Supplementary Information

Expanding Biocatalysis for Organosilane Functionalization: Enantioselective Nitrene Transfer to Benzylic Si–C–H Bonds

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Table of Contents

1. General Procedures

Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, VWR, Alfa Aesar, and Combi-Blocks) and used without further purification. Silica-gel chromatography was carried out using AMD Silica Gel 60, 230–400 mesh. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument in $CDCl_3$ and are referenced to residual proton solvent signals.[1] Sonication was performed using a Qsonica Q500 sonicator. High-resolution mass spectra HRMS were acquired from the Caltech Mass Spectral Facility with JEOL JMS-T2000GC AccuTOF using field ionization and field desorption (FI and FD). High-performance liquid-chromatography mass spectroscopy (HPLC-MS) for analysis was carried out using Agilent 1200 series instruments, with C18 (Kromasil 4.6 × 50 mm, 5 μm) column. Water and acetonitrile containing 0.1% acetic acid were used as eluents. Gas chromatography (GC) was performed on an Agilent Technologies 7820A GC system equipped with a split-mode capillary injection system and flame ionization detectors (FID) with Column #26 HP-5 (Agilent) as a stationary phase. Chiral supercritical fluid chromatography (SFC) was performed on an Agilent Technologies 1260 Infinity II system, with Daicel CHIRALPAK (4.6 × 50 mm, 5 μm) column. Distilled water was utilized for growth media while double distilled water was employed for all buffer preparations.

The M9-N buffer described in this study was prepared as follows:

A 5X stock solution was prepared by dissolving Na_2HPO_4 (34 g), KH_2PO_4 (15 g), and NaCl (2.5 g) in 1 L of water followed by sterilization by autoclaving. A 1X stock was then prepared by diluting 200 mL of the 5X solution to 1 L, followed by addition of 1 mL $CaCl_2$ (0.1 M) and 2 mL $MgSO_4$ (1.0 M) and adjusting the pH to 7.4 or 8.0 by the addition of 40% aqueous NaOH.

The KPi buffer described in this study was prepared as follows:

A stock solution was prepared by dissolving K_2HPO_4 (34.0 g) and KH_2PO_4 (15.0 g) in 1.0 L of double distilled water, and the pH was adjusted to 8.0.

1.1. Cloning and Mutagenesis

Electrocompetent *Escherichia coli* BL21 E. cloni (Lucigen) cells were utilized for all experiments. Luria-Bertani (LB) and Terrific-Broth (TB) media were used for growth. During growth, media was supplemented with ampicillin (100 μg/mL). TB was also supplemented with glycerol according to the manufacturer instructions. Protein induction was performed by the addition of isopropyl β-D-1-

thiogalactopyranoside (IPTG, 0.5 mM). Protein expression was supplemented with δ-aminolevulinic acid (ALA, 1.0 mM).

Expression vector pET-22b(+) (Novagen) was used for cloning and expression for all the variants described in this paper. Site-saturation mutagenesis (SSM) was performed using a modified QuikChange mutagenesis protocol using the 22-codon trick.[2] The PCR products were purified with a gel purification kit (New England Biolabs), and the gaps were repaired using Gibson Mix.[3] Without further purification, 1 μL of the Gibson product was used to transform 30 μL of electrocompetent *E. coli* cells. The sequence of the primers used for SSM are given below:

Primers	**Sequence (5' → 3')**
UniF	GAA ATA ATT TTG TTT AAC TTT AAG AAG GAG ATA TAC ATA TG
UniR	GCC GGA TCT CAG TGG TGG TGG TGG TGG TGC TCG AG
70F1	GAT GAA TCA CGC TTT GAT AAA NDT TTA AGT CAA CAG CTG AAA
70F2	GAT GAA TCA CGC TTT GAT AAA VHG TTA AGT CAA CAG CTG AAA
70F3	GAT GAA TCA CGC TTT GAT AAA TGG TTA AGT CAA CAG CTG AAA
70R	TTT ATC AAA GCG TGA TTC ATC GCA TGC TTC TTT AAT TAG
263F1	CAA ATT ATT ACA TTC TTA NDT GCG GGA CAC GAA GGT ACA
263F2	CAA ATT ATT ACA TTC TTA VHG GCG GGA CAC GAA GGT ACA
263F3	CAA ATT ATT ACA TTC TTA TGG GCG GGA CAC GAA GGT ACA
263R	TAA GAA TGT AAT AAT TTG ATA GCG AAT GTT CAC GTC
436F1	GAG CTC GAT ATT AAA GAA NDT TTA ACG TTA AAA CCT AAA
436F2	GAG CTC GAT ATT AAA GAA VHG TTA ACG TTA AAA CCT AAA
436F3	GAG CTC GAT ATT AAA GAA TGG TTA ACG TTA AAA CCT AAA
436R	TTC TTT AAT ATC GAG CTC GTA GTT TGT ATG ATC TTC

Random mutations were introduced using error-prone PCR with the addition of 200–500 μM $MnCl_2$ to a *Taq* PCR method as previously reported using the primers described below.[4]

Primers	**Sequence (5' → 3')**
005	GAA ATA ATT TTG TTT AAC TTT AAG AAG GAG ATA TAC ATA TG
006	GCC GGA TCT CAG TGG TGG TGG TGG TGG TGC TCG AG

007	CAT ATG TAT ATC TCC TTC TTA AAG TTA AAC AAA ATT ATT TC
008	CTC GAG CAC CAC CAC CAC CAC CAC TGA GAT CCG GC

Primers 005 and 006 were used to amplify the region coding for the enzyme (full length) using *Taq* polymerase and the parent DNA template. Mutations within the full-length fragment were introduced by varying the concentration of $MnCl_2$ during PCR amplification. Similarly, primers 007 and 008 were used to amplify the backbone (i.e., pET-22(b)+ vector) fragment using *Phusion* polymerase and the parent DNA template. The excess plasmid DNA templates were digested with *Dpn*I, and the amplified fragments were purified by gel electrophoresis (1% agarose gel). Both fragments were then assembled into a circular plasmid using Gibson assembly. Libraries generated with 200, 300, 400, and 500 μM $MnCl_2$ were test screened (one 96-well plate each) to determine which library gave the optimal balance of high diversity and low inactivation (approximately 50% variants are inactive and 50% have similar or better activity compared to the parent). The library that had the best ratio of inactive to active variants was then screened further.

Staggered extension process (StEP) recombination was conducted by pooling the plasmids encoding for the variants to be recombined in equimolar fashion and using this mixture as the template DNA.[5] A PCR was conducted by varying the annealing temperature and also by using a shorter extension time to reshuffle the mutations present in the variants. A second PCR was performed to amplify the recombined fragment. The recombined insert library was purified and inserted into the pET-22(b)+ vector as described above.

1.2. Determination of Hemeprotein Concentration

Heme protein concentration in the cell was determined using hemochrome assay on the cell lysate.[6] Lysate was obtained by sonication (6 minutes total time, 1 second on, 2 second off, 35% amplitude, on wet ice). The cell debris was removed by centrifugation (14,000*g*, 10 minutes, 4 °C). To a cuvette, 500 μL of the lysate and 500 μL of solution I [0.2 M NaOH, 40% (v/v) pyridine, 0.5 mM $K_3Fe(CN)_6$] were added. The UV-Vis spectrum (380–620 nm) of the oxidized state Fe(III) was recorded immediately. Sodium dithionite (10 μL of 0.5 M solution in water) was added, and the UV-Vis spectrum of the reduced state Fe(II) was recorded immediately. The protein concentration was calculated using the extinction coefficient and dilution factor (2× dilution in volume): $\varepsilon[557_{reduced} - 540_{oxidized}] = 23.98\ mM^{-1}cm^{-1}$. [Note

that TTN values are lower bounds since the hemochrome assay detects the levels of heme; however, heme concentration closely approximates enzyme concentration.]

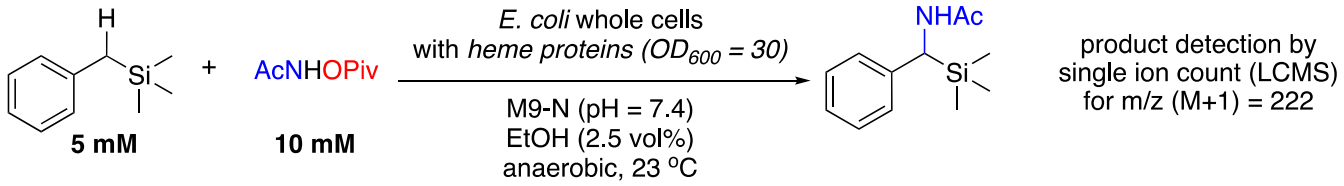
1.3. Reaction Screening in 96-Well Plate Whole-Cell Format

After single-site saturation mutagenesis (SSM) or error-prone PCR (epPCR) libraries were generated, 88 single colonies were randomly picked and cultured in 400 μL of LB medium with 0.1 mg/mL ampicillin (LB_{amp}) in a sterilized 96-well culture plate. The plate typically contained six wells inoculated with single colonies expressing the parent enzyme, and two sterile wells. The cultures were grown at 37 °C, 230 rpm, and 80% relative humidity for 8–12 hours. A separate, sterilized 96-well culture plate was filled with 950 μL of TB medium containing 0.1 mg/mL ampicillin (TB_{amp}) in each well. Likewise, a glycerol stock replica plate of the preculture (100 μL of preculture added to 100 μL of 50% glycerol per well) was also prepared and stored at -80 °C for future reference. The plate with TB_{amp} was inoculated with the LB preculture (50 μL/well) and incubated at 37 °C, 230 rpm, and 80% relative humidity for 3 hours. The plate was cooled on an ice bath for 30 minutes, induced with 0.5 mM IPTG and 1 mM ALA (final concentrations), and then expressed at 22 °C and 230 rpm for 22–24 hours. The cells were pelleted (4,000*g*, 5 minutes), and 390 μL of M9-N (pH 7.2, 20 mM D-glucose) were added to each well. After cells were fully resuspended by shaking at 500 rpm, to each well were added 10 μL of a reactant stock solution in ethanol (200 mM of organosilane and 400 mM of nitrene precursor dissolved together). Thus, the final reactant concentrations in each well were 5 mM (organosilane) and 10 mM (nitrene precursor). The plate was sealed with aluminum foil tape and shaken at 600 rpm at room temperature overnight.

Once the plate seal was removed, acetonitrile (800 μL/well) was added. The resulting suspension in the wells was mixed by pipetting. The plate was then centrifuged (4,500*g*, 10 minutes) to precipitate proteins and cell debris. The supernatant (200 μL/well) was transferred to a shallow 96-well plate for reverse-phase HPLC-MS analysis. The MS product signals from each well were compared, and wells showing signals higher than the parent wells were identified. These ‘hits’ were re-cultured using the wells from the frozen replica glycerol stock plate and then sequenced. Among these, the activities of candidates showing mutations at the targeted site were revalidated in analytical scale reactions. In this way, the best variant was selected and was used as the parent for the next round of mutagenesis.

2. Discovery of Initial Activity

The Arnold lab collection of heme proteins (including P450s, P411s, protoglobins, and cytochromes *c*) were screened in 96-well plate, whole-cell reactions for product formation (see section 1.3). The collection consisted of nearly 500 distinct variants accumulated from prior directed evolution campaigns. For amination under anaerobic conditions, only one variant showed product formation as assayed by HPLC-MS.[7] When the reaction was screened under aerobic conditions, the same variant demonstrated product formation as assayed by HPLC-MS. No product was detected in control reactions with $P450_{BM3}$ and with free heme. [Note: The directed evolution campaign was performed under aerobic conditions.]



Variant showing product formation and the control reactions

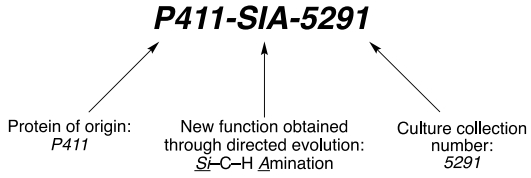
Variant	Ion Count
$P450_{BM3}$ (WT)	0
uAmD5-5117 (anaerobic)	39923
uAmD5-5117 (aerobic)	59578
Heme (50 μM)	0
Heme (50 μM) + sodium dithionite (5 mM)	0

3. Evolution for Si–C–H Amination (*P411-SIA* Lineage)

3.1. Summary of Directed Evolution

SSM Sites Tested	Leading Variant	Mutation Method	Mutations obtained	TTN	[Product] / μM	Product % Yield
-	*uAmD5-5117*	-	-	2	4 ± 1	0.1
-	*P411-SIA-5285*	epPCR and StEP	T328A, N573D, E839G	20 ± 1	68 ± 15	1.3 ± 0.3
-	*P411-SIA-5286*	epPCR and StEP	R47H, E143K, F910L, K921E, H961Q	20 ± 5	61 ± 14	1.2 ± 0.3
-	*P411-SIA-5287*	epPCR	F77S	26 ± 5	97 ± 40	1.9 ± 0.8
-	*P411-SIA-5288*	epPCR and StEP	F662C, K670I	68 ± 9	182 ± 22	3.6 ± 0.4
-	*P411-SIA-5289*	epPCR and truncation	T327P, ΔFAD	111 ± 21	625 ± 137	12.5 ± 2.7
29, 70, 78, 82, 142, 177, 186, 188, 261, 263, 330, 366, 401	*P411-SIA-5290*	SSM and StEP	S70M, V263L	194 ± 24	696 ± 28	13.9 ± 0.5
72, 74, 75, 86, 266, 267, 268, 269, 436, 437, 438	*P411-SIA-5291*[*]	SSM	T436A	250 ± 12	1719 ± 49	34.4 ± 0.9

[*] The naming system for the variants obtained from directed evolution:



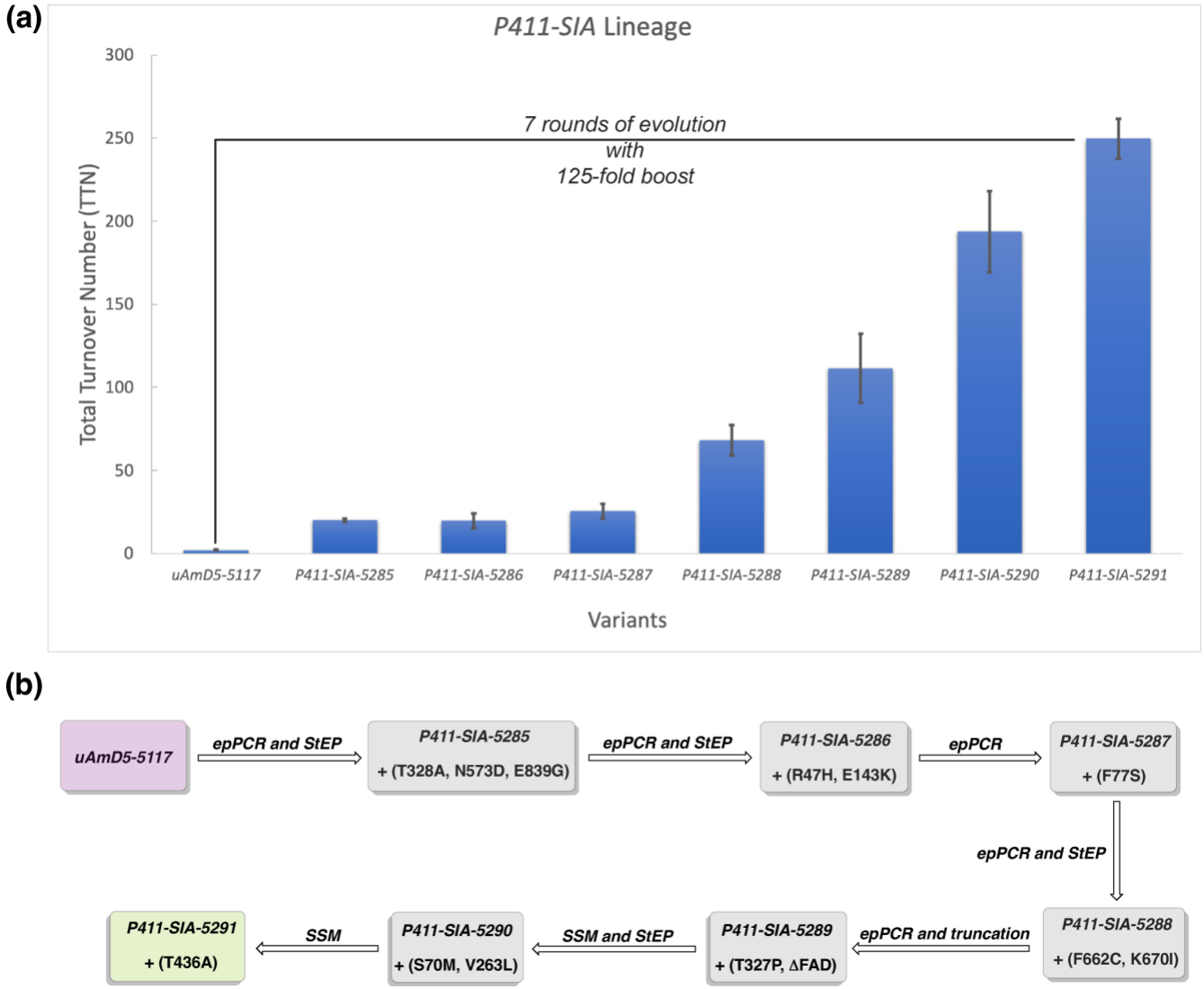


Figure. S1. Summary of the directed evolution campaign. (a) Comparison of the total turnover number (TTN) of the starting *uAmD5-5117* variant and the final *P411-SIA-5291* variant, obtained through directed evolution, reveals a 125-fold increase in TTN. (b) Evolutionary trajectory showing the mutations accumulated and the methods used for introducing them.

3.2. Comparison of Evolved Variant with Wild Type

Variants	Mutations relative to wild-type $P450_{BM3}$	Mutations relative to *uAmD5-5117*
uAmD5-5117	N70S, A74Q, V78L, A82L, F87A, P142S, T175I, A184V, S226T, H236Q, E252V, I263V, T268Q, A290V, A328T, L353V, I366V, C400A, I401L, E442K, VSGEAWSGYGEYK(849-861)RLRRSVERIWRI*[1]	-
P411-SIA-5285	N70S, A74Q, V78L, A82L, F87A, P142S, T175I, A184V, S226T, H236Q, E252V, I263V, T268Q, A290V, L353V, I366V, C400A, I401L, E442K, N573D, E839G, VSGEAWSGYGEYK(849-861)RLRRSVERIWRI*[1]	T328A, N573D, E839G
P411-SIA-5286	R47H, N70S, A74Q, V78L, A82L, F87A, P142S, E143K, T175I, A184V, S226T, H236Q, E252V, I263V, T268Q, A290V, L353V, I366V, C400A, I401L, E442K, N573D, E839G, VSGEAWSGYGEYK(849-861)RLRRSVERIWRI*[1]	R47H, E143K, T328A, N573D, E839G
P411-SIA-5287	R47H, N70S, A74Q, F77S, V78L, A82L, F87A, P142S, E143K, T175I, A184V, S226T, H236Q, E252V, I263V, T268Q, A290V, L353V, I366V, C400A, I401L, E442K, N573D, E839G, VSGEAWSGYGEYK(849-861)RLRRSVERIWRI*[1]	R47H, F77S, E143K, T328A, N573D, E839G
P411-SIA-5288	R47H, N70S, A74Q, F77S, V78L, A82L, F87A, P142S, E143K, T175I, A184V, S226T, H236Q, E252V, I263V, T268Q, A290V, L353V, I366V, C400A, I401L, E442K, N573D, F662C, K670I, E839G, VSGEAWSGYGEYK(849-861)RLRRSVERIWRI*[1]	R47H, F77S, E143K, T328A, N573D, F662C, K670I, E839G
P411-SIA-5289	R47H, N70S, A74Q, F77S, V78L, A82L, F87A, P142S, E143K, T175I, A184V, S226T, H236Q, E252V, I263V, T268Q, A290V, T327P, L353V, I366V, C400A, I401L, E442K, N573D, ΔFAD	R47H, F77S, E143K, T327P, T328A, N573D, ΔFAD
P411-SIA-5290	R47H, N70M, A74Q, F77S, V78L, A82L, F87A, P142S, E143K, T175I, A184V, S226T, H236Q, E252V, I263L, T268Q, A290V, T327P, L353V, I366V, C400A, I401L, E442K, N573D, ΔFAD	R47H, S70M, F77S, E143K, V263L, T327P, T328A, N573D, ΔFAD
P411-SIA-5291	R47H, N70M, A74Q, F77S, V78L, A82L, F87A, P142S, E143K, T175I, A184V, S226T, H236Q, E252V, I263L, T268Q, A290V, T327P, L353V, I366V, C400A, I401L, T436A, E442K, N573D, ΔFAD	R47H, S70M, F77S, E143K, V263L, T327P, T328A, T436A, N573D, ΔFAD

[1] Here the '*' denotes stop codon

3.3. DNA and Protein Sequences

3.3.1. uAmD5-5117 DNA Sequence:

ACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAA
ACACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAA
ATTCGAGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCA
TGCGATGAATCACGCTTTGATAAAAGTTTAAGTCAACAGCTGAAATTTCTGCGTGATTTTCT
TGGAGACGGGTTAGCCACAAGCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATAT
CTTACTTCCAAGCTTTAGTCAGCAGGCAATGAAAGGCTATCATGCGATGATGGTCGATATC
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GCATGCGTTTAAACCGTTTGGAAACGGTCAGCGTGCGGCGCTTGGTCAGCAGTTCGCTCTTC
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TTCAACAGCCAGGCAGTGCACGAAGCACGCGACATCTTGAAATTGAACTTCCAAAAGAAG
CTTCTTATCAAGAAGGAGATCATTTAGGTGTTATTCCTCGCAACTATGAAGGAATAGTAAA
CCGTGTAACAGCAAGGTTCGGCCTAGATGCATCACAGCAAATCCGTCTGGAAGCAGAAGA
AGAAAAATTAGCTCATTTGCCACTCGCTAAAACAGTATCCGTAGAAGAGCTTCTGCAATAC
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GCCCGCCGCATAAAGTAGAGCTTGAAGCCTTGCTTGAAAAGCAAGCCTACAAAGAACAAG
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3.3.2. uAmD5-5117 Protein Sequence:
TIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDES
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NPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKATGEQSDDLLTQMLNGKDPETGEPLDDVNI
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3.3.3. P411-SIA-5291 DNA Sequence:
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GCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACTTTAACCTCGA
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3.3.4. P411-SIA-5291 Protein Sequence:
TIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGHVTRYLSSQRLIKEACDES
RFDKMLSQQLKSLRDFLGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAMMVDIAVQL
VQKWERLNADEHIEVSKDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFIISMVRALDEVMNKLQ
RANPDDPAYDENKRQFQEDIKVVNDLVDKIIADRKATGEQSDDLLTQMLNGKDPETGEPLDDV
NIRYQIITFLLAGHEGTSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVG
MVLNEALRLWPPAPAFSLYAKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPE
RFENPSAIPQHAFKPFGNGQRAALGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKEALTLK
PKGFVVKAKSKKIPLGGIPSPSTEQSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIA
MSKGFAPQVATLDSHAGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSV
FGCGDKDWATTYQKVPAFIDETLAAKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAY
FNLDIENSEDNKSTLSLQFVDSAADLEHHHHHH*[1]

[1] Here the '*' denotes stop codon

3.4. Product Quantitation and TTNs

3.4.1. Analytical-Scale Reaction Setup

Single colonies from *E. coli* BL21(DE3) cells, transformed with plasmids encoding *P411-SIA* variants were picked from LB_{amp} agar plates using sterile toothpicks and grown in 5 mL of LB_{amp} in 12-mL sterile tubes at 37 °C (at 220 rpm and 80% humidity) for 16–18 hours. The preculture (1 mL) was used to inoculate 50 mL of TB_{amp} medium in 125-mL Erlenmeyer flasks. The expression culture plate was incubated at 37 °C (at 230 rpm and 80% humidity) for 3 hours (OD_{600} = 1.2–1.4) and then chilled on ice for 30 minutes. After that, 50 μL IPTG (0.5 mM final concentration) and 50 μL ALA (1.0 mM final concentration) were added, and the proteins were expressed at 22 °C (at 160 rpm) for 22–24 h. Cells were pelleted at 20 °C and 4,000*g* for 5 min. The cells were resuspended with KPi buffer (pH 8.0) containing 50 mM glucose (OD_{600} = 40). In 2-mL screw cap vials, 780 μL of the resuspended cells were charged and set up in triplicates. A stock solution (20 μL) of the mixture of organosilane (200 mM dissolved in isopropanol) and *N*-(pivaloyloxy)acetamide (800 mM dissolved in isopropanol) were added to each of the vials. The vials were shaken at 700 rpm under ambient conditions for 12–16 hours. The remainder of the cell suspension was used for protein quantitation with the hemochrome assay (section 1.3).

Work-up for HPLC-MS Analysis: Acetonitrile (1600 μL/ vial) was added, the resulting suspension was mixed by vortexing and then centrifuged (14,000 *g* for 15 min) to remove the cell debris. The supernatant (800 μL) was transferred into a 2-mL screw cap vial for HPLC-MS analysis.

Work-up for GC-FID Analysis: To the reaction mixture was added 1:1 EtOAc/hexane (800 μL/ vial), the resulting suspension was mixed by vortexing, and then centrifuged (14,000*g* for 15 min) to remove the cell debris. The organic layer (400 μL) was transferred into a 2-mL screw cap vial with an insert for GC-FID analysis. Product formation was quantified by GC-FID based on the calibration curve of the corresponding racemic reference compound.

3.4.2. Calibration Curves for Standard Products and Reaction Product Quantitation

Enzymatic reactions on an analytic scale were performed following the general procedure described in section 3.4.1. Product formation was quantified by GC-FID based on the calibration curve of the corresponding reference compound. TTN is defined as the concentration of product divided by the concentration of heme protein measured by the hemochrome assay as described in section 1.3. Calibration curves of the synthesized reference compounds were created for the determination of yield and TTN.

