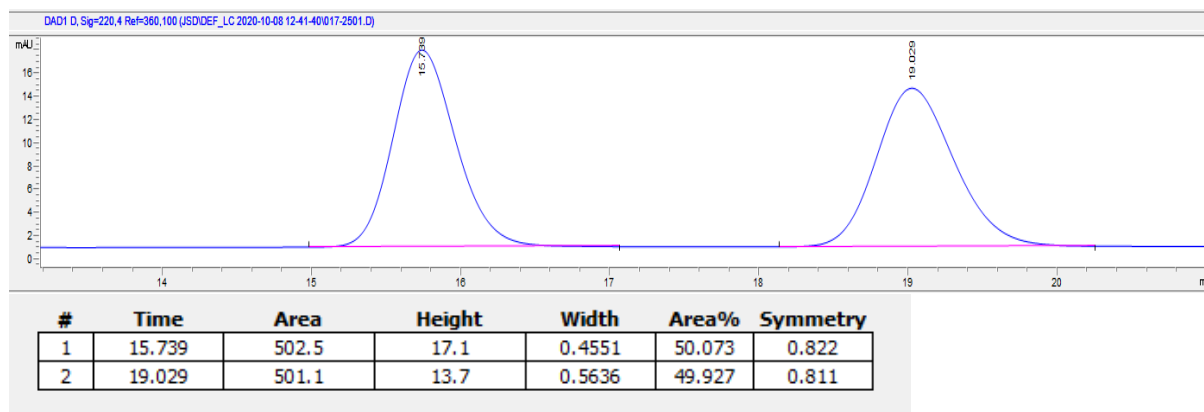


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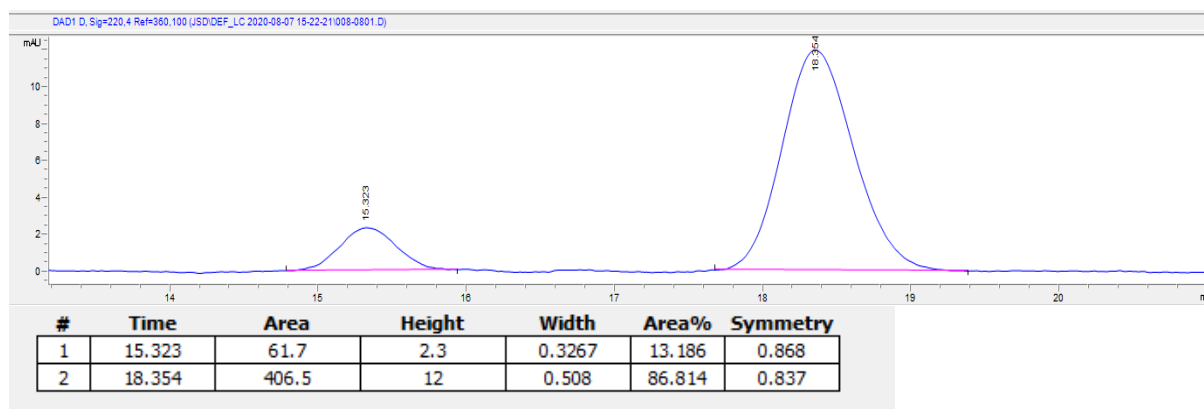




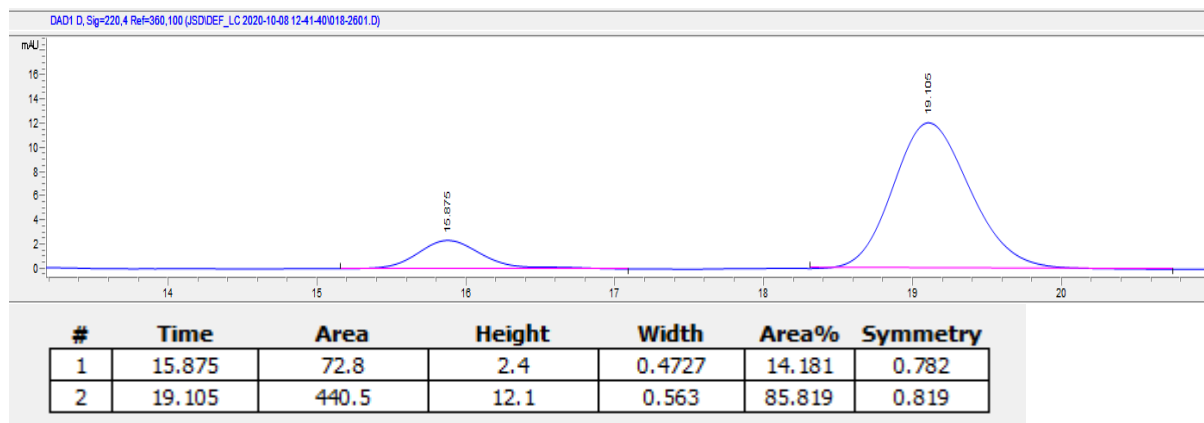
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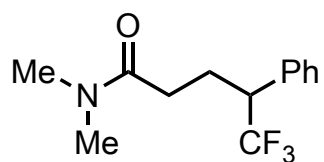


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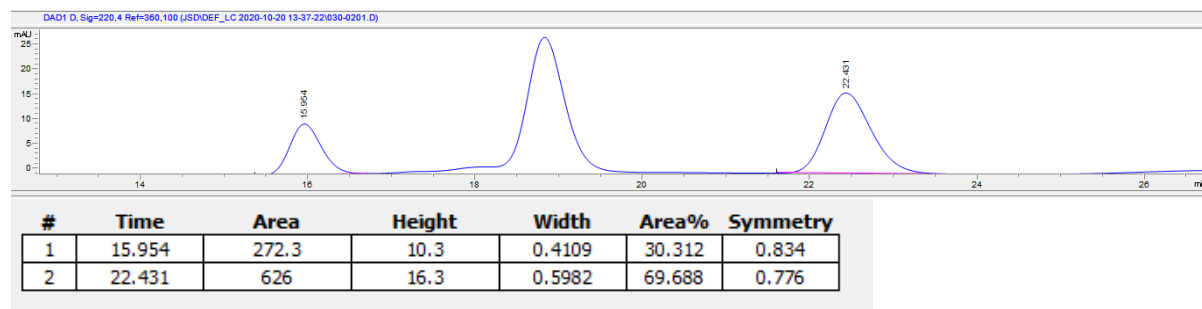


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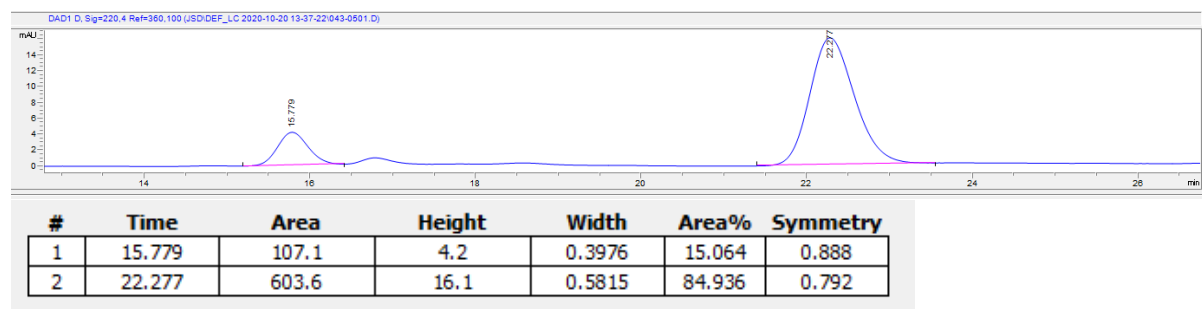




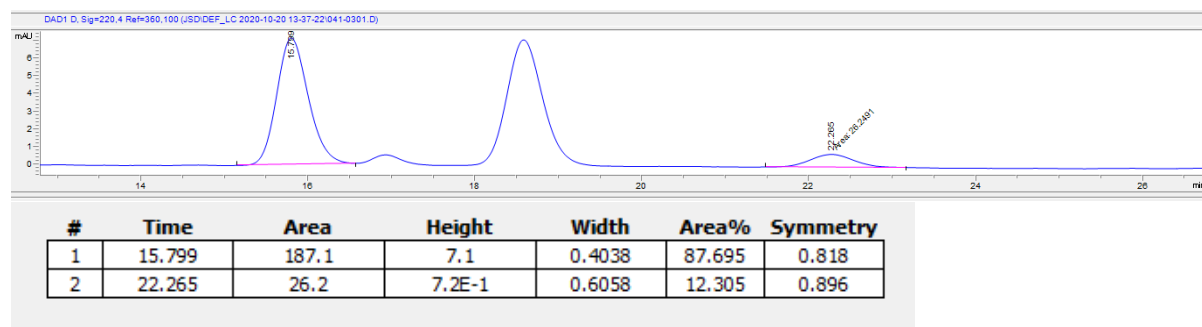
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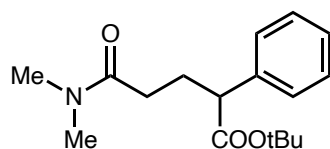


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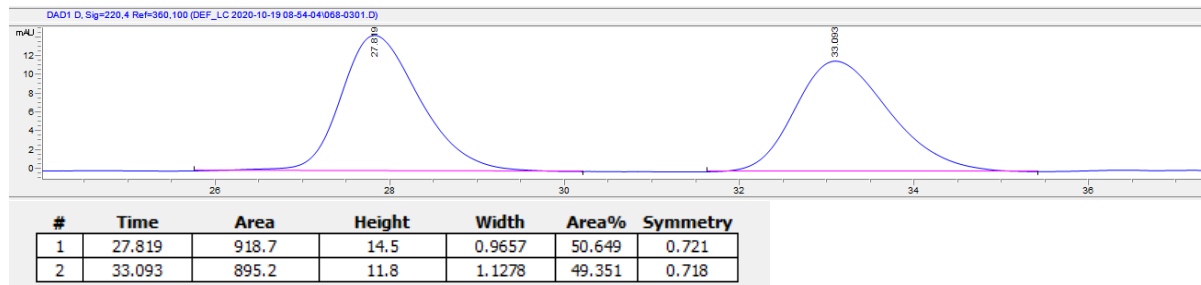


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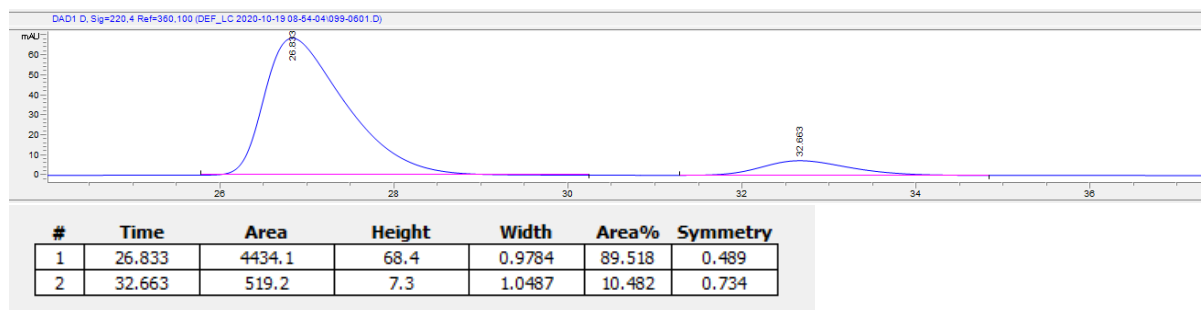




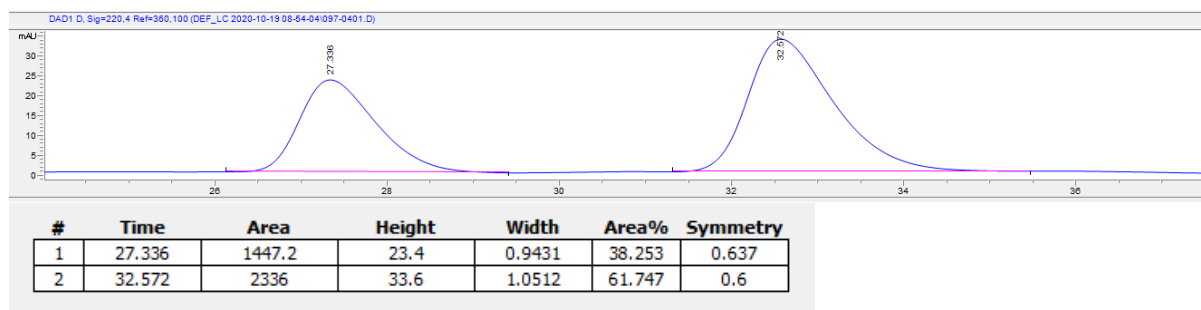
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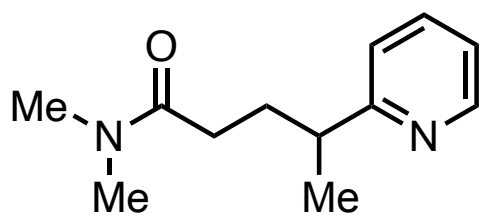


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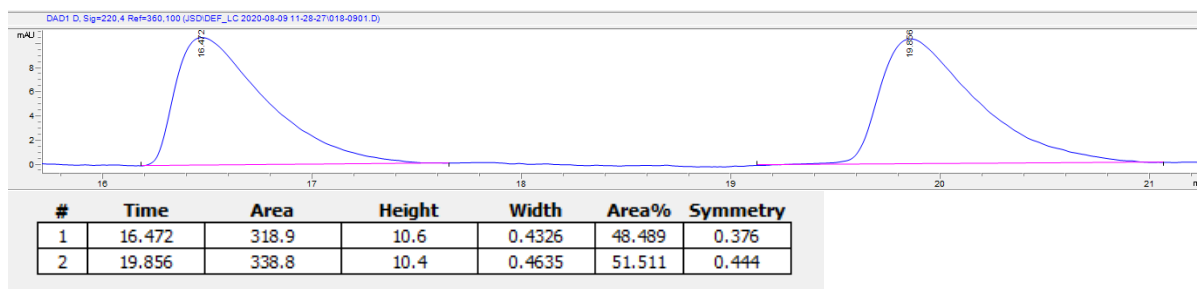


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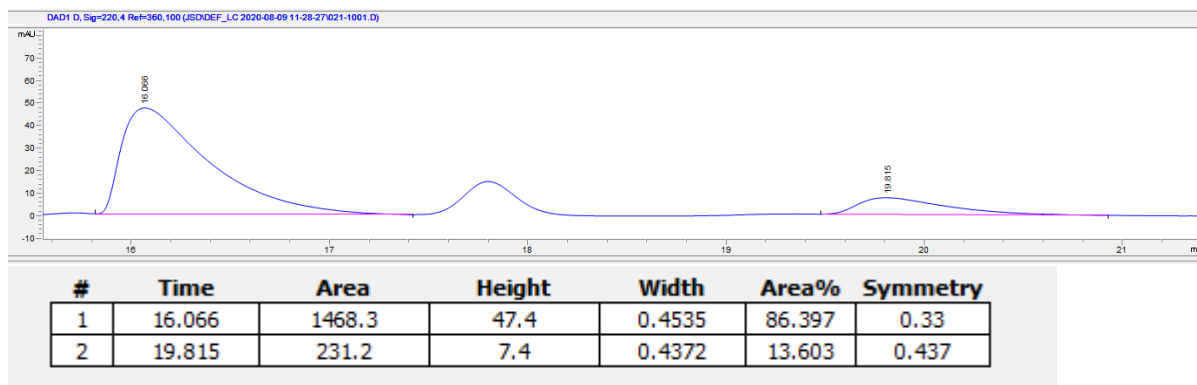




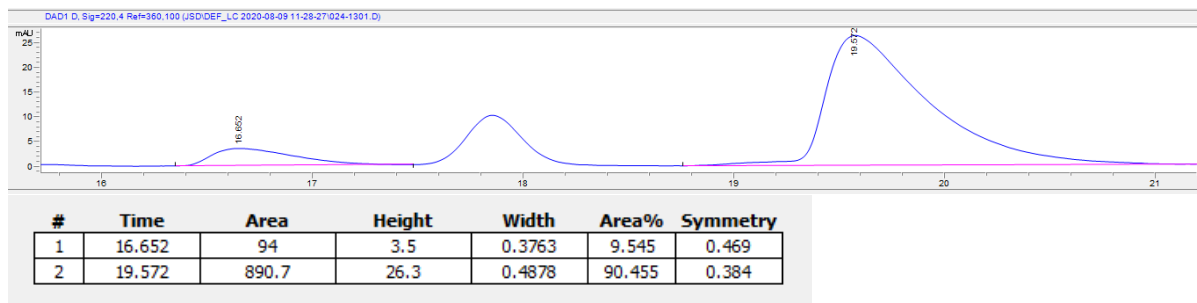
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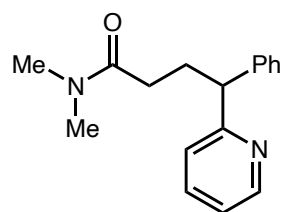


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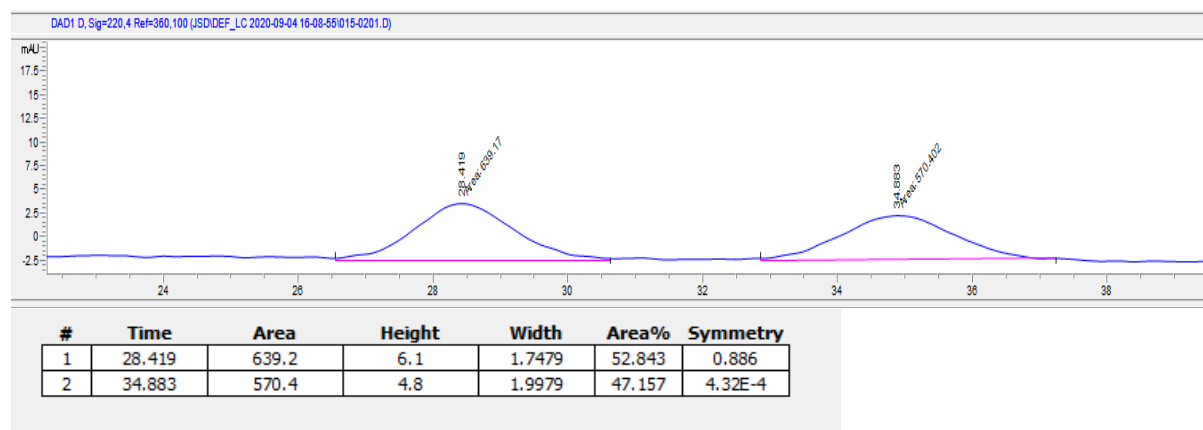


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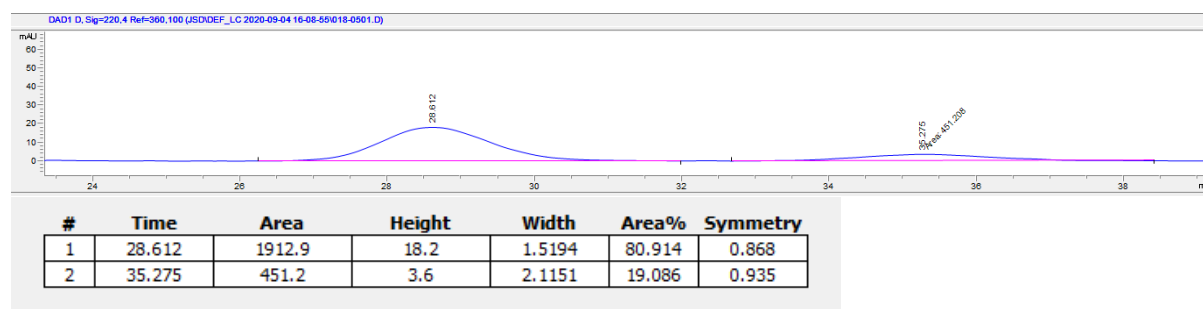




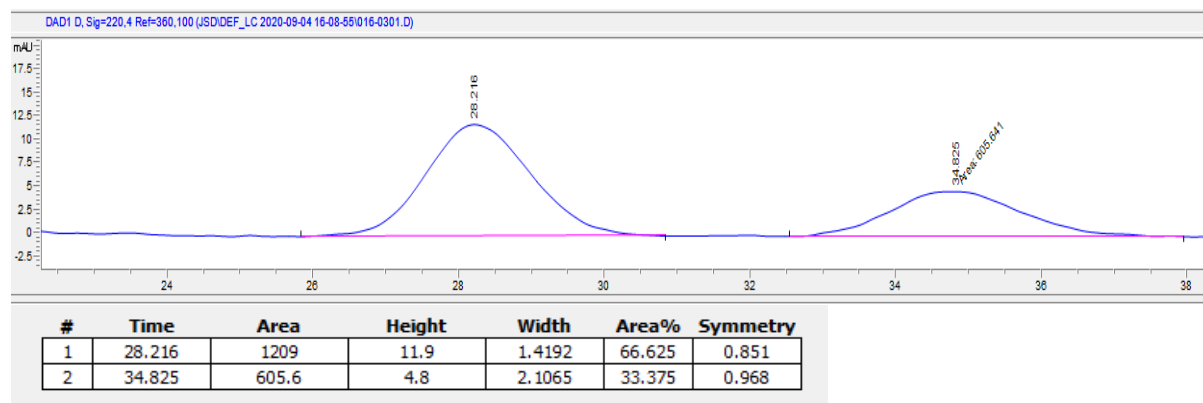
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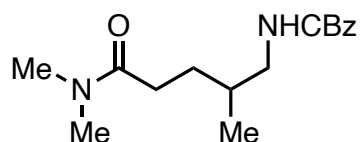


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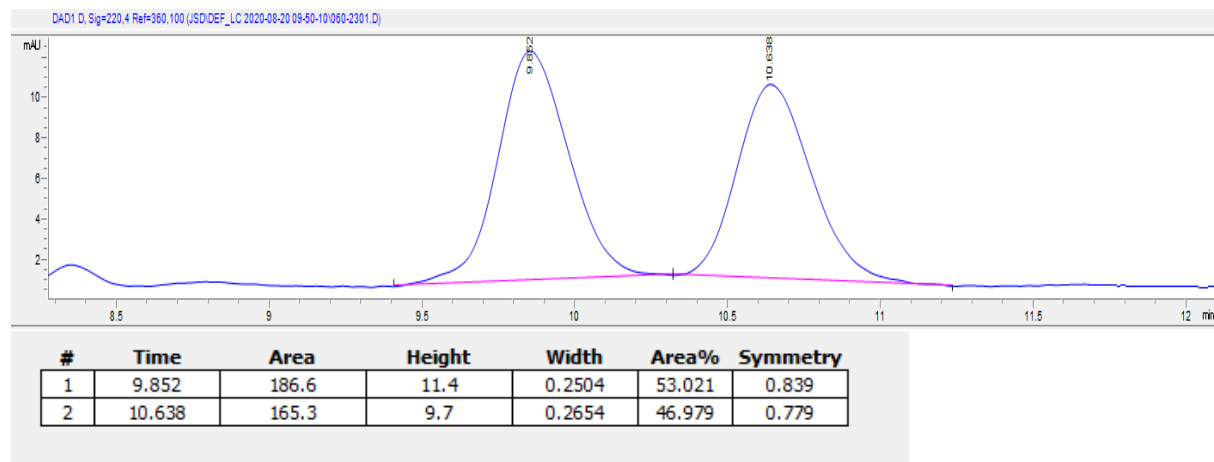


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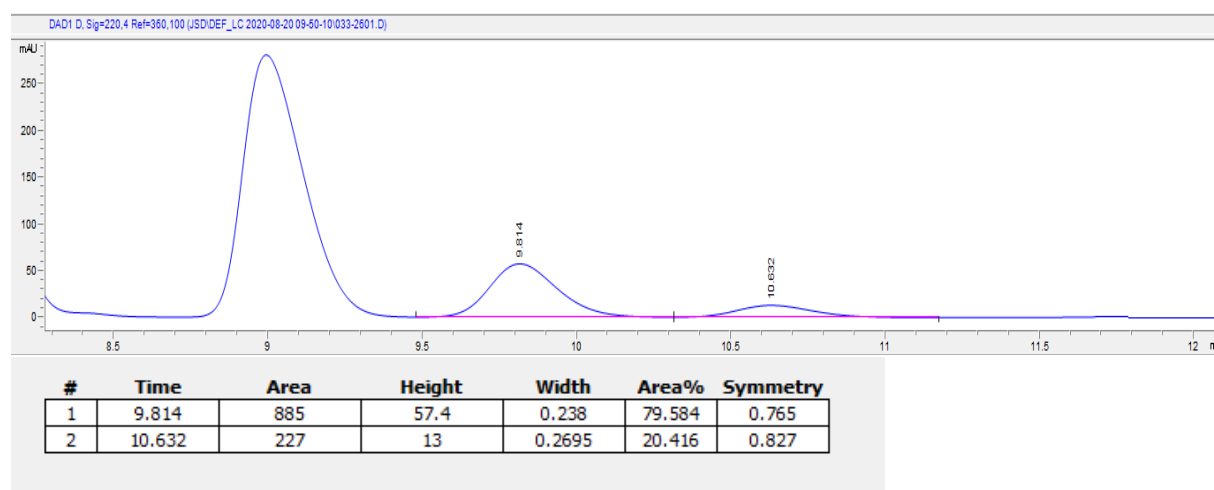




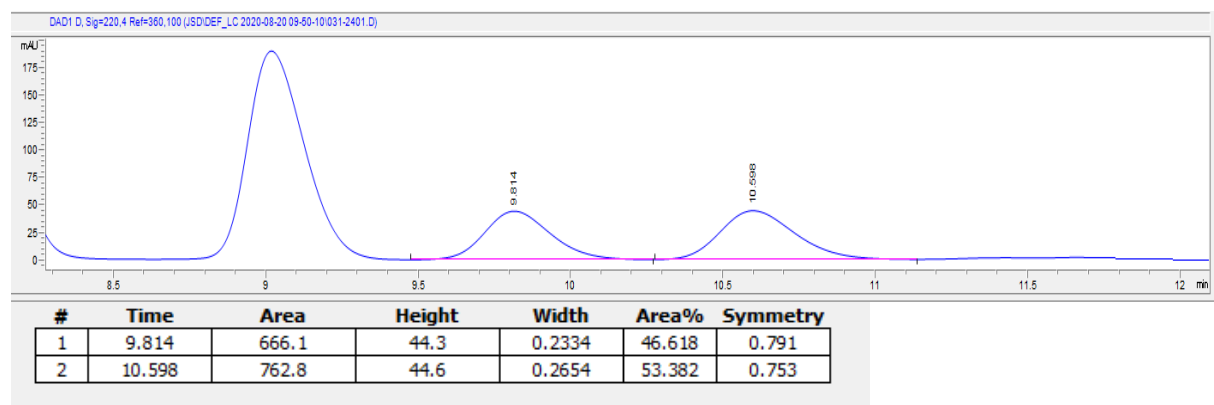
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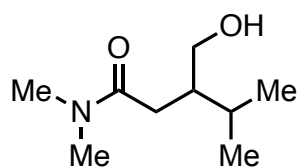


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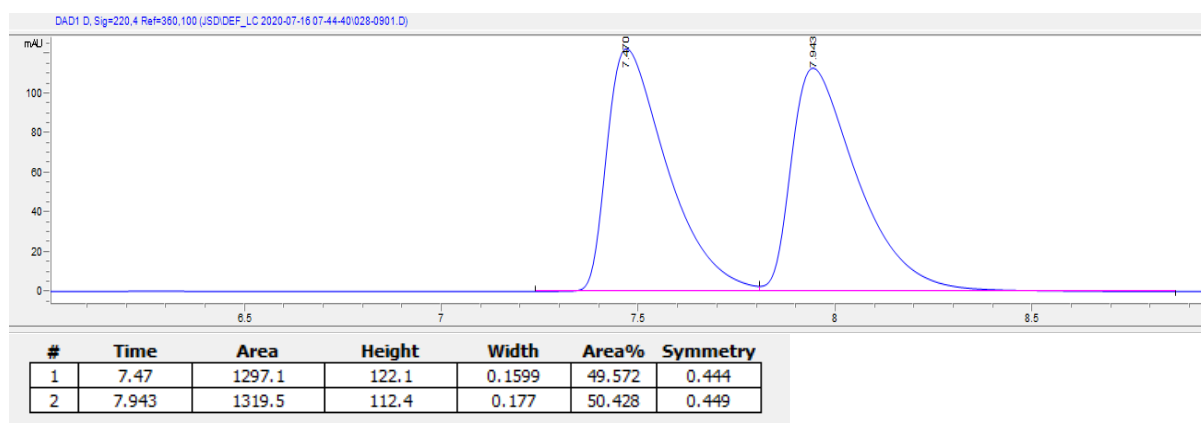


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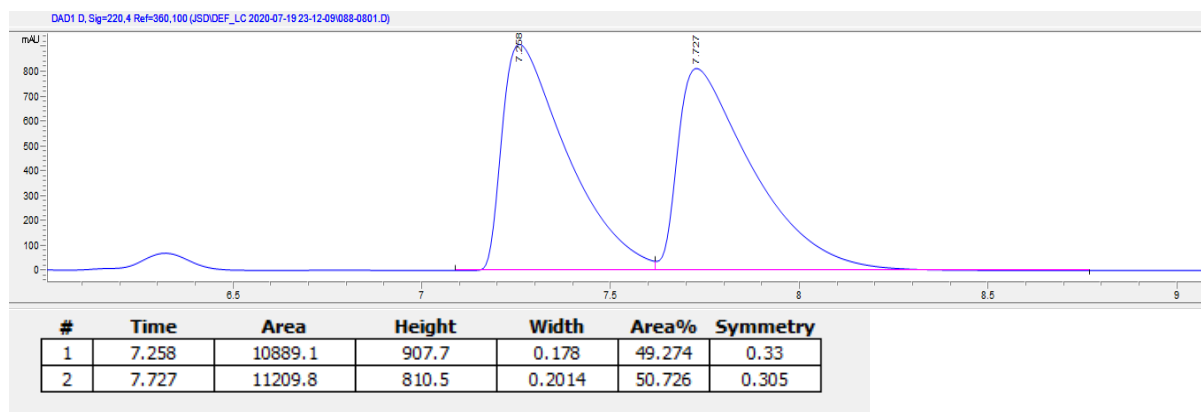