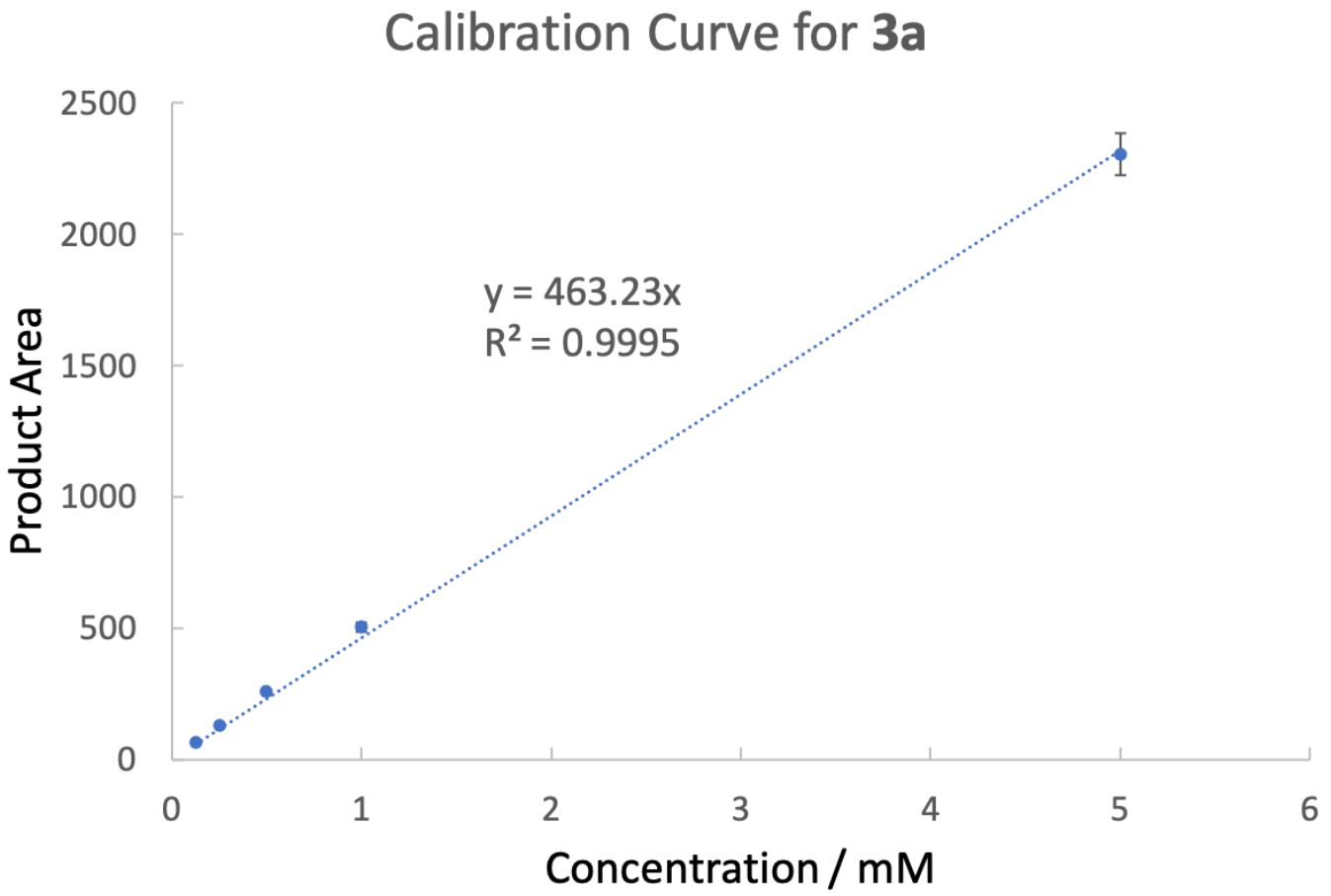
Harvested cells were resuspended with KPi buffer (pH 8.0) containing glucose (50 mM) to OD_{600} = 40. Then, 780 µL of the resuspended cells were aliquoted to 2-mL screw cap vials. Product standard stocks (20 µL, 400–50 mM stock in ethanol) were added. The mixture was vortexed and then analyzed.

For LC-MS: The ion counts at selected ion monitoring mode of product were measured. For all the analyses, water and acetonitrile containing 0.1% acetic acid were used as eluents for a Kromasil C18 column. The methods used 30–95% acetonitrile (0.3–2.0 minutes) and 95–30% (2.0–2.5 minutes) with 3-µL sample injections. The flow rate was 1.5 mL/minute, and the column was maintained at 22 °C.

For GC-FID: The total area of the racemic product peak was measured using chiral GC-FID. For all analysis, CP-Chirasil-Dex-CP (Agilent) was used as the chiral stationary phase. The method used was: start and hold at 100 °C for 2 minutes, then ramp at 50 °C/minute to 300 °C, using 3-µL sample injections.

3.4.3. Yields and TTN for amination reaction across *P411-SIA* Lineage

Based on total area for standard product (**3a**), obtained from GC-FID.



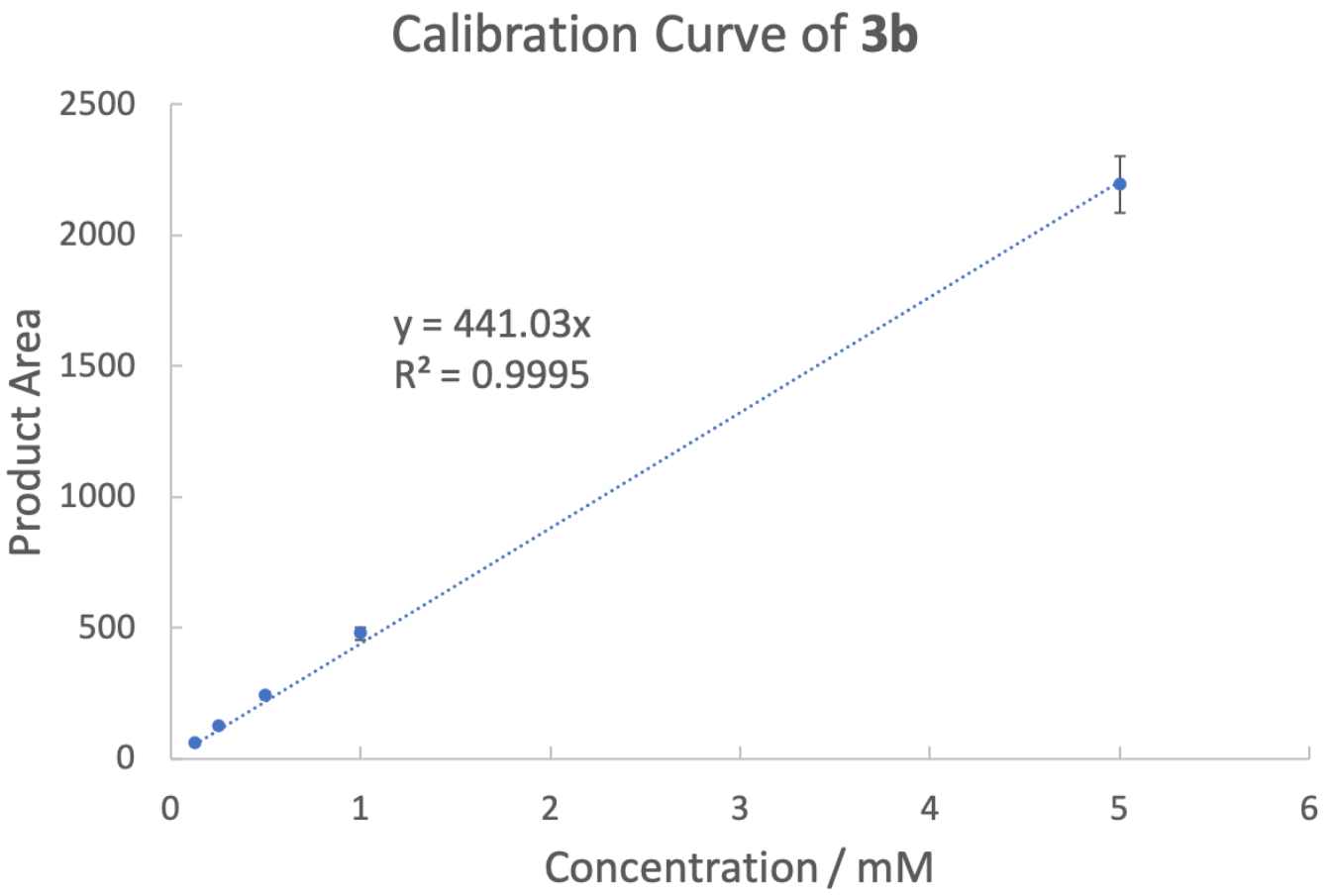
Variant	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	[Protein] / μM	TTN	Avg. TTN	Std Dev. TTN
uAmD5-55117	1.6	3.5	3.7	0.7	1.6	2.2	1.9	0.3
	1.6	3.5			1.6	2.2		
	1.3	2.8			1.6	1.8		
	2.3	4.9			2.3	2.2		
	1.7	3.7			2.3	1.6		
	1.7	3.7			2.3	1.6		
P411-SIA-5285	25.3	54.6	67.8	14.7	2.9	19.2	19.9	1.0
	26	56.1			2.9	19.7		
	24.3	52.5			2.9	18.4		
	38.3	82.7			3.9	21.2		
	37.5	80.9			3.9	20.7		

	37	79.9			3.9	20.5		
P411-SIA-5286	23.3	50.3	60.9	13.7	2.1	24.6	19.6	4.6
	21.8	47.1			2.1	23.0		
	22.4	48.4			2.1	23.7		
	35.5	76.6			4.8	16.2		
	32.7	70.6			4.8	14.9		
	33.6	72.5			4.8	15.3		
P411-SIA-5287	63.2	136.4	97.4	39.5	6.3	21.9	25.5	4.5
	61	131.7			6.3	21.1		
	61.2	132.1			6.3	21.2		
	28.7	61.9			2.1	29.9		
	29	62.6			2.1	30.2		
	27.5	59.4			2.1	28.7		
P411-SIA-5288	66.5	143.6	182.1	22.1	2.6	56.5	68.2	9.1
	84.9	183.3			2.6	72.2		
	98.4	212.4			2.6	83.7		
	83.7	180.7			2.8	64.3		
	85.6	184.8			2.8	65.8		
	86.9	187.6			2.8	66.8		
P411-SIA-5289	189.1	408.2	625.4	136.7	4.3	96.0	111.4	20.8
	279.2	602.7			4.3	141.7		
	259.2	559.5			4.3	131.6		
	304.5	657.3			7.4	90.0		
	370.7	800.3			7.4	109.6		
	335.5	724.3			7.4	99.2		
P411-SIA-5290	332.5	717.8	695.7	27.8	4.2	170.7	193.7	24.4
	336.6	726.6			4.2	172.8		
	332.6	718.0			4.2	170.8		
	309.6	668.4			3.1	215.2		
	309.4	667.9			3.1	215.1		
	312.9	675.5			3.1	217.5		
P411-SIA-5291	774.6	1672.2	1718.7	49.3	6.5	259.7	249.6	11.9
	779.9	1683.6			6.5	261.5		
	774.7	1672.4			6.5	259.7		
	808	1744.3			7.4	236.6		
	828.7	1788.9			7.4	242.7		
	810.9	1750.5			7.4	237.5		

Notes: Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; [Protein] = protein concentration in reaction; TTN = total turnover number; Avg. TTN = average of total turnover number; Std. Dev. TTN = standard deviation of total turnover number.

3.4.4. Yields and TTNs for Substrates

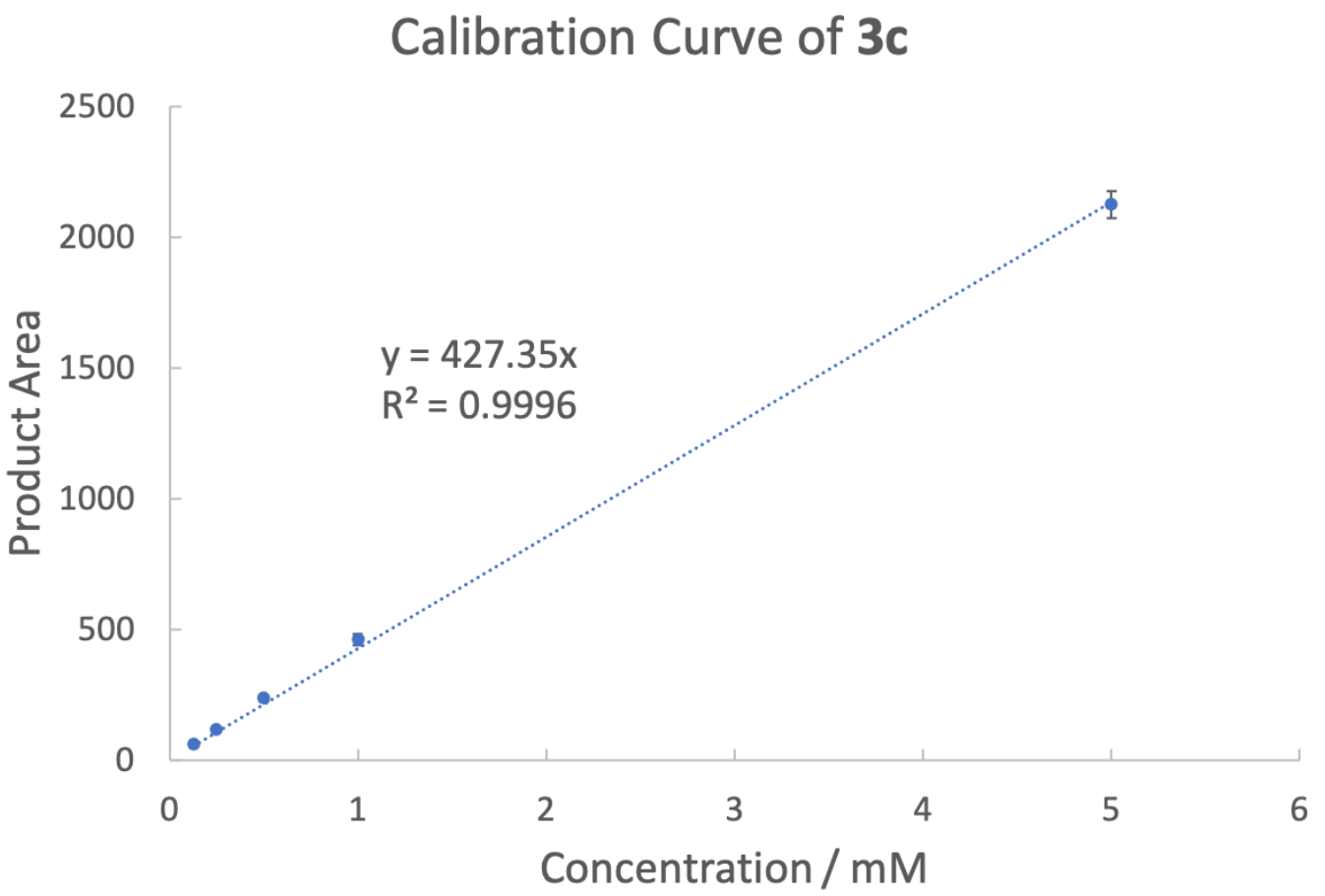
Based on total area for standard product (**3b**), obtained from GC-FID.



Variant	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	[Protein] / μM	TTN	Avg. TTN	Std Dev. TTN
P411-SIA-5291	529.3	1200.1	1199.2	2.8	3.6	333.3	333.1	1
	529.9	1201.5			3.6	333.7		
	527.5	1196.1			3.6	332.2		

Notes: Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; [Protein] = protein concentration in reaction; TTN = total turnover number; Avg. TTN = average of total turnover number; Std. Dev. TTN = standard deviation of total turnover number.

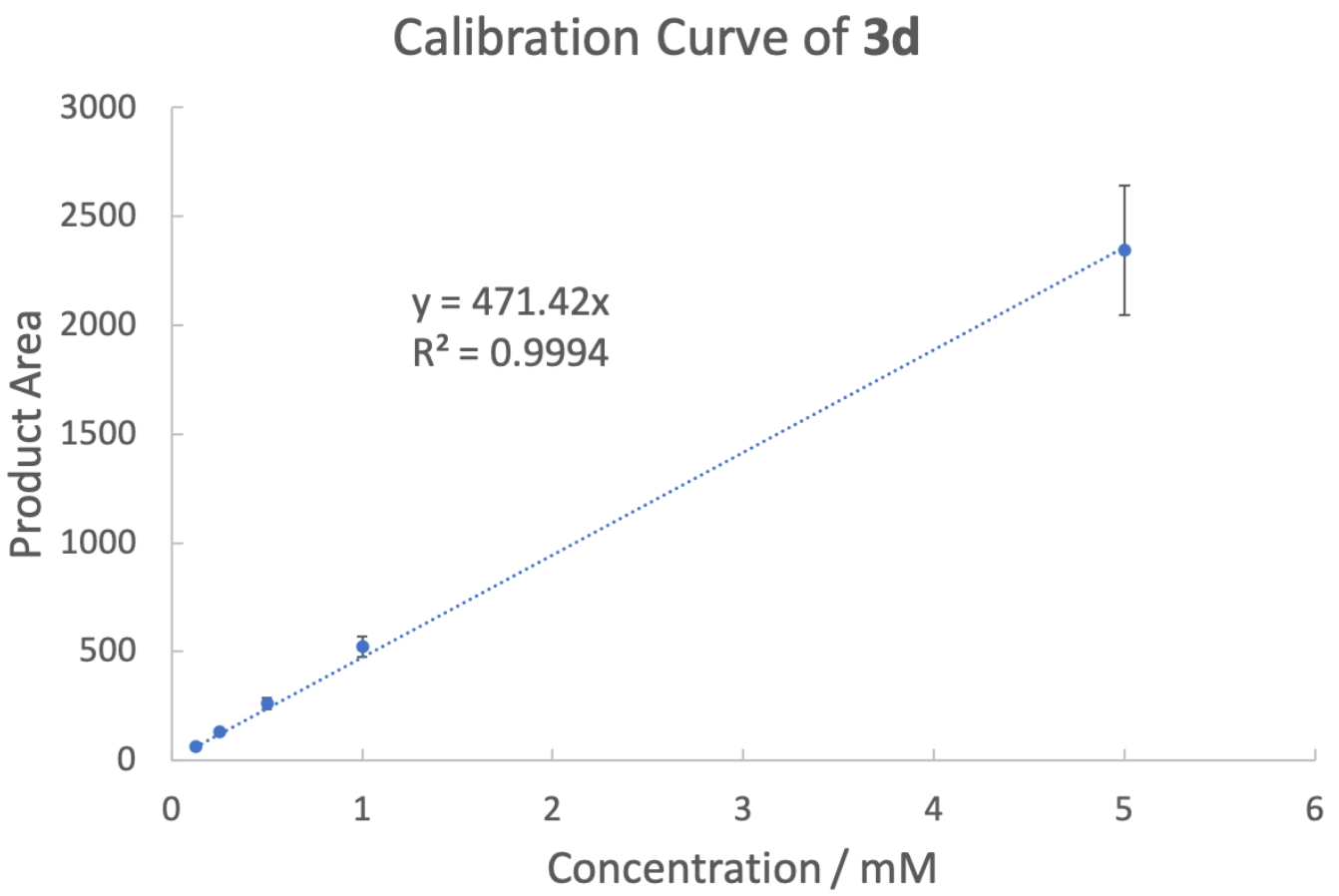
Based on total area for standard product (**3c**), obtained from GC-FID.



Variant	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	[Protein] / μM	TTN	Avg. TTN	Std Dev. TTN
P411-SIA-5291	255.7	598.3	600.4	1.9	3.6	166.2	166.8	1
	256.7	600.6			3.6	166.8		
	257.3	602.1			3.6	167.3		

Notes: Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; [Protein] = protein concentration in reaction; TTN = total turnover number; Avg. TTN = average of total turnover number; Std. Dev. TTN = standard deviation of total turnover number.

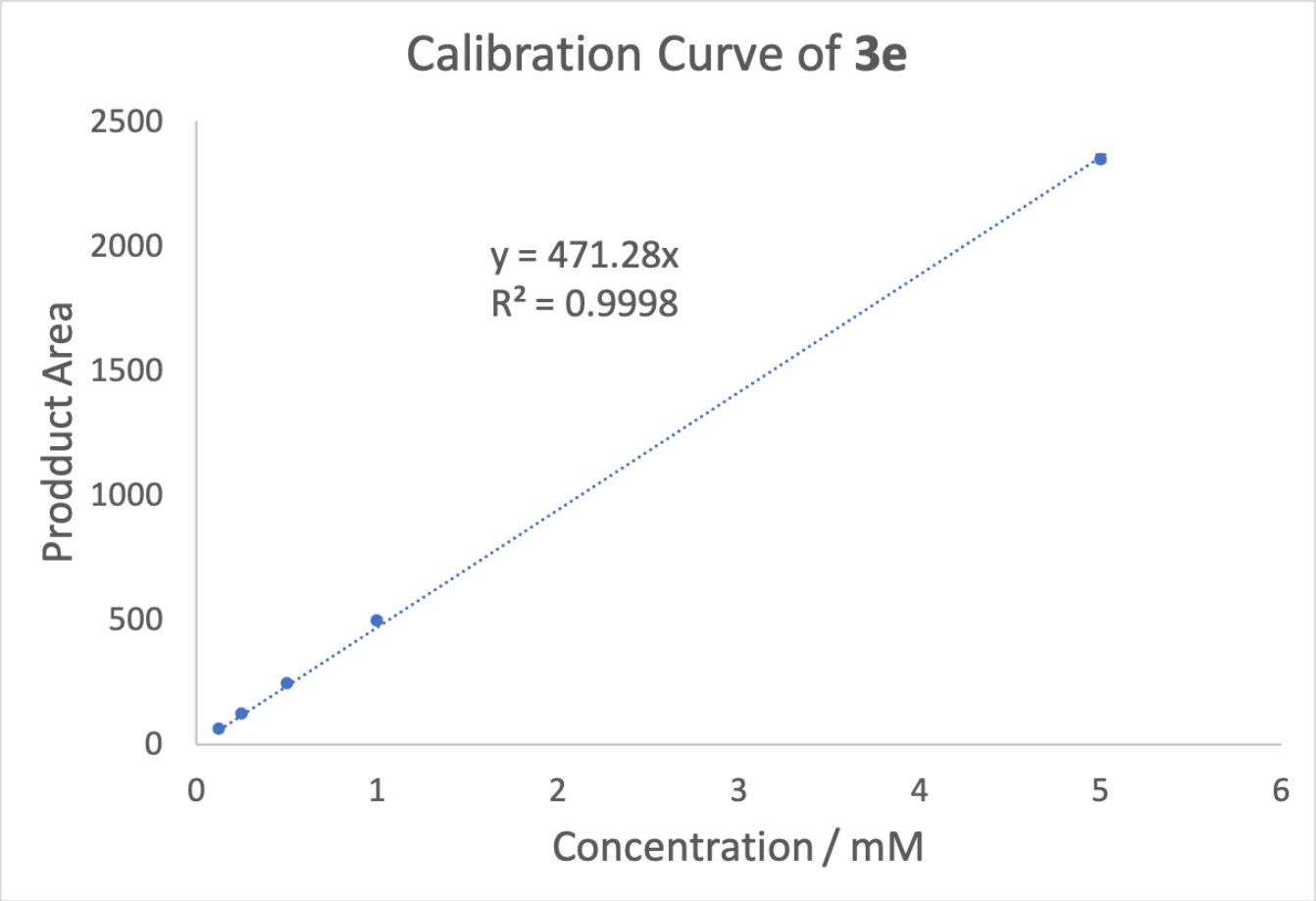
Based on total area for standard product (**3d**), obtained from GC-FID.



Variant	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	[Protein] / μM	TTN	Avg. TTN	Std Dev. TTN
P411-SIA-5291	253.1	536.9	532.9	8.1	3.6	149.1	148	2.3
	253.7	538.2			3.6	149.5		
	246.8	523.5			3.6	145.4		

Notes: Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; [Protein] = protein concentration in reaction; TTN = total turnover number; Avg. TTN = average of total turnover number; Std. Dev. TTN = standard deviation of total turnover number.

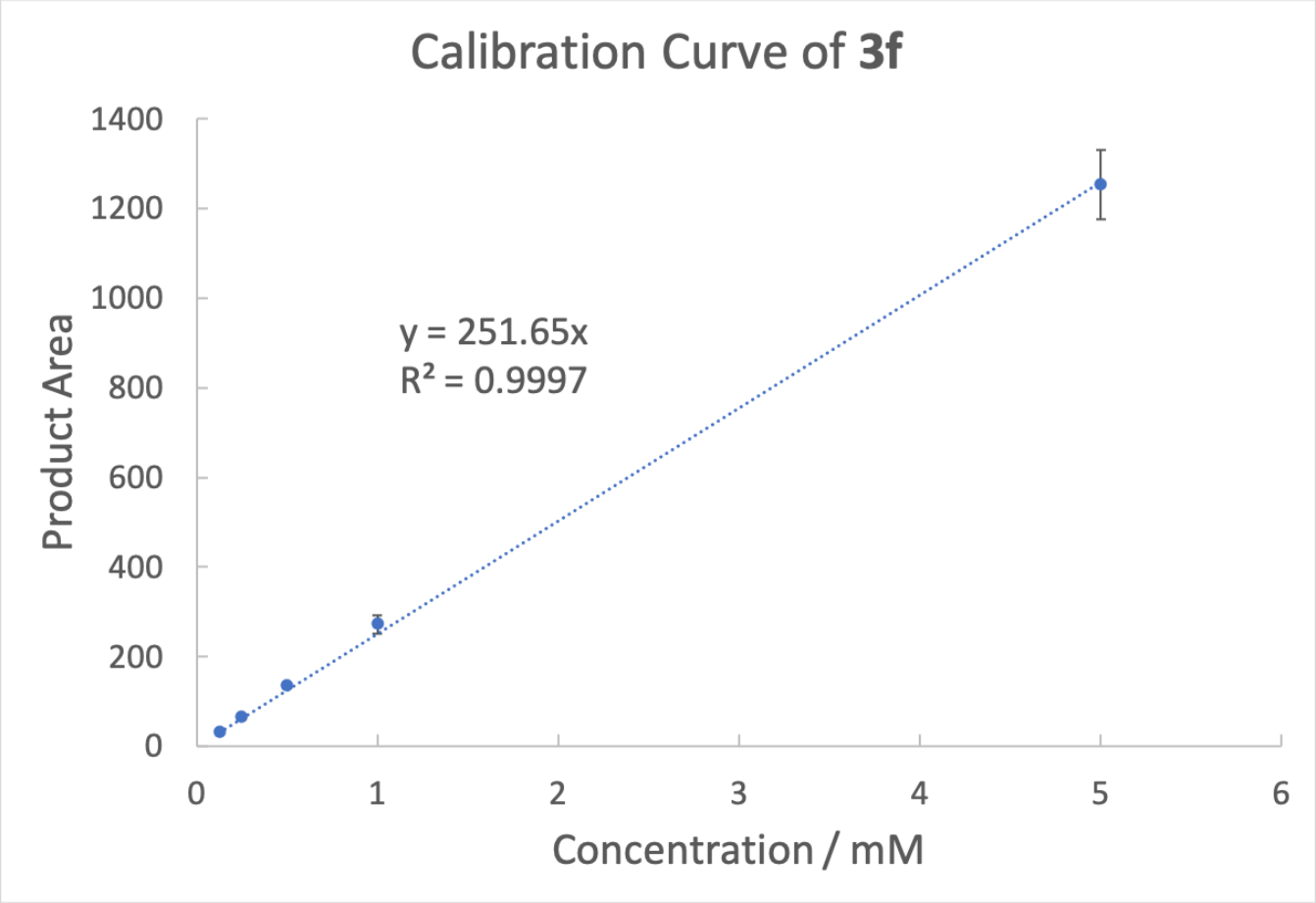
Based on total area for standard product (**3e**), obtained from GC-FID.



Variant	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	[Protein] / μM	TTN	Avg. TTN	Std Dev. TTN
P411-SIA-5291	110.7	234.9	234.5	0.4	3.6	65.2	65.1	0.1
	110.6	234.7			3.6	65.2		
	110.3	234.0			3.6	65.0		

Notes: Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; [Protein] = protein concentration in reaction; TTN = total turnover number; Avg. TTN = average of total turnover number; Std. Dev. TTN = standard deviation of total turnover number.

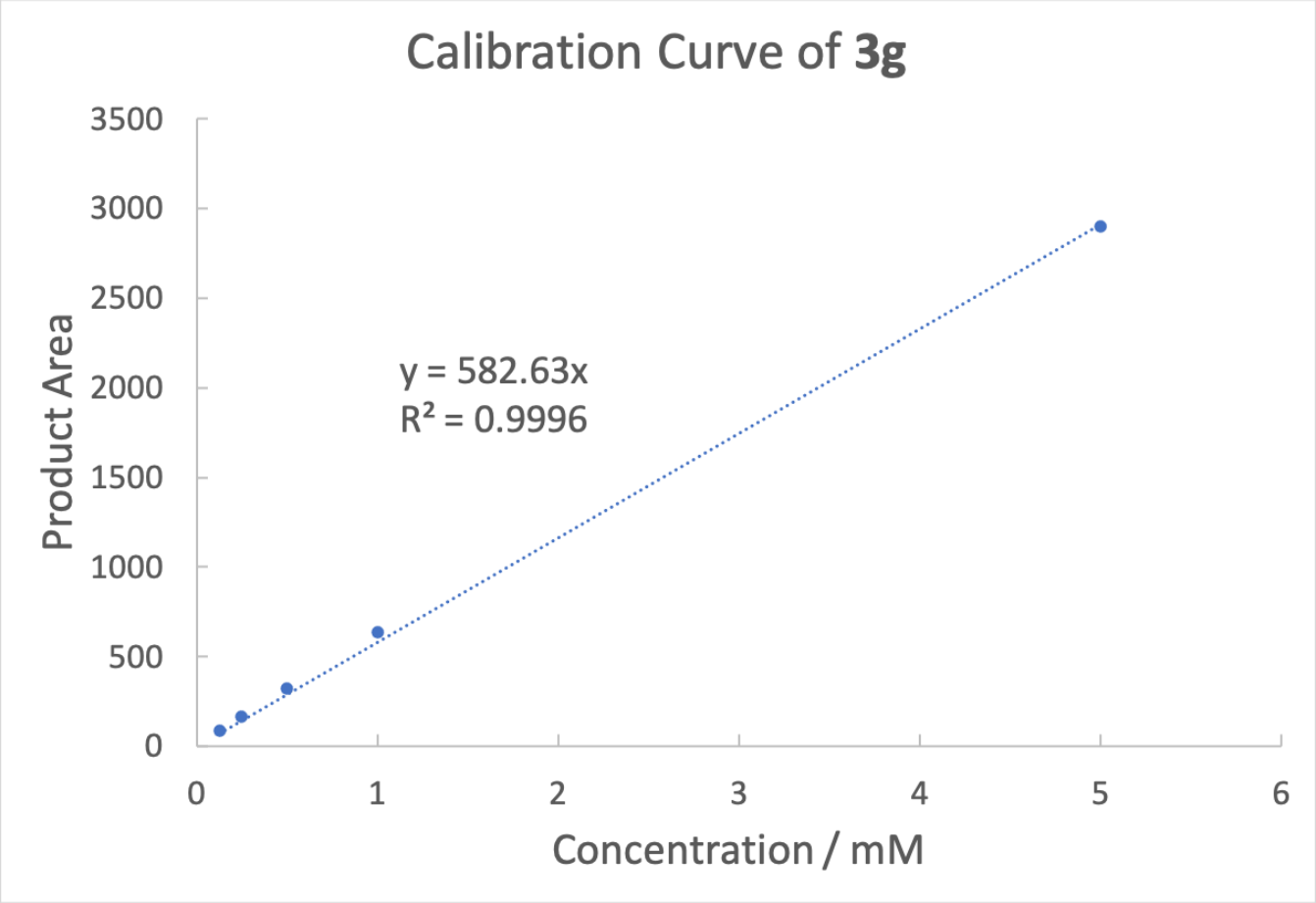
Based on total area for standard product (**3f**), obtained from GC-FID.



Variant	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	[Protein] / μM	TTN	Avg. TTN	Std Dev. TTN
P411-SIA-5291	165.1	656.1	659.5	3.2	3.6	182.2	183.2	1
	166.7	662.4			3.6	183.9		
	166.1	660			3.6	183.3		

Notes: Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; [Protein] = protein concentration in reaction; TTN = total turnover number; Avg. TTN = average of total turnover number; Std. Dev. TTN = standard deviation of total turnover number.

Based on total area for standard product (**3g**), obtained from GC-FID.



Variant	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	[Protein] / μM	TTN	Avg. TTN	Std Dev. TTN
P411-SIA-5291	313	537.2	535.7	10	3.6	149.2	148.8	2.8
	317.5	544.9			3.6	151.4		
	305.9	525			3.6	145.8		

Notes: Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; [Protein] = protein concentration in reaction; TTN = total turnover number; Avg. TTN = average of total turnover number; Std. Dev. TTN = standard deviation of total turnover number.

3.4.5. Optimization of the Enzymatic Reaction

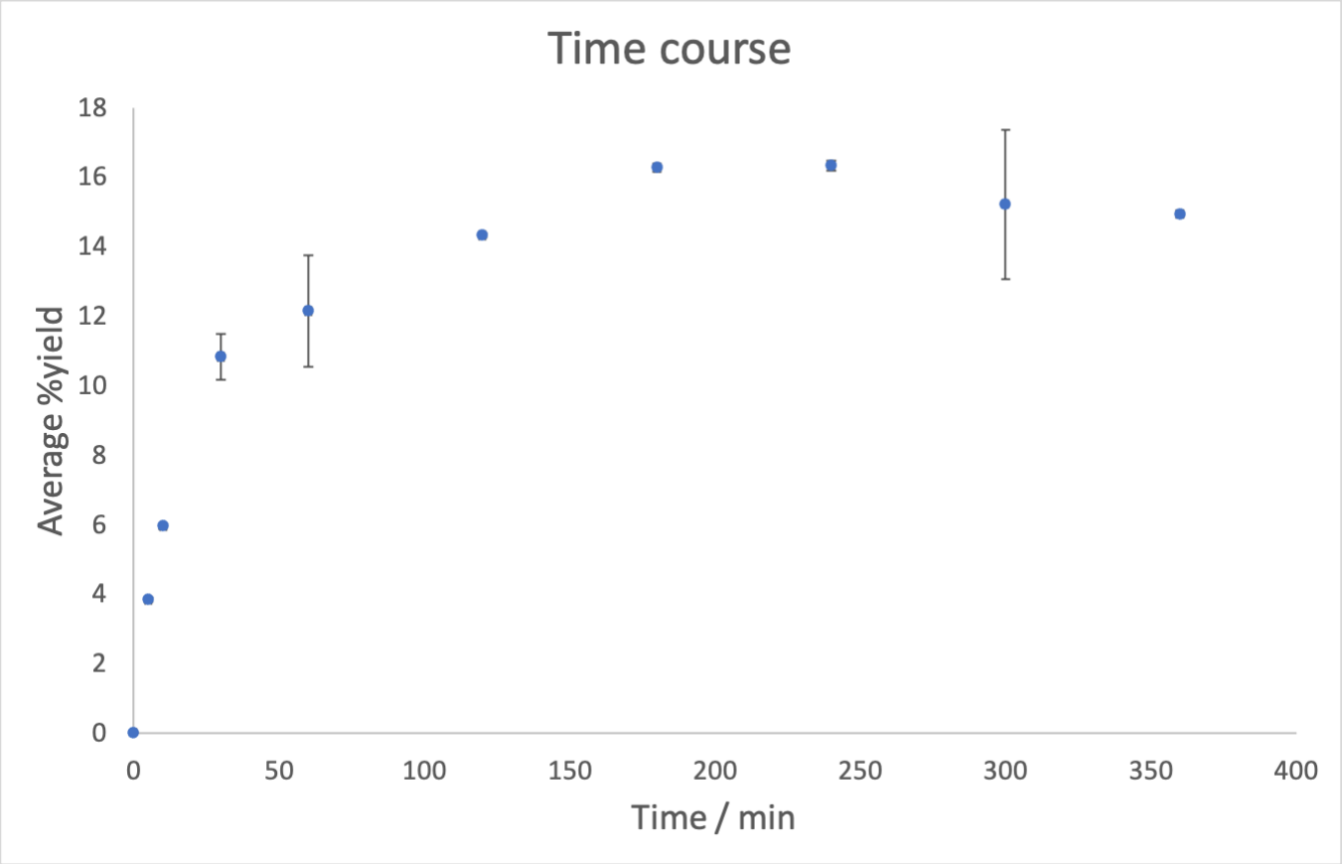


Figure S2. Monitoring the reaction over time shows that the reaction stops after 5 h. Reaction conditions are as follows: *P411-SIA-5289* variant in whole *E. coli* cells (OD_{600} = 40), 5.0 mM substrate **1a**, 20 mM nitrene precursor **2a**, 2.5 vol% 2-PrOH co-solvent in KPi (pH = 8.0) buffer, 800 μL total reaction volume at 23 °C under aerobic conditions for 6 h, each of the reaction time points were setup in duplicates.

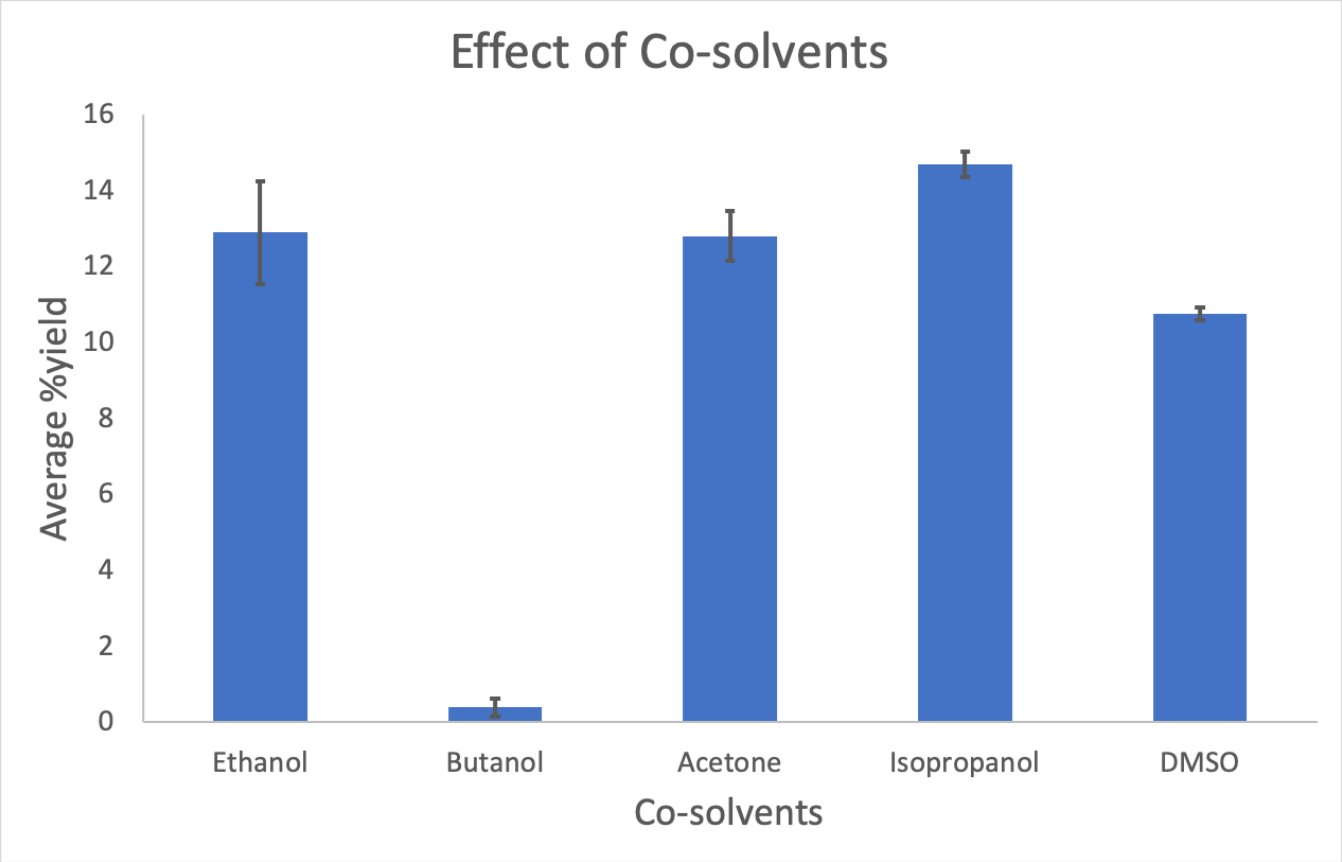


Figure S3. The activity of the enzymatic reaction is dependent on the co-solvent. Isopropanol is a slightly better co-solvent than ethanol. Reaction conditions are as follows: *P411-SIA-5289* variant in whole *E. coli* cells (OD_{600} = 40), 5.0 mM substrate **1a**, 20 mM nitrene precursor **2a**, 2.5 vol% co-solvent in Kpi (pH = 8.0) buffer, 800 µL total reaction volume at 23 °C under aerobic conditions for 6 h, each of the reaction time points were setup in duplicates.

Table S1. Varying the concentration and the stoichiometry of the organosilane substrate and nitrene precursor affects the enzymatic reaction. While **entry 4** gives the highest yield, **entry 8** gives the highest amount of product.

Entry	[Substrate] / mM	[Nitrene] / mM	Pdt Area	[Pdt] / μM	Avg. [Pdt] / μM	Std. Dev. [Pdt] / μM	%yield	Avg. %yield	Std. Dev. %yield
1	1.25	1.25	45.2	97.6	78.9	26.4	7.8	6.3	2.1
			27.9	60.2			4.8		
2	1.25	2.5	62.3	134.5	130.7	5.3	10.7	10.5	0.4
			58.8	126.9			10.2		
3	1.25	5.0	117.8	254.3	228.3	36.8	20.3	18.3	2.9
			93.7	202.3			16.2		
4	1.25	20	212.8	459.4	505.5	65.2	36.7	40.4	5.2
			255.5	551.6			44.1		
5	2.5	10	288.5	622.8	574.7	67.9	24.9	22.9	2.7
			244	526.7			21.1		
6	2.5	20	268.1	578.7	559.5	27.2	23.2	22.4	1.1
			250.3	540.3			21.6		
7	5	10	302.3	652.6	632.7	28.1	13.1	12.6	0.6
			283.9	612.9			12.3		
8	5	20	379.4	819	787.4	44.7	16.4	15.7	0.9
			350.1	755.8			15.1		
9	10	10	123	265.5	282.8	24.4	2.7	2.8	0.2
			139	300.1			3		
10	10	20	295	636.8	535.2	143.8	6.4	5.4	1.4
			200.8	433.4			4.3		

Notes: [Substrate] = organosilane substrate concentration in reaction; [Nitrene] = nitrene concentration in reaction; Pdt Area = integrated product area; [Pdt] = product concentration in reaction; Avg. [Pdt] = average of product concentration; Std. Dev. [Pdt] = standard deviation of product concentration; %yield = percent yield of the product; Avg. %yield = average yield; Std. Dev. %yield = standard deviation of the yield. Reaction conditions are as follows: *P411-SIA-5289* variant in whole *E. coli* cells (OD_{600} = 40), x mM substrate **1a**, x mM nitrene precursor **2a**, 2.5 vol% *2-PrOH* in Kpi (pH = 8.0) buffer, 800 μL total reaction volume at 23 °C under aerobic conditions for 6 h, each of the reaction time points were set up in duplicates

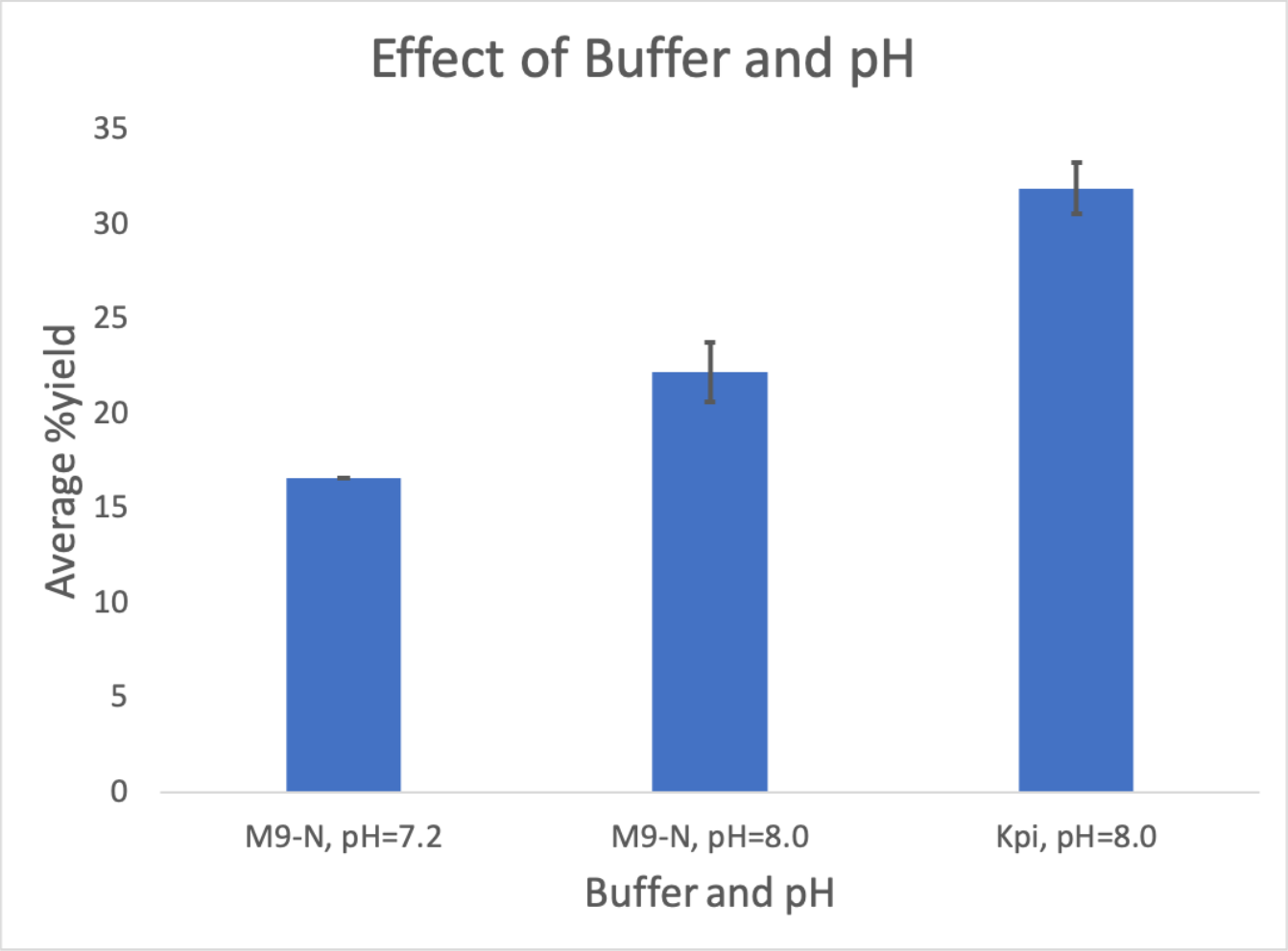


Figure S4. Varying the reaction buffer and the pH affects the enzymatic activity. Kpi (pH = 8.0) is the most suitable reaction buffer for the enzymatic reaction. Reaction conditions are as follows: *P411-SIA-5291* variant in whole *E. coli* cells (OD_{600} = 40), 5.0 mM substrate **1a**, 20 mM nitrene precursor **2a**, 2.5 vol% *2-PrOH* in buffer, 800 μL total reaction volume at 23 °C under aerobic conditions for 6 h, each of the reaction time points were setup in duplicates.

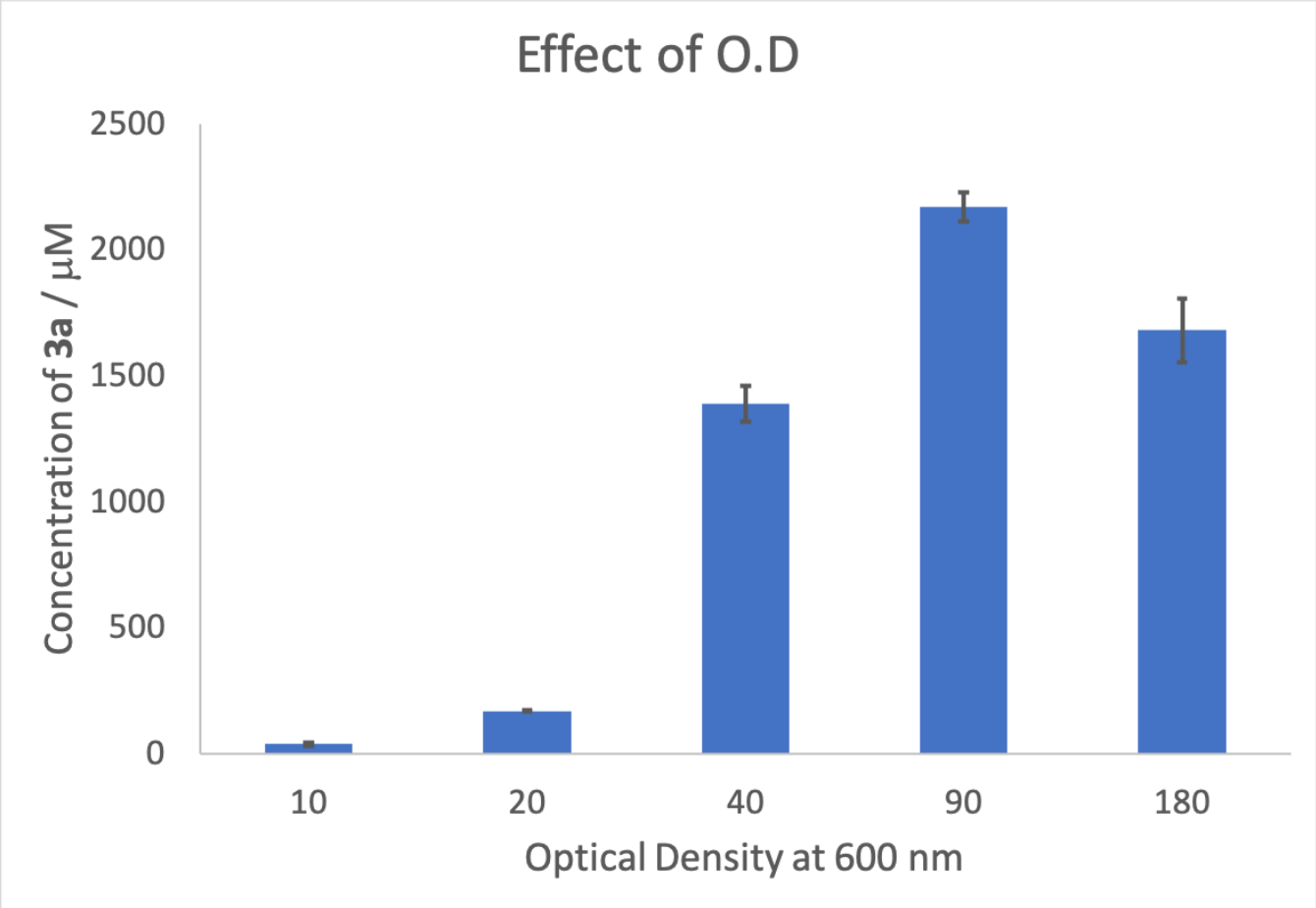


Figure S5. The enzymatic activity can be optimized by varying the OD_{600} (i.e. amount of cells containing the heme protein). OD_{600} = 90 gives the highest amount of product from the enzymatic reaction. Reaction conditions are as follows: *P411-SIA-5291* variant in whole *E. coli* cells (OD_{600} = x), 5.0 mM substrate **1a**, 20 mM nitrene precursor **2a**, 2.5 vol% 2-PrOH in Kpi (pH = 8.0), 800 µL total reaction volume at 23 °C under aerobic conditions for 6 h, each of the reaction time points were setup in triplicates.

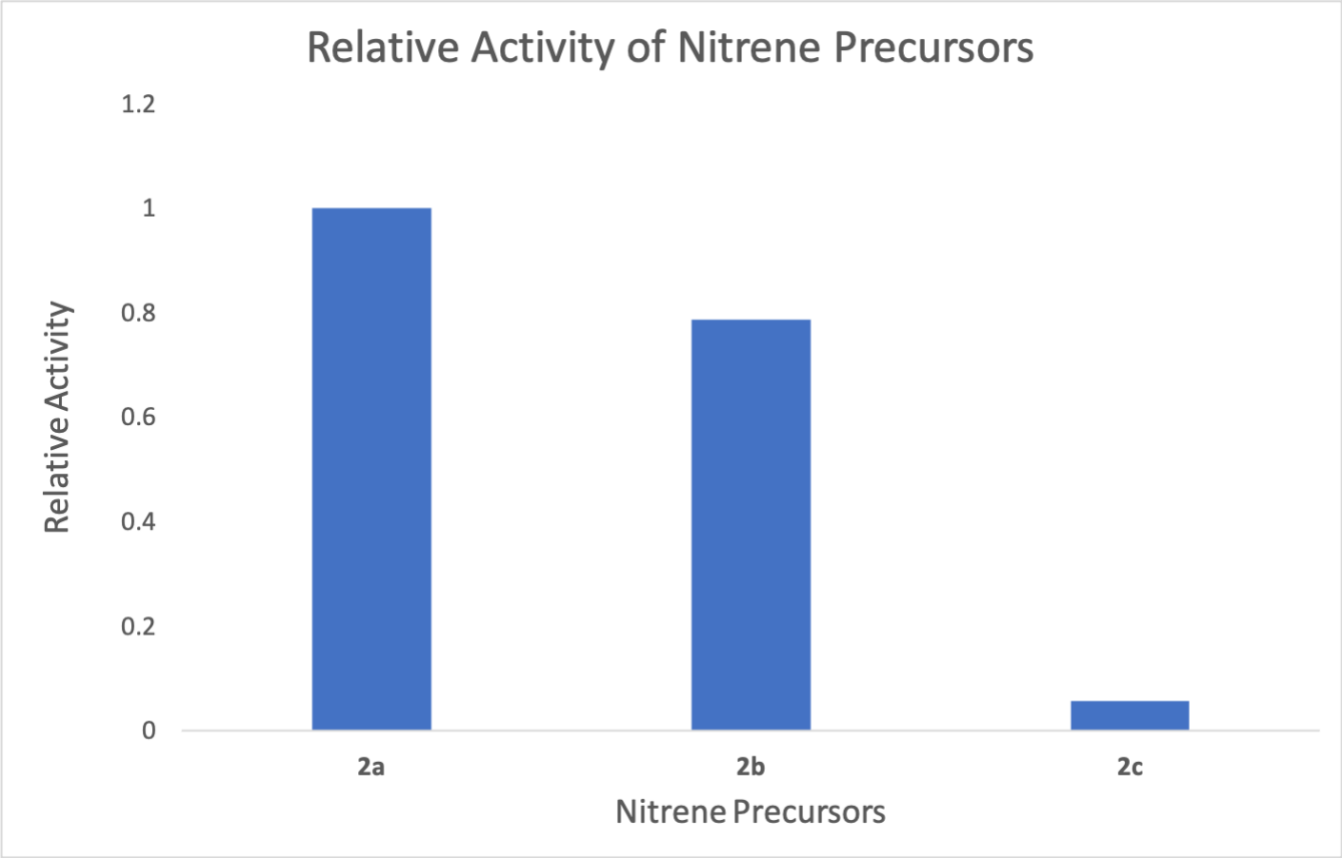
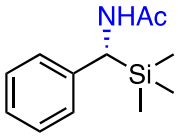


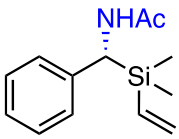
Figure S6. Comparison of the relative activity of different nitrene precursors generating **3a**. The *P411-SIA-5291* enzyme has a preference for **2a**, however, its promiscuity indicates that it can be evolved to work with other nitrene precursors as well.

3.4.6. Preparative-Scale Enzymatic Reaction

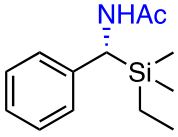
A single colony from *E. coli* BL21(DE3) cells, transformed with plasmid encoding the *P411-SIA-5291* variant was picked from an LB_{amp} agar plate using a sterile toothpick and grown in 50 mL of LB_{amp} in a 125-mL Erlenmeyer flask at 37 °C (at 220 rpm and 80% humidity) for 16–18 hours. The preculture (20 mL) was used to inoculate 1 L of TB_{amp} medium in 2.5-L Erlenmeyer flasks. The expression culture plate was then incubated at 37 °C (at 200 rpm and 80% humidity) for 3 hours (OD_{600} = 1.2–1.4) and then chilled on ice for 30 minutes. After that, 1 mL IPTG (0.5 mM final concentration) and 1 mL ALA (1.0 mM final concentration) were added, and the protein was expressed at 22 °C (at 160 rpm) for 22–24 h. Cells were then pelleted at 20 °C and 4,000*g* for 5 min. The cells were resuspended with KPi buffer (pH 8.0) containing 50 mM glucose (OD_{600} = 90). In a 250-mL screw cap Erlenmeyer flask, 195 mL of the resuspended cells were charged. A solution (5 mL) of the mixture of organosilane (1 mmol dissolved in 2-PrOH) and *N*-(pivaloyloxy)acetamide (4 mmol dissolved in 2-PrOH) were added. The flask was then shaken at 700 rpm under ambient conditions for 6 hours. After that, EtOAc (200 mL) was added to the reaction mixture and the resulting suspension was mixed by vortexing, and then centrifuged (14,000*g* for 15 min) to remove the cell debris. The organic phase was transferred, dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography to afford the corresponding enzymatic products.