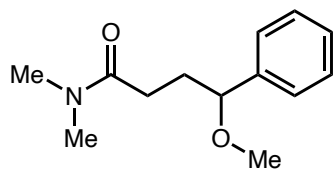


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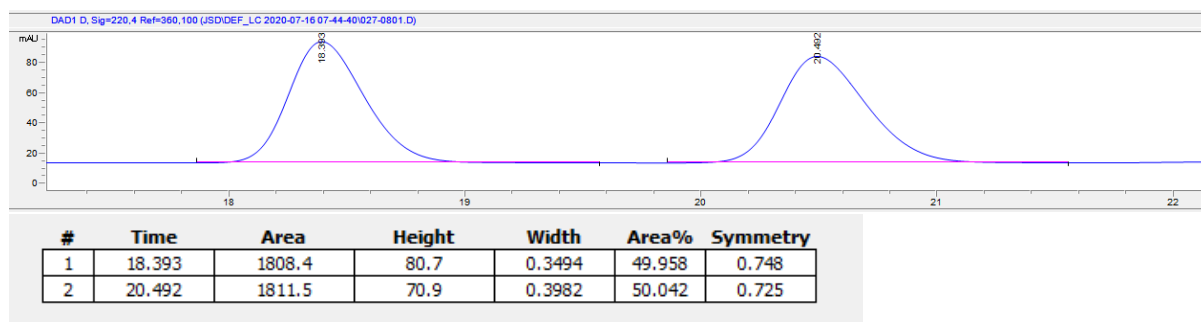


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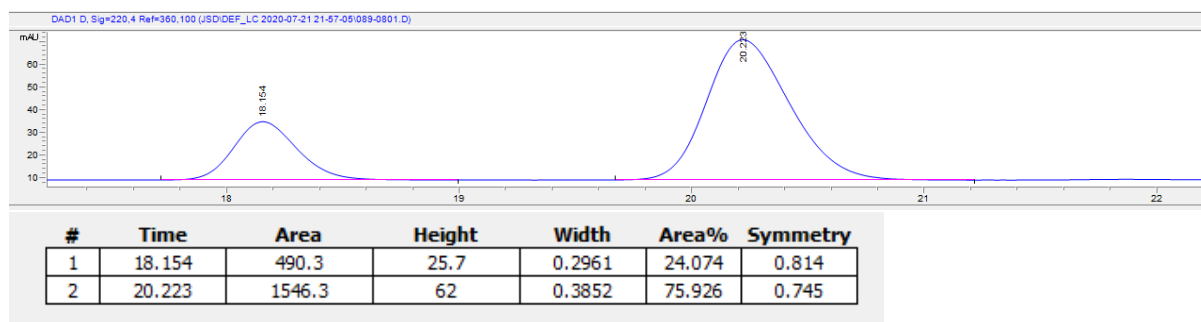




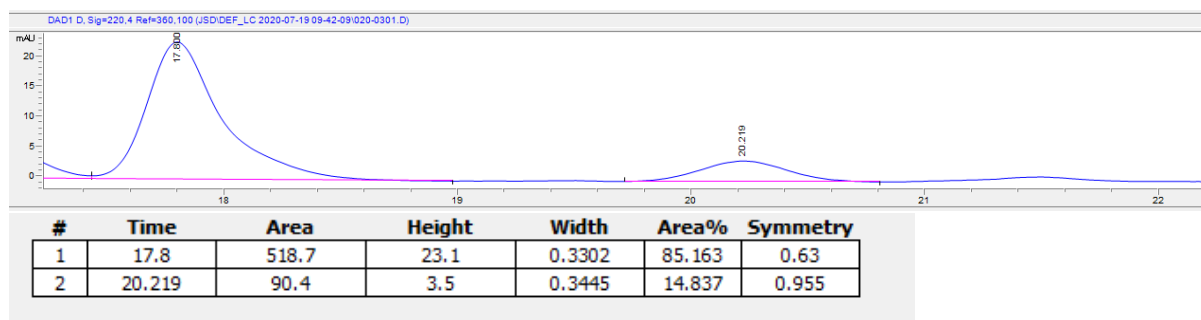
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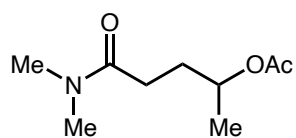


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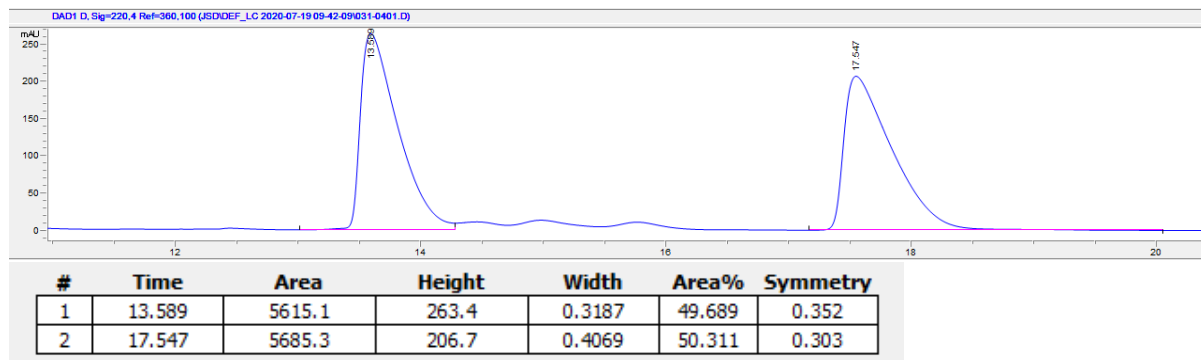


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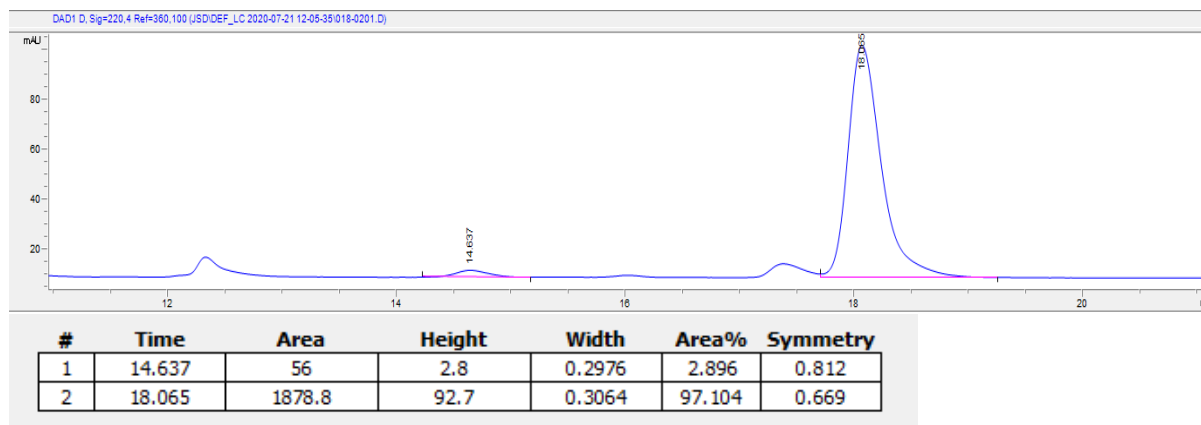




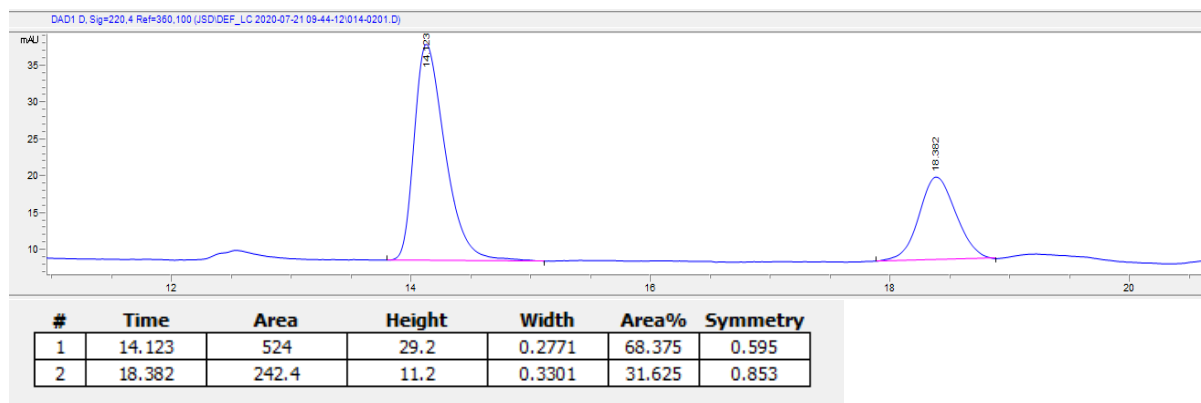
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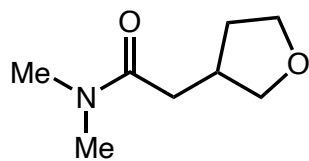


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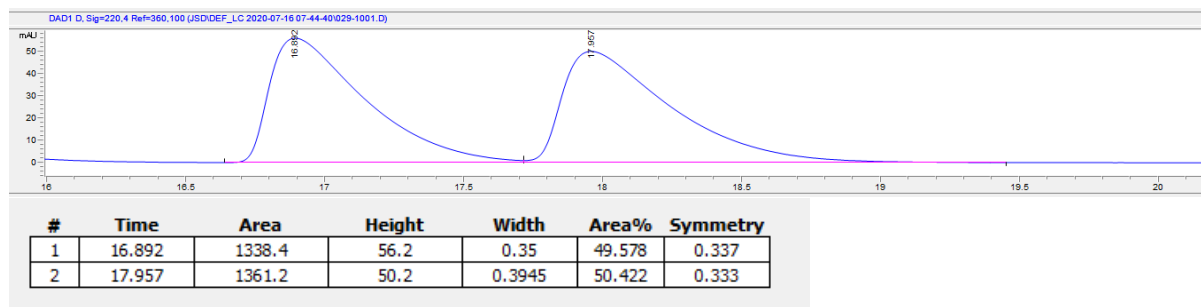


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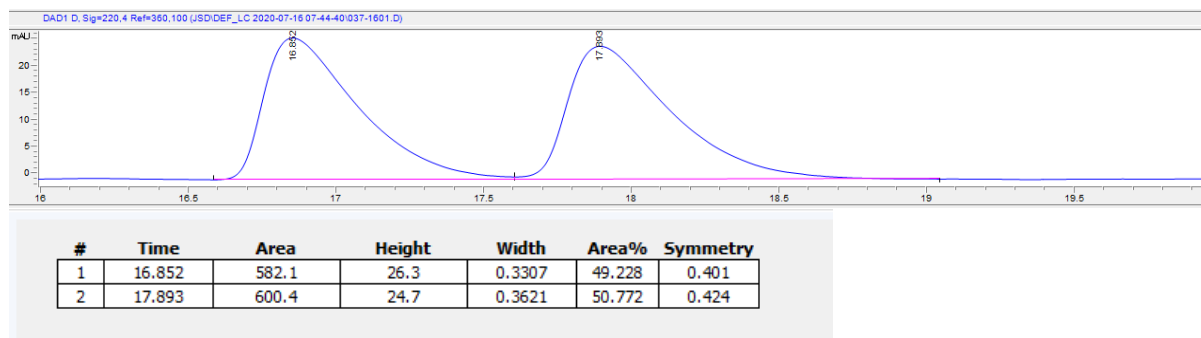




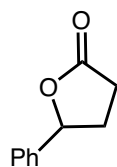
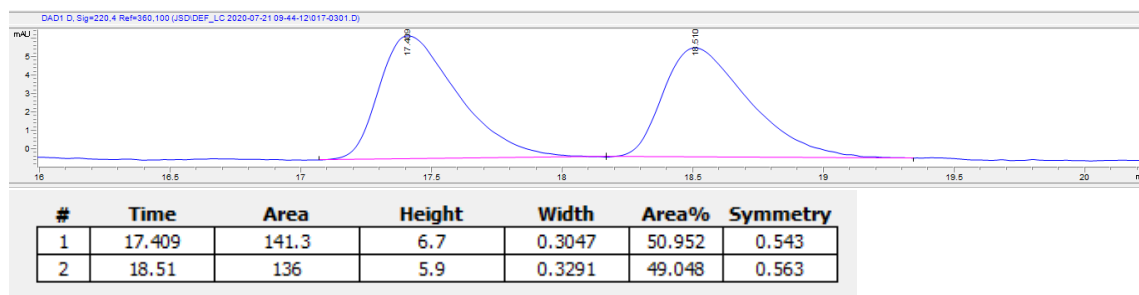
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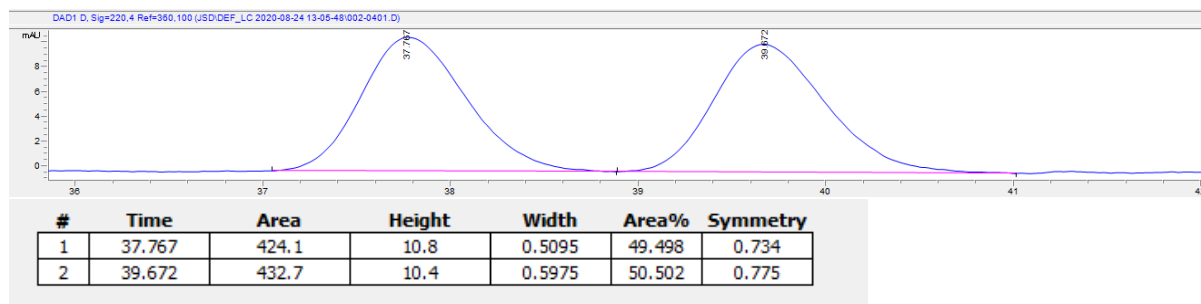
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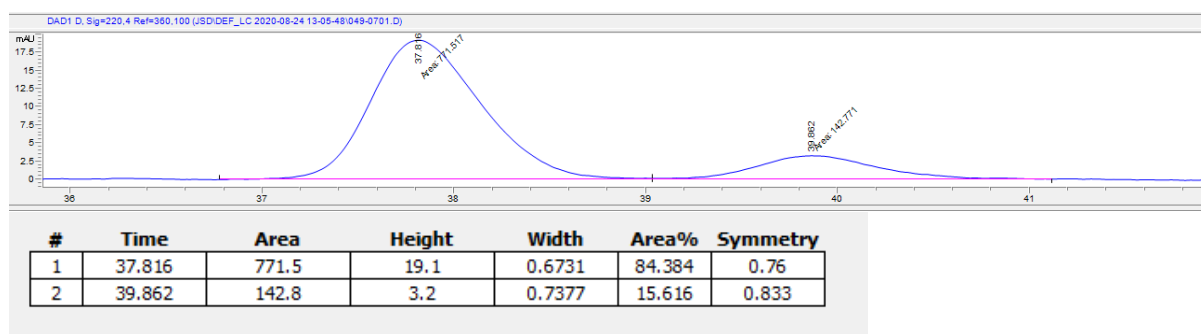
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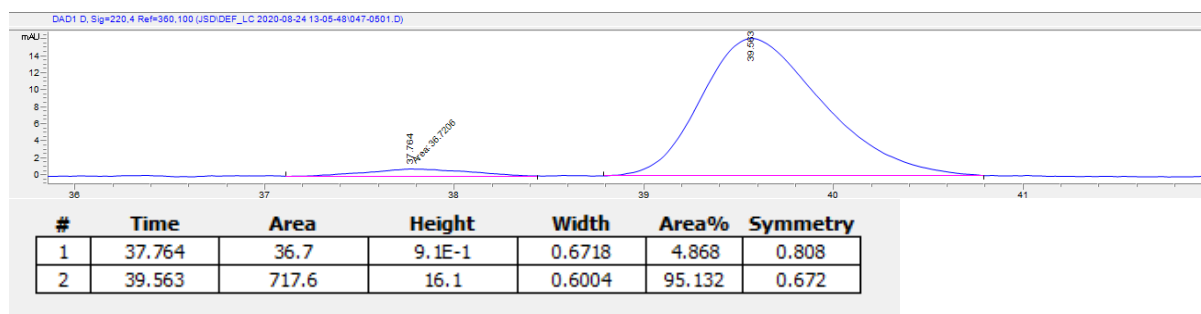
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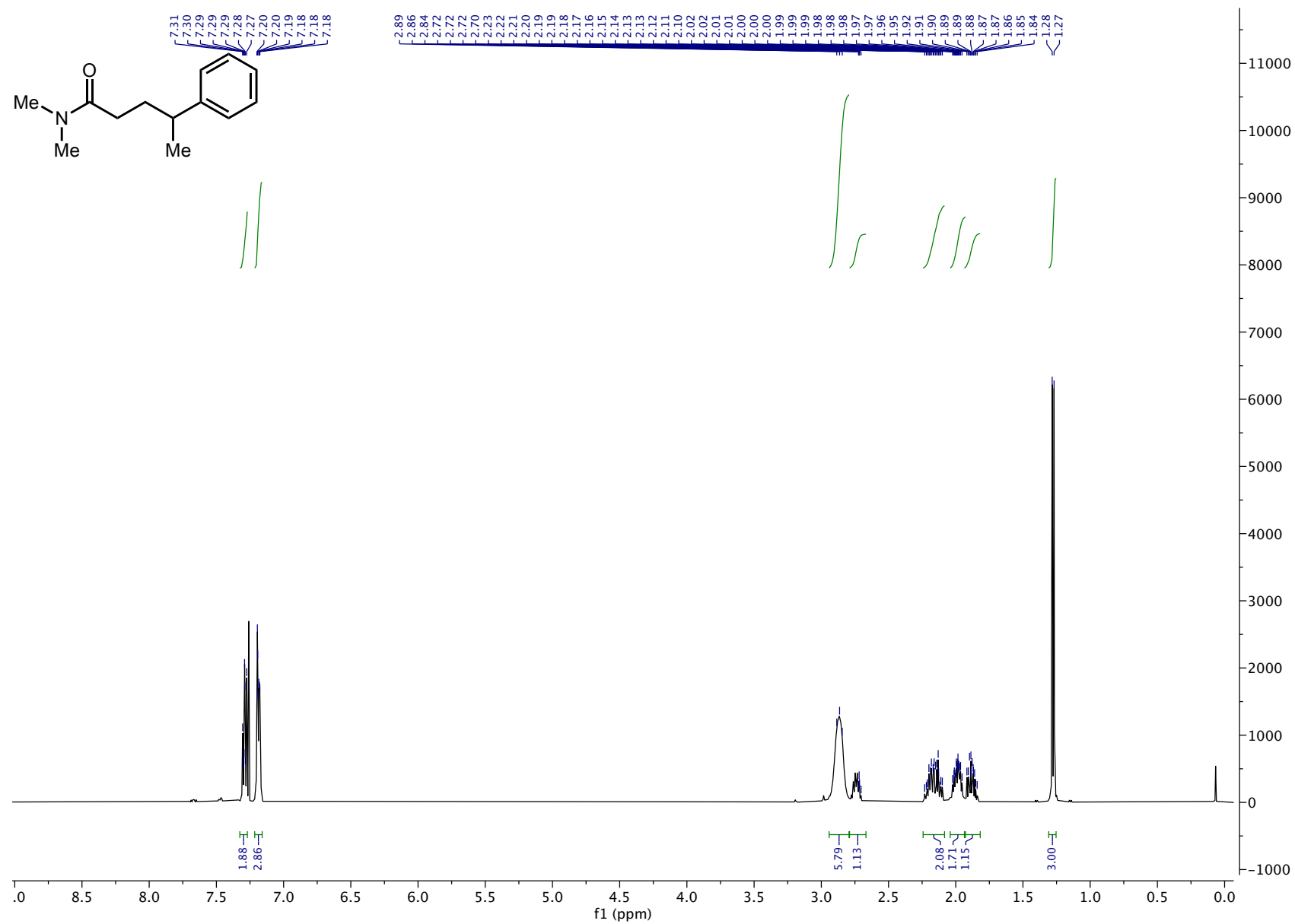
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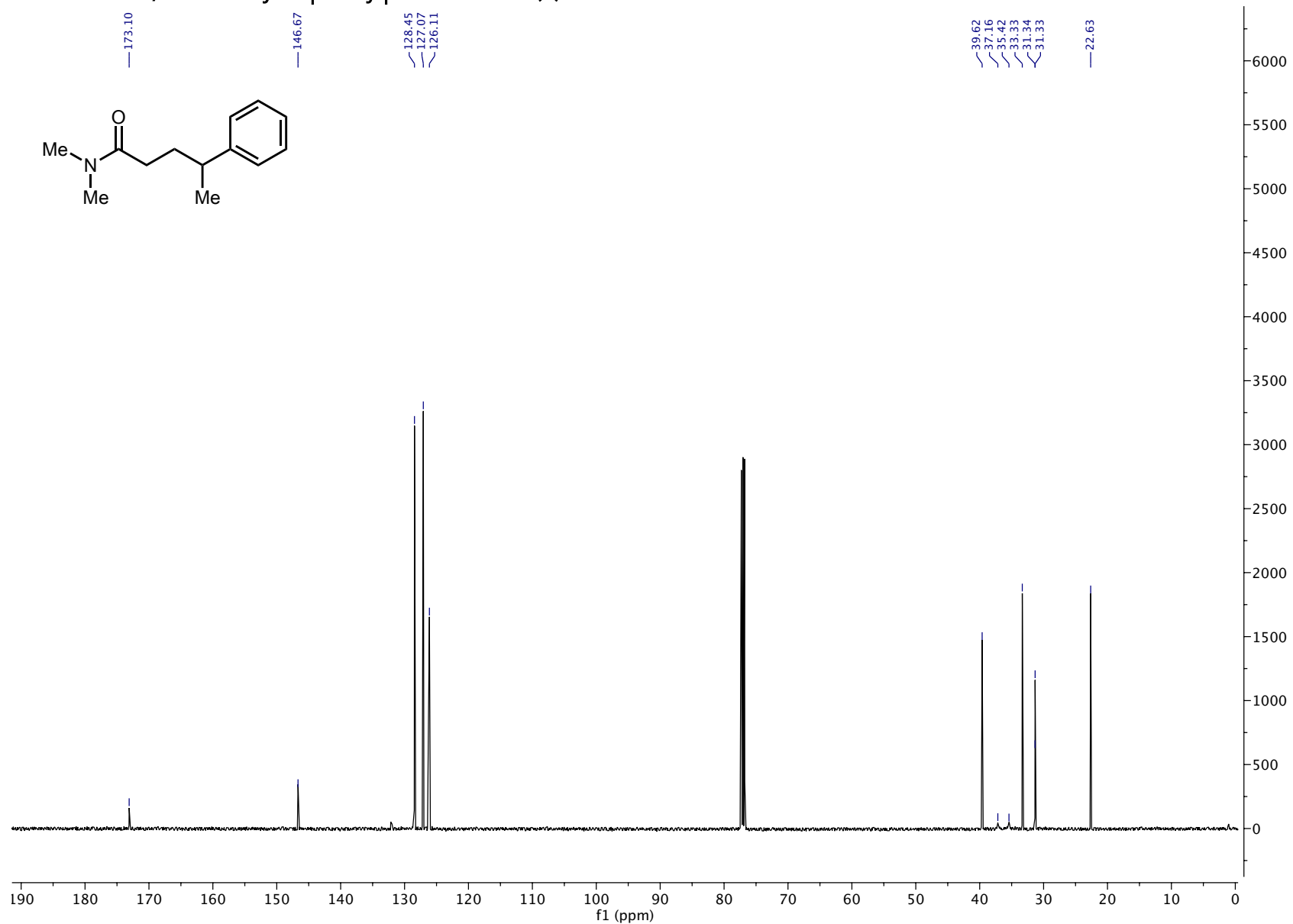
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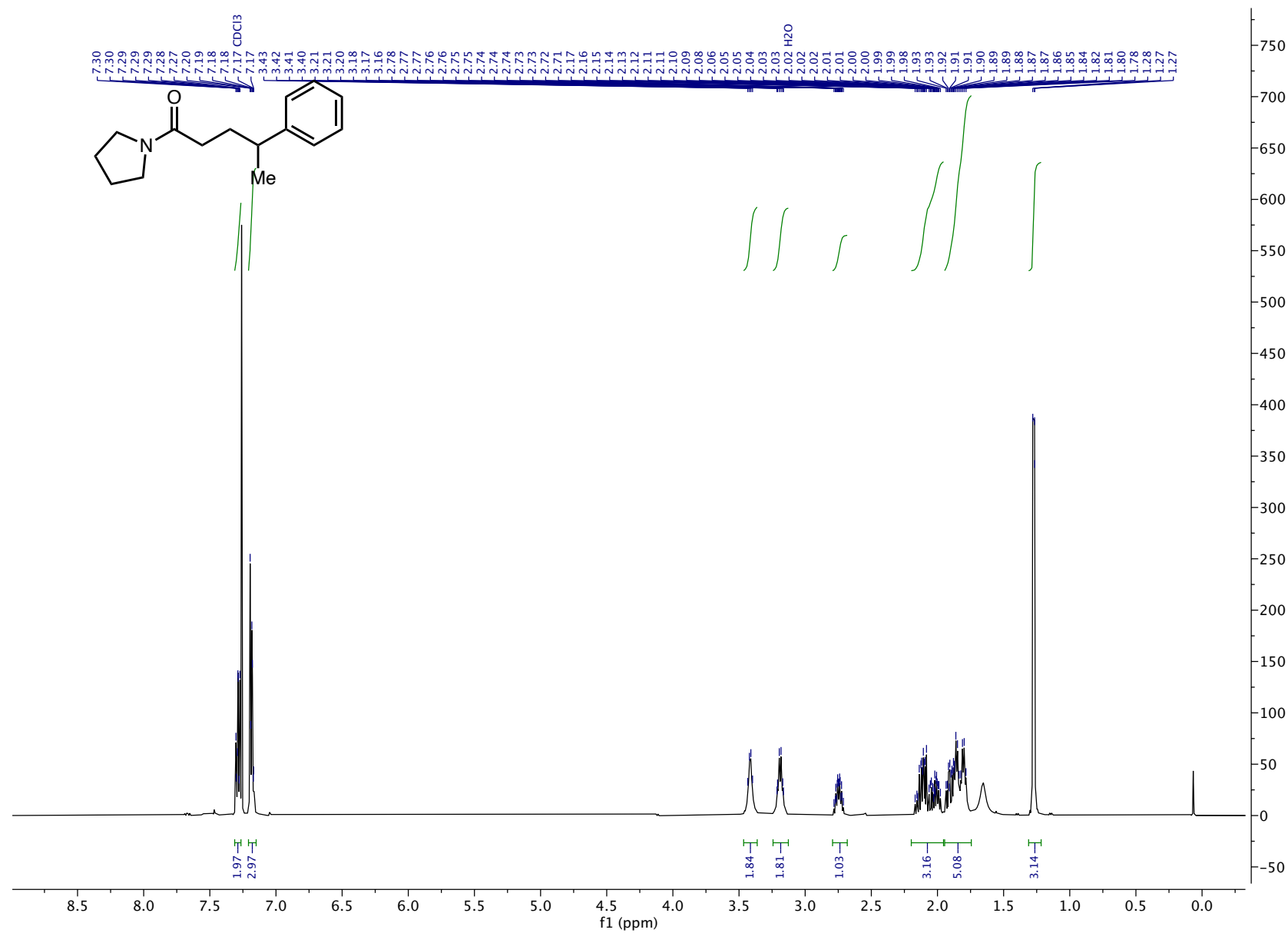
¹H NMR of *N,N*-dimethyl-4-phenylpentanamide (3)



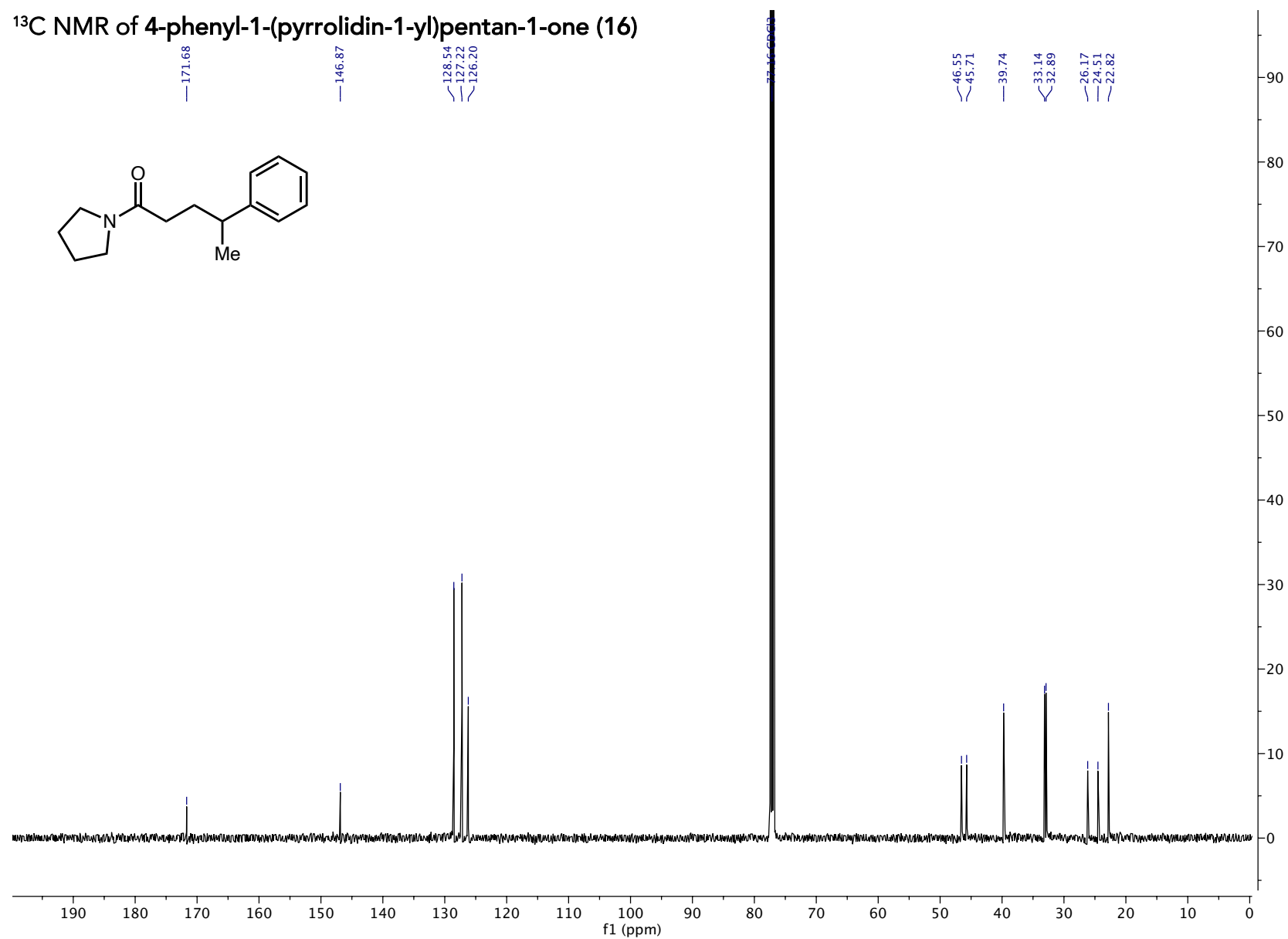
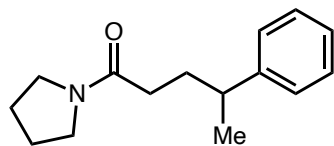
¹³C NMR of *N,N*-dimethyl-4-phenylpentanamide (3)