***N*-(phenyl(trimethylsilyl)methyl)acetamide (3a):** The compound was purified by flash column chromatography (10% to 60% EtOAc/hexanes) as a white solid (1 mmol scale, 115 mg, 52% yield, >99% ee). ^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.28 (t, *J* = 6 Hz, 3H), 7.15 (t, *J* = 8 Hz, 1H), 7.08 (d, *J* = 8 Hz, 2H), 6.00 (br, 1H), 4.63 (d, *J* = 8 Hz, 1H), 2.04 (s, 3H), 0.02 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 169.9, 141.4, 128.5, 126.1, 125.8, 46.8, 23.4, 3.2. HRMS: 221.1236 (*calc.*), 221.1235 (*expt.*).

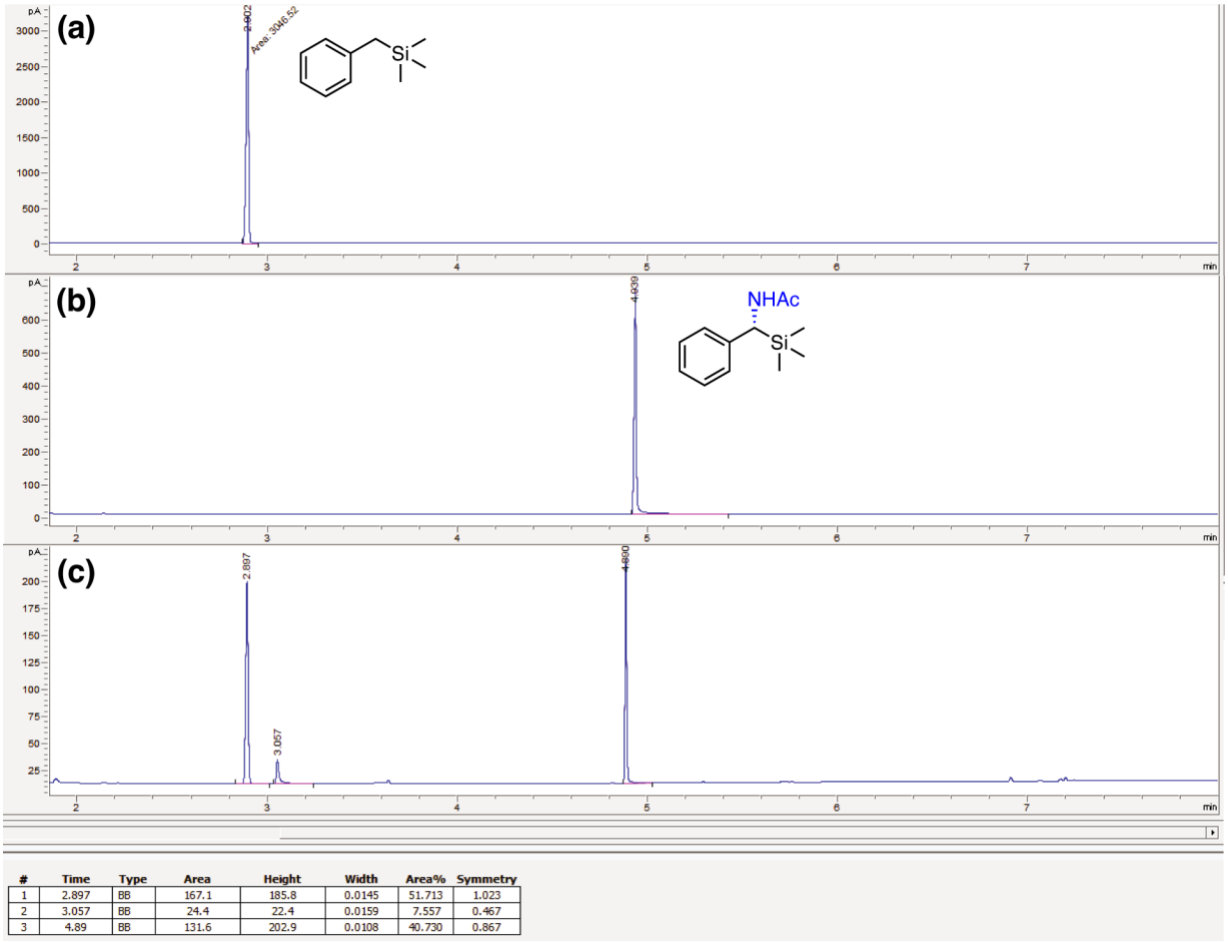


***N*-((dimethylvinylsilyl)(phenyl)methyl)acetamide (3f):** The compound was purified by preparative TLC (40% EtOAc/hexanes) as a white solid (0.1 mmol scale, 7.5 mg, 32% yield). ^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.26 (t, *J* = 8 Hz, 3H), 7.16 (d, *J* = 4 Hz, 1H), 7.09 (d, *J* = 4 Hz, 2H), 6.09–6.06 (m, 2H), 5.77–5.71 (m, 1H), 4.67 (d, *J* = 8 Hz, 1H), 2.07 (s, 3H), 0.10 (d, *J* = 16 Hz, 6H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 169.7, 141.2, 135.4, 134.7, 128.5, 126.2, 125.9, 45.8, 38.5, 27.1, 23.5, 19.9, 4.9. HRMS: 233.1236 (*calc.*), 233.1232 (*expt.*).



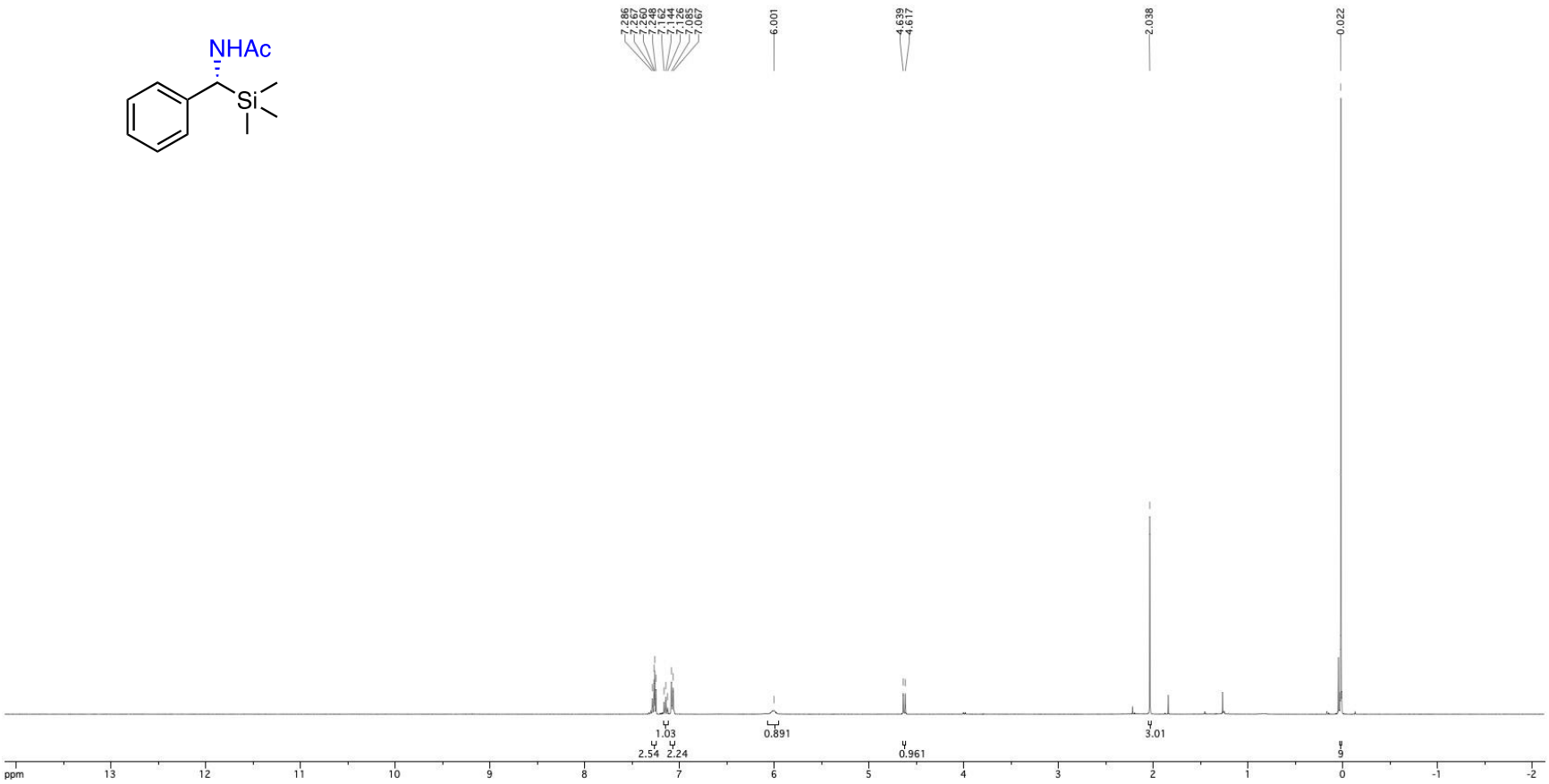
***N*-((ethyldimethylsilyl)(phenyl)methyl)acetamide (3g):** The compound was purified by preparative TLC (40% EtOAc/hexanes) as a light yellow oil that solidified upon cooling (0.1 mmol scale, 5.8 mg, 25% yield, >99% ee). ^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.28-7.24 (m, 3H), 7.16-7.11 (m, 3H), 4.68 (d, *J* = 8Hz, 1H), 2.12 (s, 3H), 0.89 (t, *J* = 8 Hz, 3H), 0.54 (t,

J = 8 Hz, 2H), 0.02 (d, *J* = 16 Hz, 6H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 169.7, 141.6, 128.5, 126.1, 125.8, 45.8, 38.4, 27.3, 23.6, 19.9, 7.3, 5.3, -5.4, -5.5. HRMS: 235.1392 (*calc.*), 235.1394 (*expt.*).

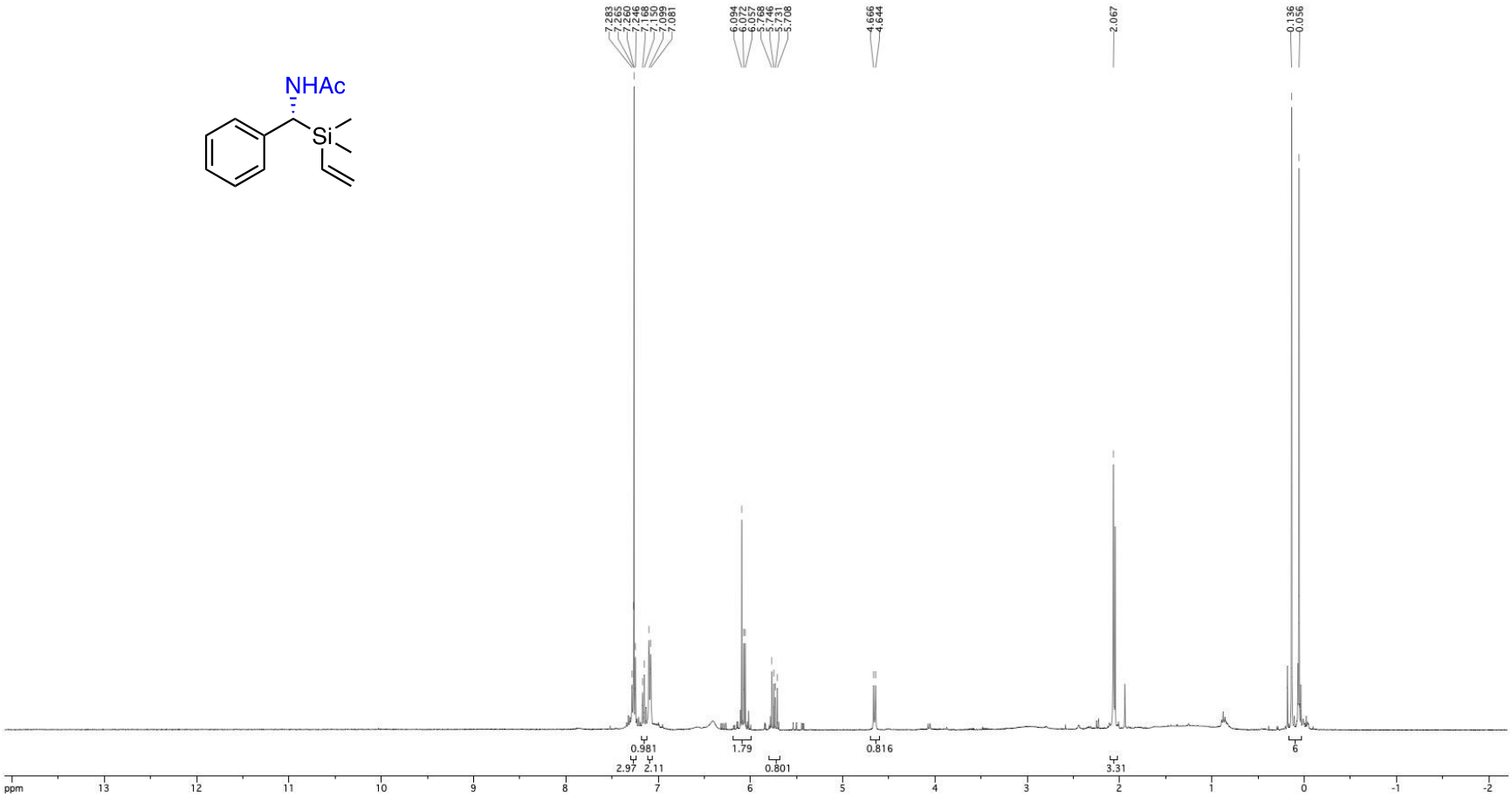


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	2.897	BB	167.1	185.8	0.0145	51.713	1.023
2	3.057	BB	24.4	22.4	0.0159	7.557	0.467
3	4.89	BB	131.6	202.9	0.0108	40.730	0.867

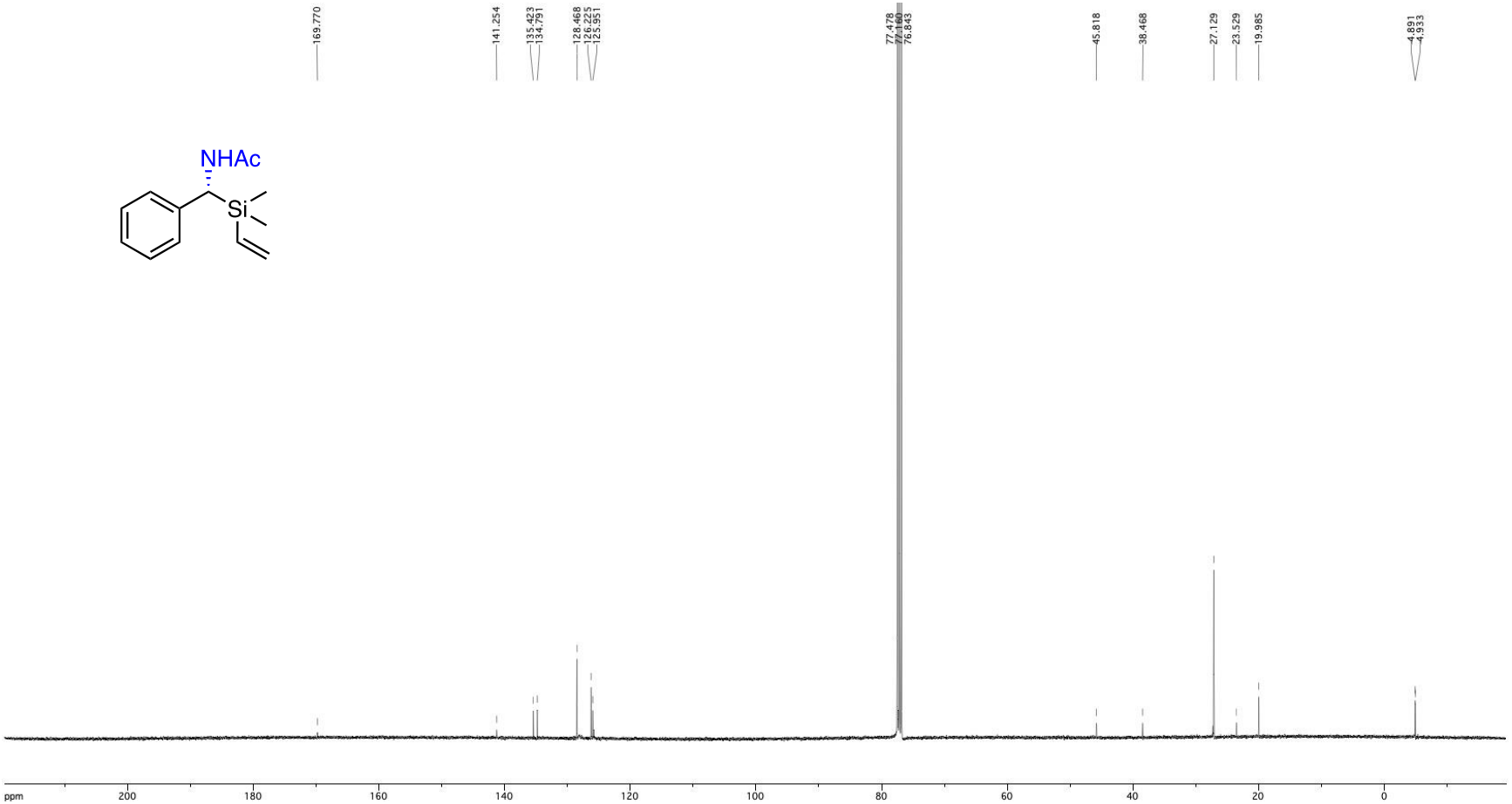
Figure S7. Comparison of the GC-FID traces of the preparative-scale reaction of benzyltrimethylsilane (**1a**) with *N*-(pivaloyloxy)acetamide (**2a**) to form *N*-(phenyl(trimethylsilyl)methyl)acetamide (**3a**). **(a)** GC-FID trace of unreacted **1a** after flash column chromatographic purification. **(b)** GC-FID trace of the product **3a** after flash column chromatographic purification. **(c)** GC-FID trace of the crude reaction mixture. The peak at 3.057 min corresponds to unreacted **2a**.



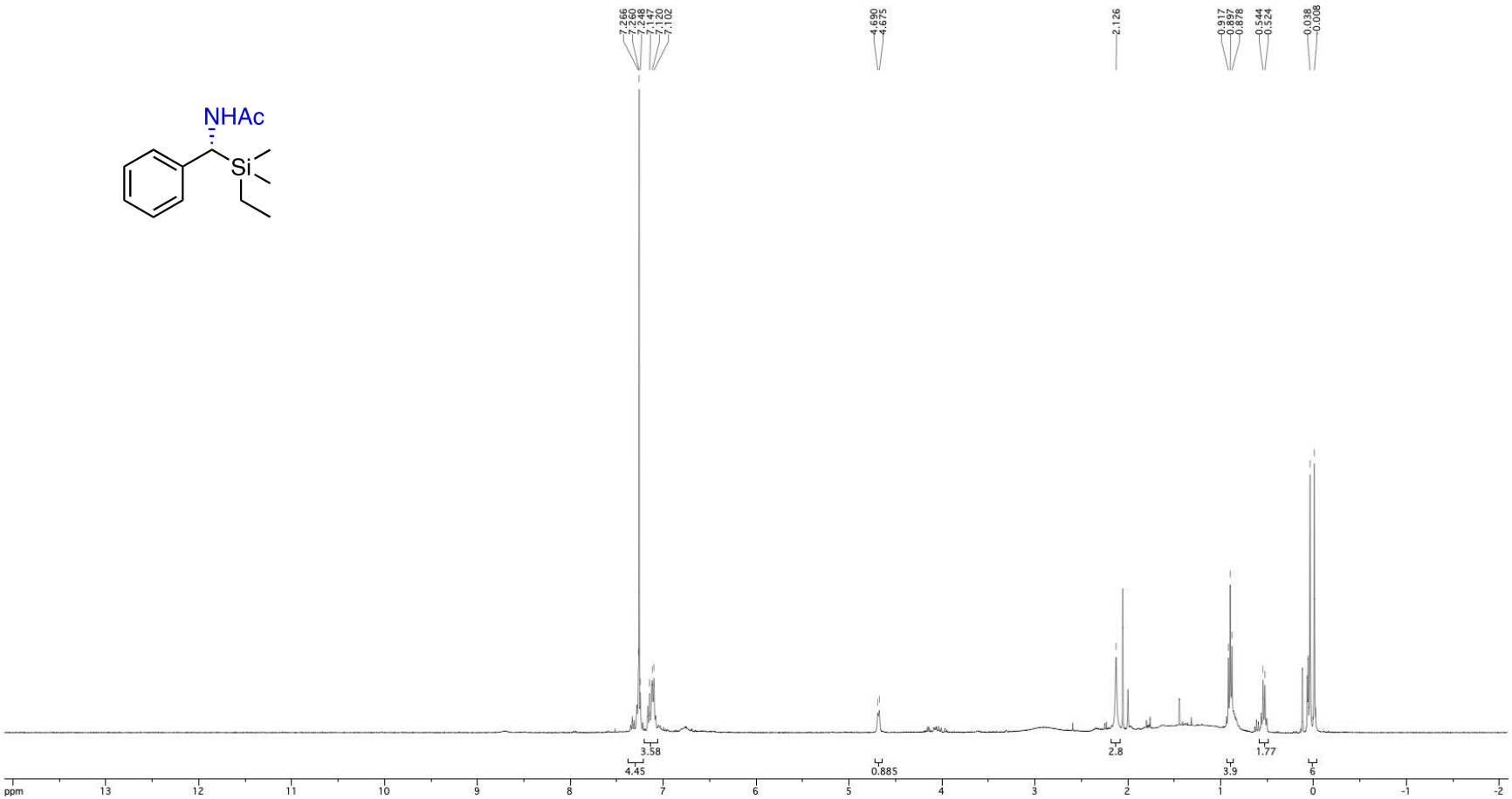
^{1}H-NMR spectrum of *N*-(phenyl(trimethylsilyl)methyl)acetamide **3a** enzymatic product obtained in $CDCl_3$ at 23 °C.



^{1}H-NMR spectrum of *N*-((dimethylvinylsilyl)(phenyl)methyl)acetamide **3f** enzymatic product obtained in $CDCl_3$ at 23 °C.



^{13}C-NMR spectrum of *N*-((dimethylvinylsilyl)(phenyl)methyl)acetamide **3f** enzymatic product obtained in $CDCl_3$ at 23 °C.



^{1}H-NMR spectrum of *N*-((ethyldimethylsilyl)(phenyl)methyl)acetamide **3g** enzymatic product obtained in $CDCl_3$ at 23 °C.

4. Selectivity Details for Enzymatic Products

A single colony from *E. coli* BL21(DE3) cells, transformed with plasmid encoding the *P411-SIA-5291* variant was picked from an LB_{amp} agar plate using a sterile toothpick and grown in 5 mL of LB_{amp} in a 12-mL sterile tube at 37 °C (at 220 rpm and 80% humidity) for 16–18 hours. The preculture (1 mL) was used to inoculate 50 mL of TB_{amp} medium in 125-mL Erlenmeyer flasks. The expression culture plate was then incubated at 37 °C (at 230 rpm and 80% humidity) for 3 hours (OD_{600} = 1.2–1.4) and then chilled on ice for 30 minutes. After that, 50 μL IPTG (0.5 mM final concentration) and 50 μL ALA (1.0 mM final concentration) were added, and the proteins were expressed at 22 °C (at 160 rpm) for 22–24 h. Cells were then pelleted at 20 °C and 4,000*g* for 5 min. The cells were resuspended with Kpi buffer (pH 8.0) containing 50 mM glucose (OD_{600} = 40). In 7-mL screw cap vials, 3.9 mL of the resuspended cells were charged. A stock solution (100 μL) of the mixture of organosilane (200 mM dissolved in isopropanol) and *N*-(pivaloyloxy)acetamide (800 mM dissolved in isopropanol) was added to the vial. The vial was shaken at 700 rpm under ambient conditions for 6 hours.

Work-up for chiral SFC Analysis: To the reaction mixture was added 1:1 EtOAc/hexane (4.0 mL/ vial), the resulting suspension was mixed by vortexing and then centrifuged (14,000*g* for 15 min) to remove the cell debris. The organic layer was transferred into another 7-mL screw cap vial and the solvent was removed under reduced pressure. The mixture was dissolved in 1 mL 2-PrOH and the enantioselectivity was determined by chiral SFC methods based on the corresponding racemic reference compounds.

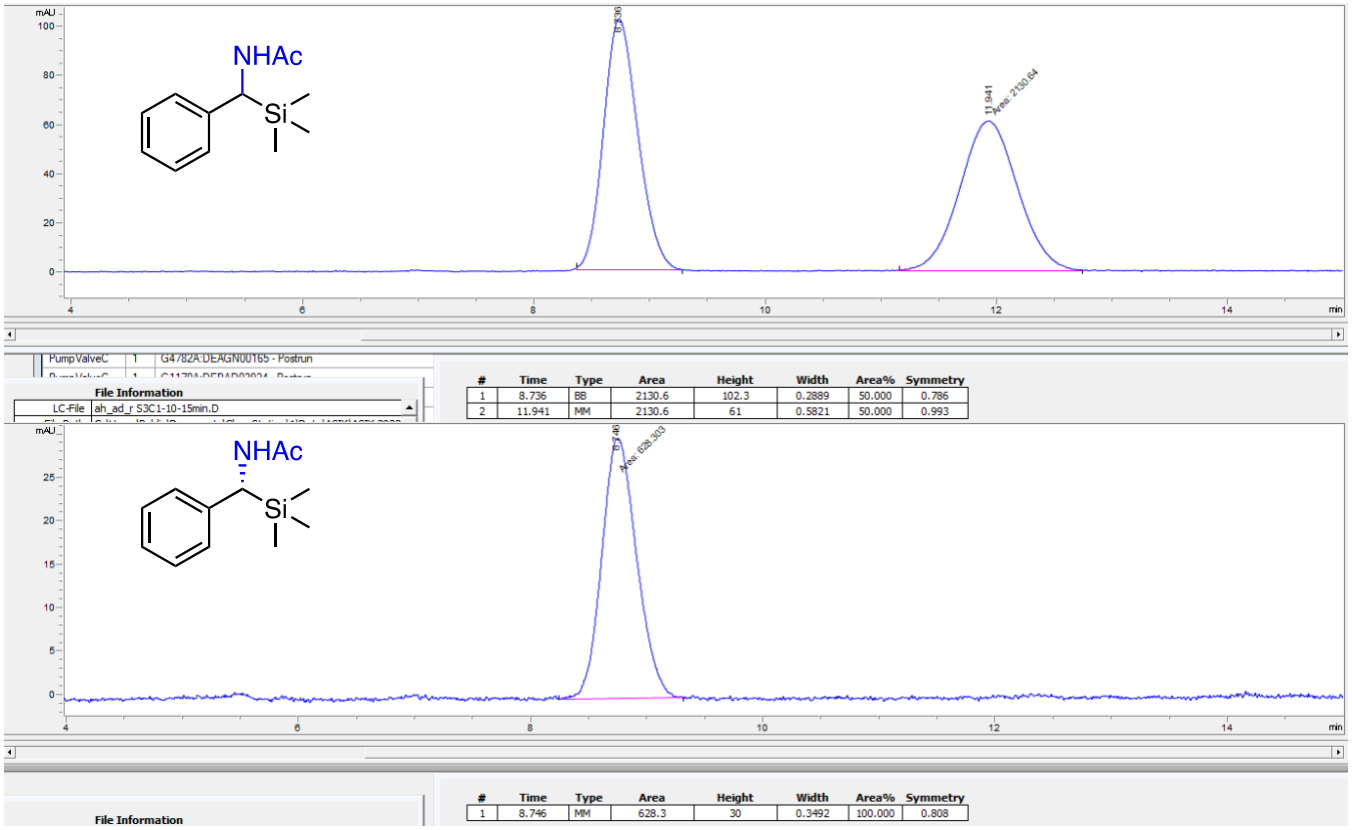


Figure S8. Comparison of chiral SFC traces of racemic **3a** (top) with the enzymatic **3a** (bottom). The enzymatic reaction has >99% *ee*. Determination of the absolute stereochemistry from single crystal X-ray crystallography reveals that it the (*R*)-enantiomer. The *ee* was determined on a CHIRALPAK IC-3 column (10% 2-PrOH in supercritical CO_2 at 2.5 mL/min). SFC peaks: 8.736 and 11.941 (*racemic*), 8.746 (*enzymatic*).

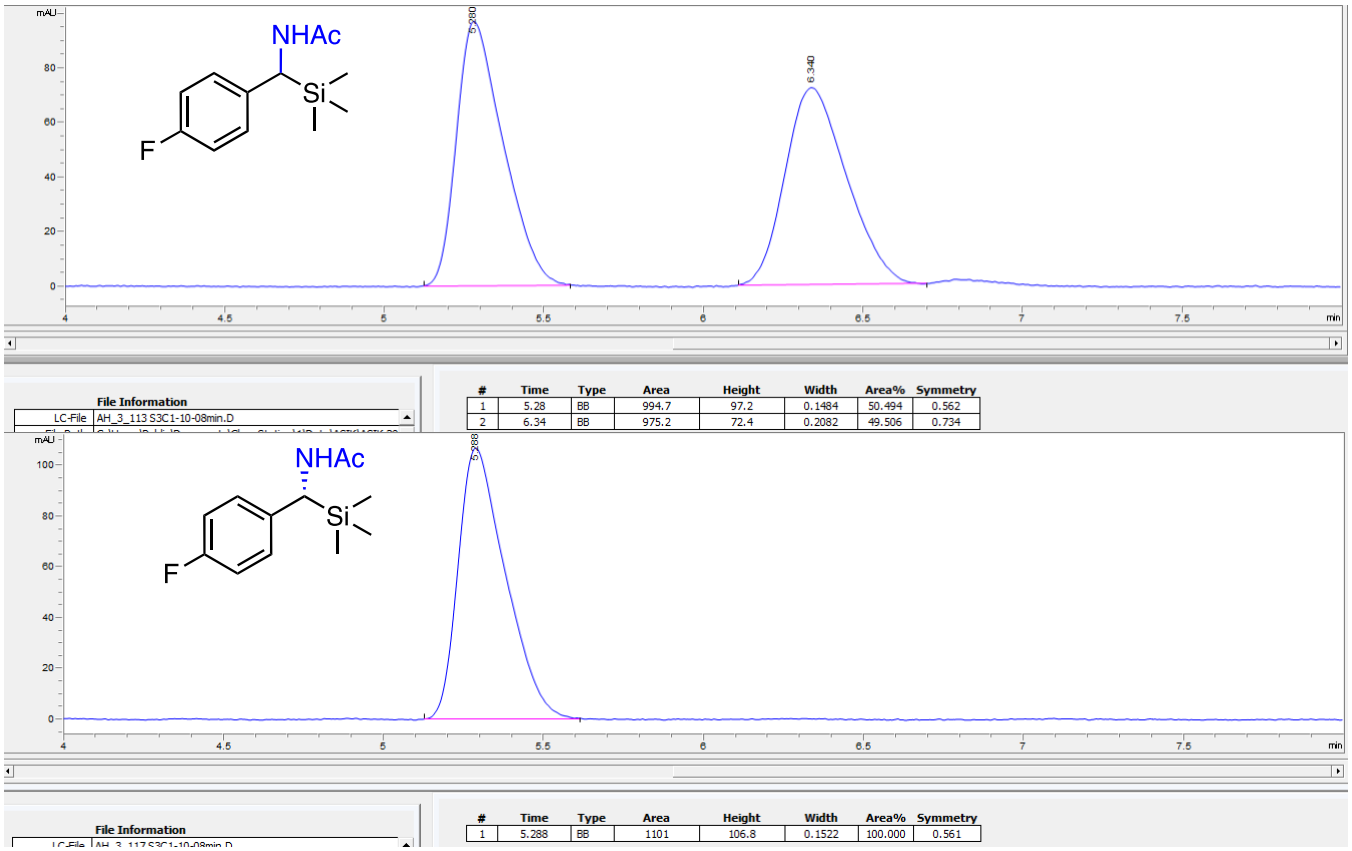


Figure S9. Comparison of chiral SFC traces of racemic **3b** (top) with the enzymatic **3b** (bottom). The enzymatic reaction has >99% *ee*. The *ee* was determined on a CHIRALPAK IC-3 column (10% 2-PrOH in supercritical CO_2 at 2.5 mL/min). SFC peaks: 5.280 and 6.340 (*racemic*), 5.288 (*enzymatic*).

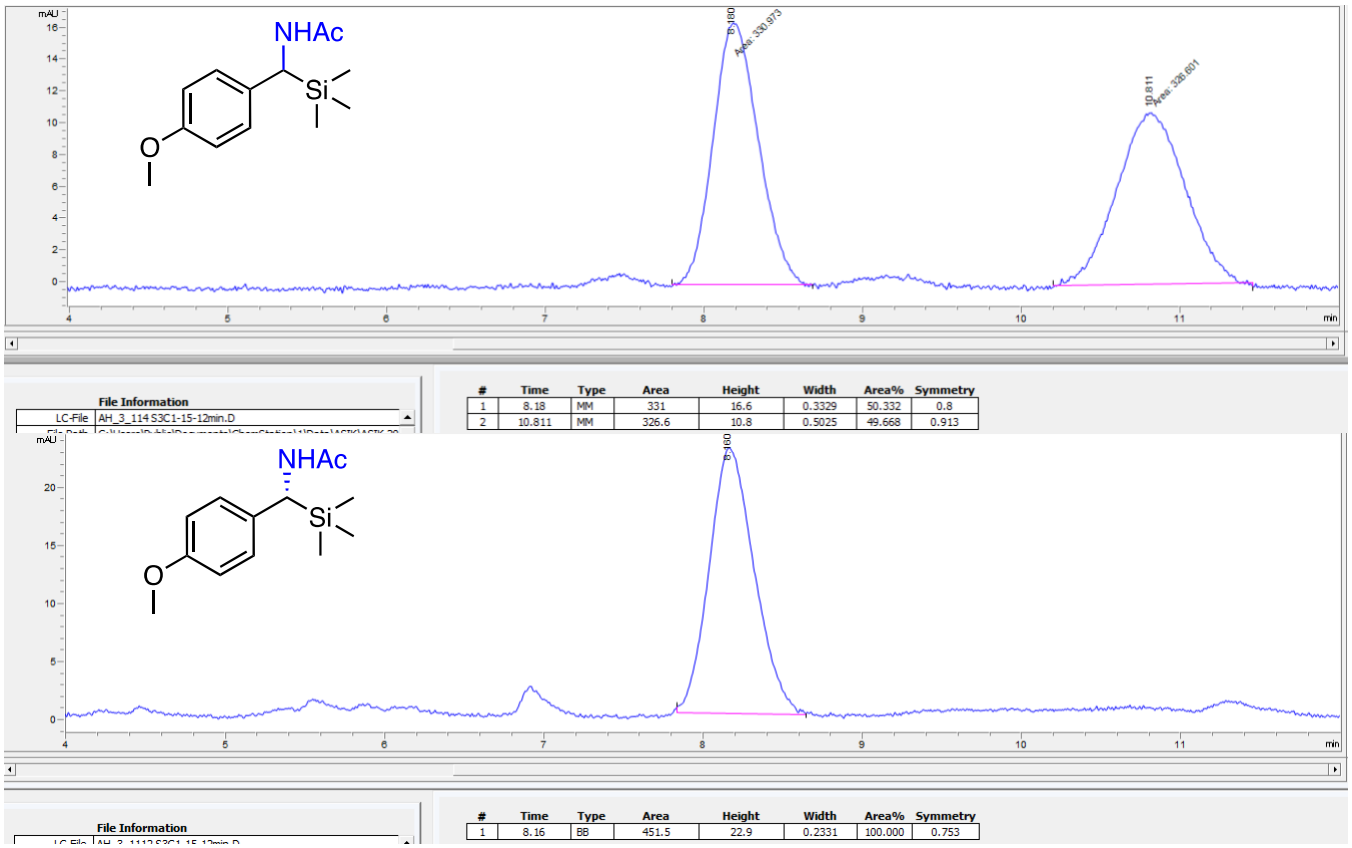


Figure S10. Comparison of chiral SFC traces of racemic **3c** (top) with the enzymatic **3c** (bottom). The enzymatic reaction has >99% *ee*. The *ee* was determined on a CHIRALPAK IC-3 column (15% 2-PrOH in supercritical CO_2 at 2.5 mL/min). SFC peaks: 8.180 and 10.811 (*racemic*), 8.160 (*enzymatic*).

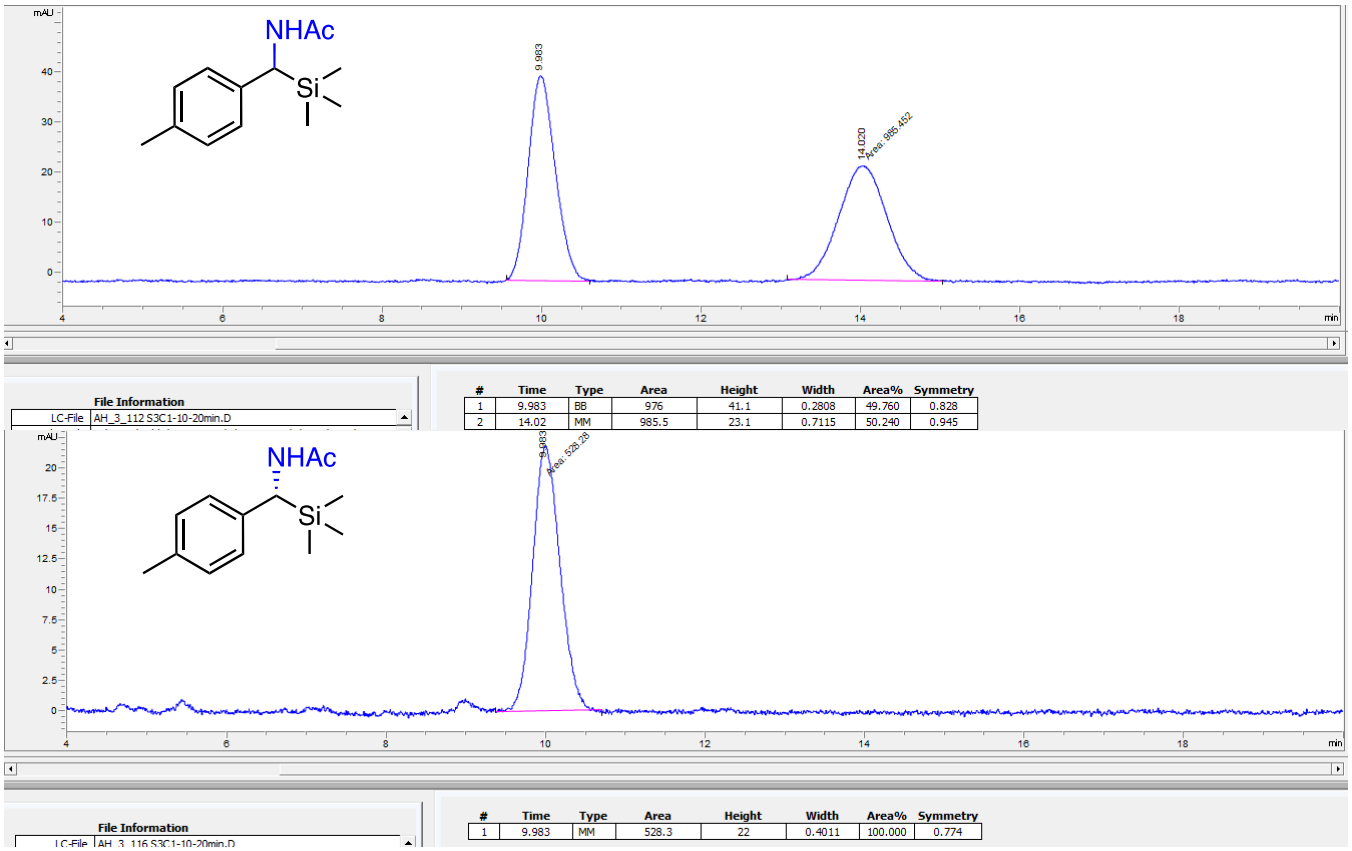


Figure S11. Comparison of chiral SFC traces of racemic **3d** (top) with the enzymatic **3d** (bottom). The enzymatic reaction has >99% *ee*. The *ee* was determined on a CHIRALPAK IC-3 column (10% 2-PrOH in supercritical CO_2 at 2.5 mL/min). SFC peaks: 9.983 and 14.020 (*racemic*), 9.983 (*enzymatic*).

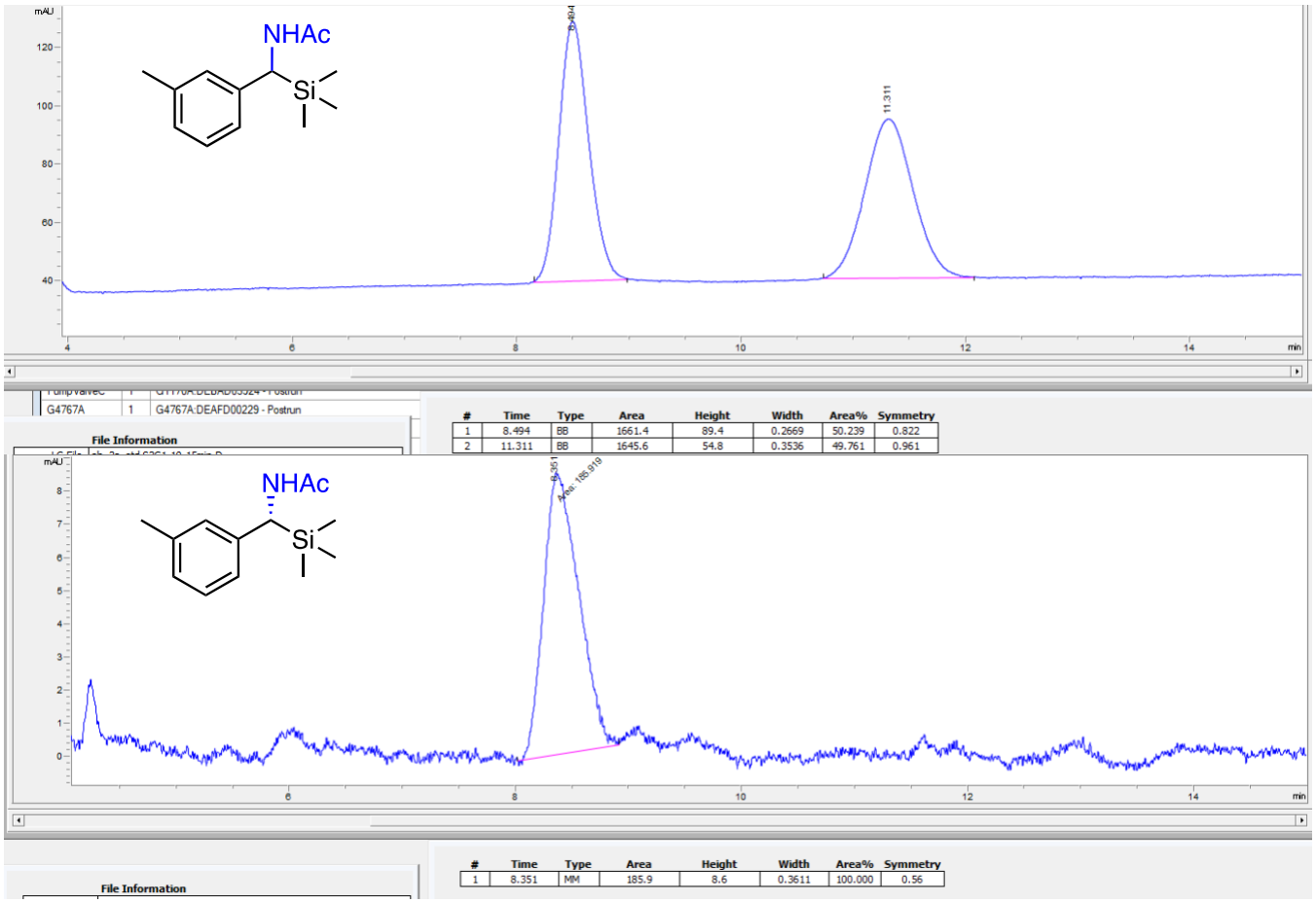


Figure S12. Chiral SFC traces of racemic **3e** (top) with the enzymatic **3e** (bottom). The enzymatic reaction has >99% *ee*. The *ee* was determined on a CHIRALPAK IC-3 column (10% 2-PrOH in supercritical CO_2 at 2.5 mL/min). SFC peaks: 8.494 and 11.311 (*racemic*), 8.351 (*enzymatic*).

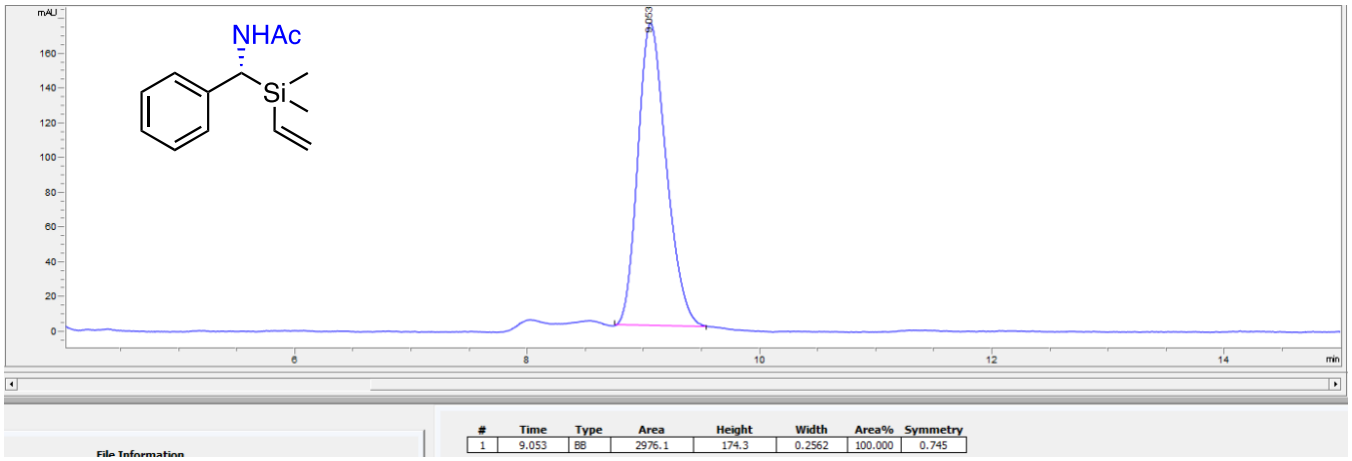


Figure S13. Chiral SFC trace of the purified **3f** obtained from the preparative scale enzymatic reaction. The *ee* could not be determined as the racemic standard could not be synthesized. The sample was measured using a CHIRALPAK IC-3 column (10% 2-PrOH in supercritical CO_2 at 2.5 mL/min). SFC peaks: 9.053 (*enzymatic*).

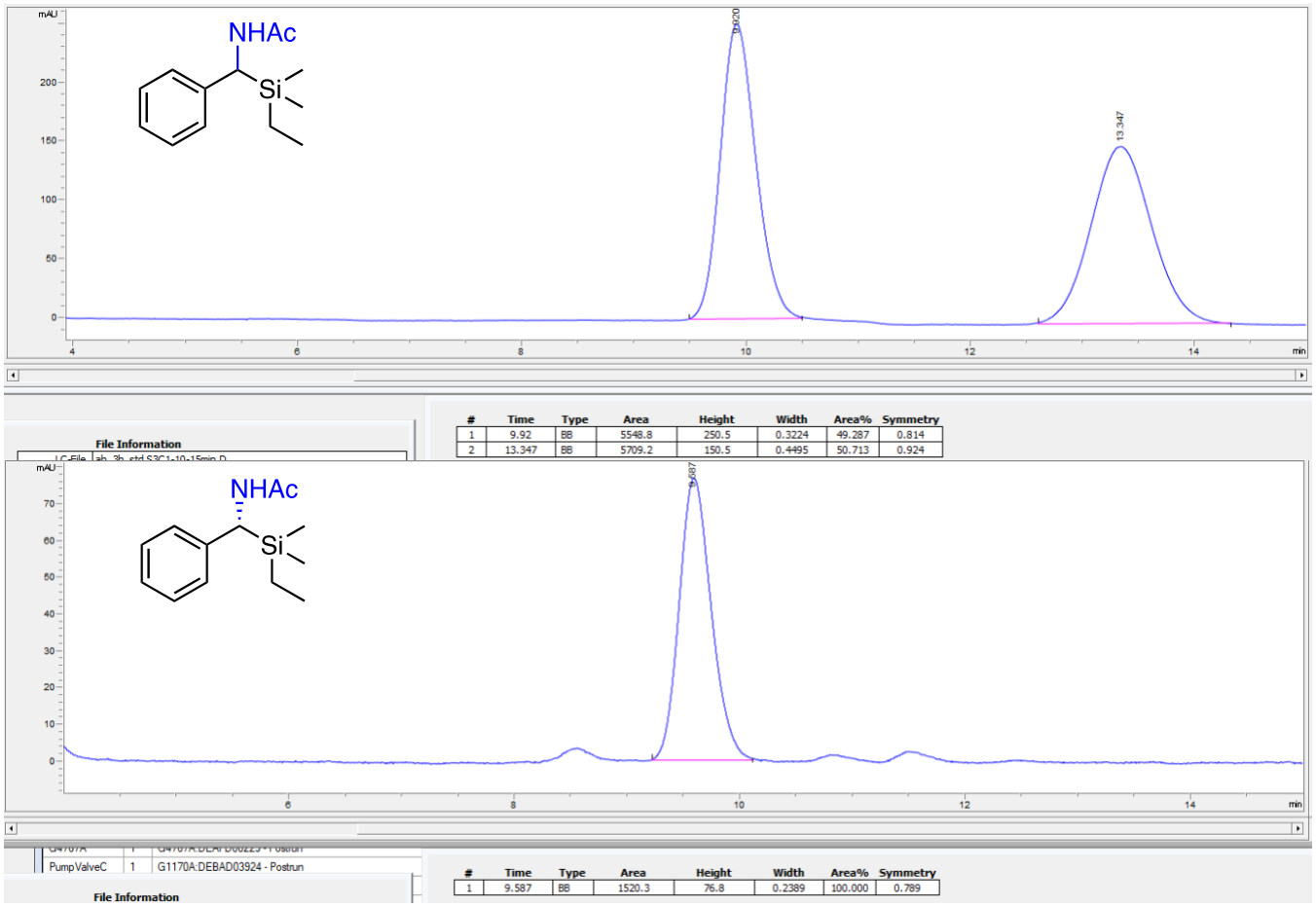
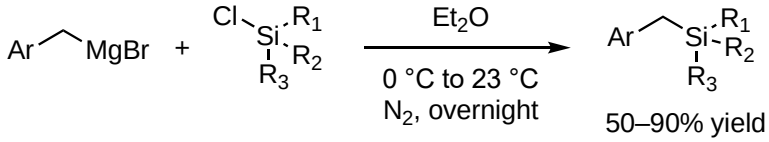


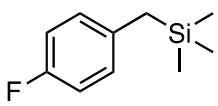
Figure S14. Comparison of chiral SFC traces of racemic **3g** (top) with the enzymatic **3g** (bottom). The enzymatic reaction has >99% *ee*. The *ee* was determined on a CHIRALPAK IC-3 column (10% 2-PrOH in supercritical CO_2 at 2.5 mL/min). SFC peaks: 9.920 and 13.347 (*racemic*), 9.587 (*enzymatic*).

5. Synthesis and Characterization of Compounds

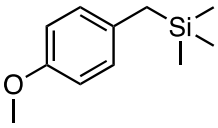
5.1. Synthesis of organosilane substrates (1b–h)



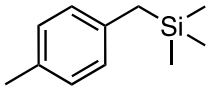
The compounds were prepared according to the following modification of the literature method.[8] A 100-mL, oven-dried round-bottom flask was charged with the Grignard reagent ArMgBr (10.0 mmol) under nitrogen. The solution was diluted with 20 mL dry diethyl ether and then cooled to 0 °C. Trialkylsilyl chloride was added to the mixture dropwise, and then it was stirred overnight. After that, it was quenched with saturated ammonium chloride solution (10 mL) and water (30 mL). The phases were separated, and the aqueous phase was extracted with Et_2O (3 x 20 mL). The combined organic phases were dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography, with hexanes as the eluent, to afford the corresponding organosilanes **1b**–**g**.



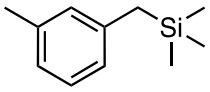
(4-fluorobenzyl)trimethylsilane (1b)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 6.95-6.89 (m, 4H), 2.06 (s, 2H), 0.00 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 161.6, 159.2, 136.1, 129.1, 115.1, 114.9, 26.2, 1.9.



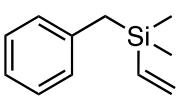
(4-methoxybenzyl)trimethylsilane (1c)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 6.92 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 3.78 (s, 3H), 2.01 (s, 2H), 0.02 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 156.6, 132.5, 130.0, 128.9, 113.8, 55.4, 25.8, 1.8.



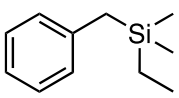
(4-methylbenzyl)trimethylsilane (1d)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.03 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8 Hz, 2H), 2.29 (s, 3H), 2.04 (s, 2H), 0.01 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 137.3, 133.2, 128.9, 128.1, 26.6, 21.1, 1.8.



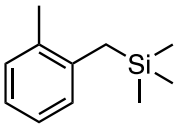
(3-methylbenzyl)trimethylsilane (1e)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.11 (t, *J* = 8 Hz, 1H), 6.89 (d, *J* = 8 Hz, 1H), 6.81 (d, *J* = 8 Hz, 2H), 2.31 (s, 3H), 2.05 (s, 2H), -0.01 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 140.5, 137.7, 129.0, 128.1, 125.2, 124.7, 27.0, 21.6, 1.7.



Benzyldimethyl(vinyl)silane (1f)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.31 (t, *J* = 8 Hz, 2H), 7.17 (t, *J* = 8 Hz, 1H), 7.11 (d, *J* = 8 Hz, 2H), 6.23 (dd, *J* = 16 Hz, 1H), 6.07 (dd, *J* = 4 Hz, 1H), 5.77 (dd, *J* = 4 Hz, 1H), 2.25 (s, 2H), 0.16 (s, 6H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 139.9, 138.2, 132.3, 128.4, 128.2, 124.1, 25.9, 3.6.