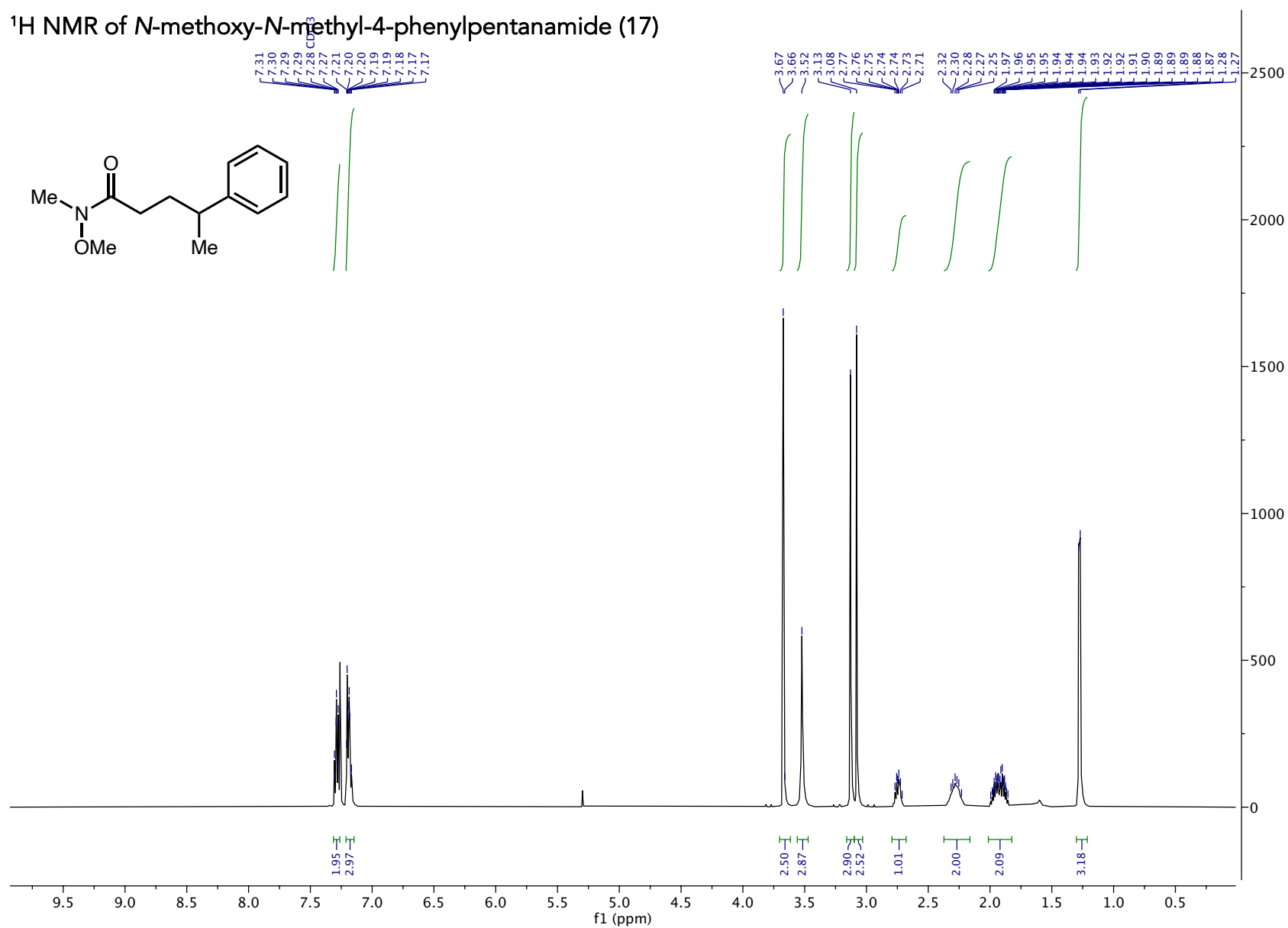
¹H NMR of 4-phenyl-1-(pyrrolidin-1-yl)pentan-1-one (16)



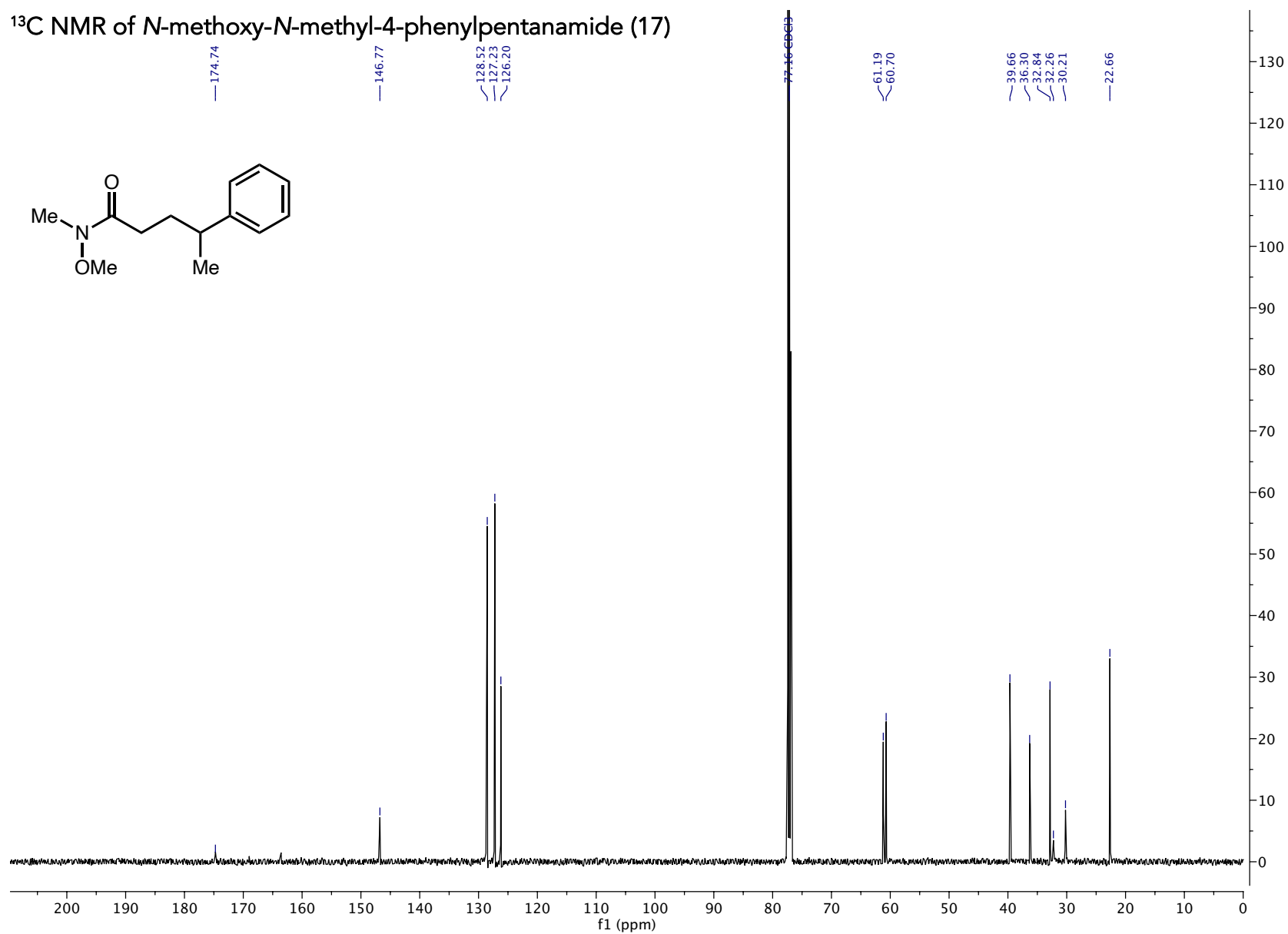
¹³C NMR of 4-phenyl-1-(pyrrolidin-1-yl)pentan-1-one (16)



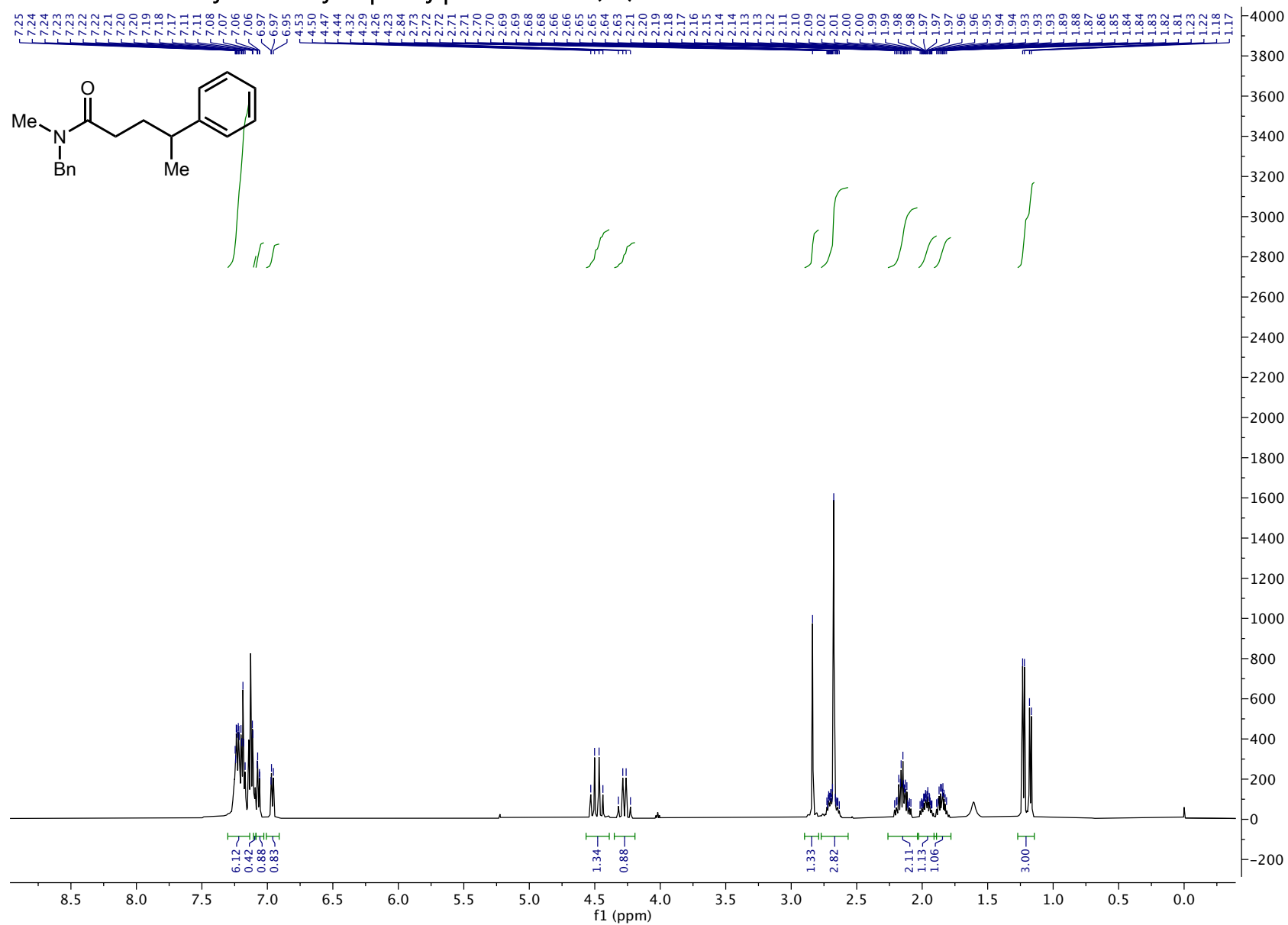
¹H NMR of *N*-methoxy-*N*-methyl-4-phenylpentanamide (17)



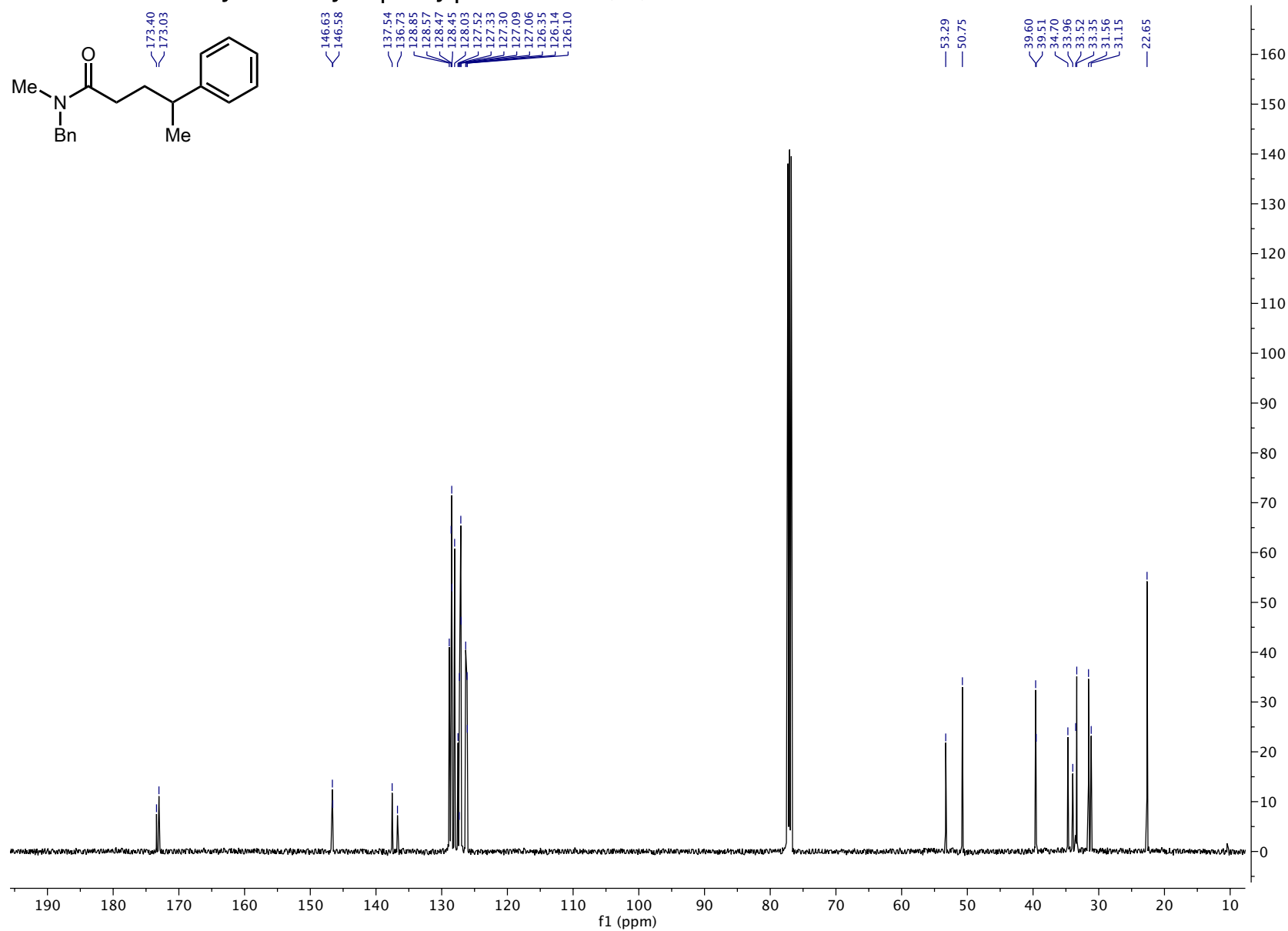
¹³C NMR of *N*-methoxy-*N*-methyl-4-phenylpentanamide (17)



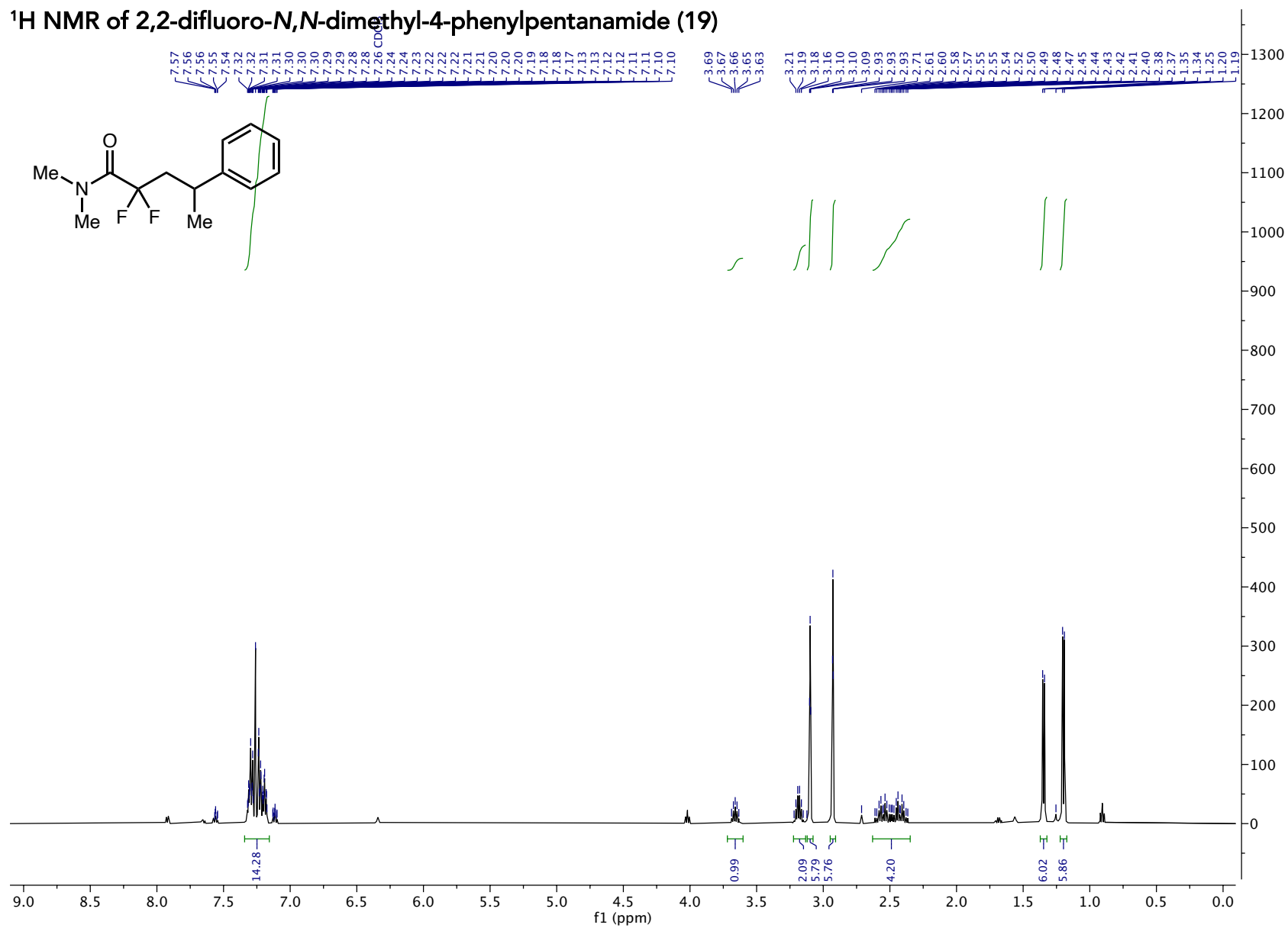
¹H NMR of *N*-benzyl-*N*-methyl-4-phenylpentanamide (18)



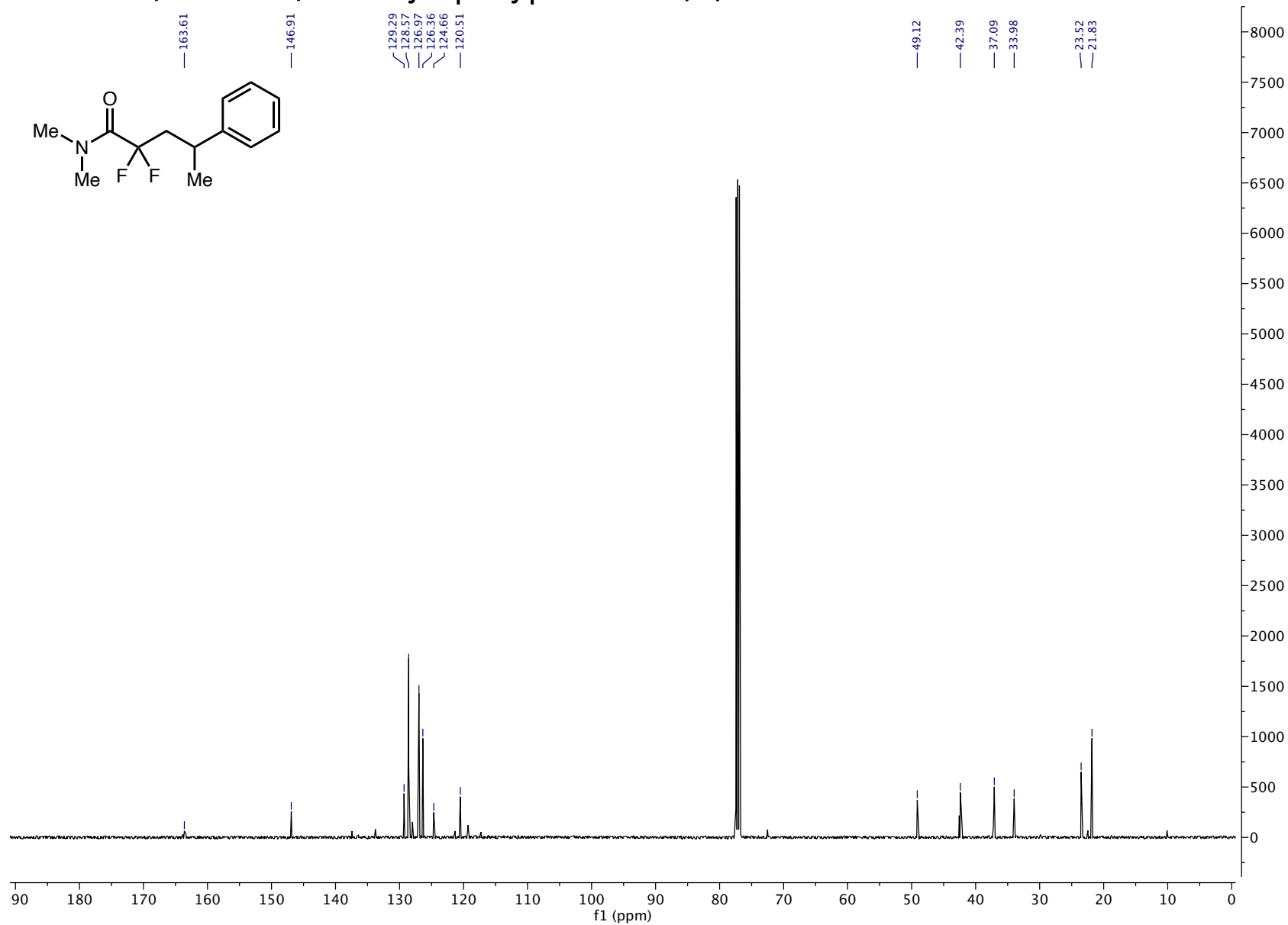
¹³C NMR of *N*-benzyl-*N*-methyl-4-phenylpentanamide (18)



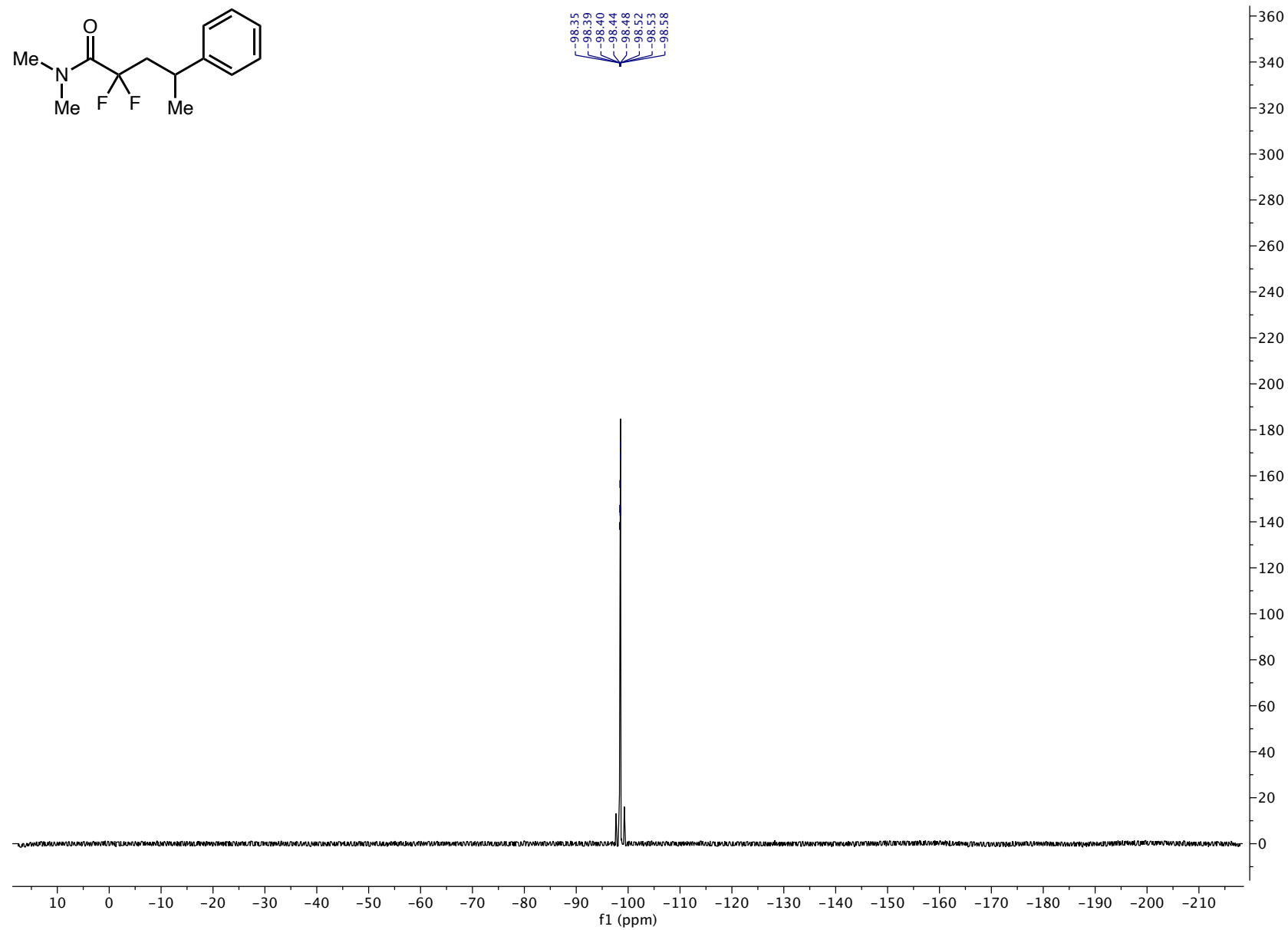
¹H NMR of 2,2-difluoro-N,N-dimethyl-4-phenylpentanamide (19)



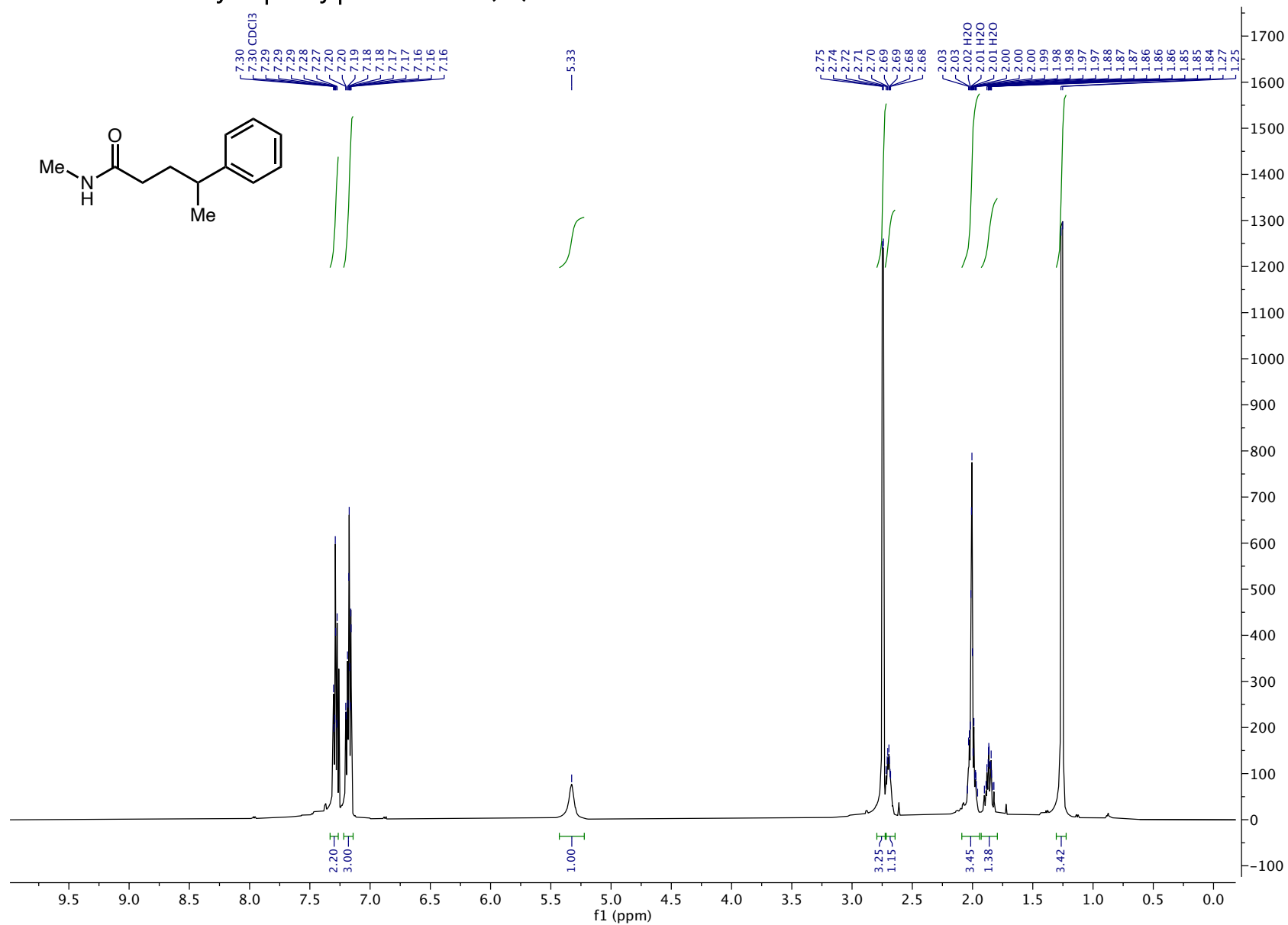
¹³C NMR of 2,2-difluoro-*N,N*-dimethyl-4-phenylpentanamide (19)