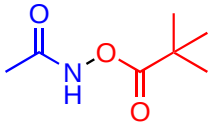
Benzyl(ethyl)dimethylsilane (1g)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.24 (t, *J* = 8 Hz, 2H), 7.07 (t, *J* = 8 Hz, 1H), 7.01 (d, *J* = 8 Hz, 2H), 2.09 (s, 2H), 0.94 (t, *J* = 8 Hz, 3H), 0.50 (q, *J* = 8 Hz, 2H), -0.04 (s, 6H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 140.6, 128.2, 123.9, 25.4, 7.4, 6.6, -3.9.



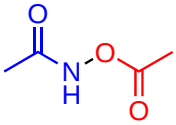
(2-methylbenzyl)trimethylsilane (1h)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.11–7.05 (m, 2H), 7.00–6.95 (m, 2H), 2.23 (s, 3H), 2.10 (s, 2H), -0.01 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 139.1, 134.7, 130.2, 128.8, 125.7, 124.2, 23.8, 20.5, 1.2.

5.2. Synthesis of nitrene precursor (2a-c)

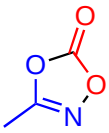
The compounds were prepared according to the following literature method.[9]



***N*-(pivaloyloxy)acetamide (2a)**
^{1}H NMR (δ, 23 °C, $CDCl_3$): 9.29 (NH, br, 1H), 2.00 (s, 3H), 1.27 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 176.8, 168.4, 38.4, 27.2, 27.1, 19.8.

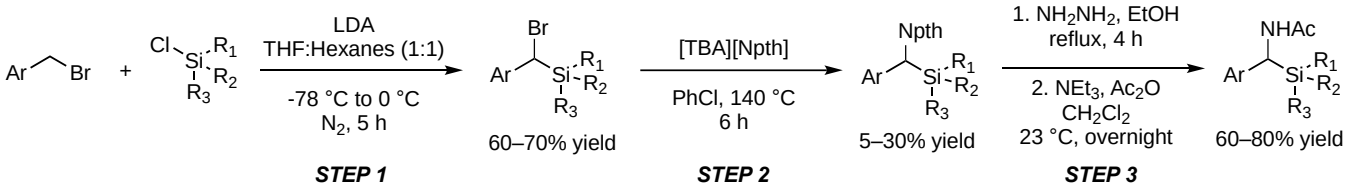


***N*-acetoxyacetamide (2b)**
^{1}H NMR (δ, 23 °C, $CDCl_3$): 2.22 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 168.9, 19.8, 18.4.



3-methyl-1,4,2-dioxazol-5-one (2c)
^{1}H NMR (δ, 23 °C, $CDCl_3$): 2.34 (s, 3H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 163.9, 154.2, 10.6.

5.3. Synthesis of α-aminosilane racemic products (3a–g)



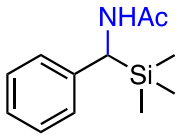
Procedure for Step 1: The compounds were prepared according to the following modification of the literature method.[10, 11] A 100-mL, oven-dried round-bottom flask was charged with the LDA (20.0 mmol) under nitrogen, diluted with hexanes (9 mL) and then cooled to -78 °C. A mixture of the benzyl bromide (20 mmol) and the silyl chloride (25 mmol) in THF (9 mL) was added to the mixture dropwise over the course oof 30 minutes. The reaction was stirred for 5 h, then warmed to 0 °C and quenched with water (30 mL). After that, it was quenched with saturated ammonium chloride solution (10 mL) and water (30

mL). The aqueous phase was extracted with Et_2O (3 x 30 mL). The combined organic phases were dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography, with hexanes as the eluent, to afford the corresponding (α-bromoaryl)organosilane.

Procedure for Step 2: The compounds were prepared according to the following modification of the literature method.[12] A 40-mL vial was charged with (α-bromoaryl)organosilane (5 mmol), tetrabutylammonium phthalimide [TBA][Npth] (6 mmol) and PhCl (20 mL). The reaction mixture was heated at 140 °C for 6 h. After that, it was cooled to room temperature and was poured into ice-water and extracted with Et_2O (2 x 50 mL). The combined organic phases were dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography (0% to 40% EtOAc/hexanes), to afford the corresponding (α-phthalimidoaryl)organosilane.[Note: The major byproduct of this reaction is the desilylated (α-phthalimidoaryl compound.]

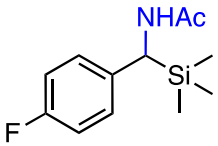
Procedure for Step 3: The compounds were prepared according to the following modification of the literature method.[12] A 20-mL vial was charged with a mixture of the (α-phthalimidoaryl)organosilane (0.5 mmol) and hydrazine (0.7 mmol) in EtOH (10 mL). The reaction mixture was refluxed for 3 h, after which concentrated HCl (0.1 mL) was added, and the mixture was refluxed for another 1 h. It was then cooled to room temperature; the precipitate was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was then treated with water, the insoluble material was removed by filtration and the filtrate was made alkaline with NaOH solution and extracted with CH_2Cl_2. The solvent was removed under reduced pressure to afford the crude free amine.

A 20-mL vial was charged with the (α-aminoaryl)organosilane (0.5 mmol), triethylamine (0.1 mL), acetic anhydride (0.1 mL), and CH_2Cl_2 (10 mL). The reaction was stirred overnight at room temperature, after which it was poured into water and extracted with CH_2Cl_2. The combined organic phases were dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography (10% to 60% EtOAc/hexanes), to afford the corresponding products (**3a-g**).



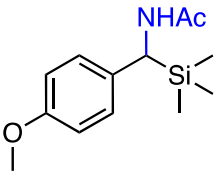
***N*-(phenyl(trimethylsilyl)methyl)acetamide (3a)**

^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.28 (t, *J* = 6 Hz, 3H), 7.15 (t, *J* = 8 Hz, 1H), 7.08 (d, *J* = 8 Hz, 2H), 4.63 (d, *J* = 8 Hz, 1H), 2.06 (s, 3H), 0.03 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 170.1, 141.2, 128.5, 126.1, 125.9, 47.0, 23.3, 3.2.



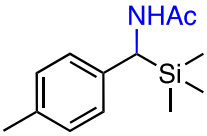
***N*-((4-fluorophenyl)(trimethylsilyl)methyl)acetamide (3b)**

^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.04 (t, *J* = 8 Hz, 2H), 6.96 (t, *J* = 8 Hz, 2H), 4.57 (d, *J* = 8 Hz, 1H), 2.05 (s, 3H), 0.03 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 170.2, 162.5, 160.0, 137.2, 127.5, 127.4, 115.5, 115.2, 46.2, 23.3, 3.3. HRMS: 239.1142 (*calc.*), 239.1146. (*expt.*).



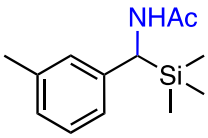
***N*-((4-methoxyphenyl)(trimethylsilyl)methyl)acetamide (3c)**

^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.01 (d, *J* = 8 Hz, 2H), 6.82 (d, *J* = 8 Hz, 2H), 4.56 (d, *J* = 8 Hz, 1H), 3.78 (s, 3H), 2.04 (s, 3H), 0.02 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 170.1, 157.9, 133.3, 127.4, 114.0, 55.4, 46.3, 23.3, 3.1. HRMS: 251.1342 (*calc.*), 251.1340. (*expt.*).



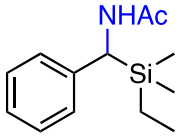
***N*-(*p*-tolyl(trimethylsilyl)methyl)acetamide (3d)**

^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.08 (d, *J* = 8 Hz, 2H), 6.97 (d, *J* = 8 Hz, 2H), 4.59 (d, *J* = 8 Hz, 1H), 2.29 (s, 3H), 2.04 (s, 3H), 0.02 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 170.1, 138.1, 135.5, 129.2, 126.2, 46.8, 23.3, 21.1, 3.2. HRMS: 235.1392 (*calc.*), 235.1391. (*expt.*).



***N*-(*m*-tolyl(trimethylsilyl)methyl)acetamide (3e)**

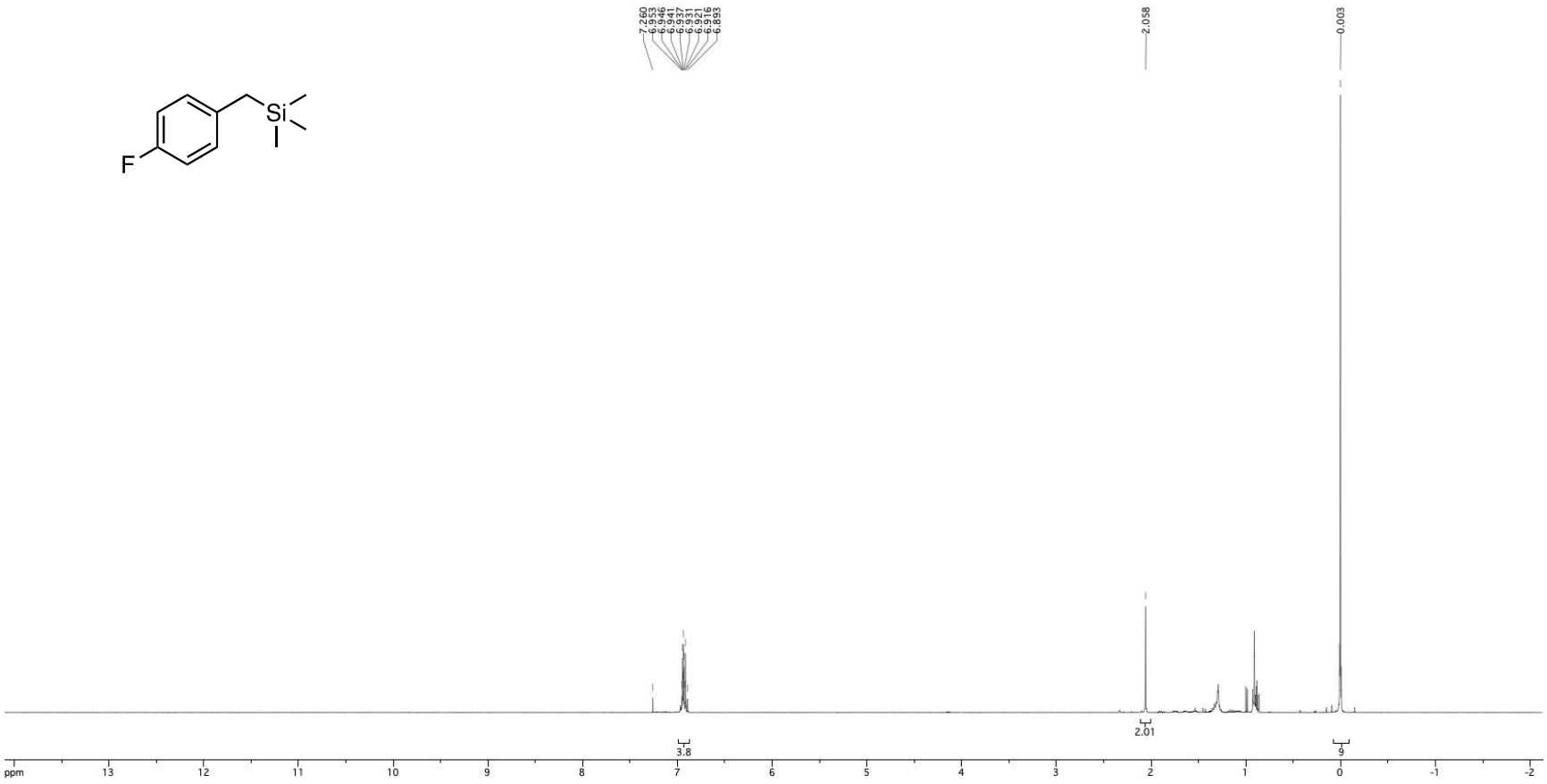
^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.15–7.12 (m, 2H), 6.97–6.91 (m, 2H), 4.69–4.59 (m, 1H), 2.31 (s, 2H), 2.09 (s, 3H), 0.03 (s, 9H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 170.1, 138.1, 135.5, 129.2, 126.2, 46.8, 23.3, 21.1, 3.2.



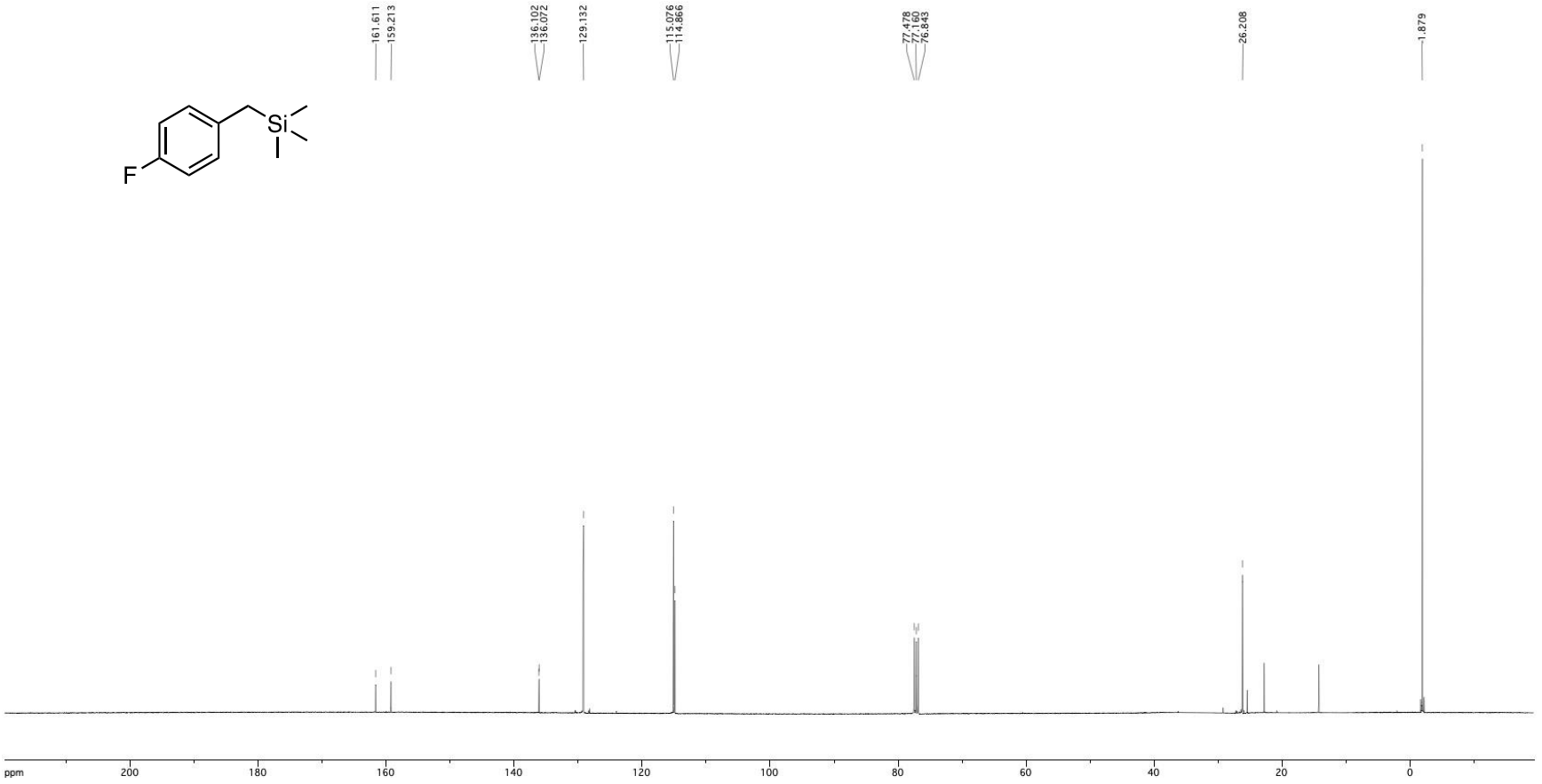
***N*-((ethyldimethylsilyl)(phenyl)methyl)acetamide (3g)**

^{1}H NMR (δ, 23 °C, $CDCl_3$): 7.28-7.24 (m, 3H), 7.16-7.11 (m, 3H), 4.68 (d, *J* = 8Hz, 1H), 2.12 (s, 3H), 0.89 (t, *J* = 8 Hz, 3H), 0.54 (t, *J* = 8 Hz, 2H), 0.02 (d, *J* = 16 Hz, 6H). ^{13}C NMR (δ, 23 °C, $CDCl_3$): 171.8, 140.2, 129.9, 129.0, 128.6, 126.4, 126.1, 125.6, 47.5, 22.1, 7.2, 5.2, -5.3, -5.5.

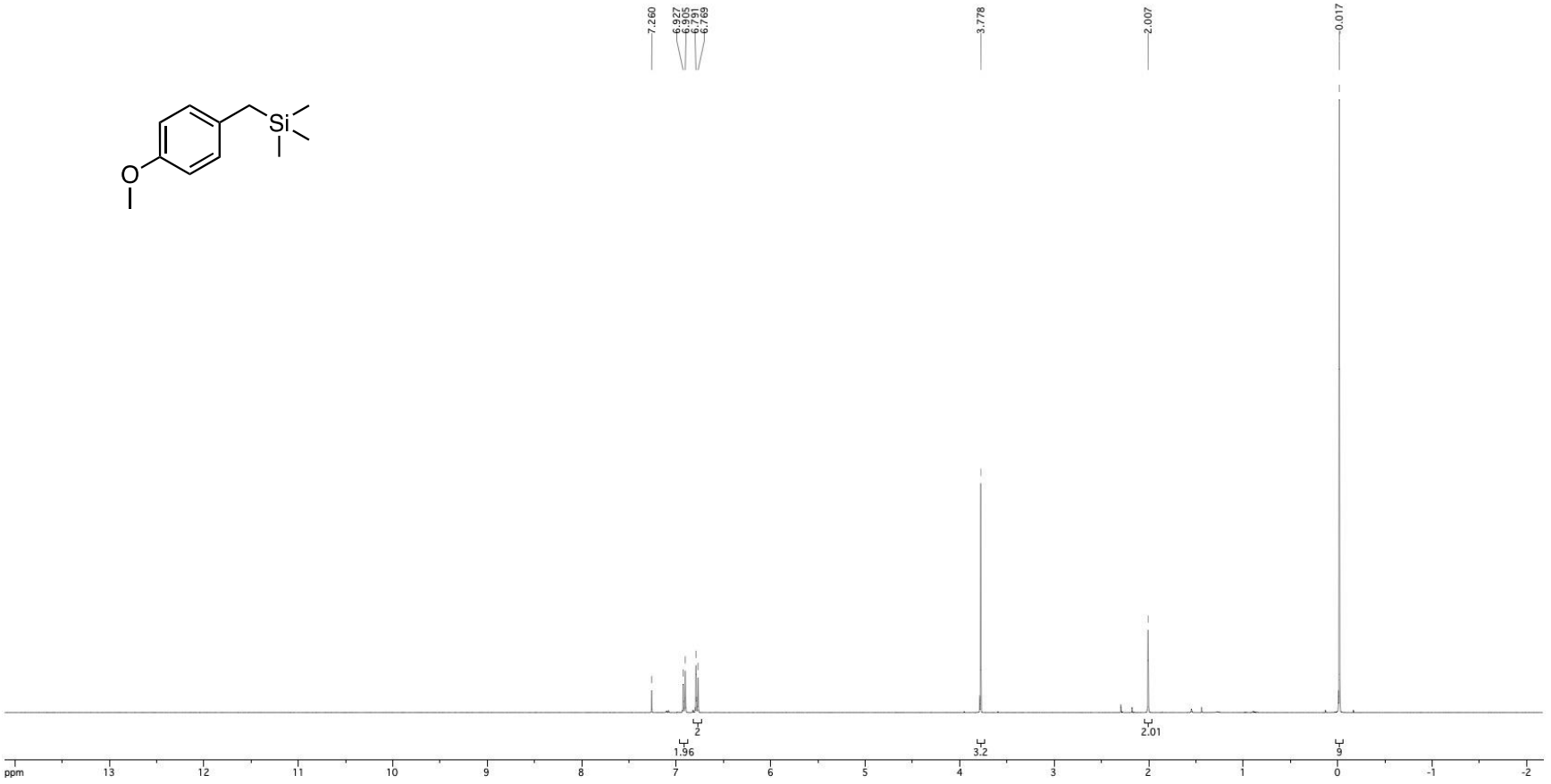
6. NMR Spectra of Compounds



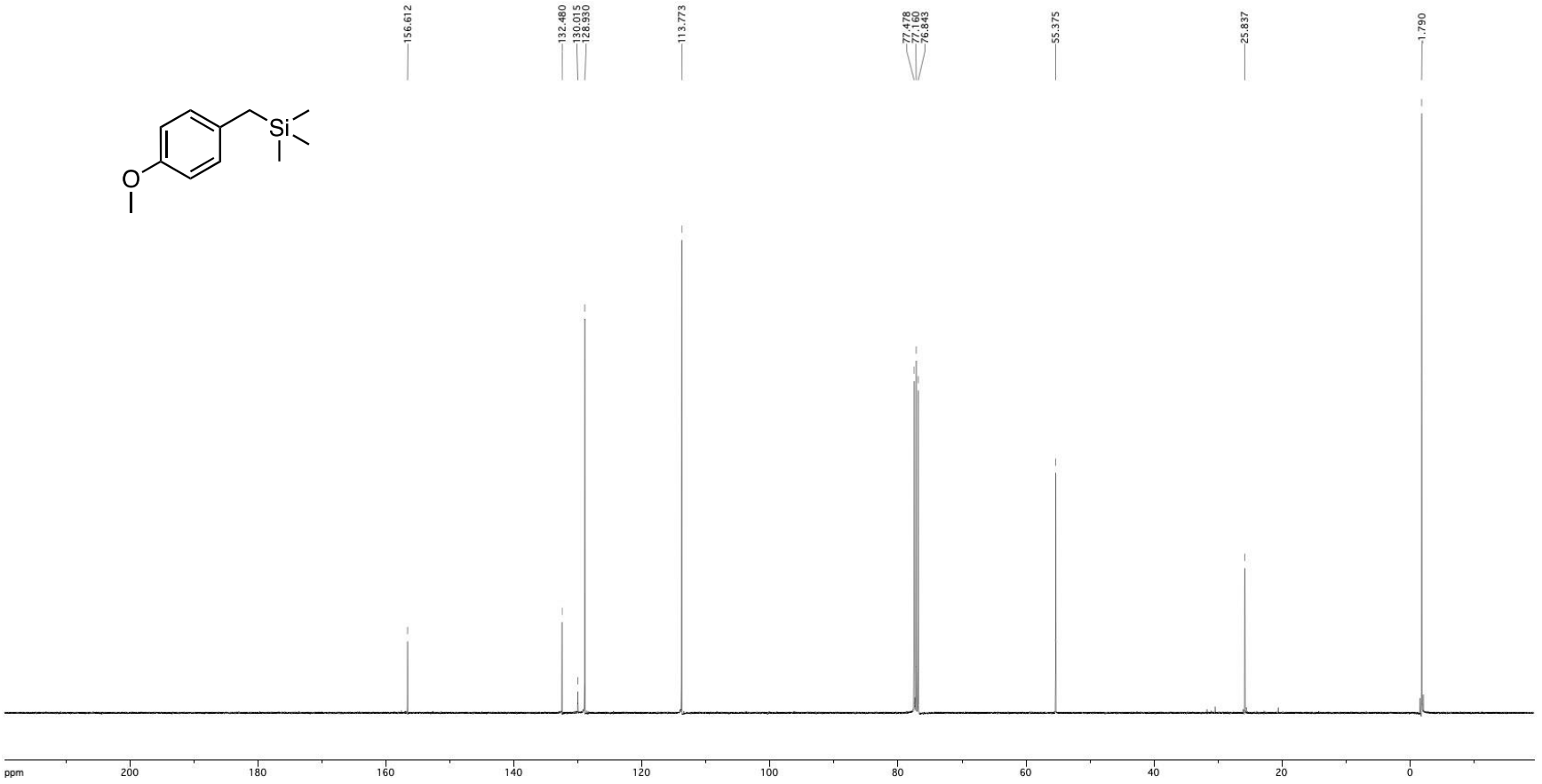
^{1}H-NMR spectrum of (4-fluorobenzyl)trimethylsilane **1b** obtained in $CDCl_3$ at 23 °C.



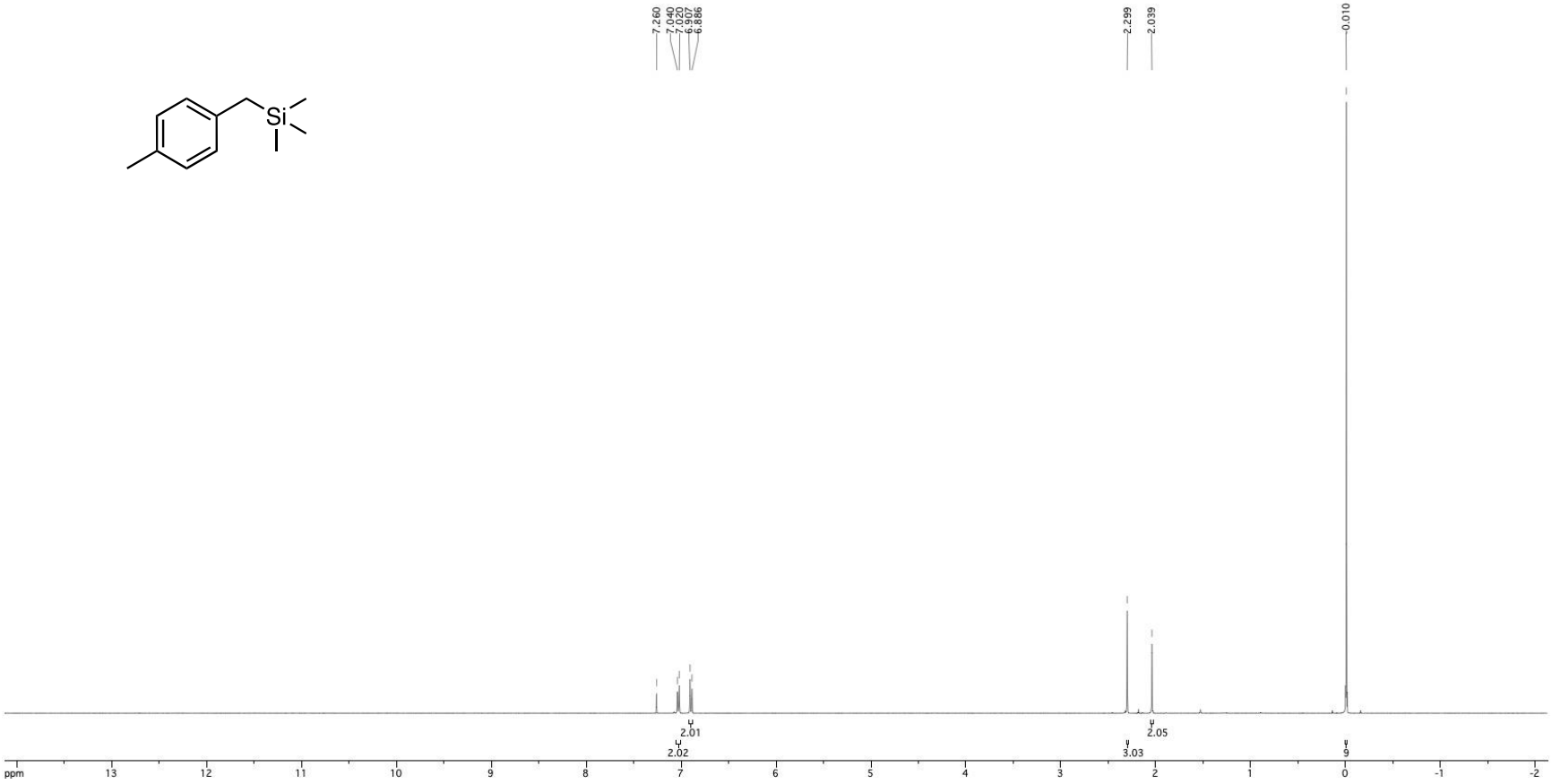
^{13}C-NMR spectrum of (4-fluorobenzyl)trimethylsilane **1b** obtained in $CDCl_3$ at 23 °C.