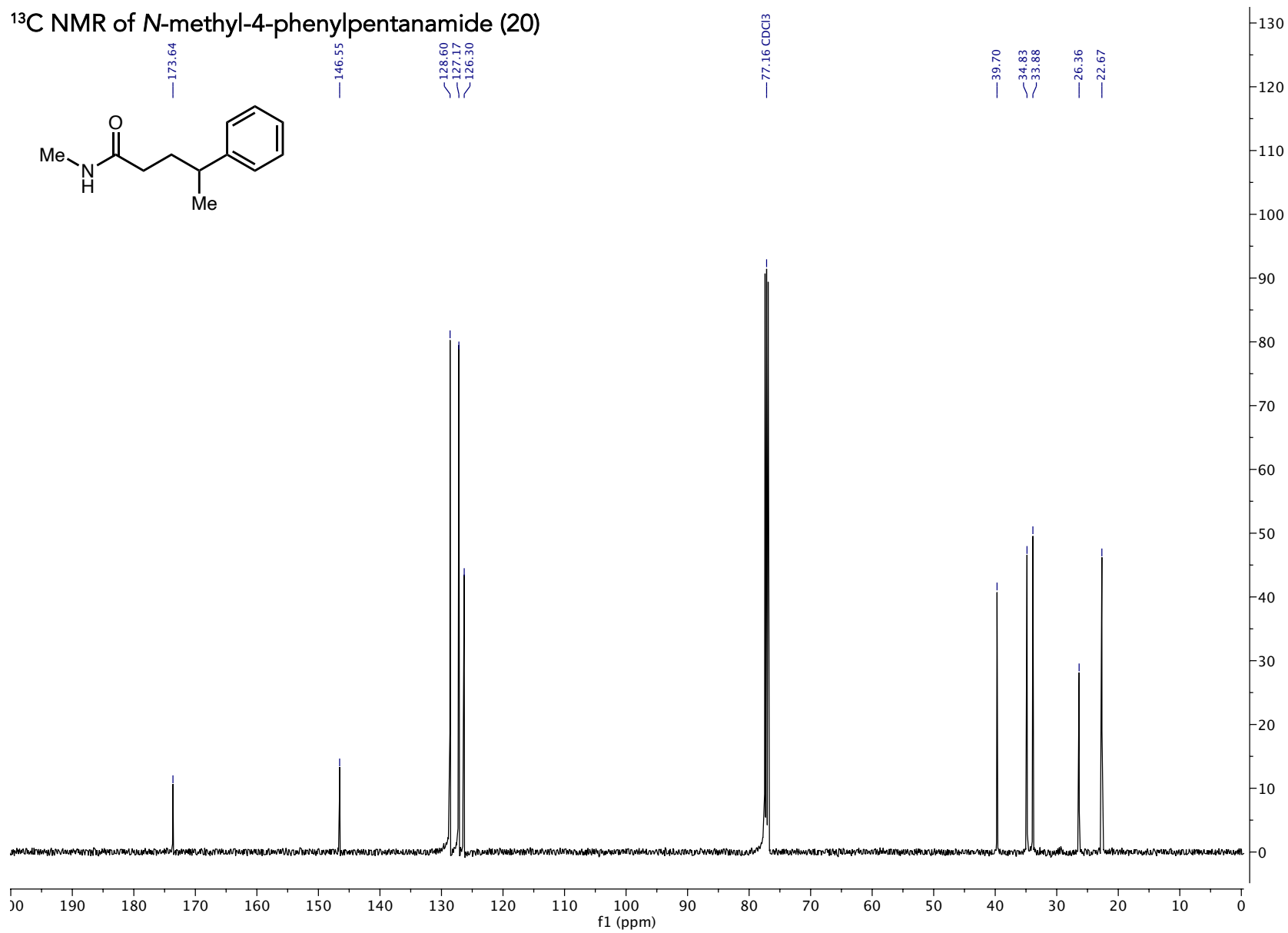
¹⁹F NMR of 2,2-difluoro-*N,N*-dimethyl-4-phenylpentanamide (19)



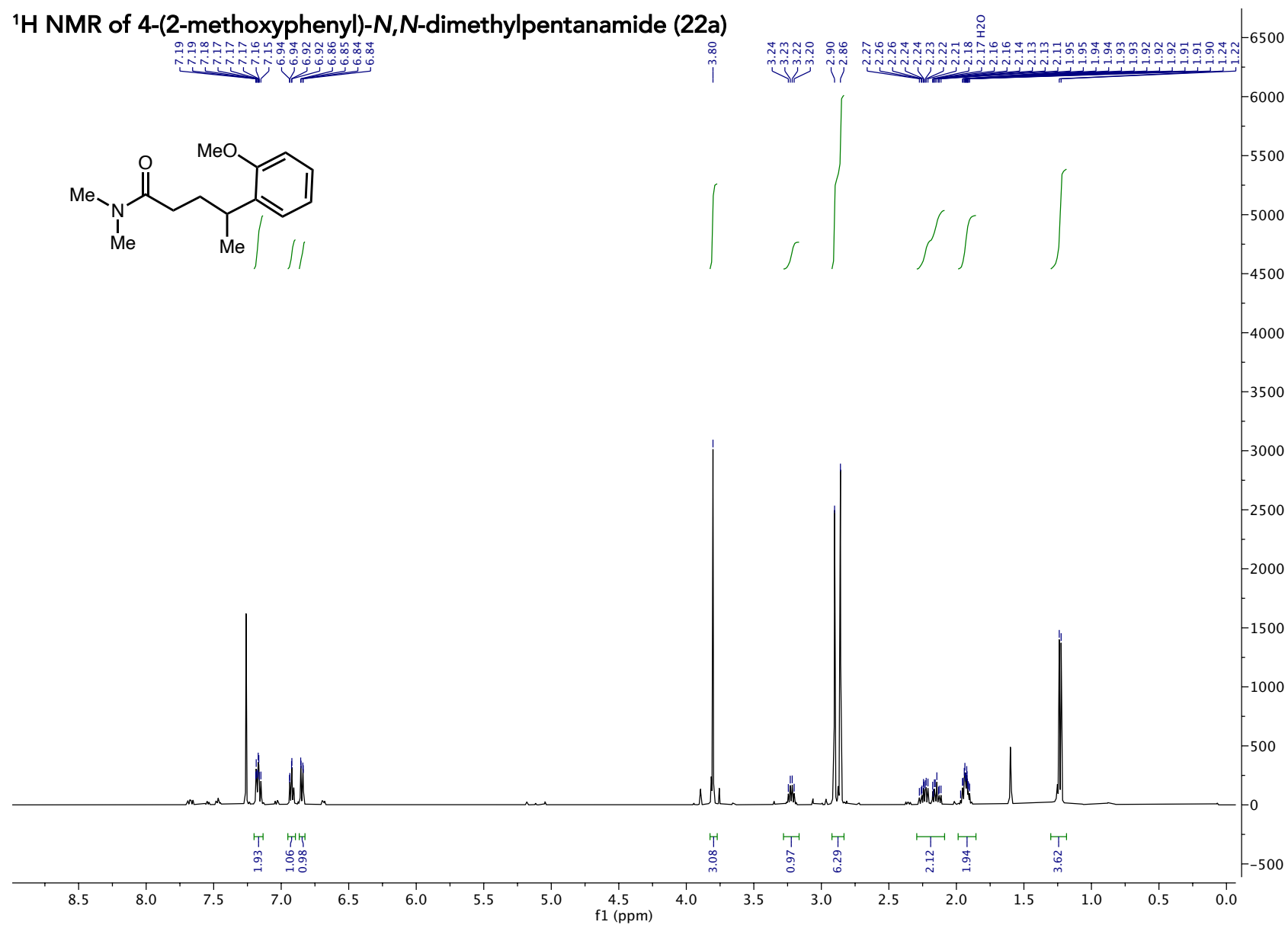
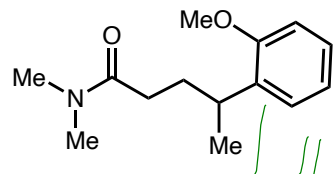
¹H NMR of *N*-methyl-4-phenylpentanamide (20)



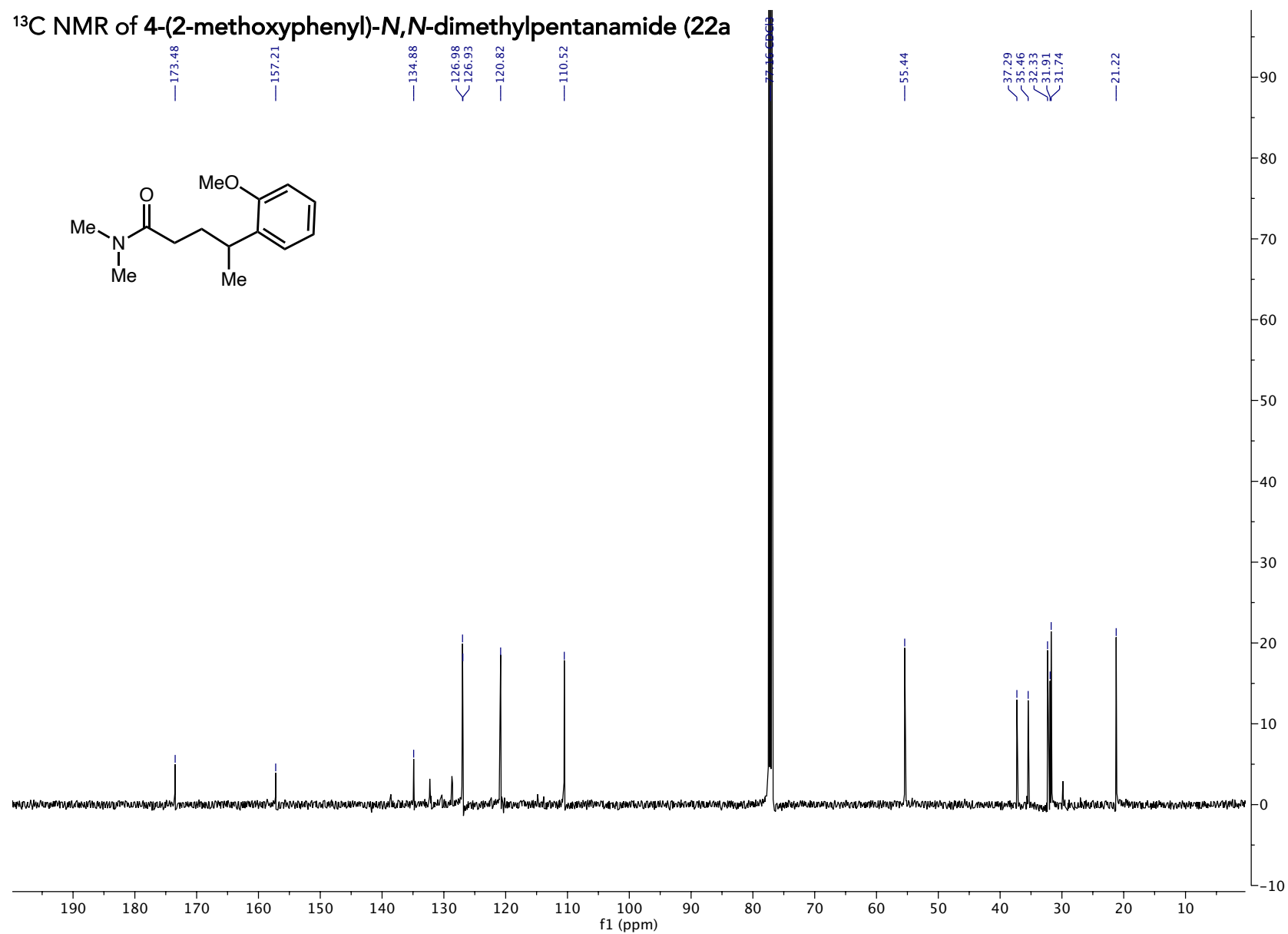
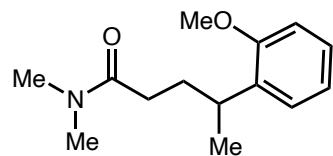
¹³C NMR of *N*-methyl-4-phenylpentanamide (20)



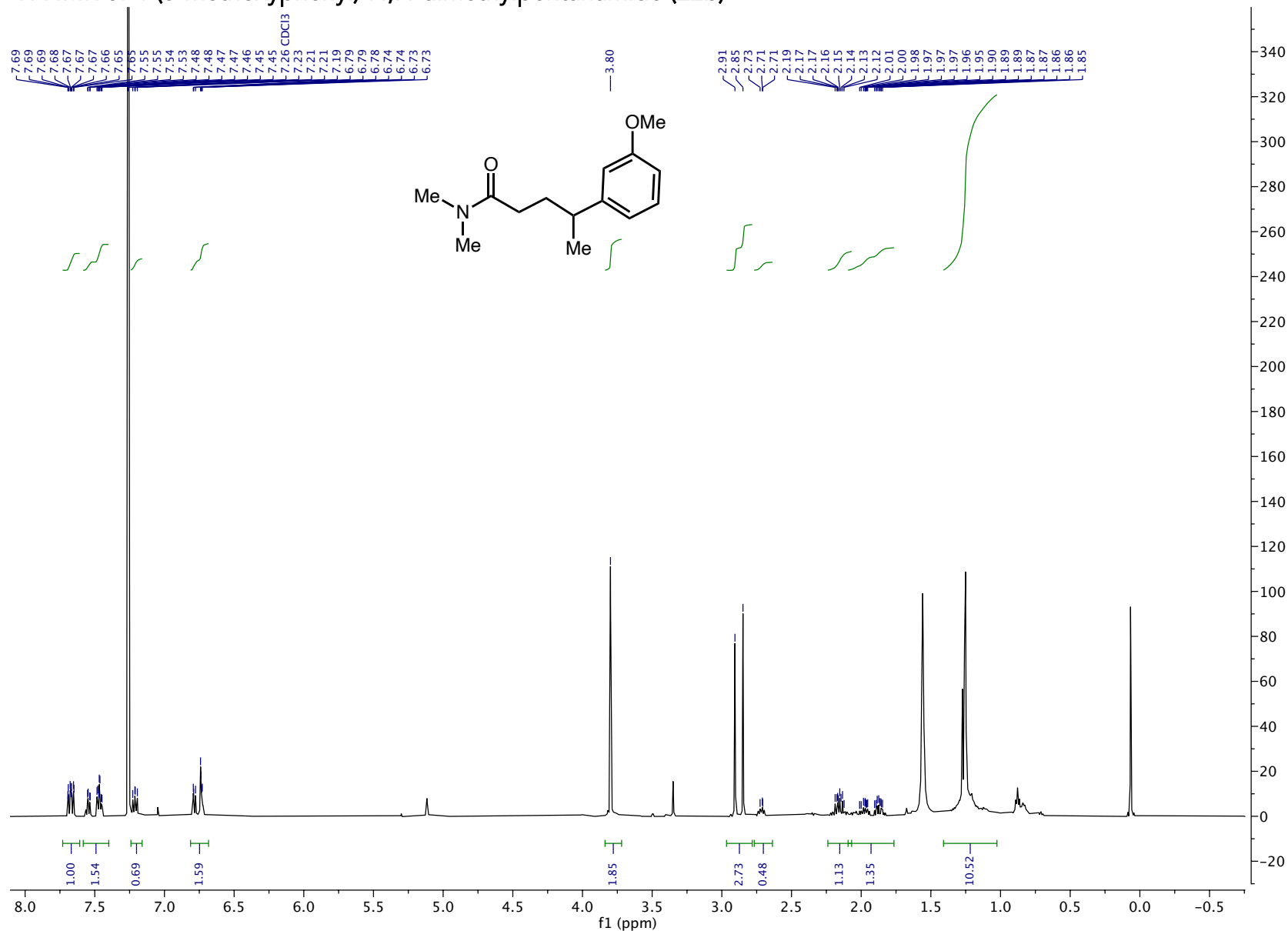
¹H NMR of 4-(2-methoxyphenyl)-N,N-dimethylpentanamide (22a)



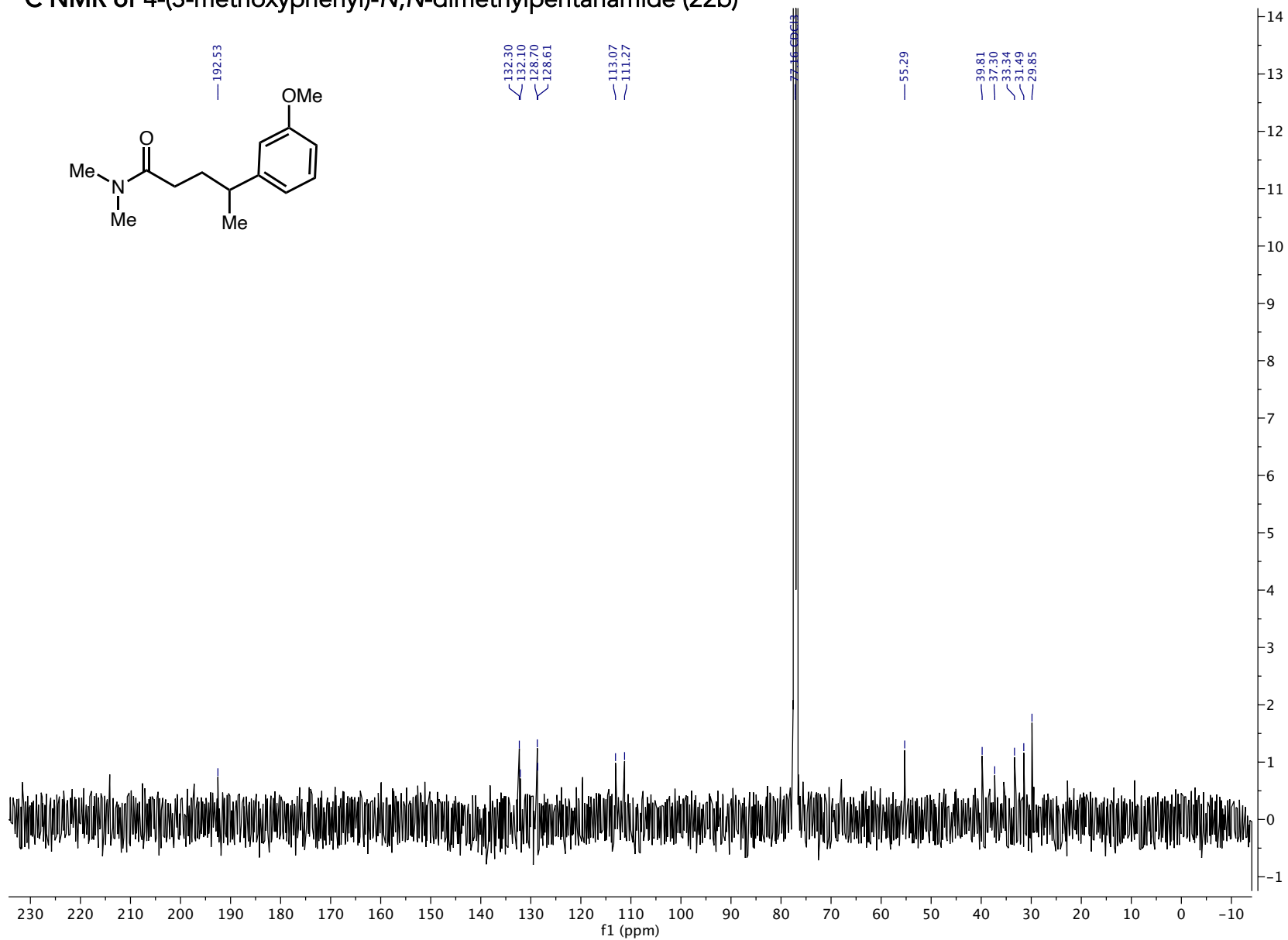
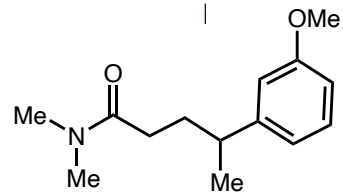
¹³C NMR of 4-(2-methoxyphenyl)-*N,N*-dimethylpentanamide (22a)



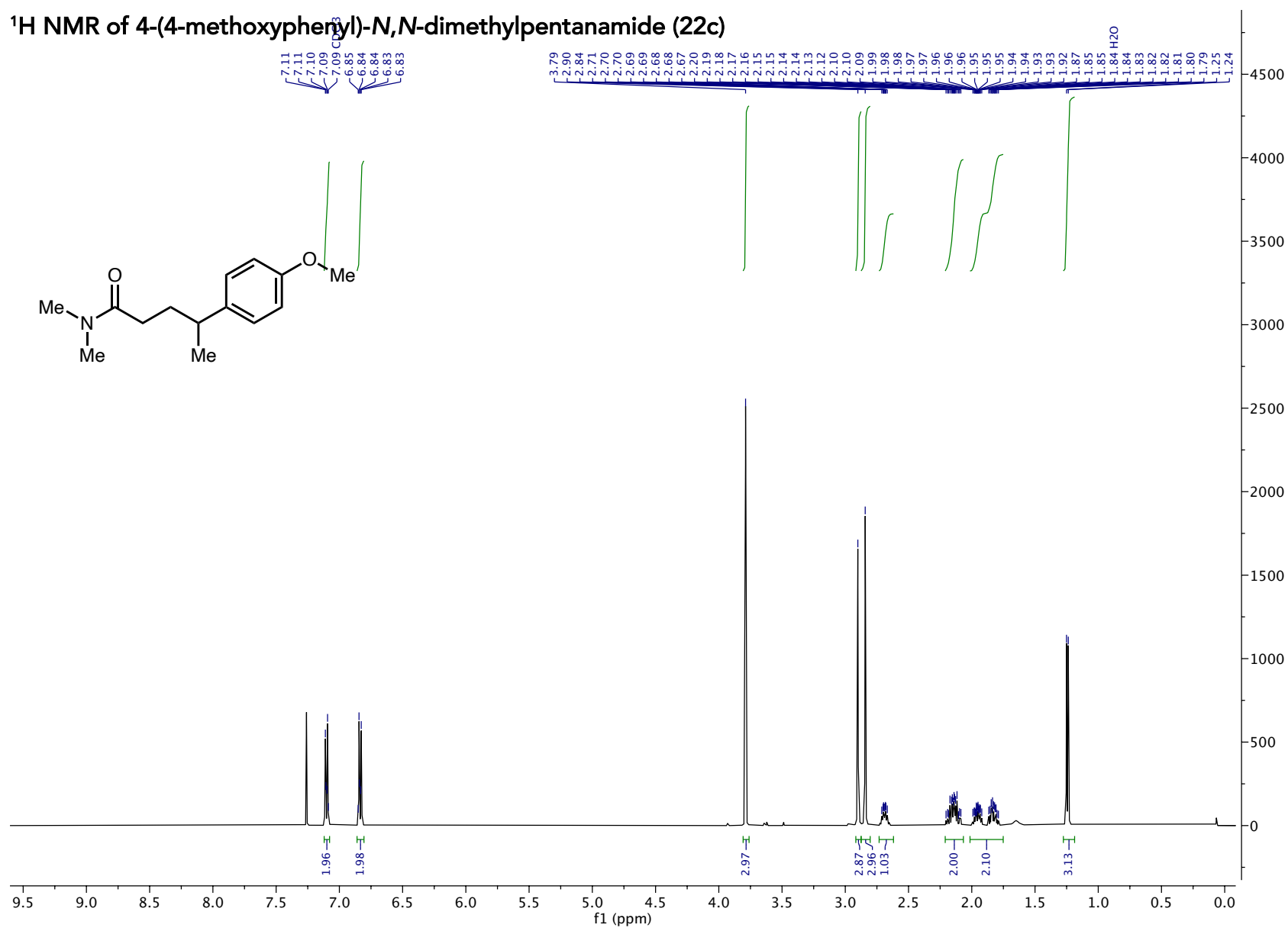
¹H NMR of 4-(3-methoxyphenyl)-*N,N*-dimethylpentanamide (22b)



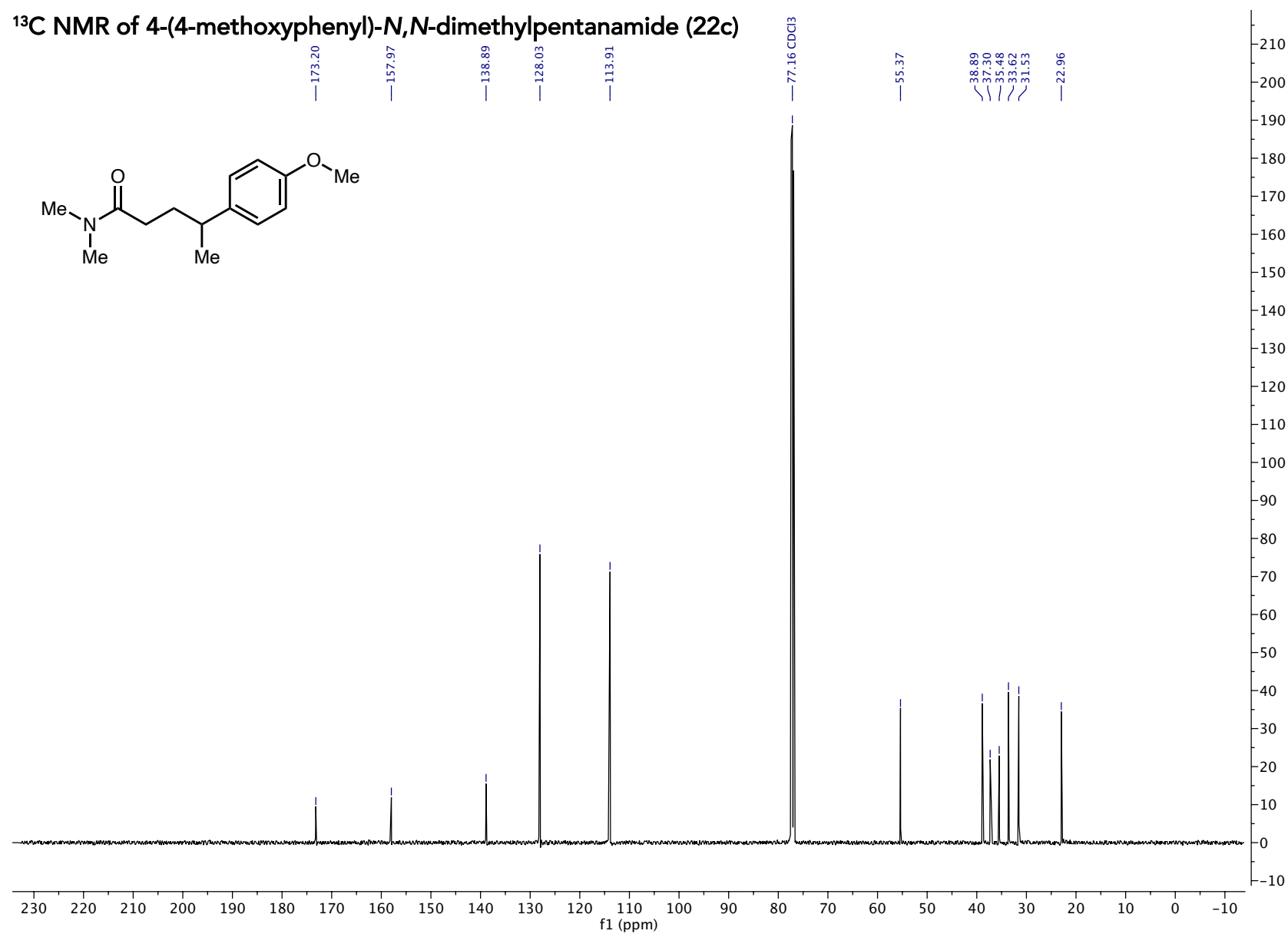
¹³C NMR of 4-(3-methoxyphenyl)-*N,N*-dimethylpentanamide (22b)



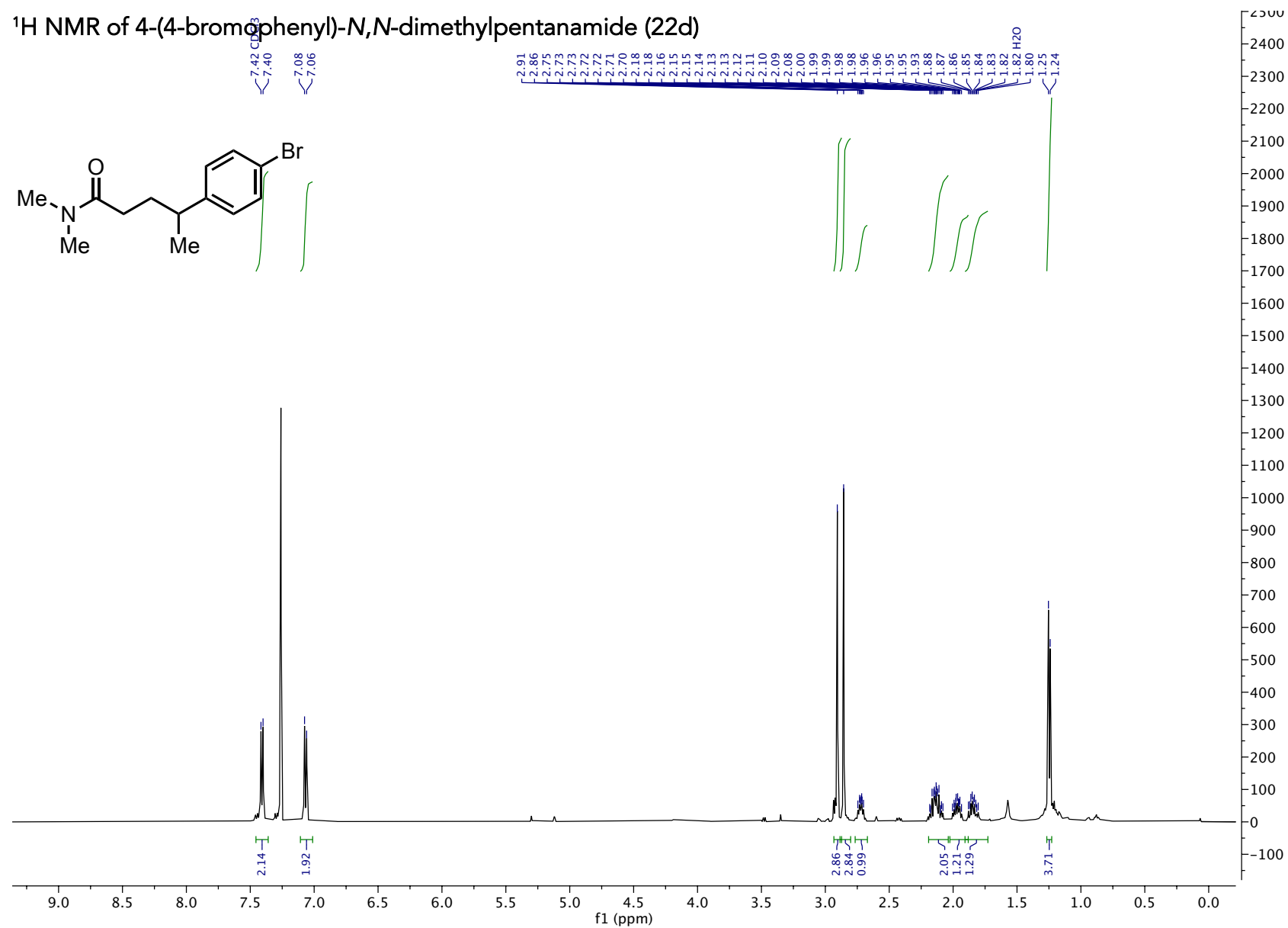
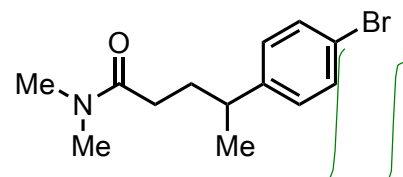
¹H NMR of 4-(4-methoxyphenyl)-*N,N*-dimethylpentanamide (22c)



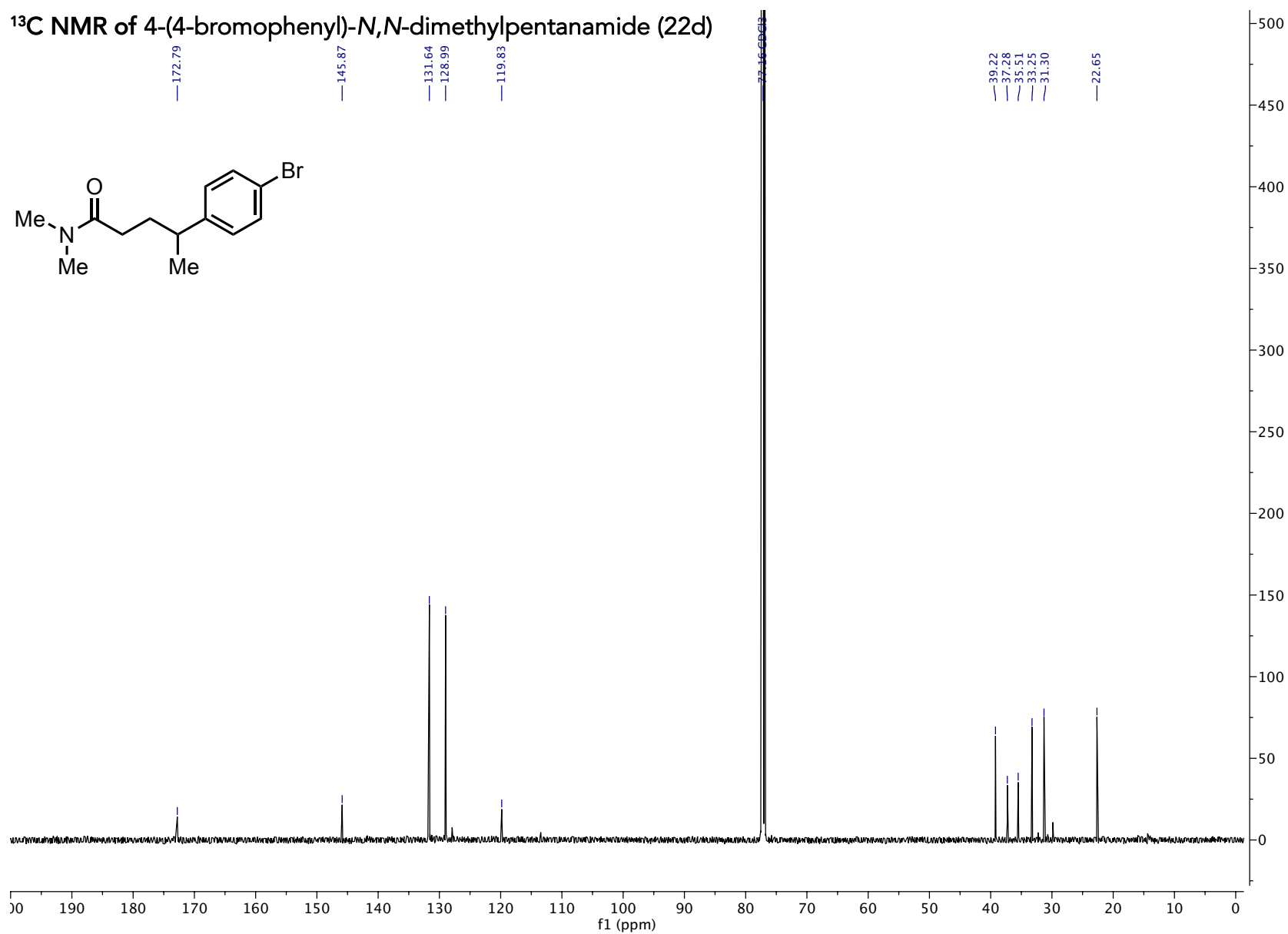
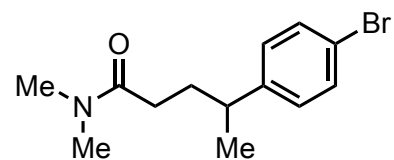
¹³C NMR of 4-(4-methoxyphenyl)-*N,N*-dimethylpentanamide (22c)



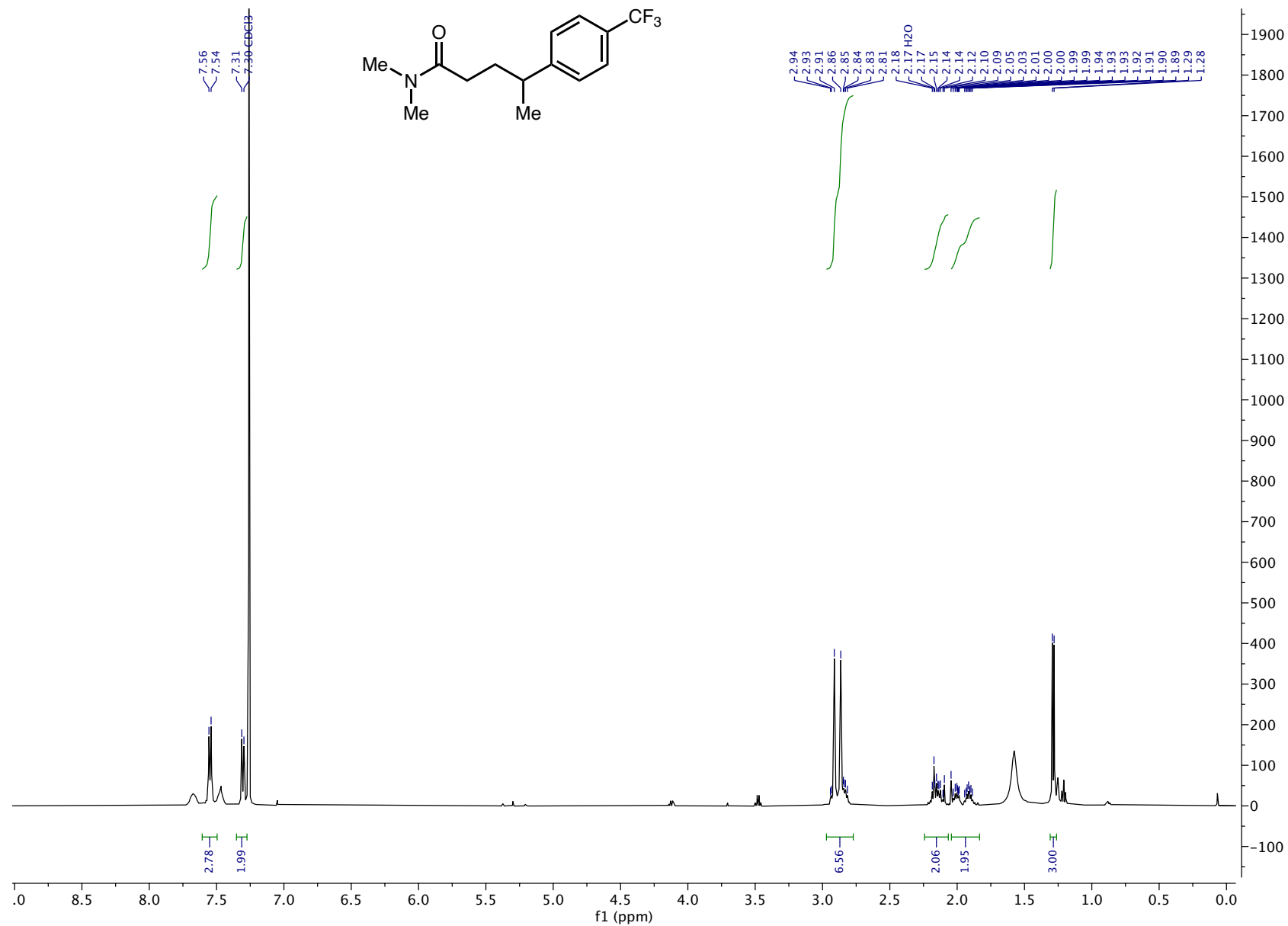
¹H NMR of 4-(4-bromophenyl)-*N,N*-dimethylpentanamide (22d)



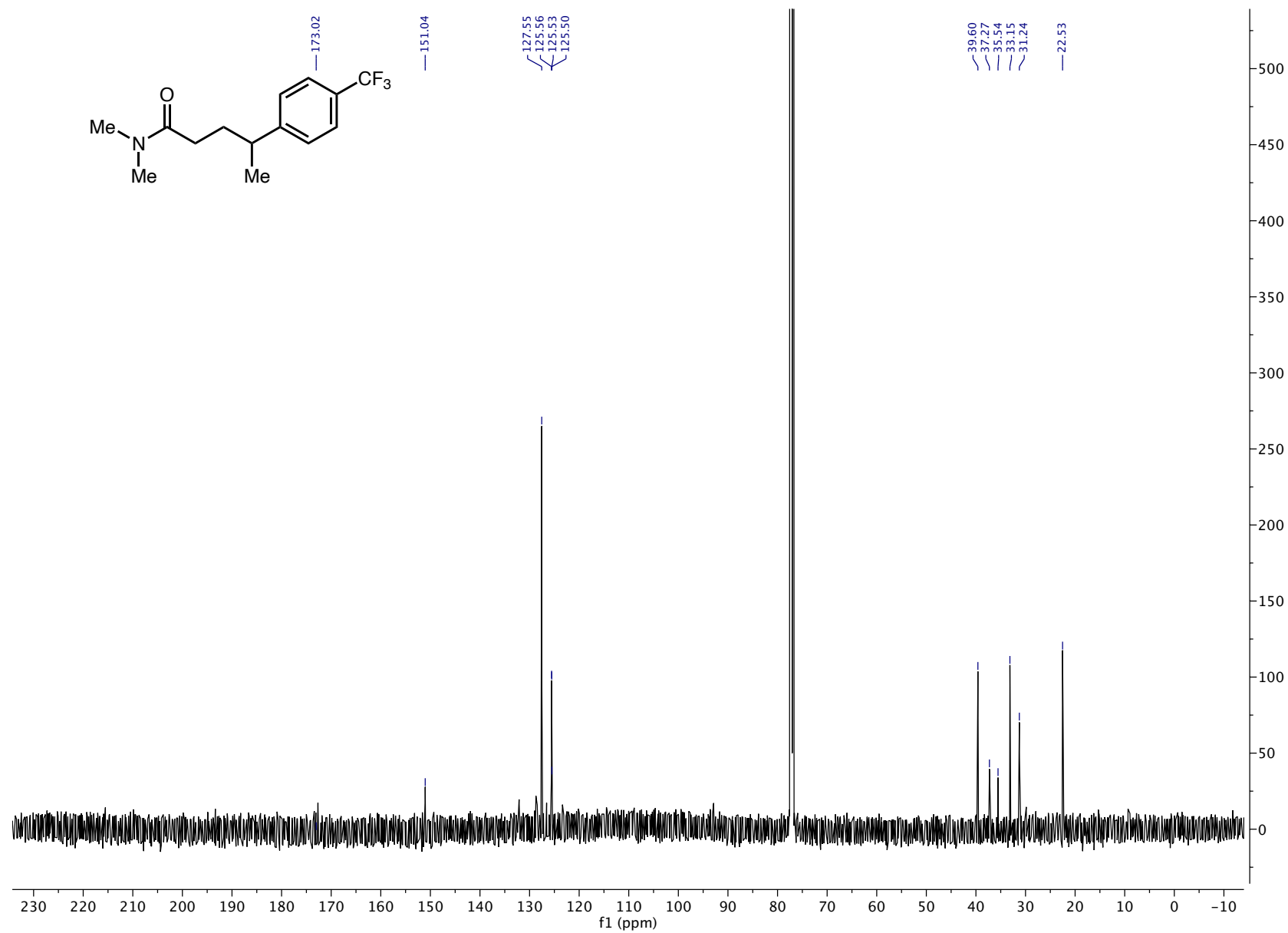
¹³C NMR of 4-(4-bromophenyl)-*N,N*-dimethylpentanamide (22d)



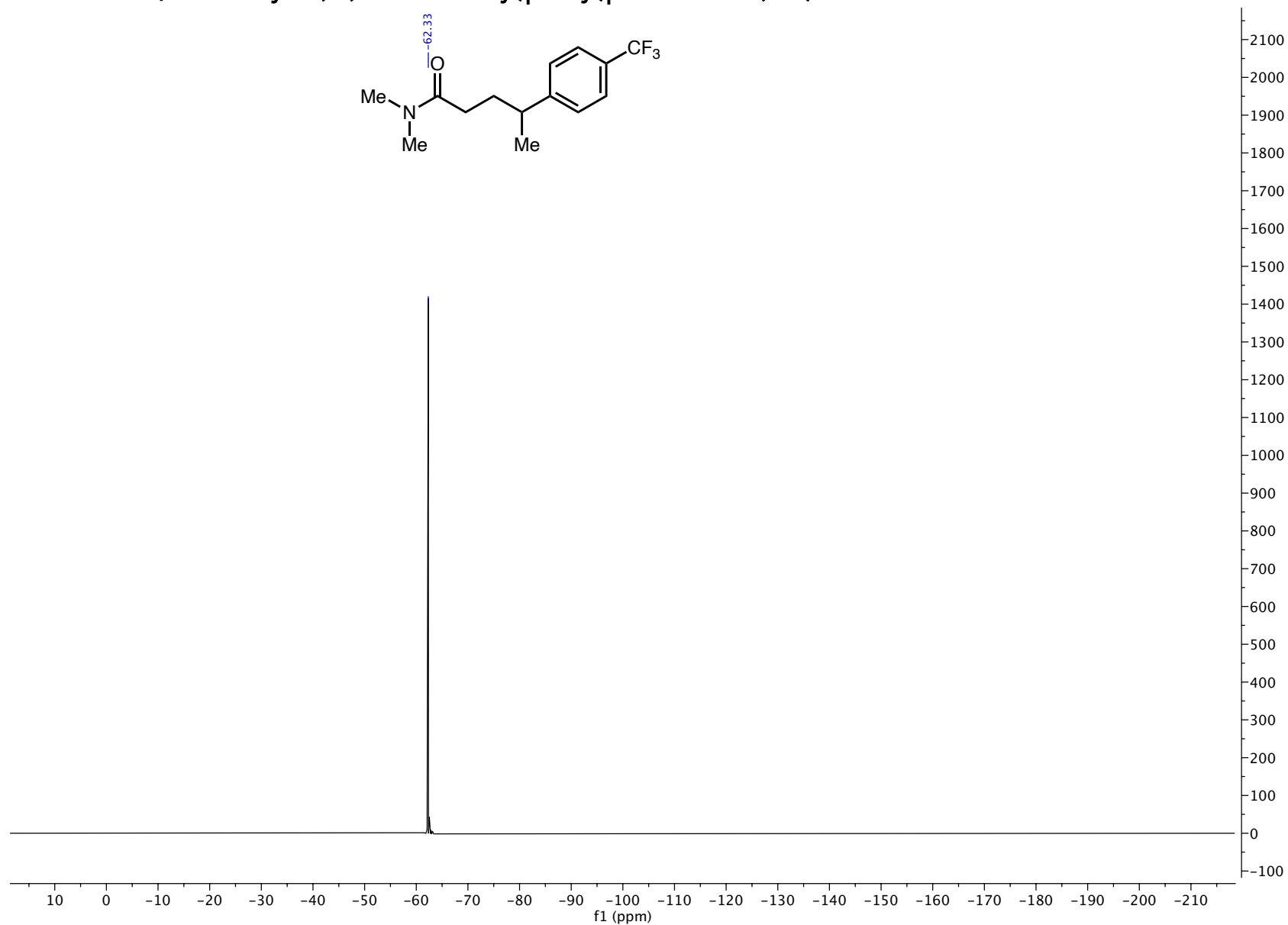
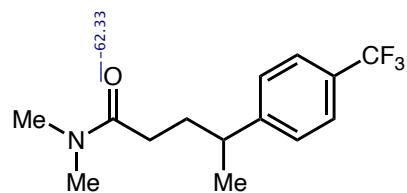
¹H NMR of *N,N*-dimethyl-4-(4-(trifluoromethyl)phenyl)pentanamide (22e)



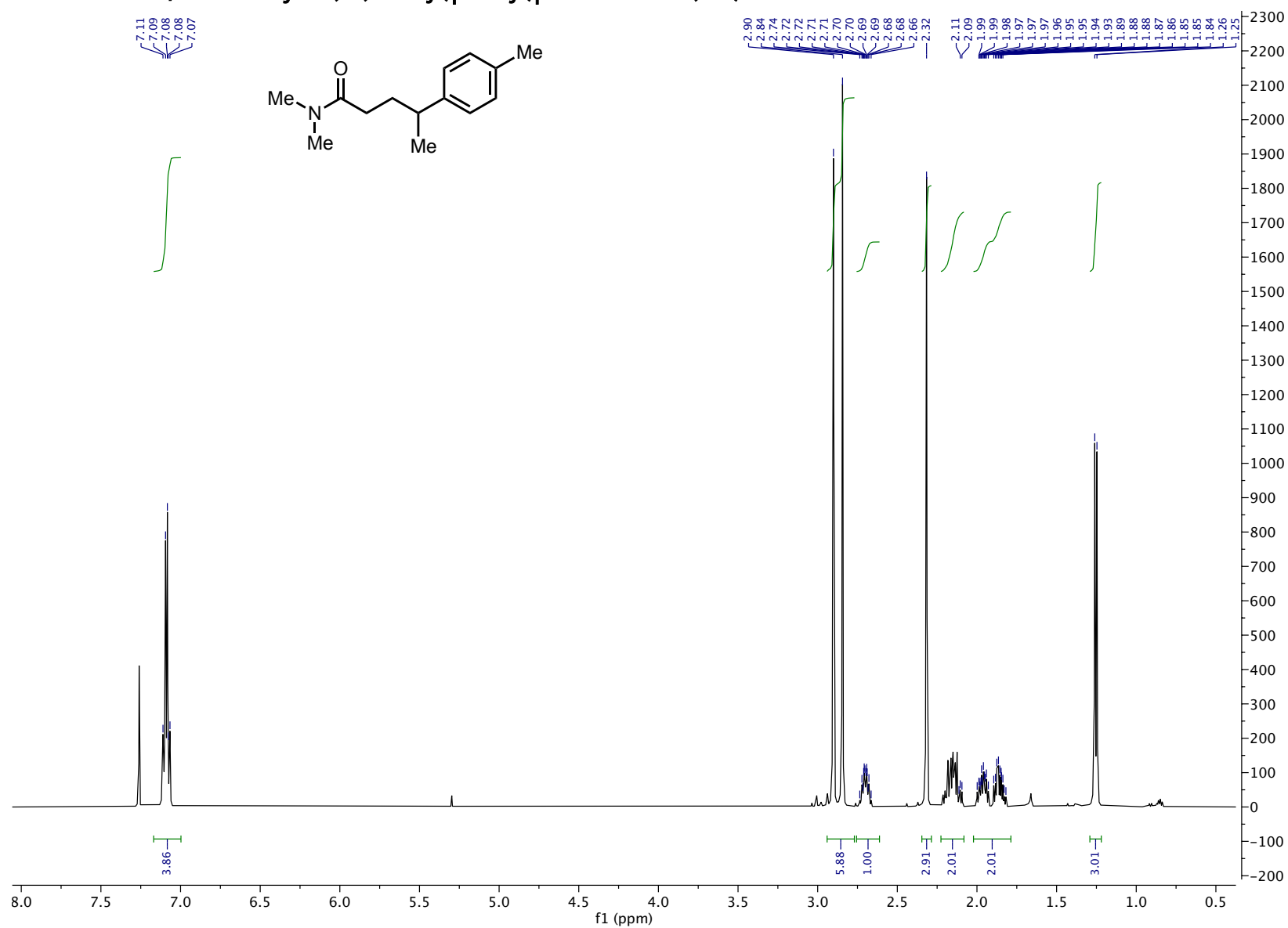
¹³C NMR of *N,N*-dimethyl-4-(4-(trifluoromethyl)phenyl)pentanamide (22e)



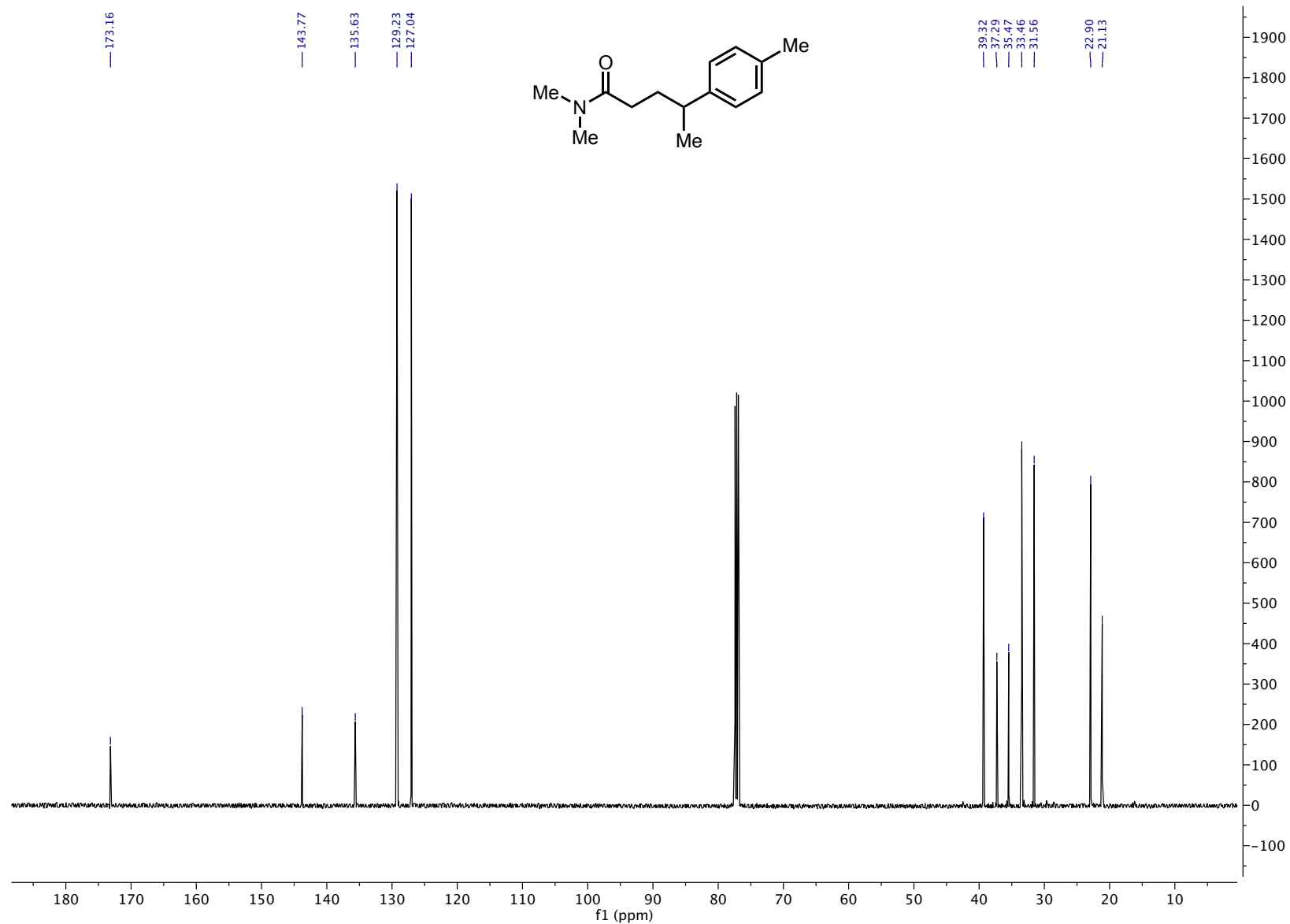
¹⁹F NMR of *N,N*-dimethyl-4-(4-(trifluoromethyl)phenyl)pentanamide (22e)



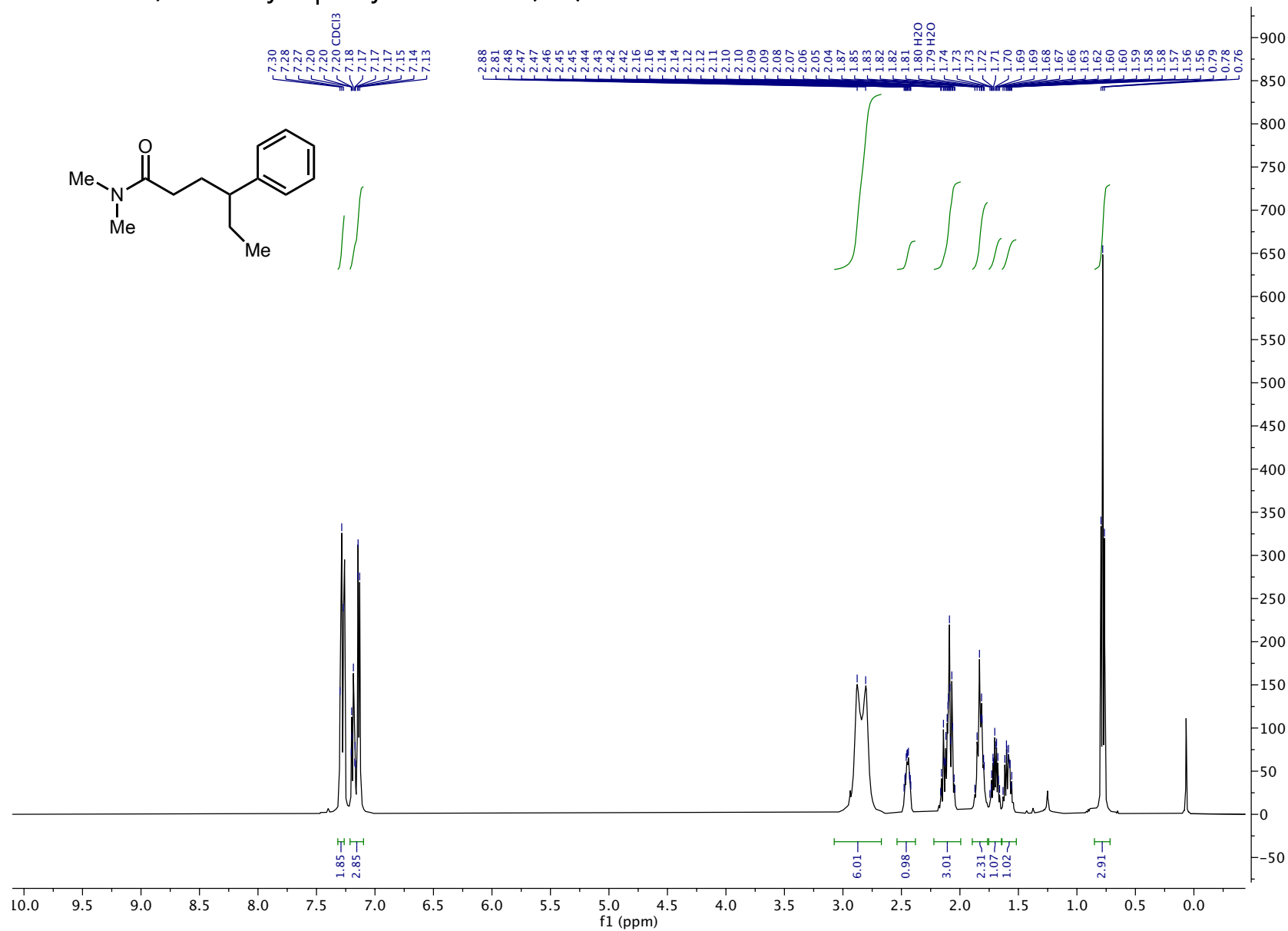
¹H NMR of *N,N*-dimethyl-4-(4-(methyl)phenyl)pentanamide (22f)



¹³C NMR of *N,N*-dimethyl-4-(4-(methyl)phenyl)pentanamide (22f)