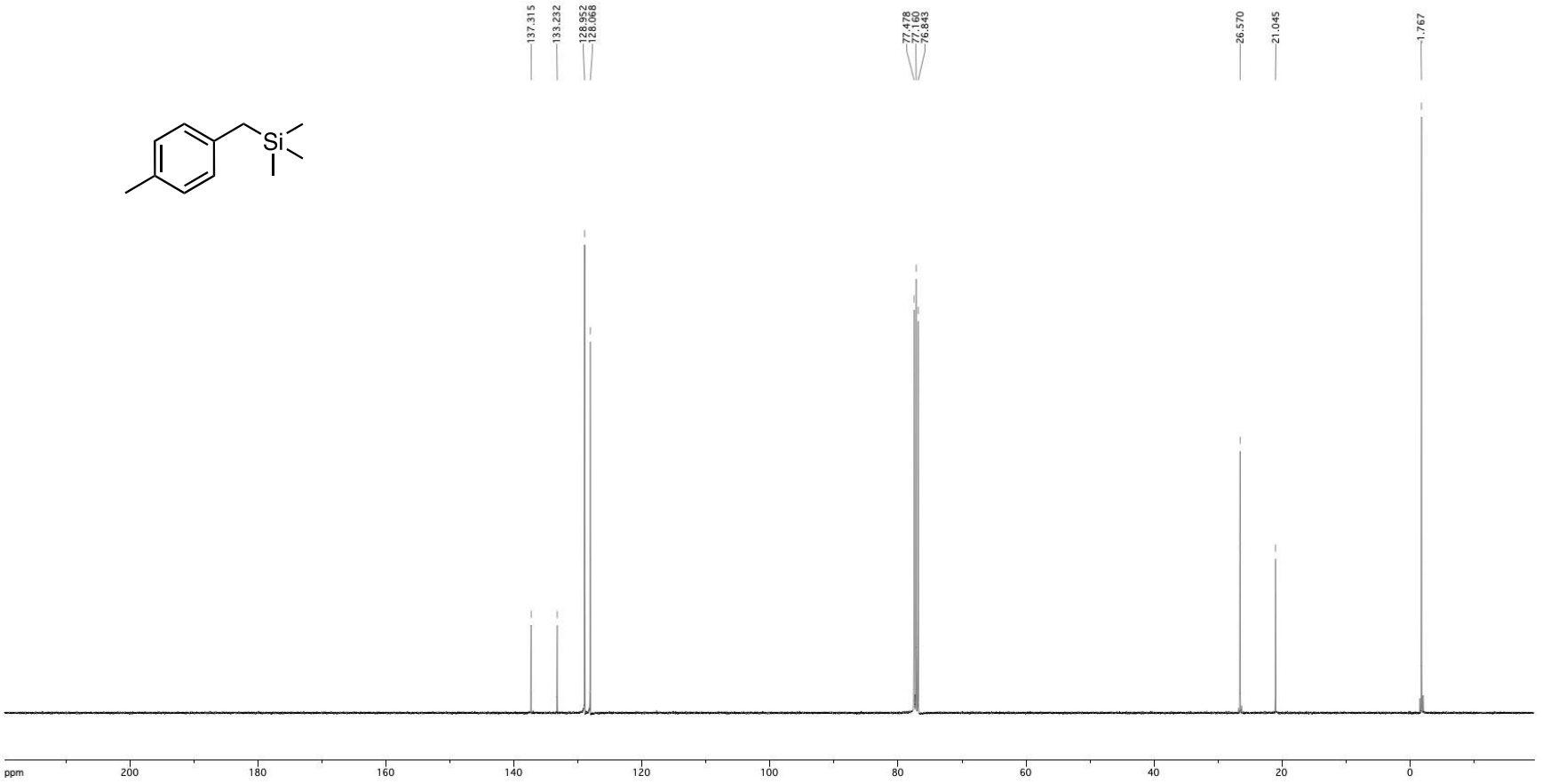
^{1}H-NMR spectrum of (4-methoxybenzyl)trimethylsilane **1c** obtained in $CDCl_3$ at 23 °C.



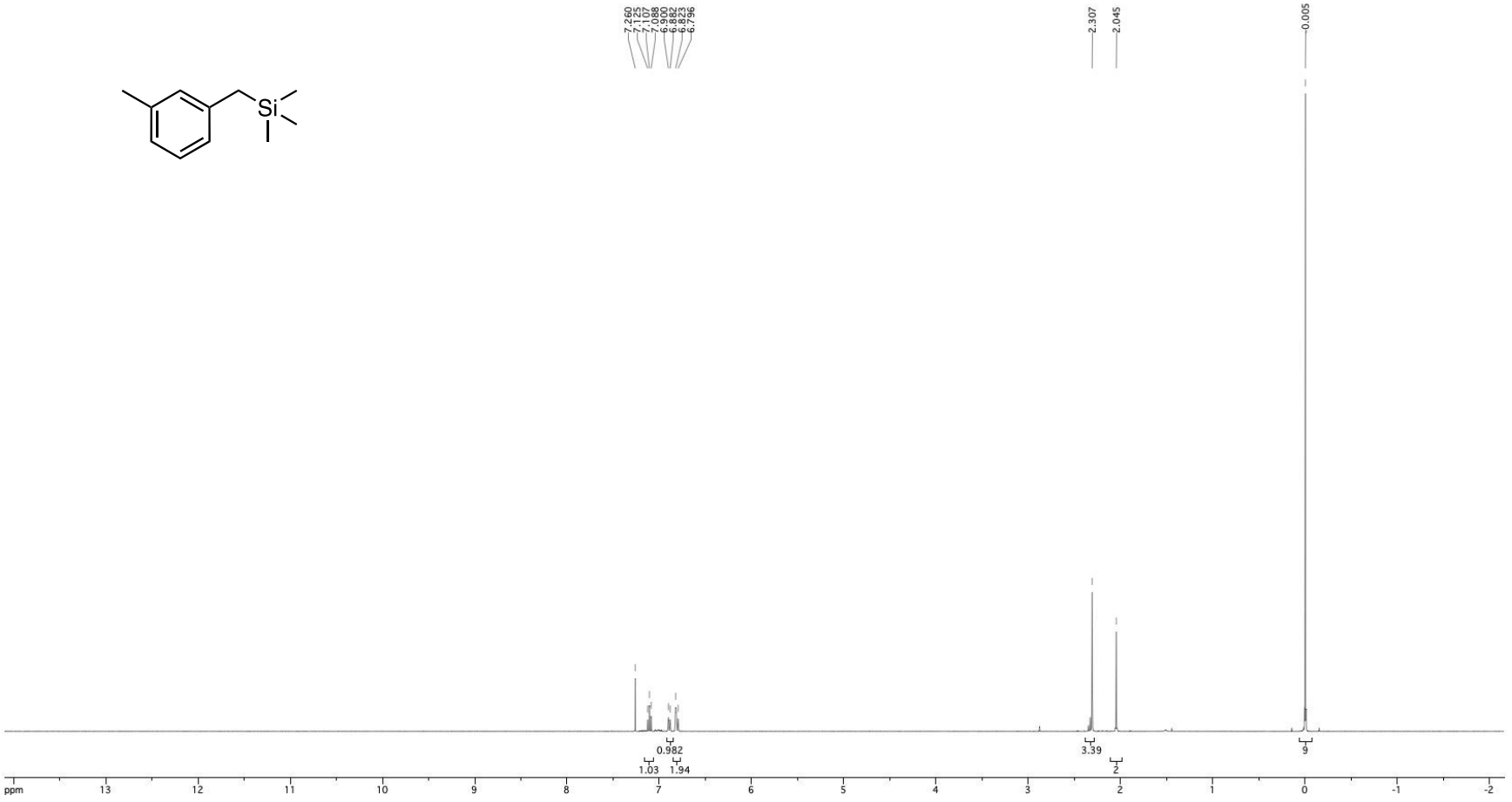
^{13}C-NMR spectrum of (4-methoxybenzyl)trimethylsilane **1c** obtained in $CDCl_3$ at 23 °C.



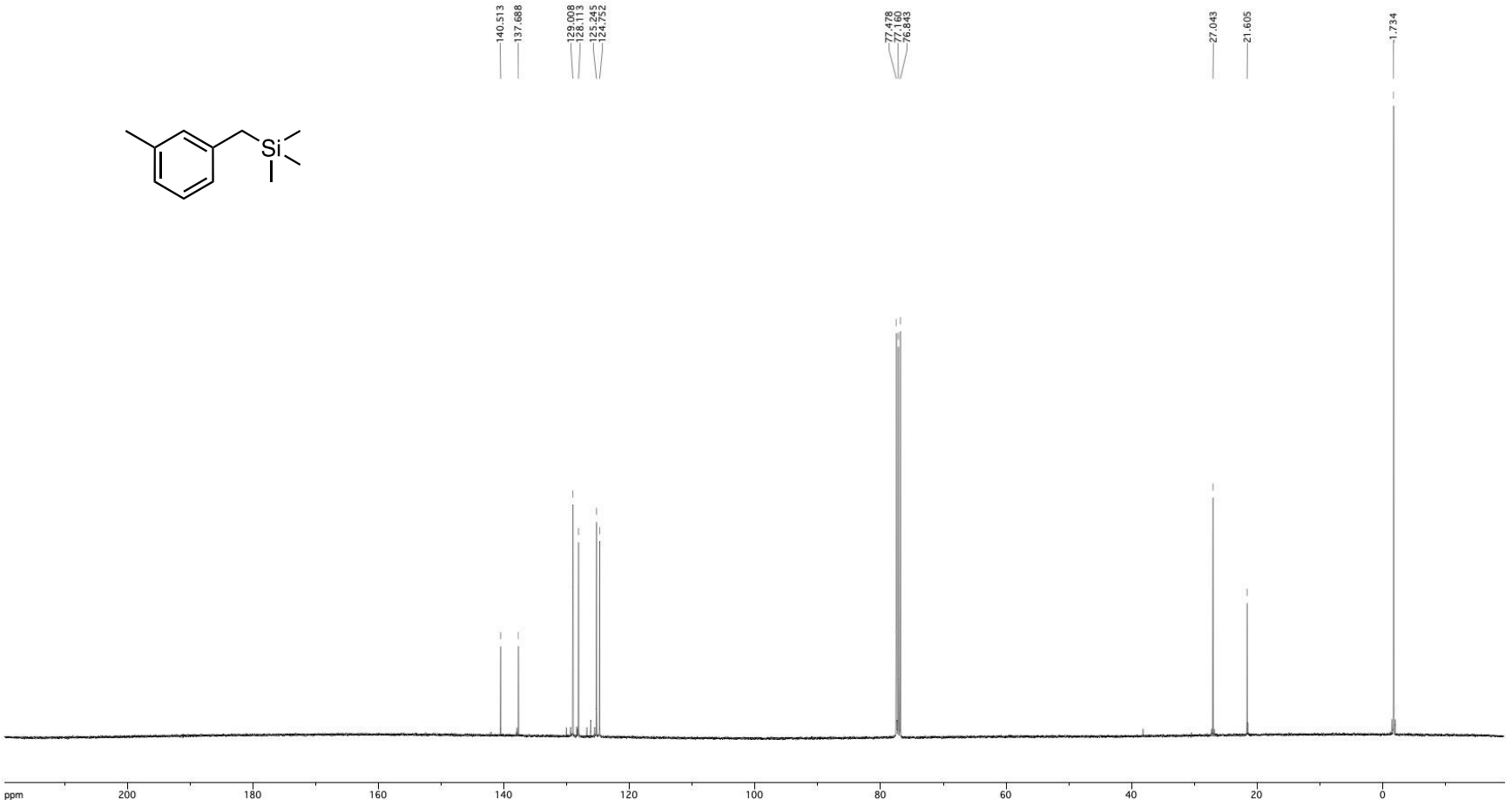
^{1}H-NMR spectrum of (4-methylbenzyl)trimethylsilane **1d** obtained in $CDCl_3$ at 23 °C.



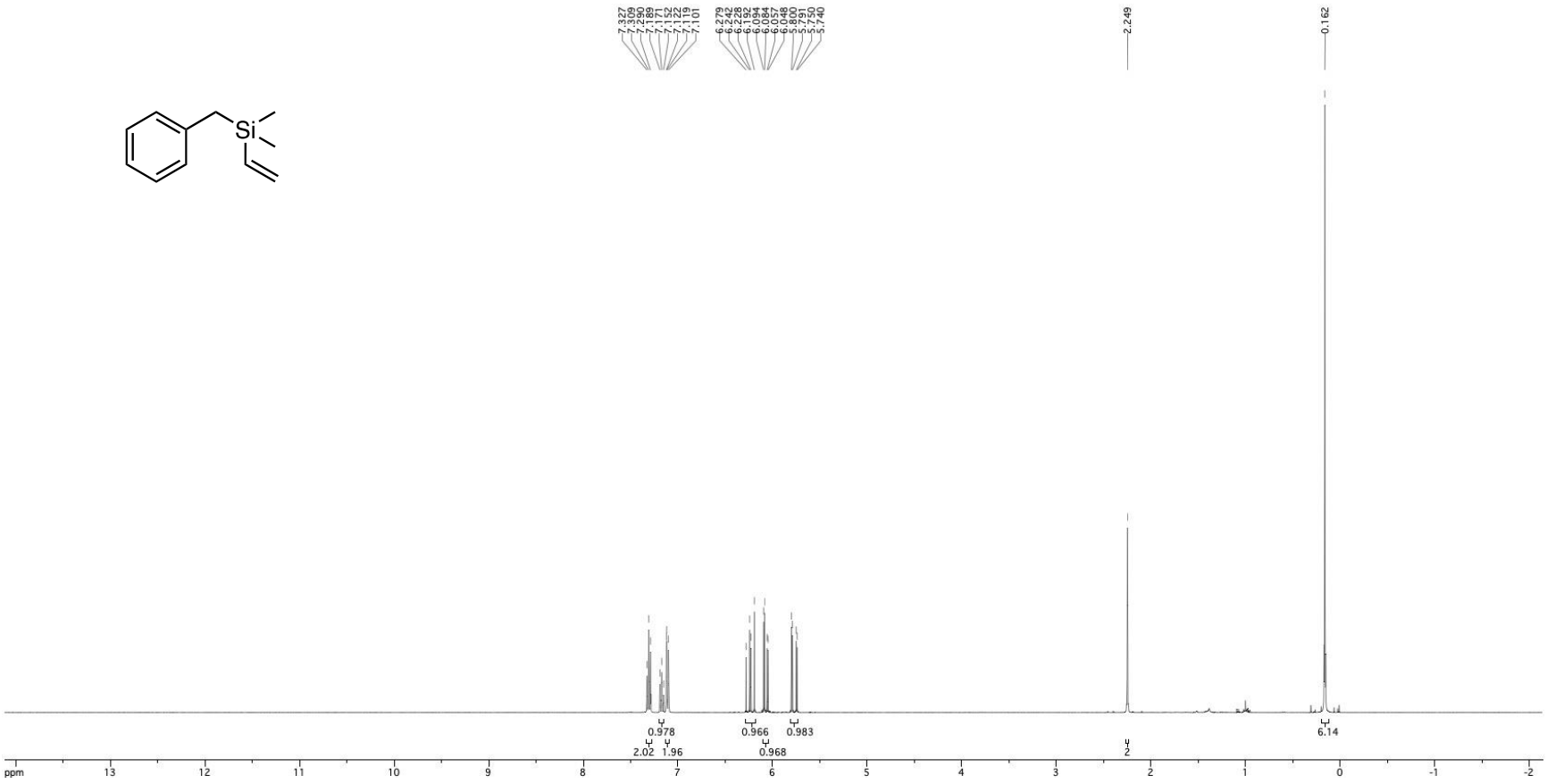
^{13}C-NMR spectrum of (4-methylbenzyl)trimethylsilane **1d** obtained in $CDCl_3$ at 23 °C.



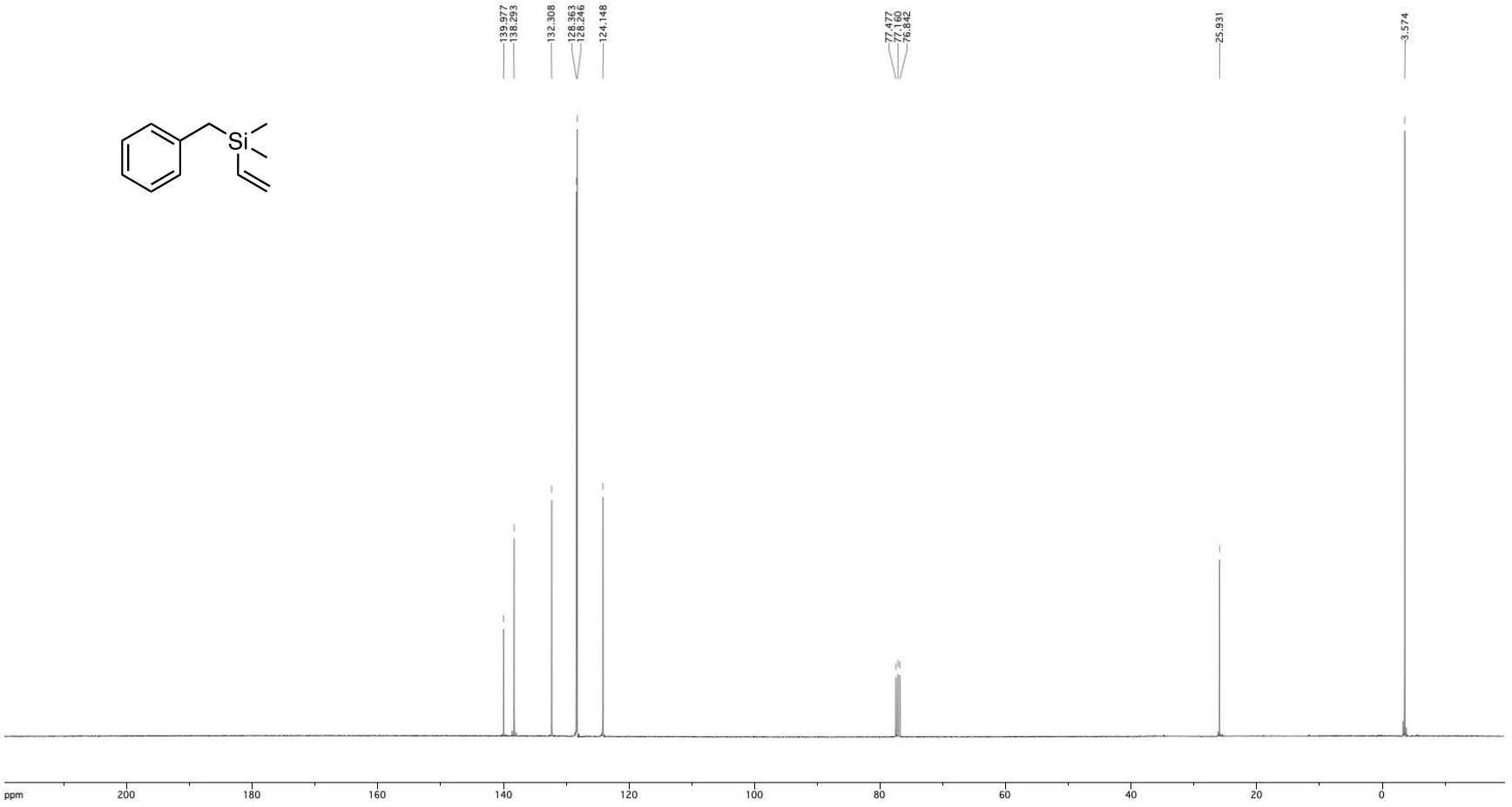
^{1}H-NMR spectrum of (3-methylbenzyl)trimethylsilane **1e** obtained in $CDCl_3$ at 23 °C.



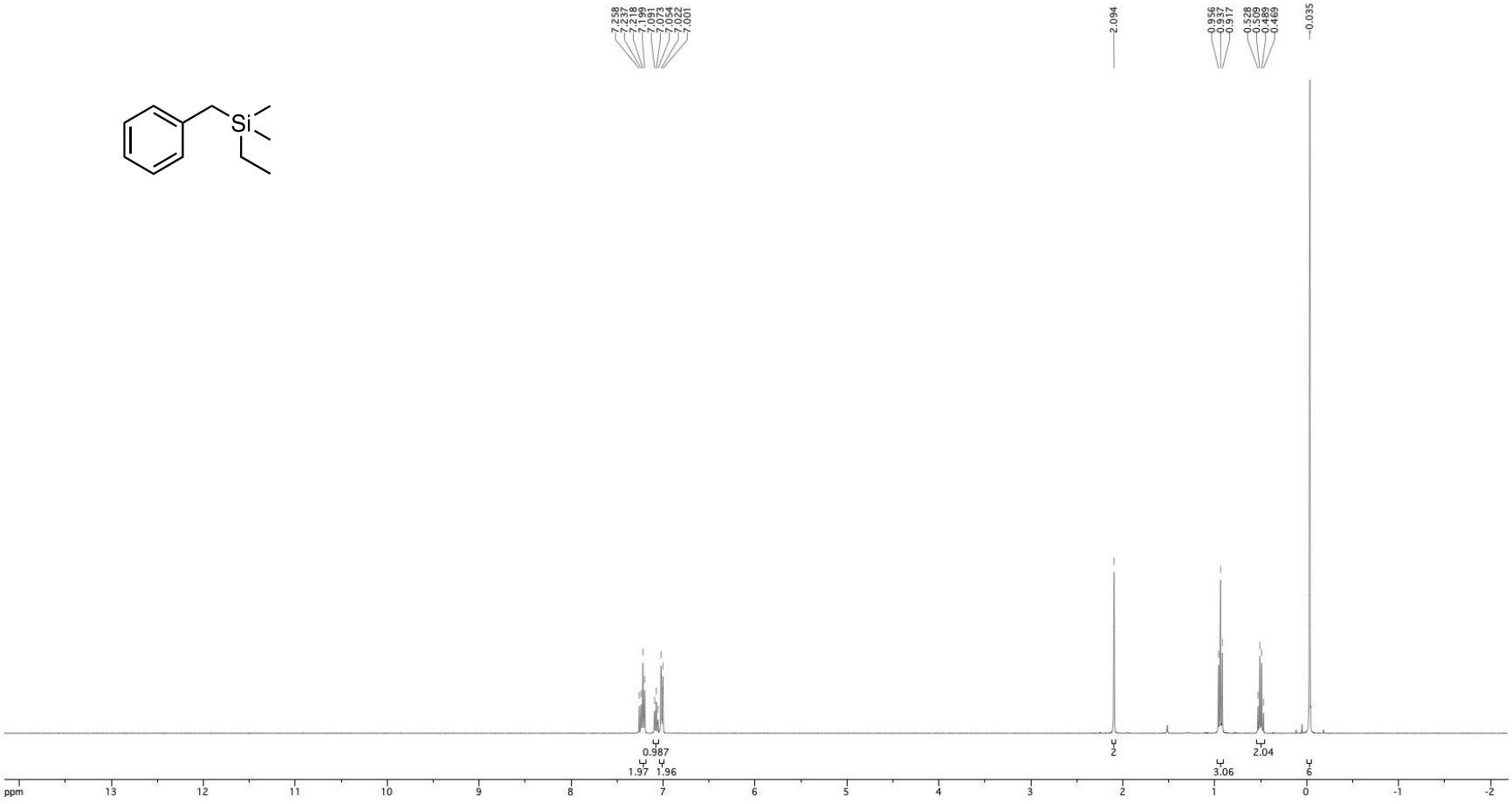
^{13}C-NMR spectrum of (3-methylbenzyl)trimethylsilane **1e** obtained in $CDCl_3$ at 23 °C.



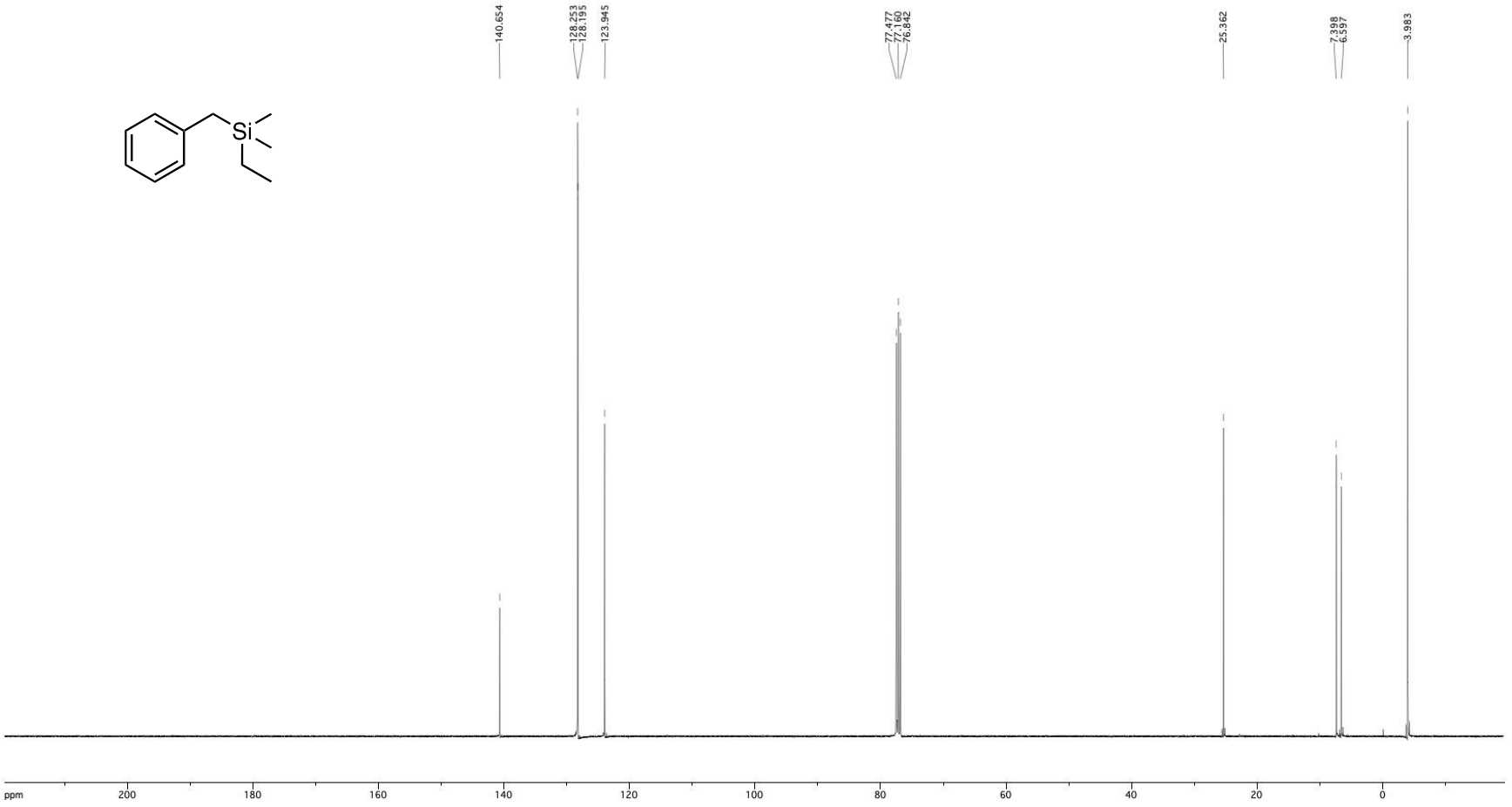
^{1}H-NMR spectrum of benzyldimethyl(vinyl)silane **1f** obtained in $CDCl_3$ at 23 °C.