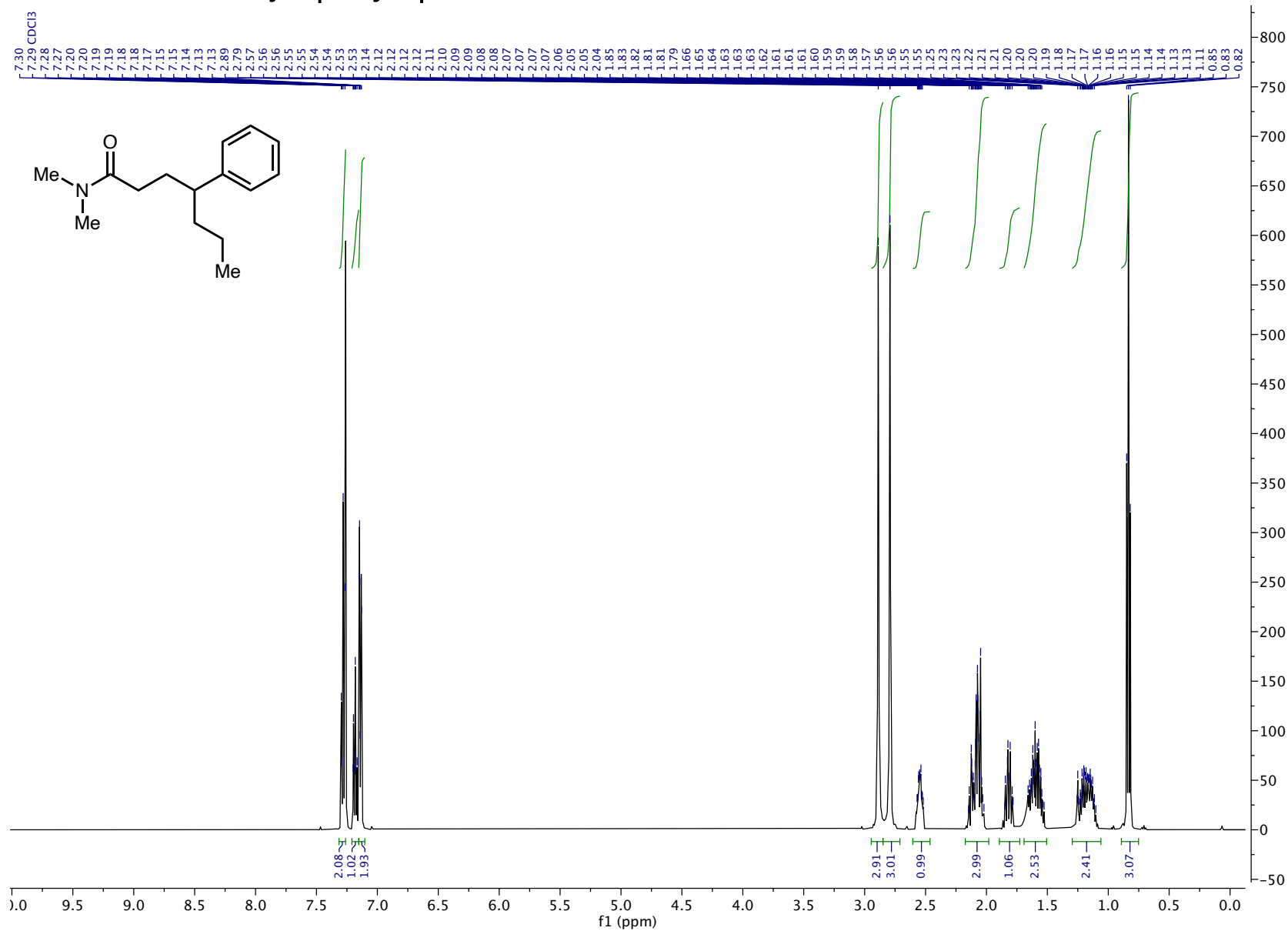
¹H NMR of *N,N*-dimethyl-4-phenylhexanamide (23a)



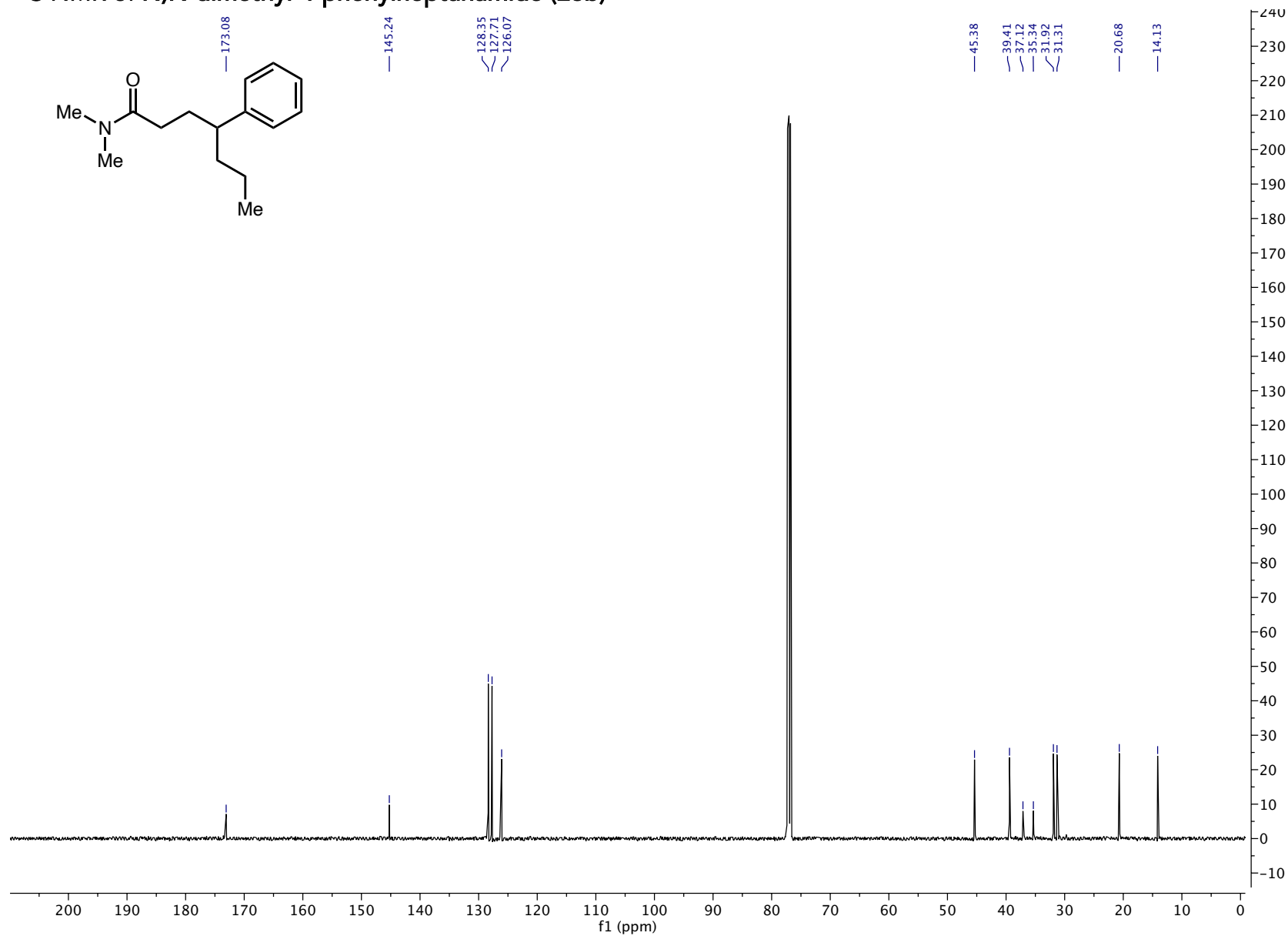
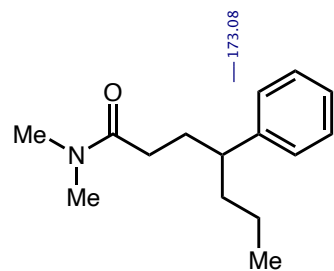
¹³C NMR of *N,N*-dimethyl-4-phenylhexanamide (23a)



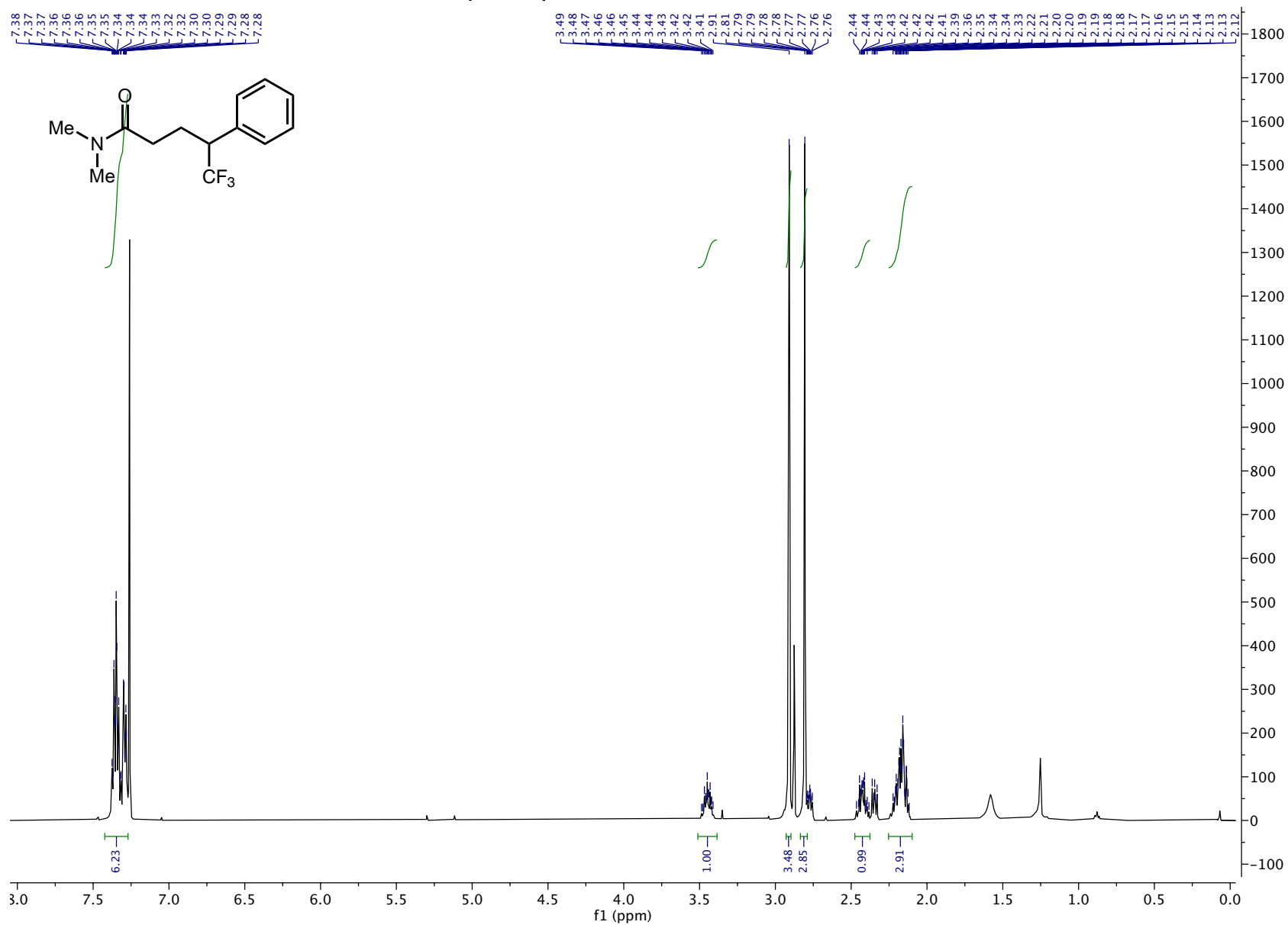
¹H NMR of *N,N*-dimethyl-4-phenylheptanamide (23b)



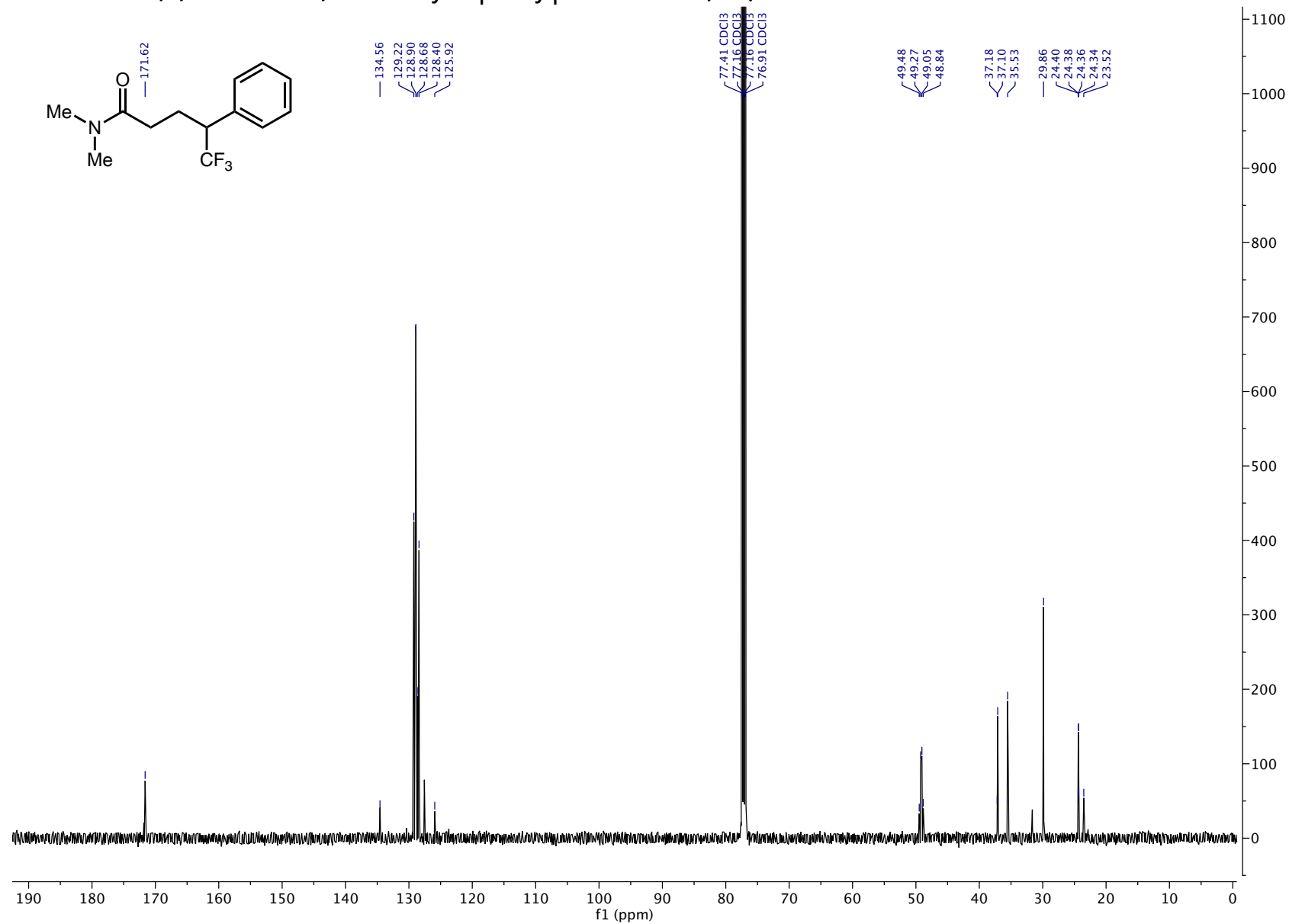
¹³C NMR of *N,N*-dimethyl-4-phenylheptanamide (23b)



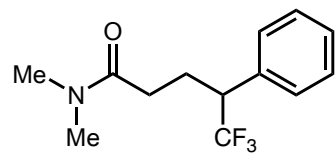
¹H NMR of 5,5,5-trifluoro-N,N-dimethyl-4-phenylpentanamide (23c)



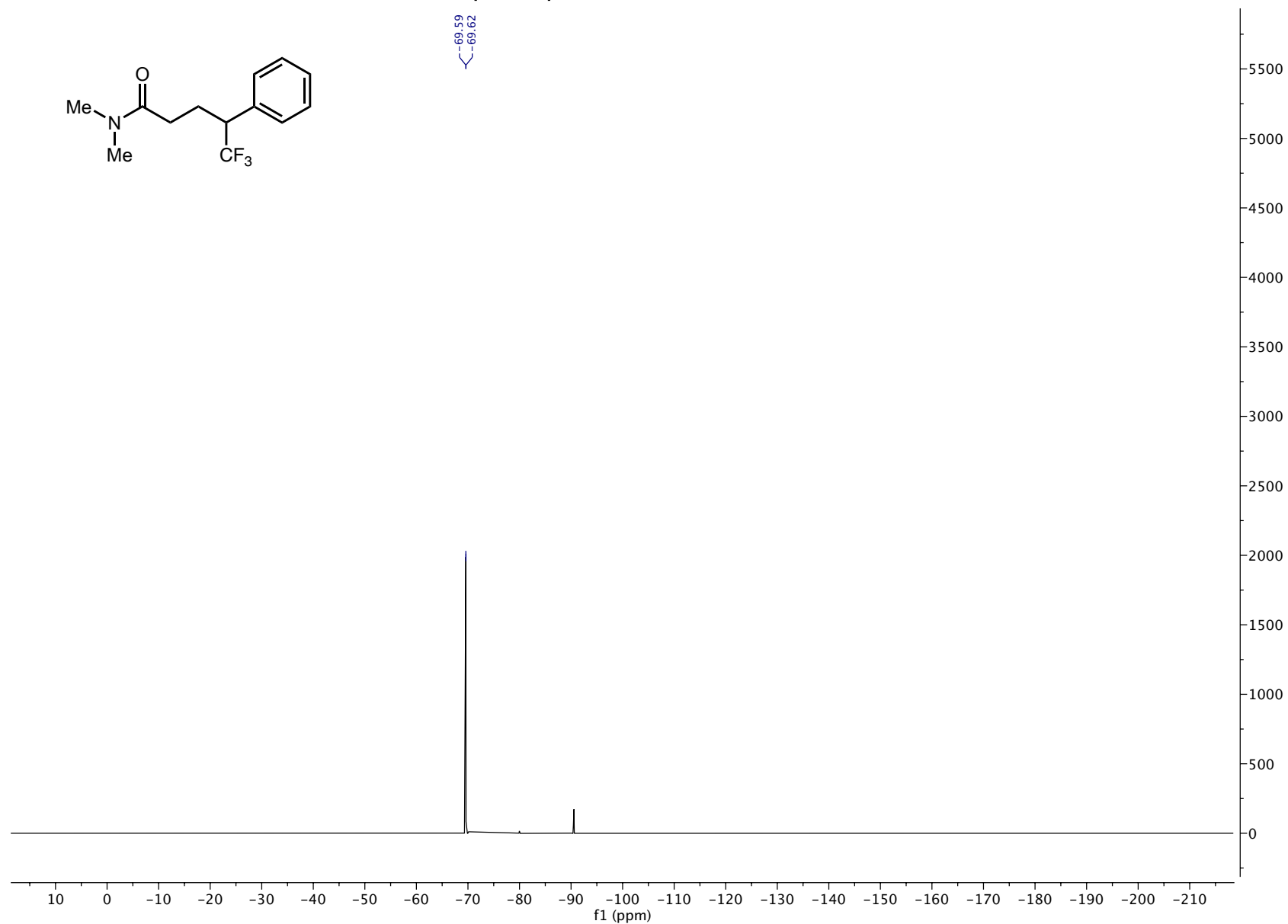
¹³C NMR of 5,5,5-trifluoro-*N,N*-dimethyl-4-phenylpentanamide (23c)



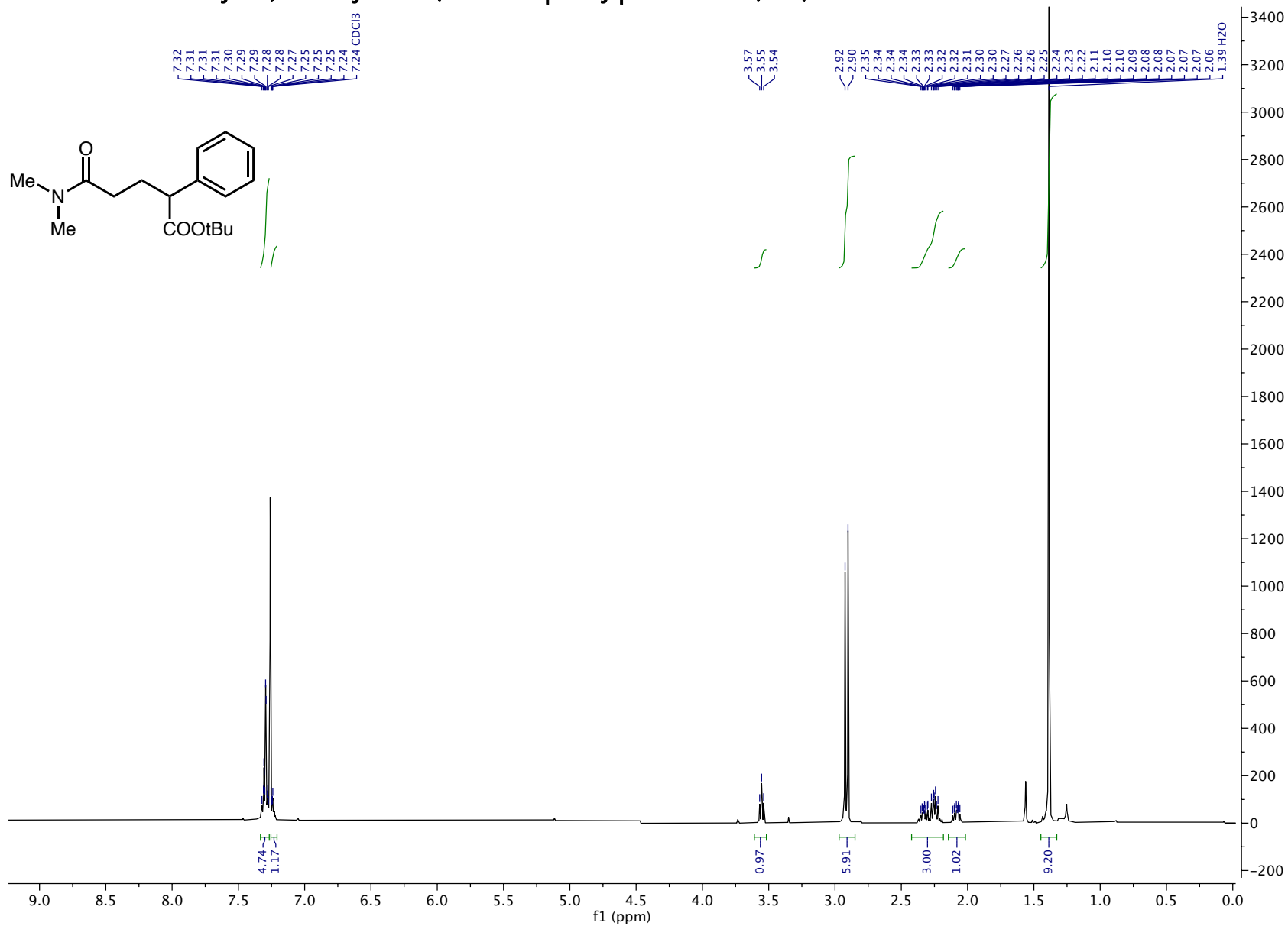
¹⁹F NMR of 5,5,5-trifluoro-*N,N*-dimethyl-4-phenylpentanamide (23c)



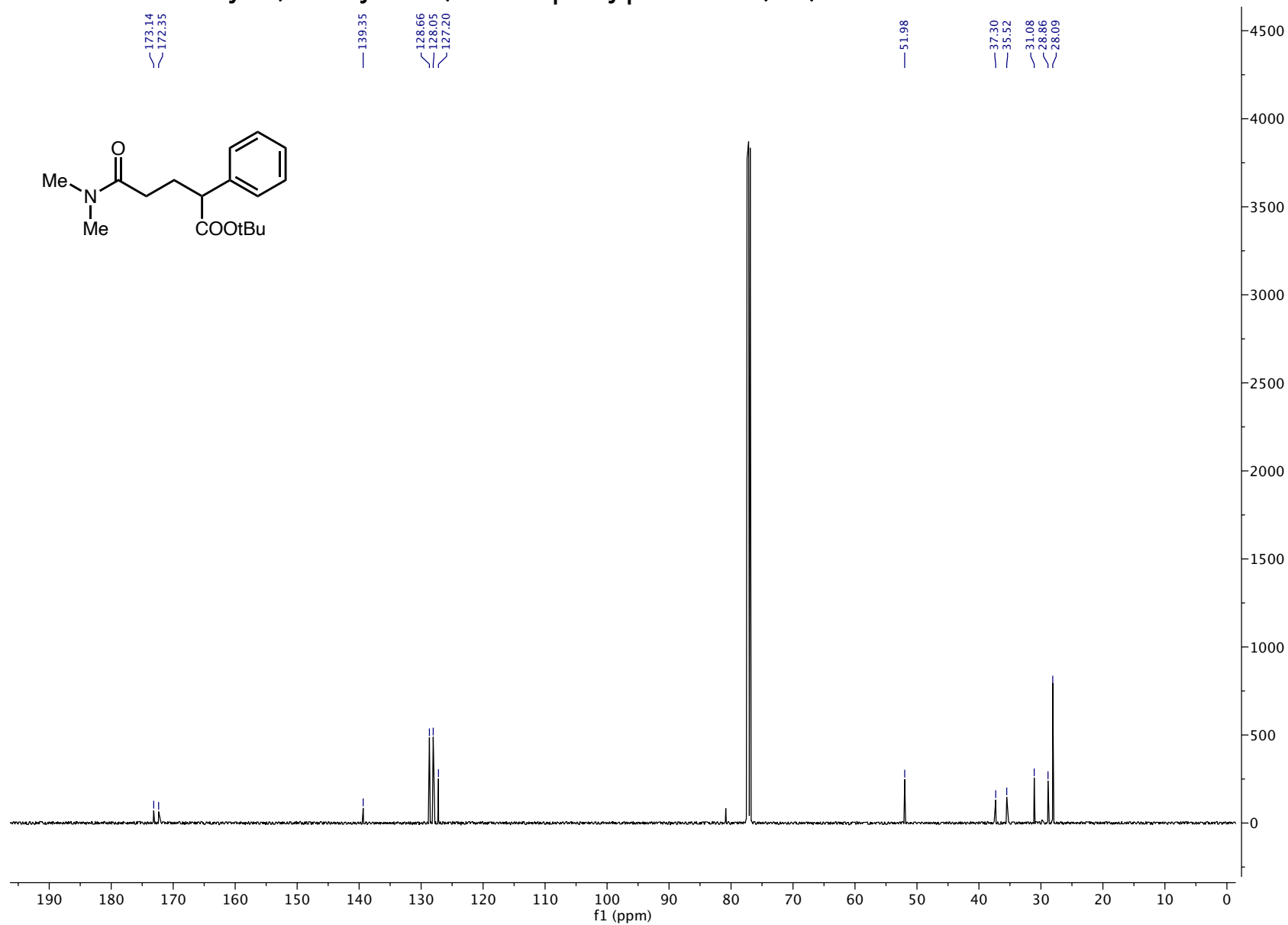
-69.59
-69.62



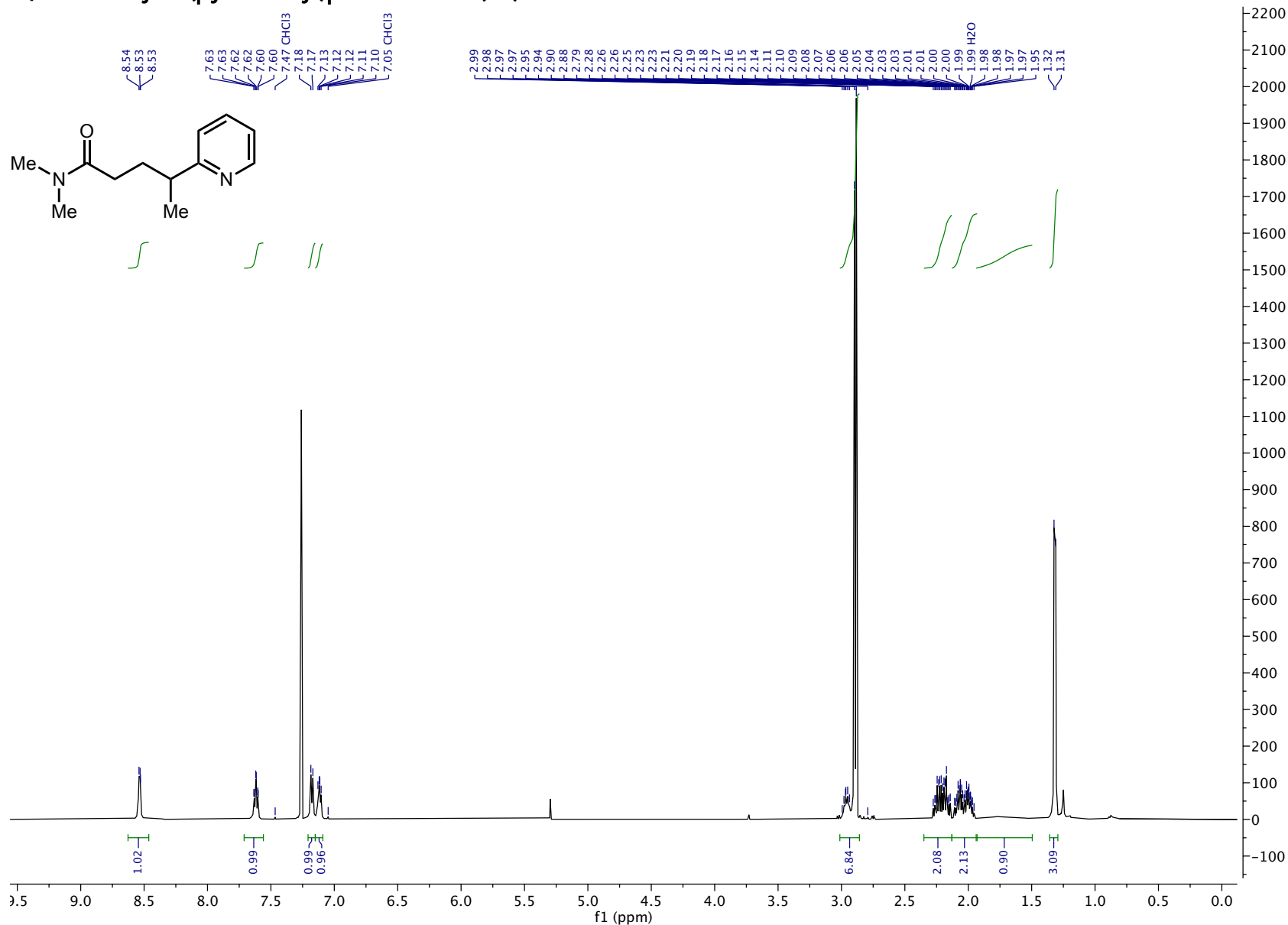
¹H NMR of tert-butyl 5-(dimethylamino)-5-oxo-2-phenylpentanoate (23d)



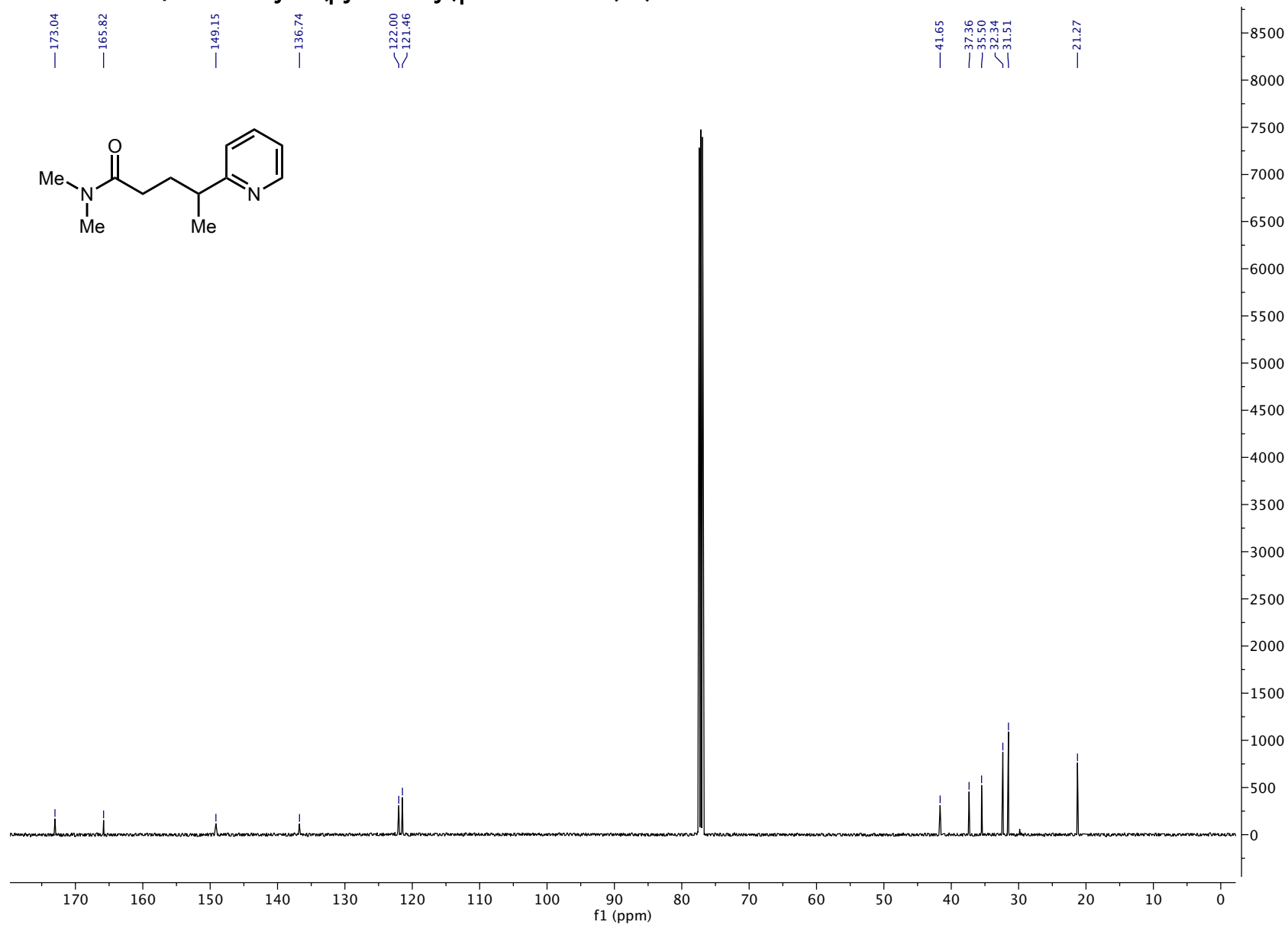
¹³C NMR of tert-butyl 5-(dimethylamino)-5-oxo-2-phenylpentanoate (23d)



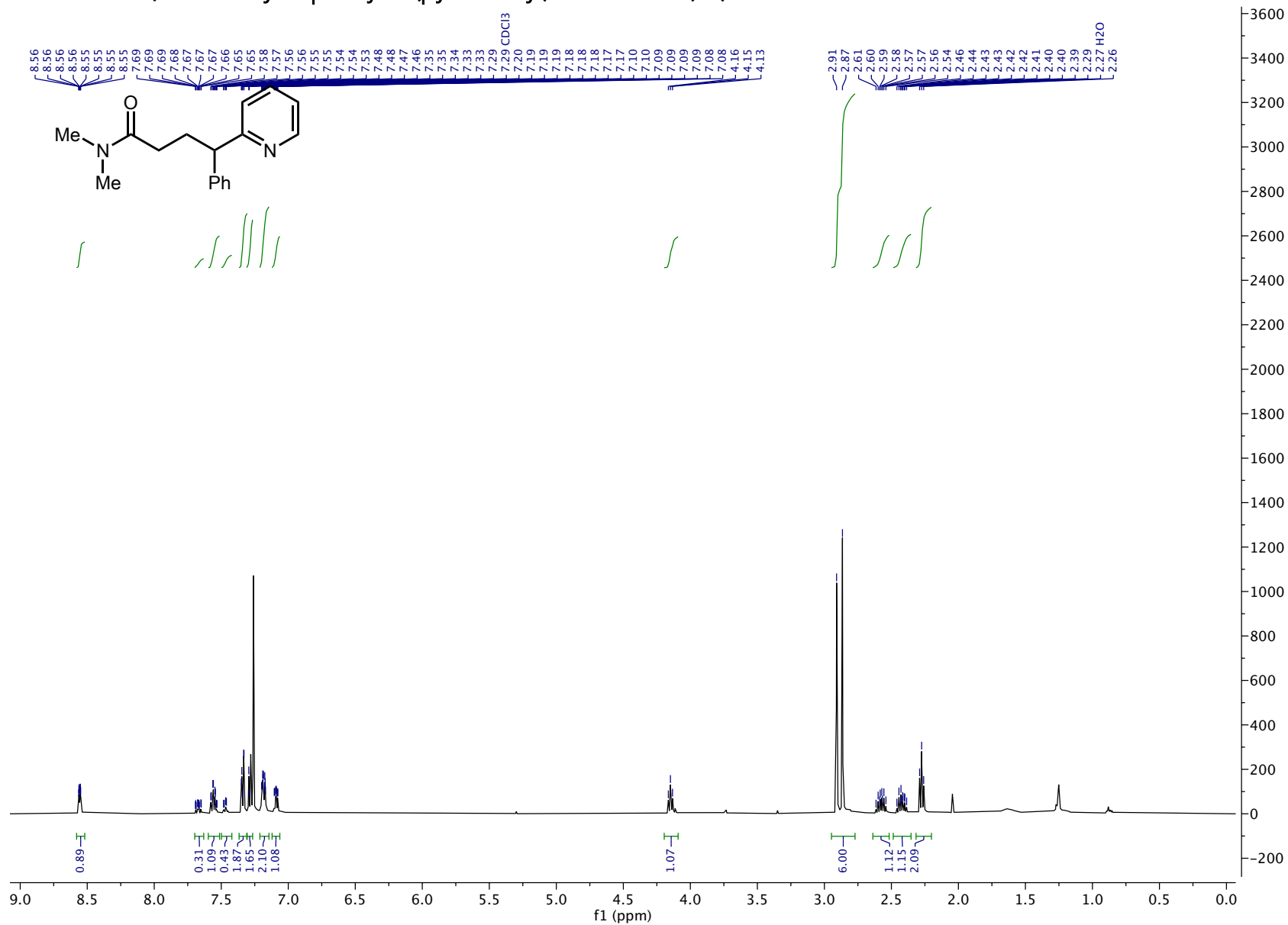
***N,N*-dimethyl-4-(pyridin-2-yl)pentanamide (25)**



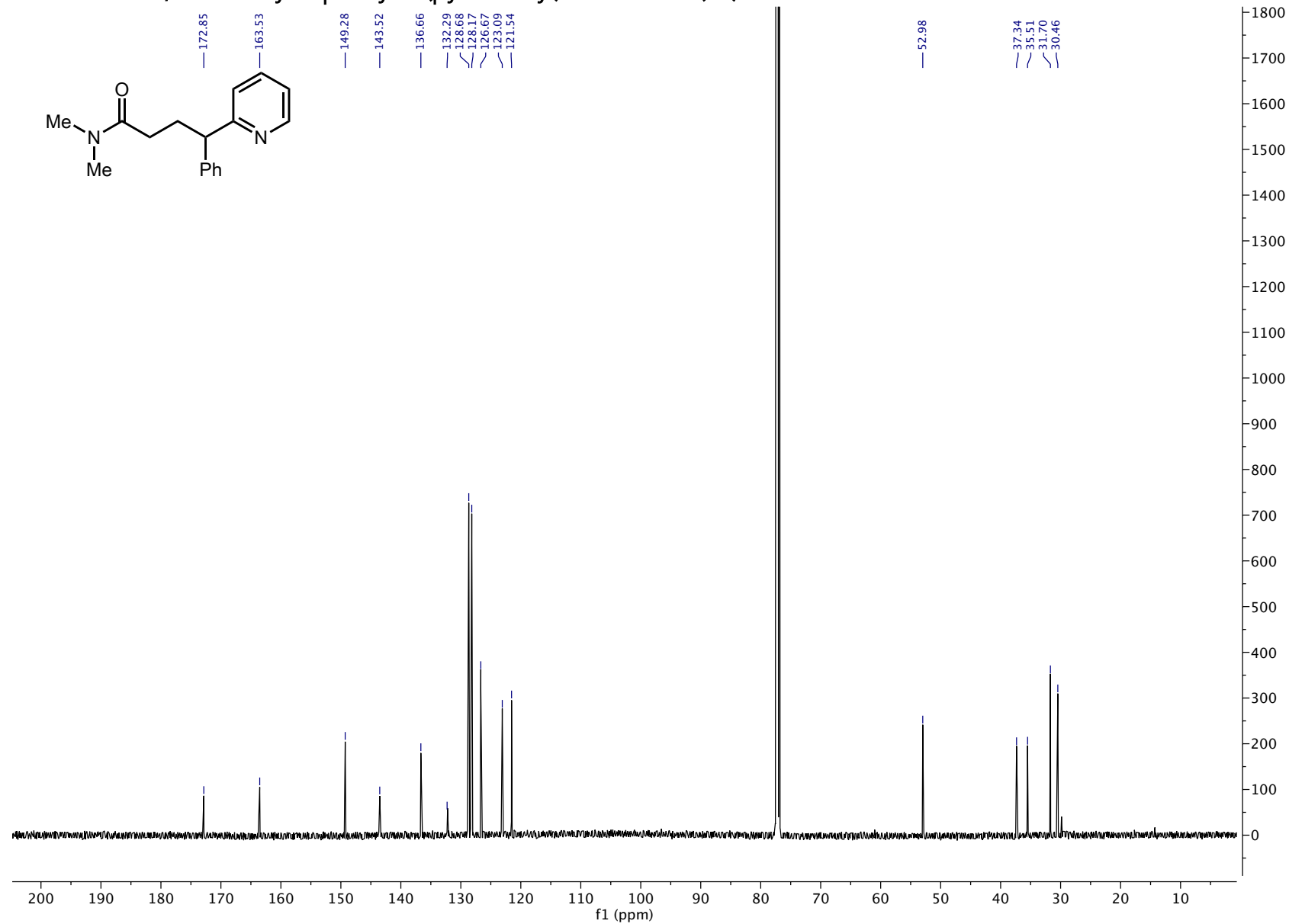
¹³C NMR of *N,N*-dimethyl-4-(pyridin-2-yl)pentanamide (25)



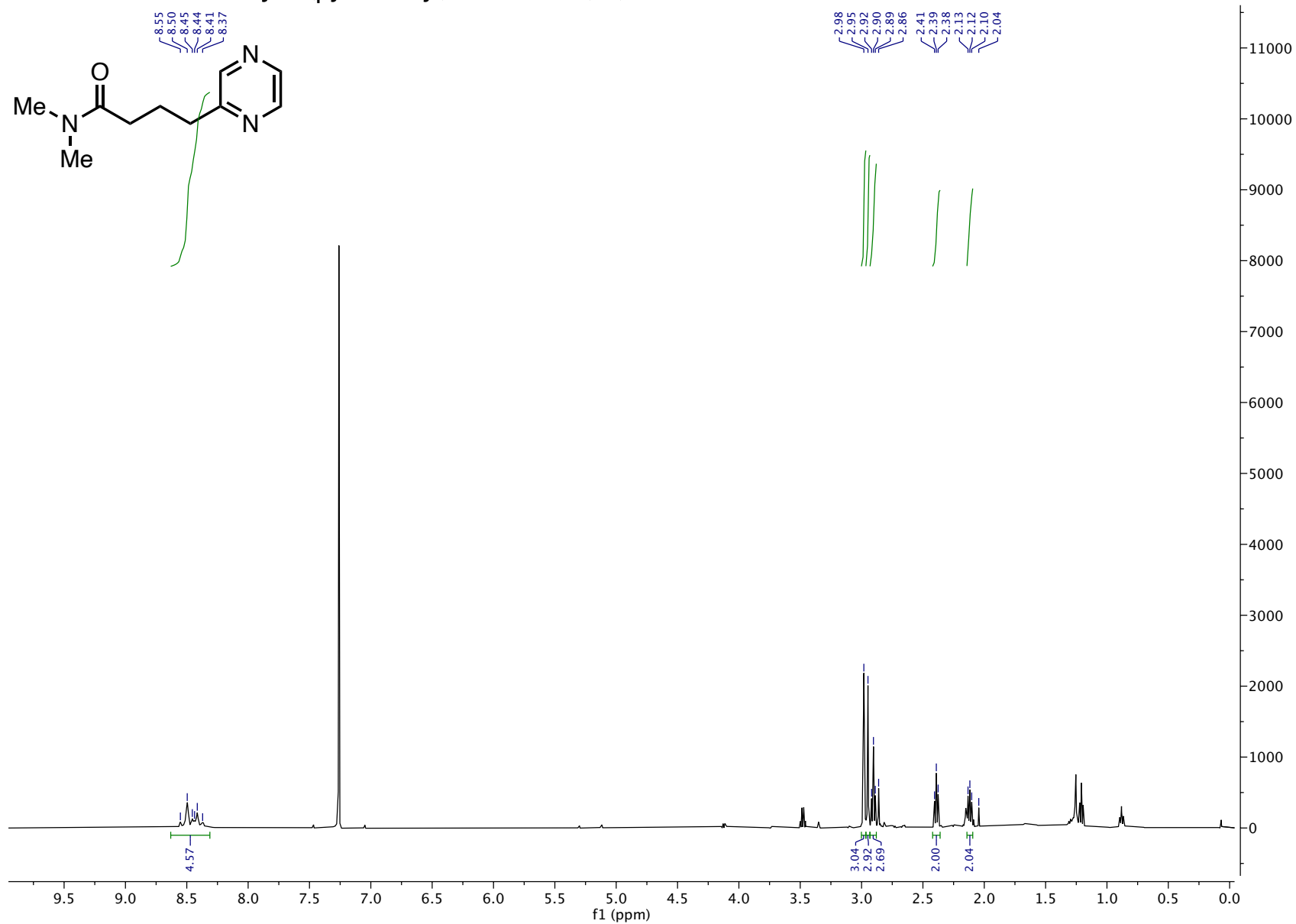
¹H NMR of *N,N*-dimethyl-4-phenyl-4-(pyridin-2-yl)butanamide (26)



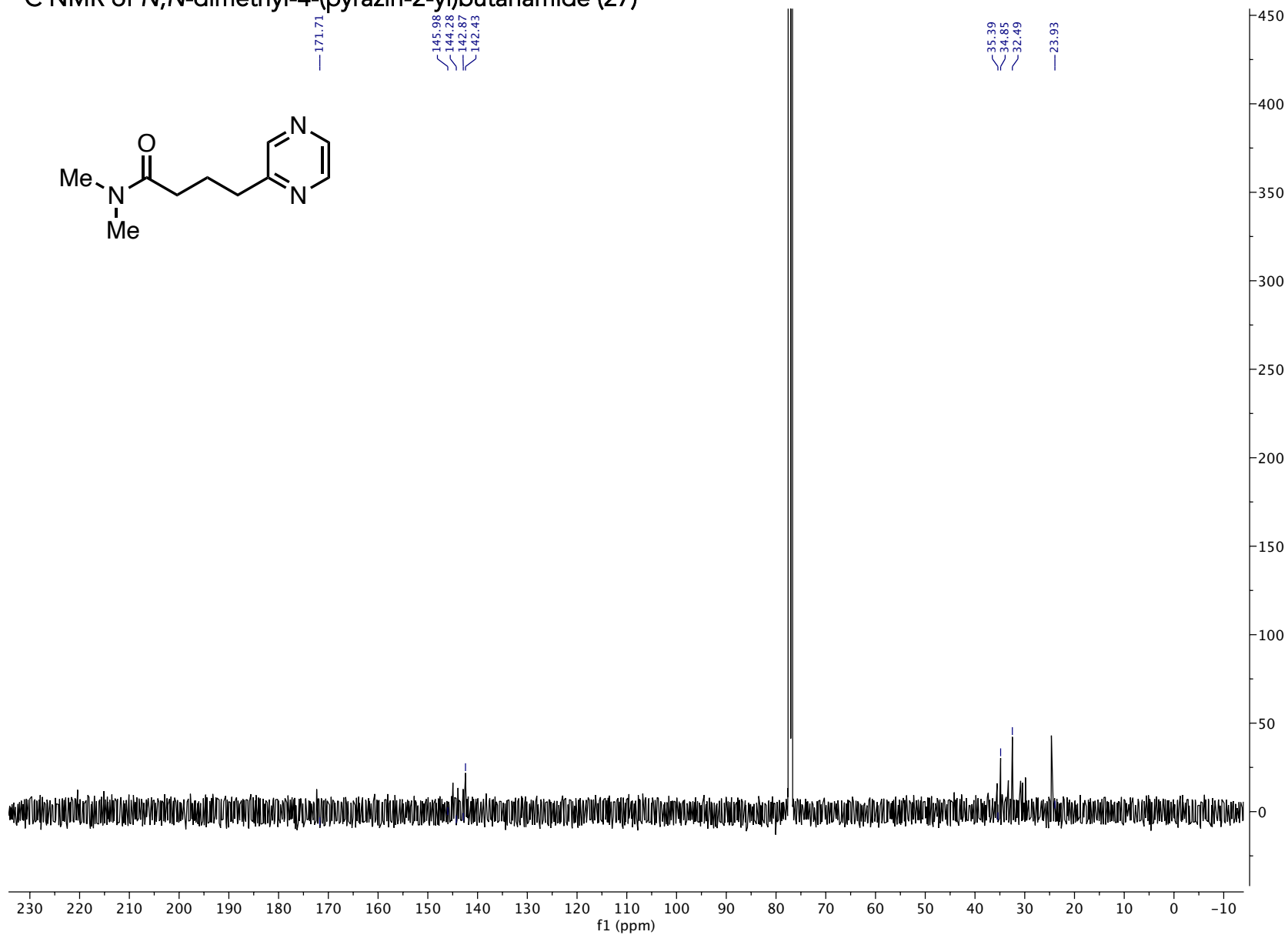
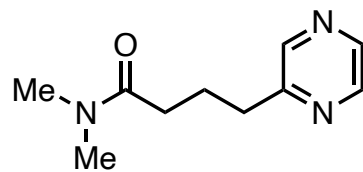
¹³C NMR of *N,N*-dimethyl-4-phenyl-4-(pyridin-2-yl)butanamide (26)



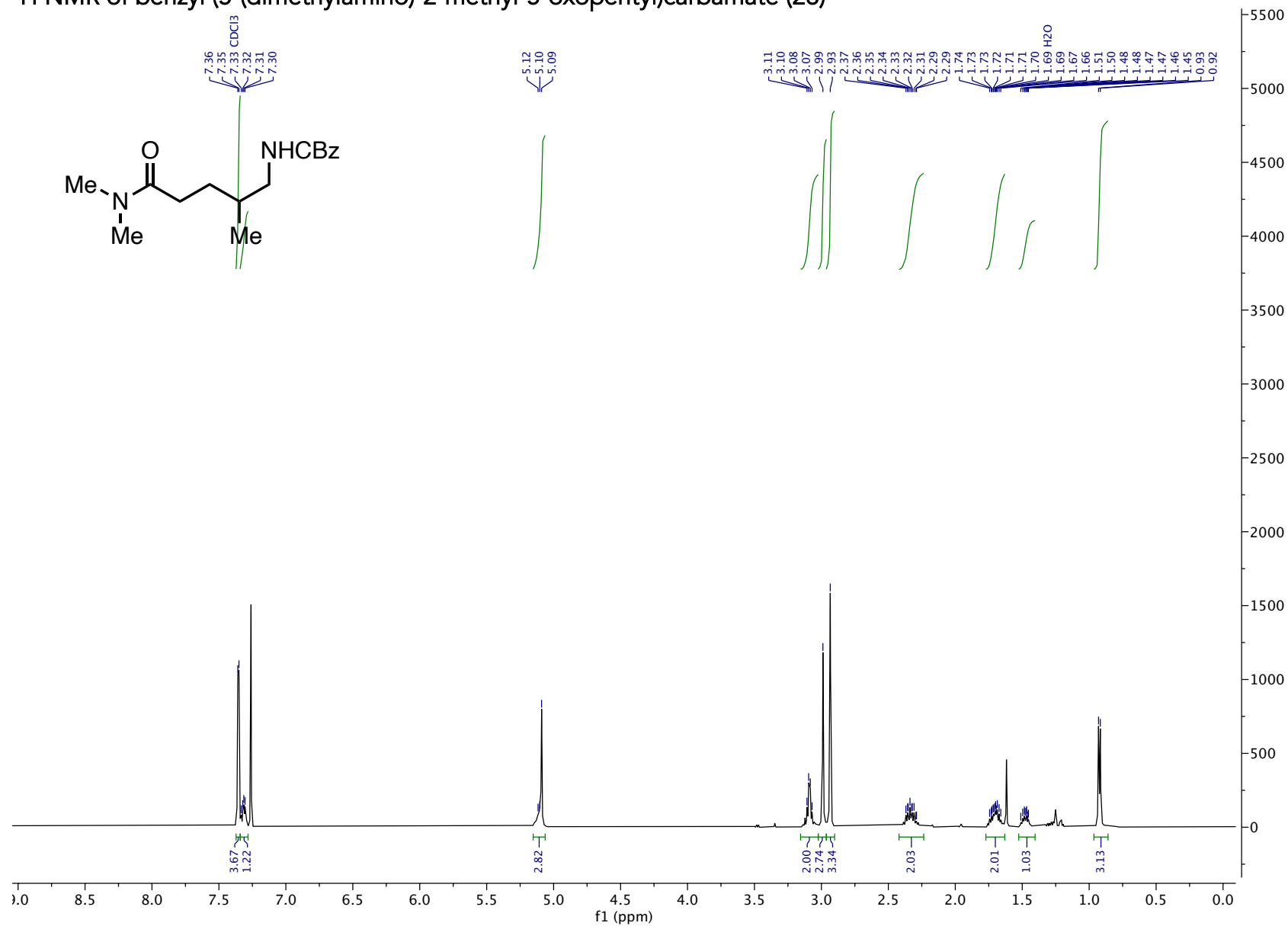
¹H NMR of *N,N*-dimethyl-4-(pyrazin-2-yl)butanamide (27)



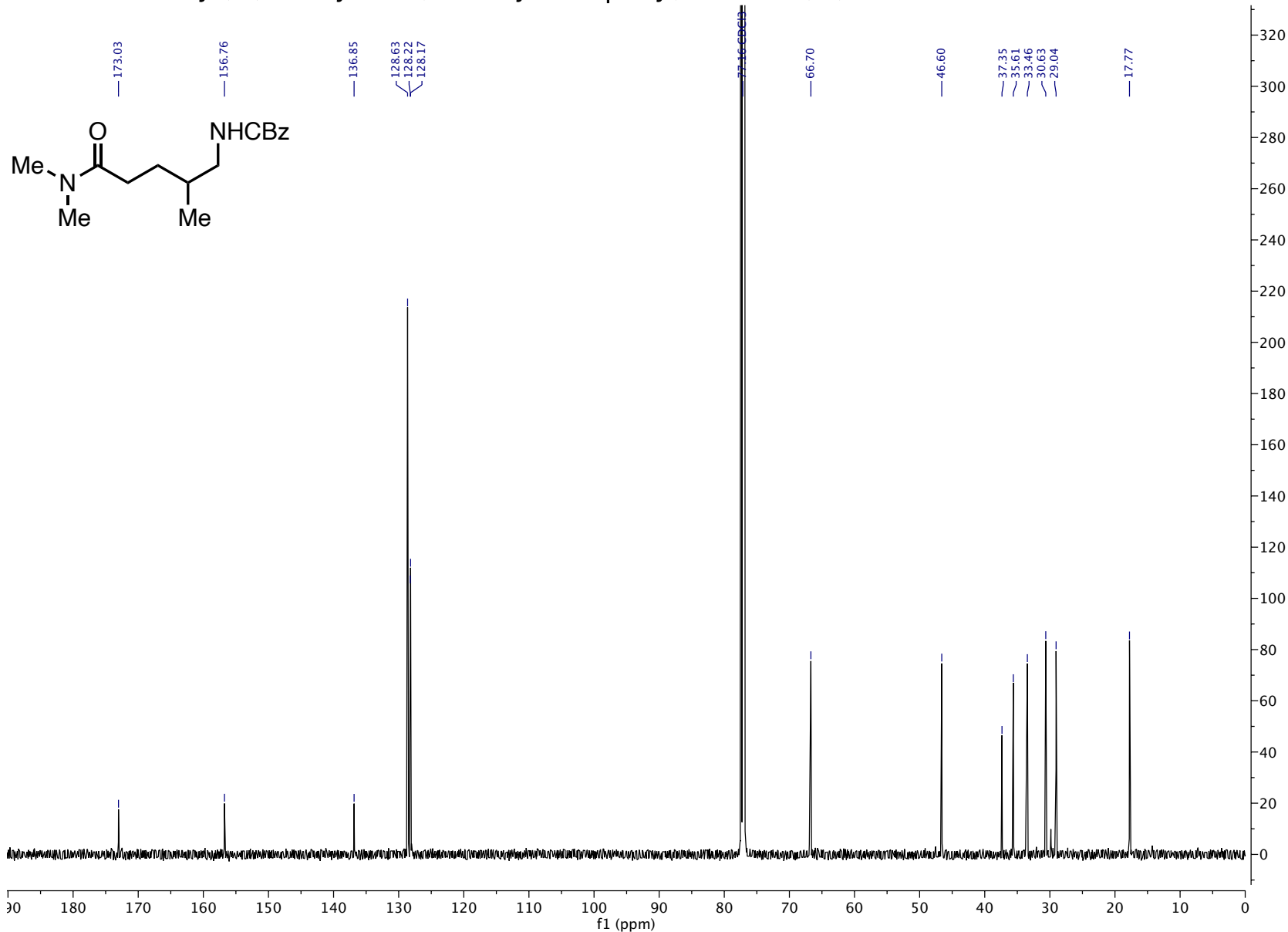
¹³C NMR of *N,N*-dimethyl-4-(pyrazin-2-yl)butanamide (27)



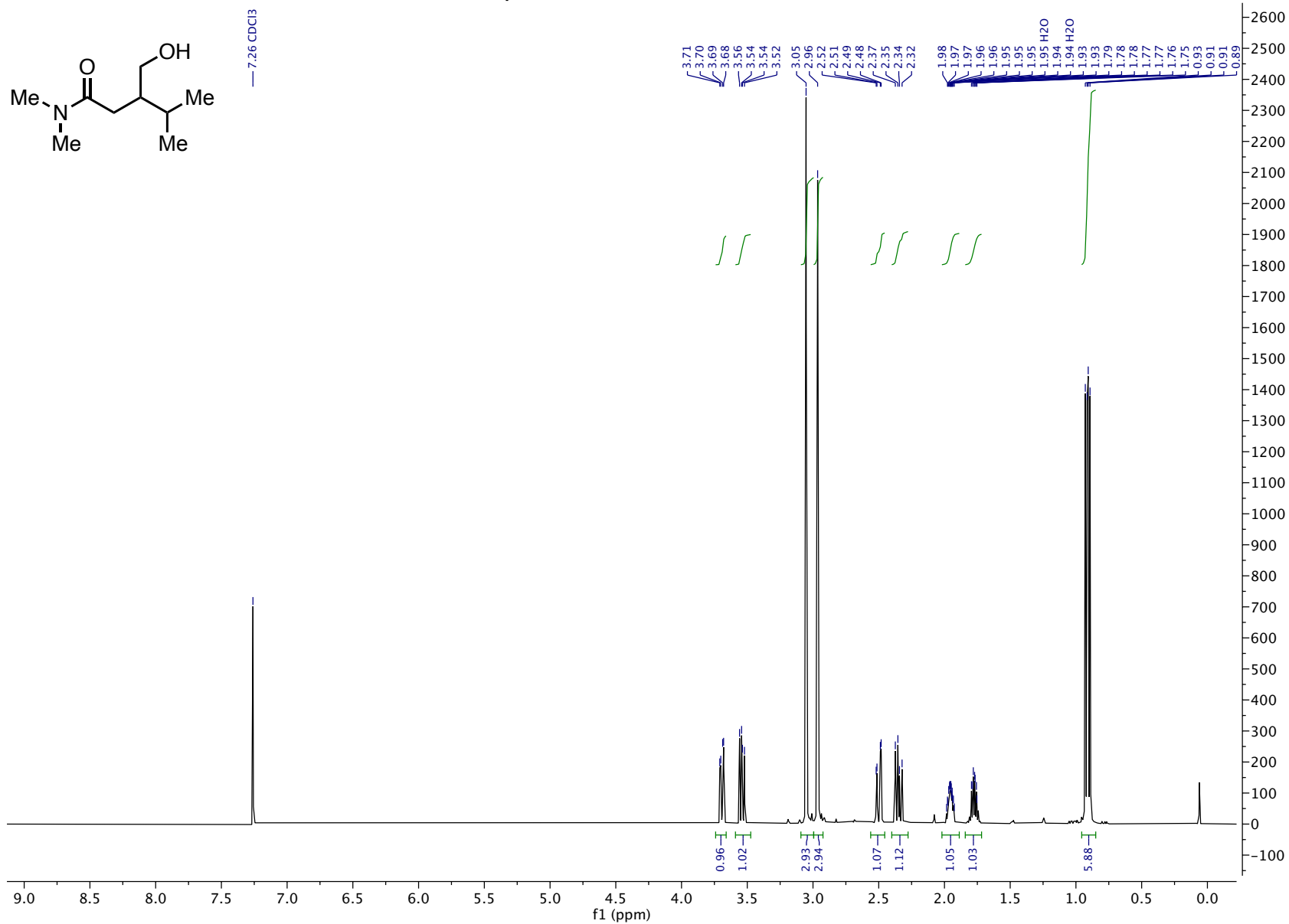
¹H NMR of benzyl (5-(dimethylamino)-2-methyl-5-oxopentyl)carbamate (28)



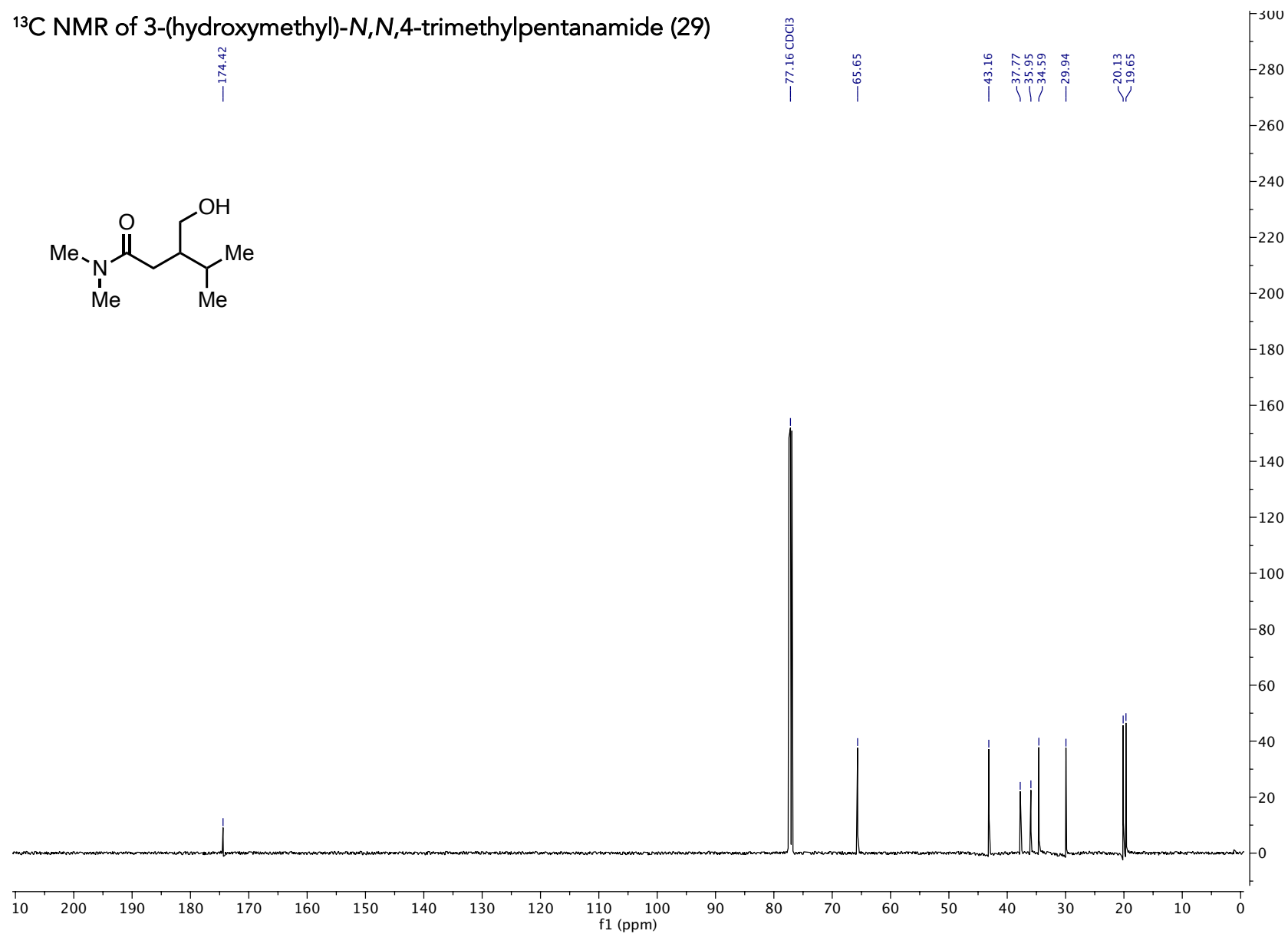
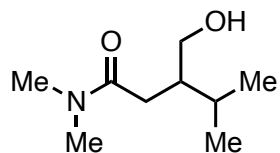
¹³C NMR of benzyl (5-(dimethylamino)-2-methyl-5-oxopentyl)carbamate (28)



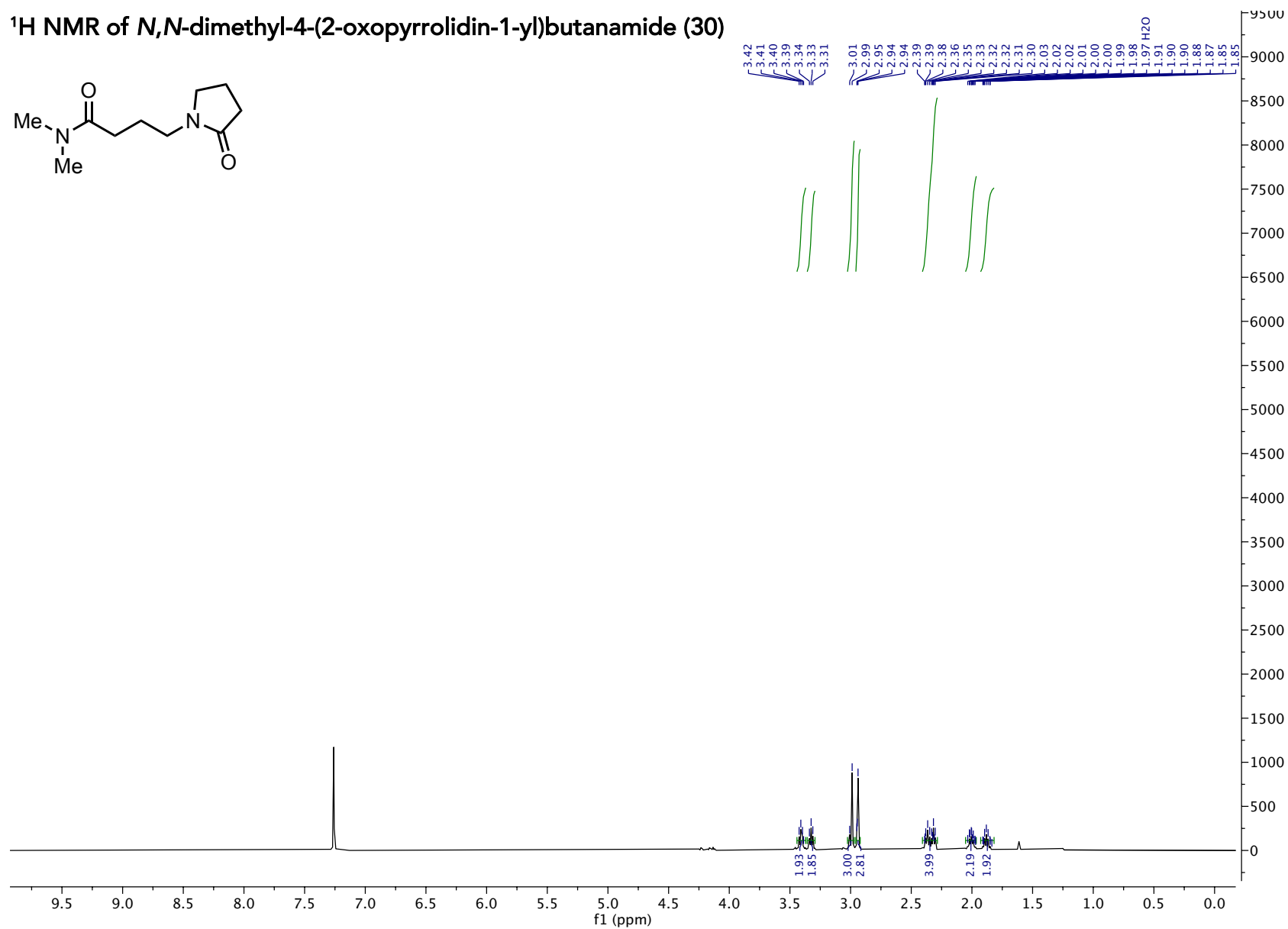
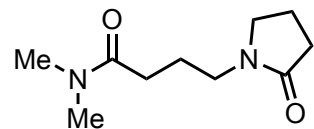
¹H NMR of 3-(hydroxymethyl)-N,N,4-trimethylpentanamide (29)



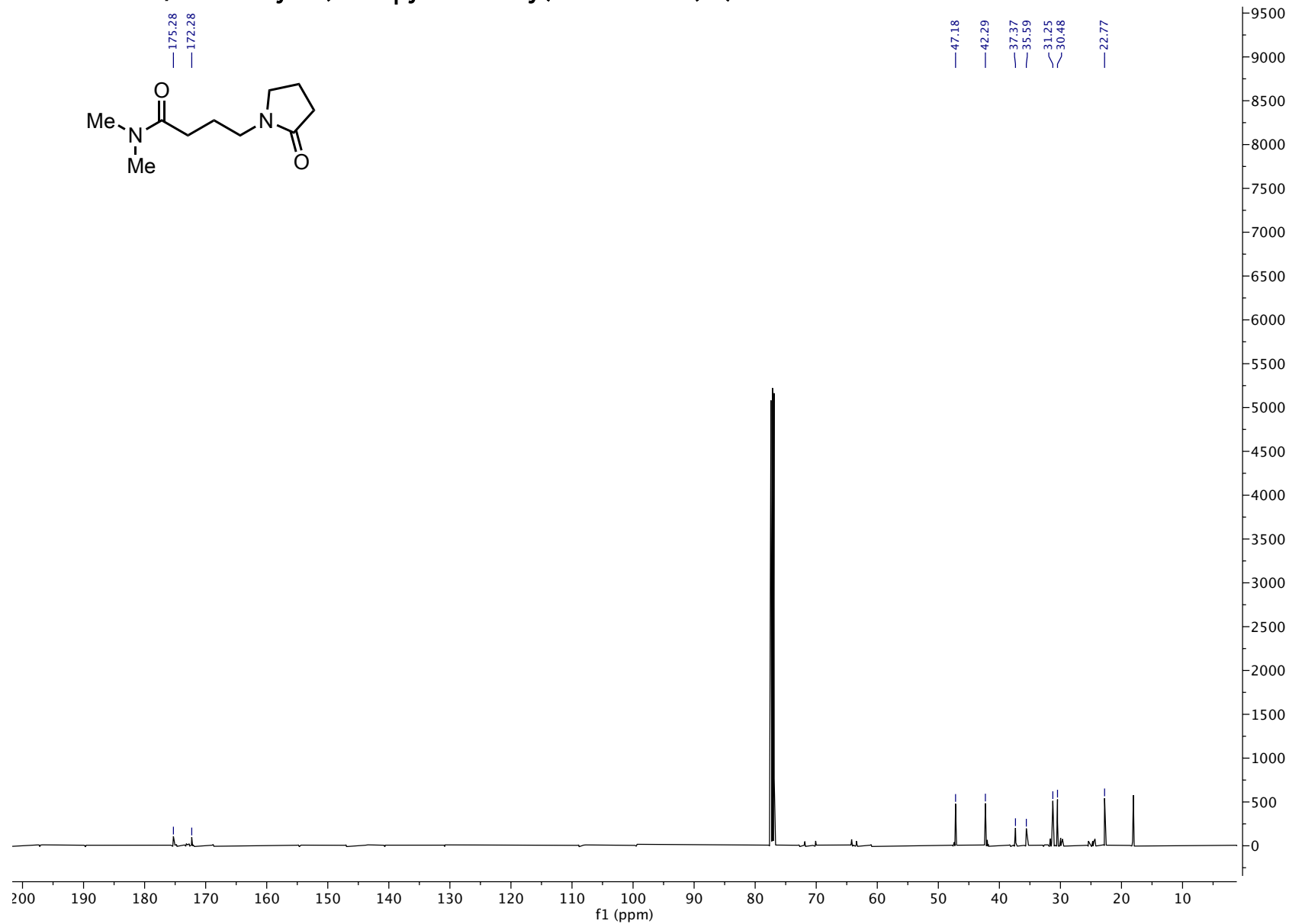
¹³C NMR of 3-(hydroxymethyl)-*N,N*,4-trimethylpentanamide (29)



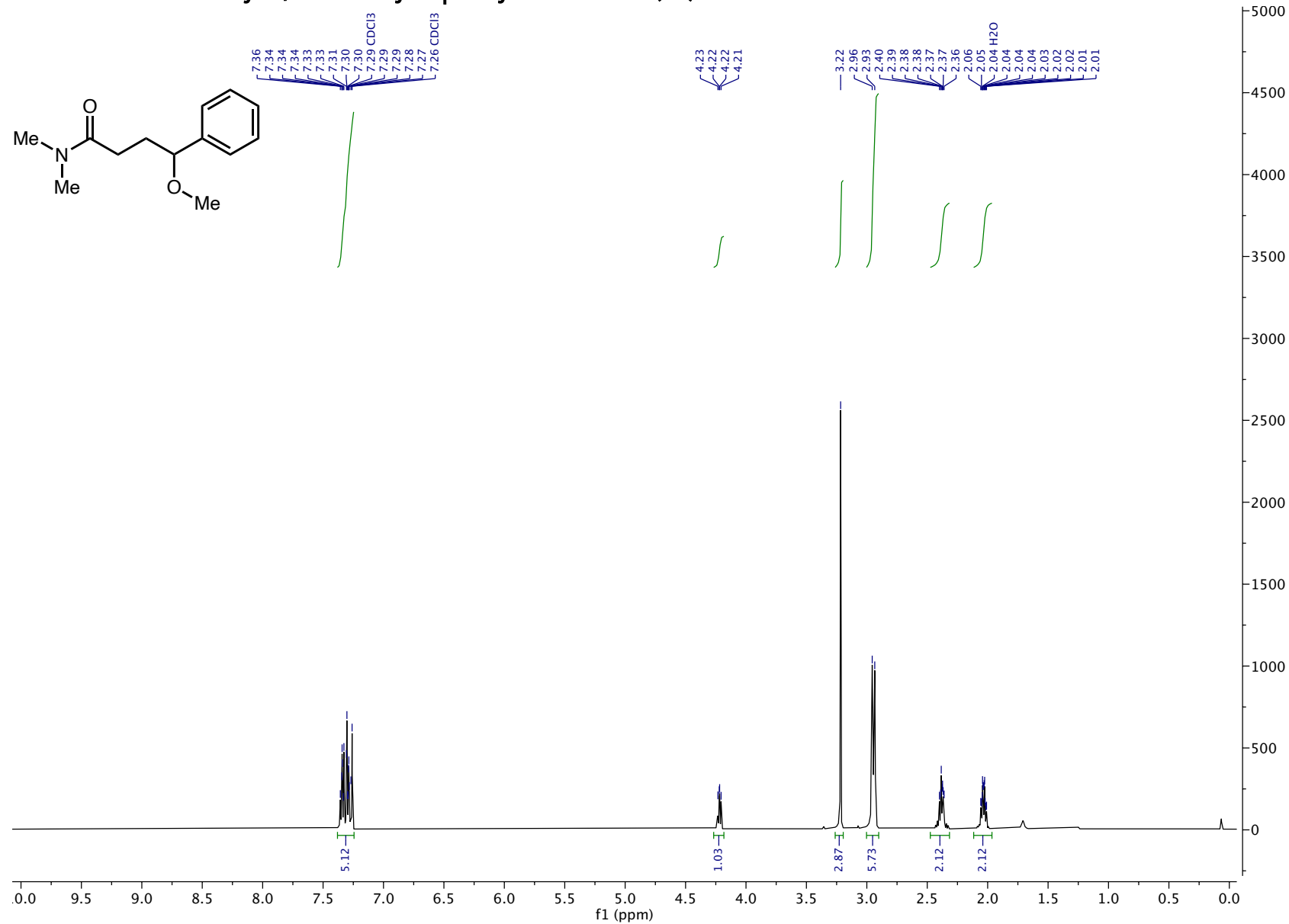
¹H NMR of *N,N*-dimethyl-4-(2-oxopyrrolidin-1-yl)butanamide (30)



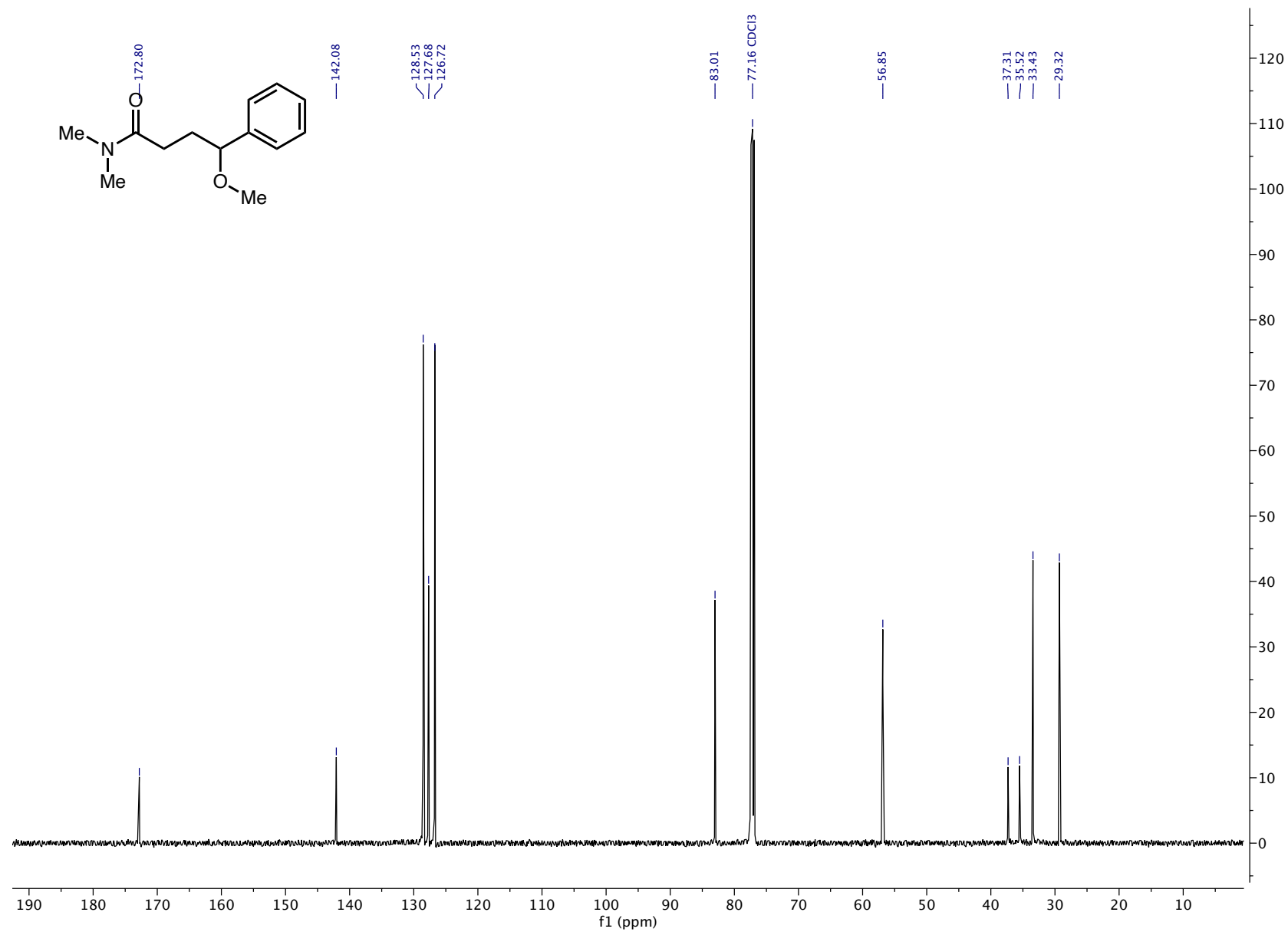
¹³C NMR of *N,N*-dimethyl-4-(2-oxopyrrolidin-1-yl)butanamide (30)



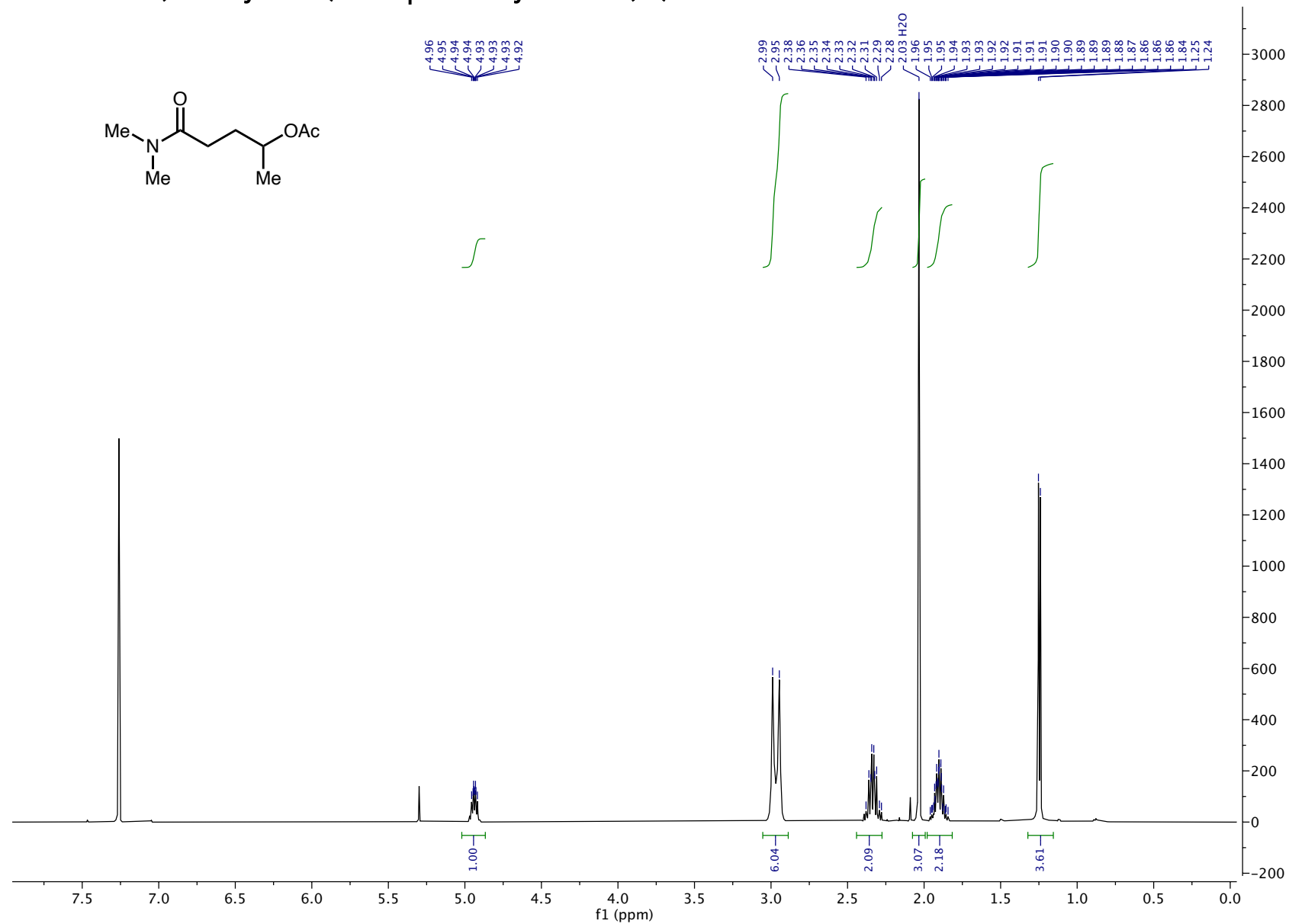
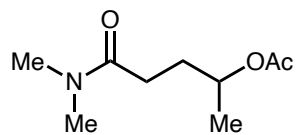
¹H NMR of 4-methoxy-*N,N*-dimethyl-4-phenylbutanamide (31)



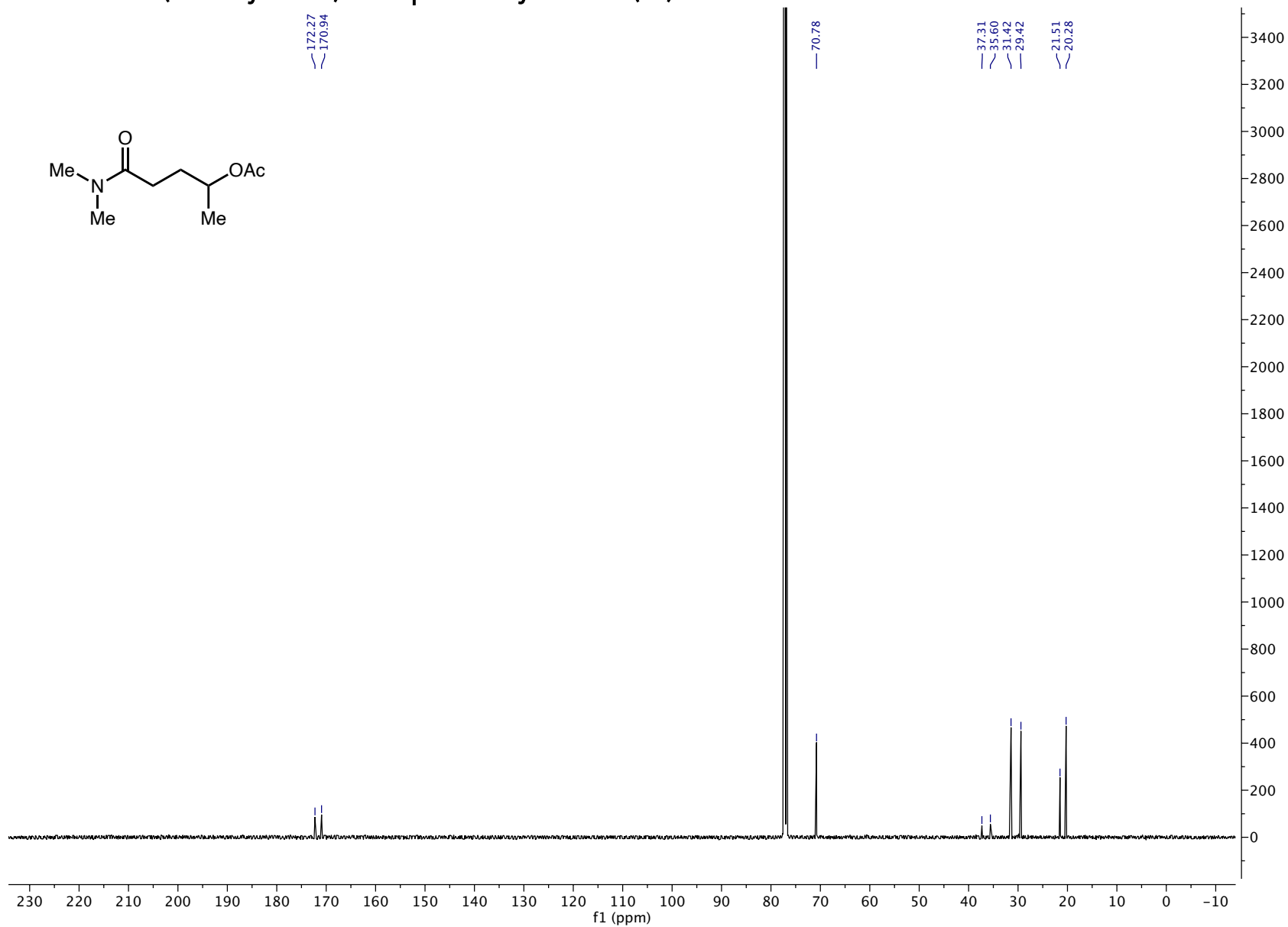
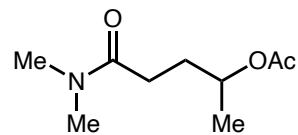
¹³C NMR of 4-methoxy-*N,N*-dimethyl-4-phenylbutanamide (31)



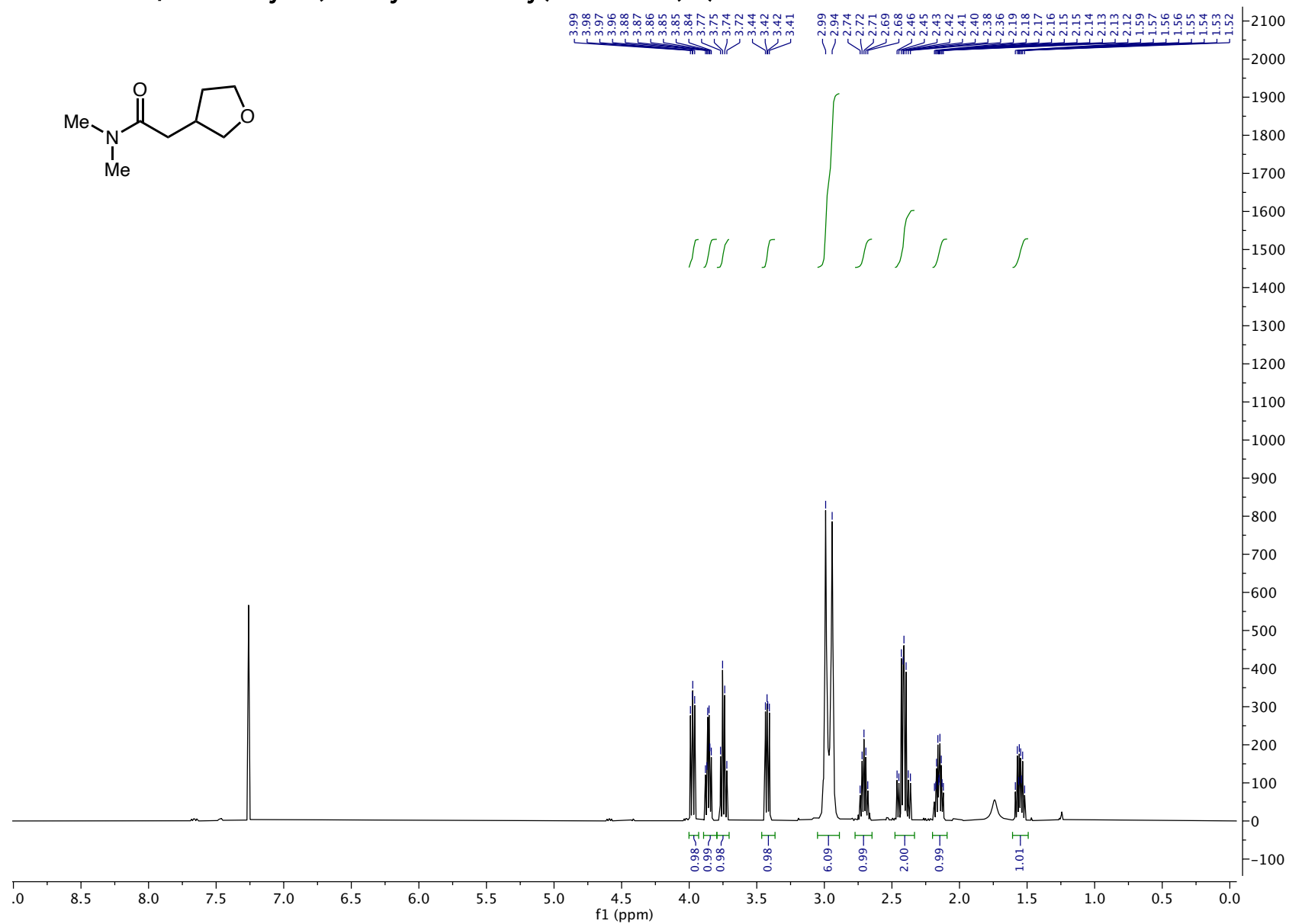
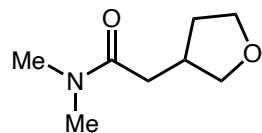
¹H NMR of 5-(dimethylamino)-5-oxopentan-2-yl acetate (32)



¹³C NMR of 5-(dimethylamino)-5-oxopentan-2-yl acetate (32)



¹H NMR of *N,N*-dimethyl-2-(tetrahydrofuran-3-yl)acetamide (33)



¹³C NMR of *N,N*-dimethyl-2-(tetrahydrofuran-3-yl)acetamide (33)