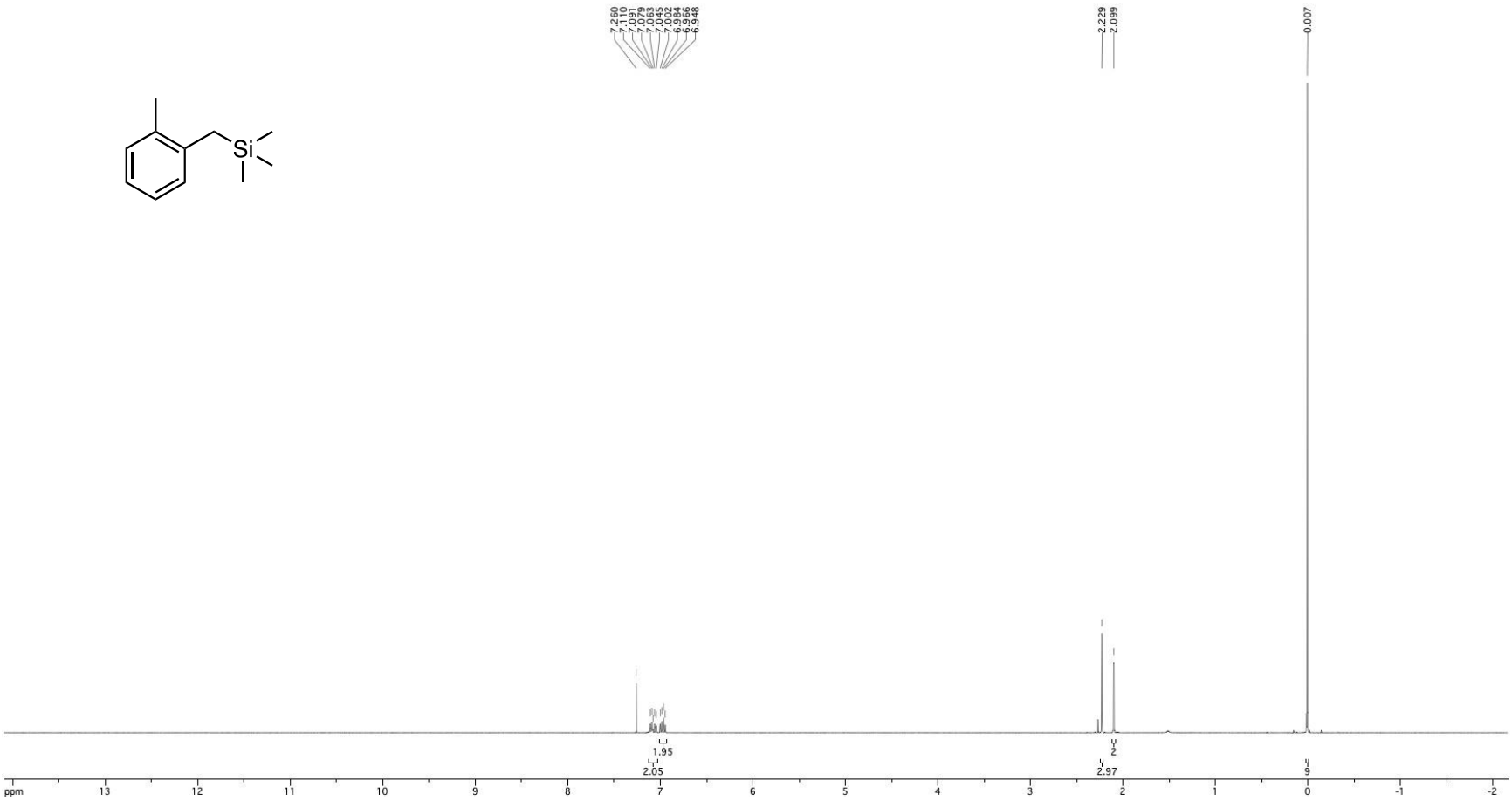
^{13}C-NMR spectrum of benzyldimethyl(vinyl)silane **1f** obtained in $CDCl_3$ at 23 °C.



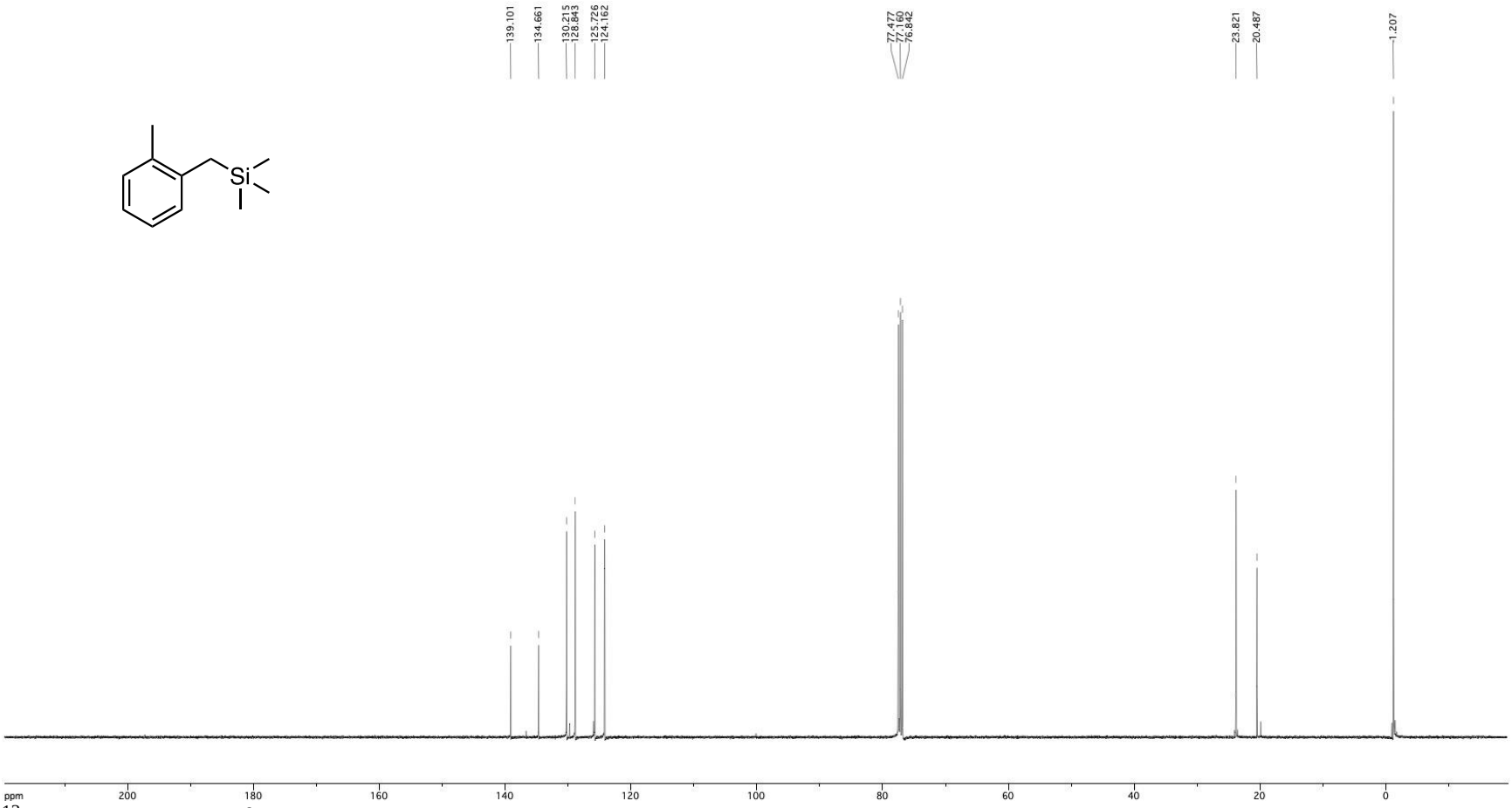
^{1}H-NMR spectrum of benzyl(ethyl)dimethylsilane **1g** obtained in $CDCl_3$ at 23 °C.



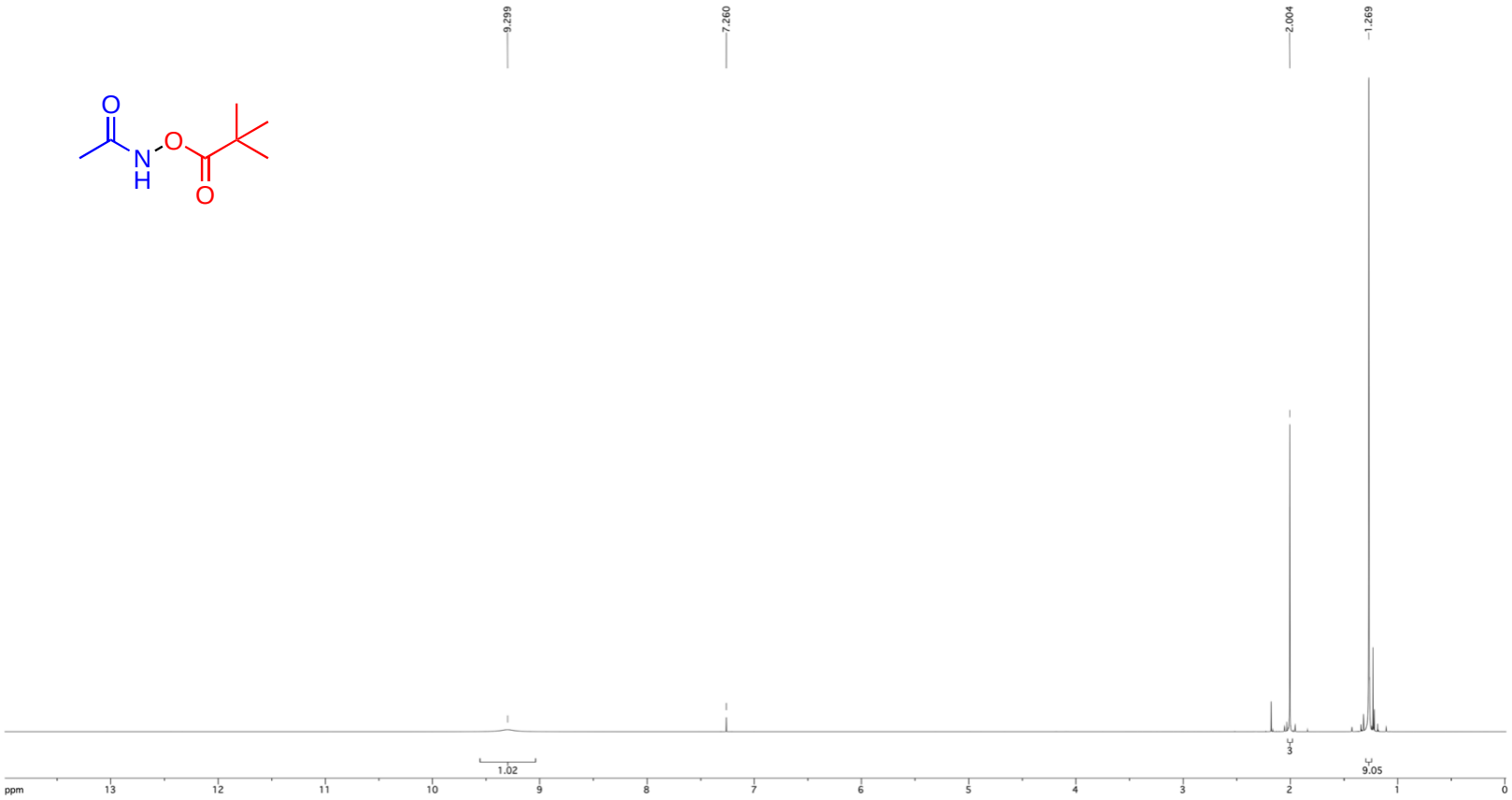
^{13}C-NMR spectrum of benzyl(ethyl)dimethylsilane **1g** obtained in $CDCl_3$ at 23 °C.



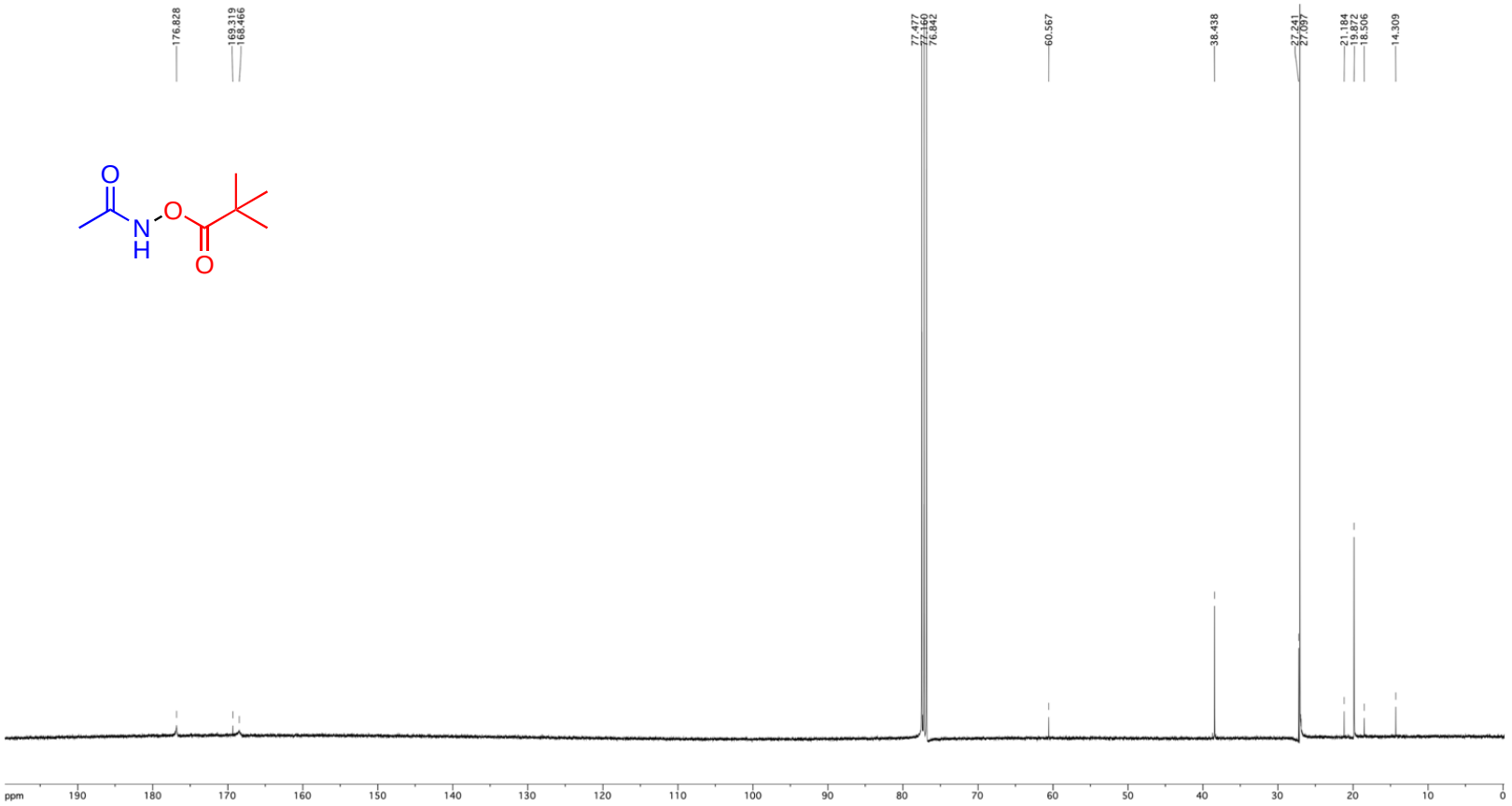
^{1}H-NMR spectrum of (2-methylbenzyl)trimethylsilane **1h** obtained in $CDCl_3$ at 23 °C.



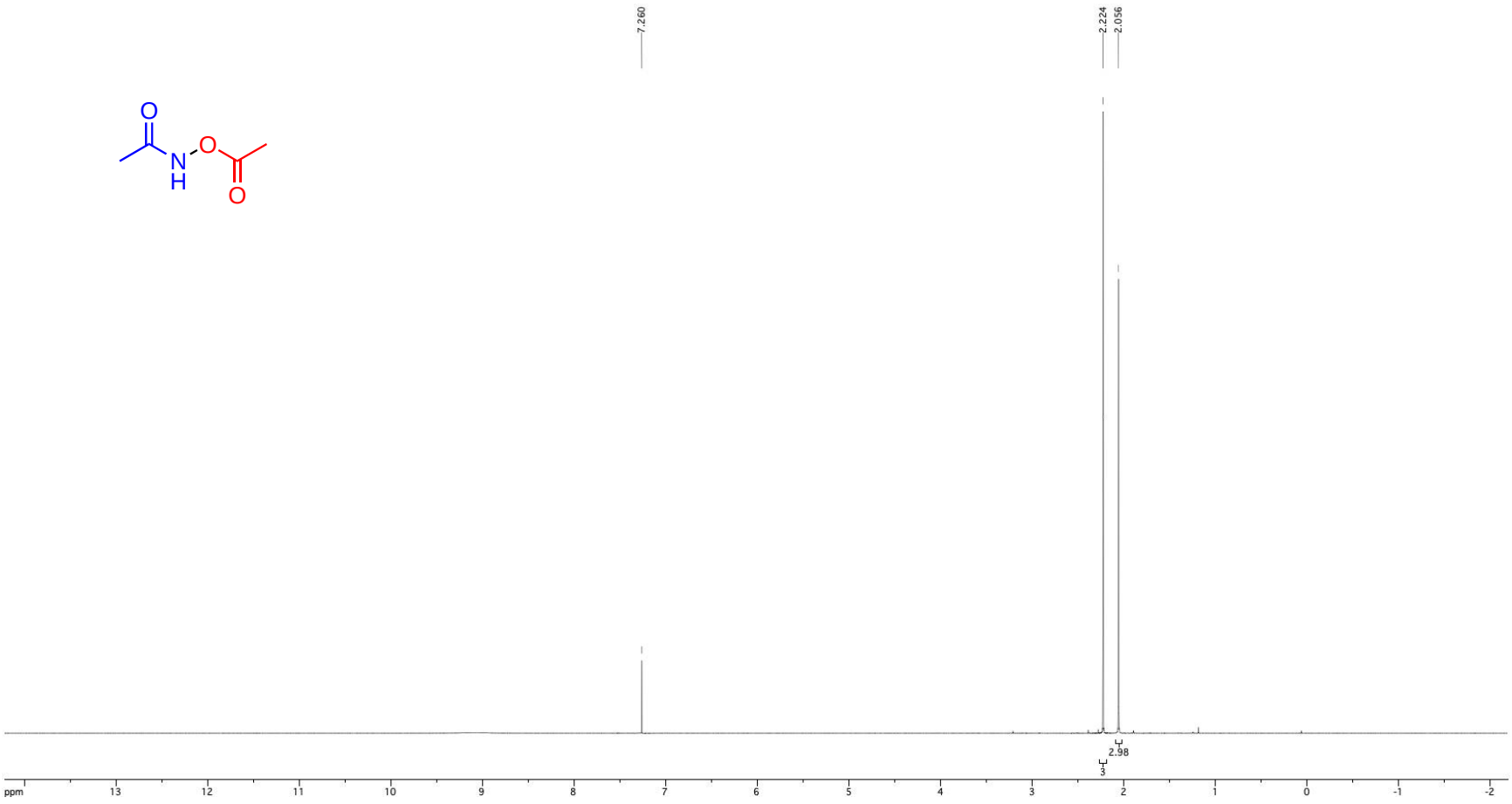
^{13}C-NMR spectrum of (2-methylbenzyl)trimethylsilane **1h** obtained in $CDCl_3$ at 23 °C.



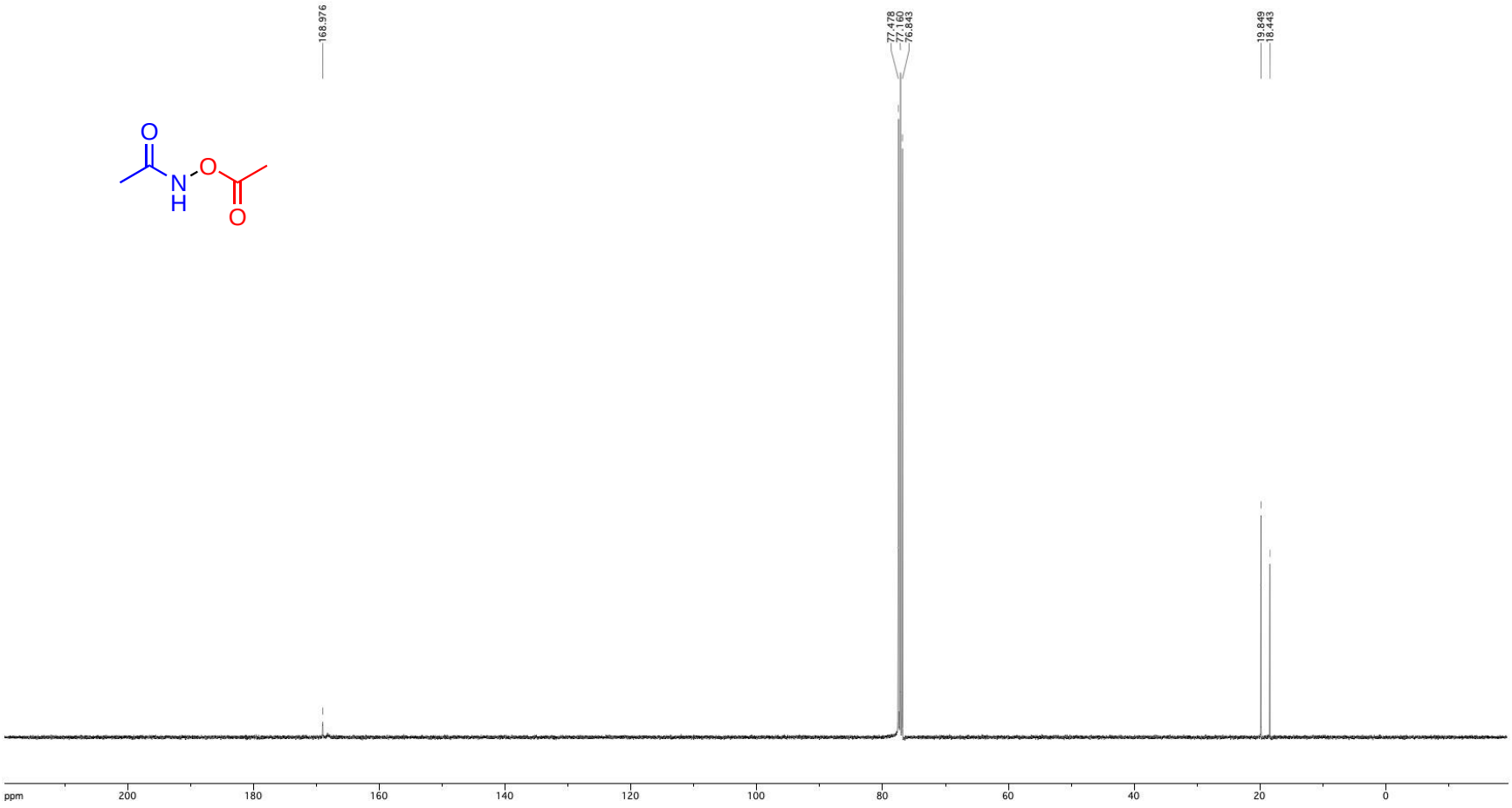
^{1}H-NMR spectrum of *N*-(pivaloyloxy)acetamide **2a** (AcNHOPiv) obtained in $CDCl_3$ at 23 °C.



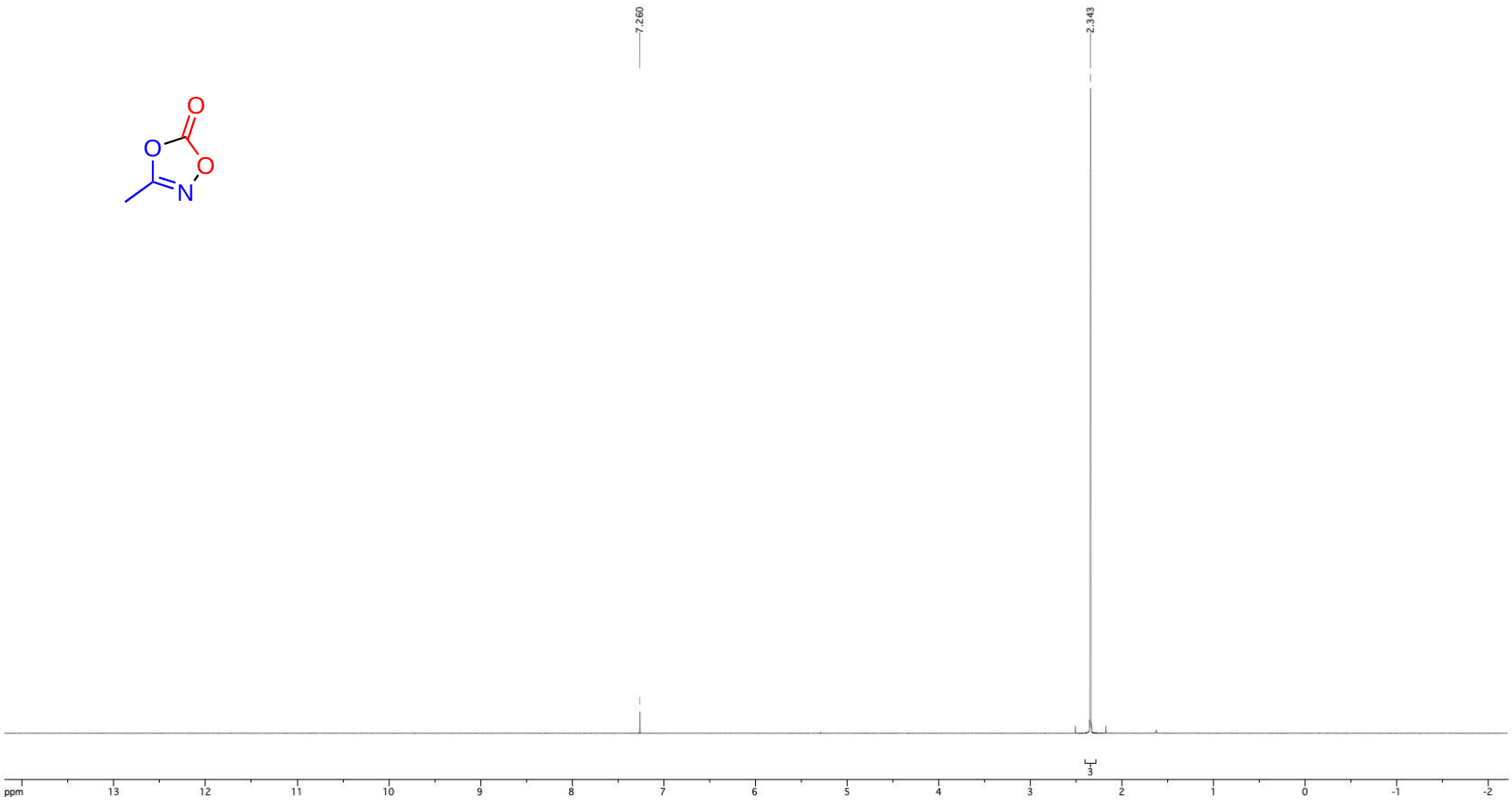
^{13}C-NMR spectrum of *N*-(pivaloyloxy)acetamide **2a** obtained in $CDCl_3$ at 23 °C.



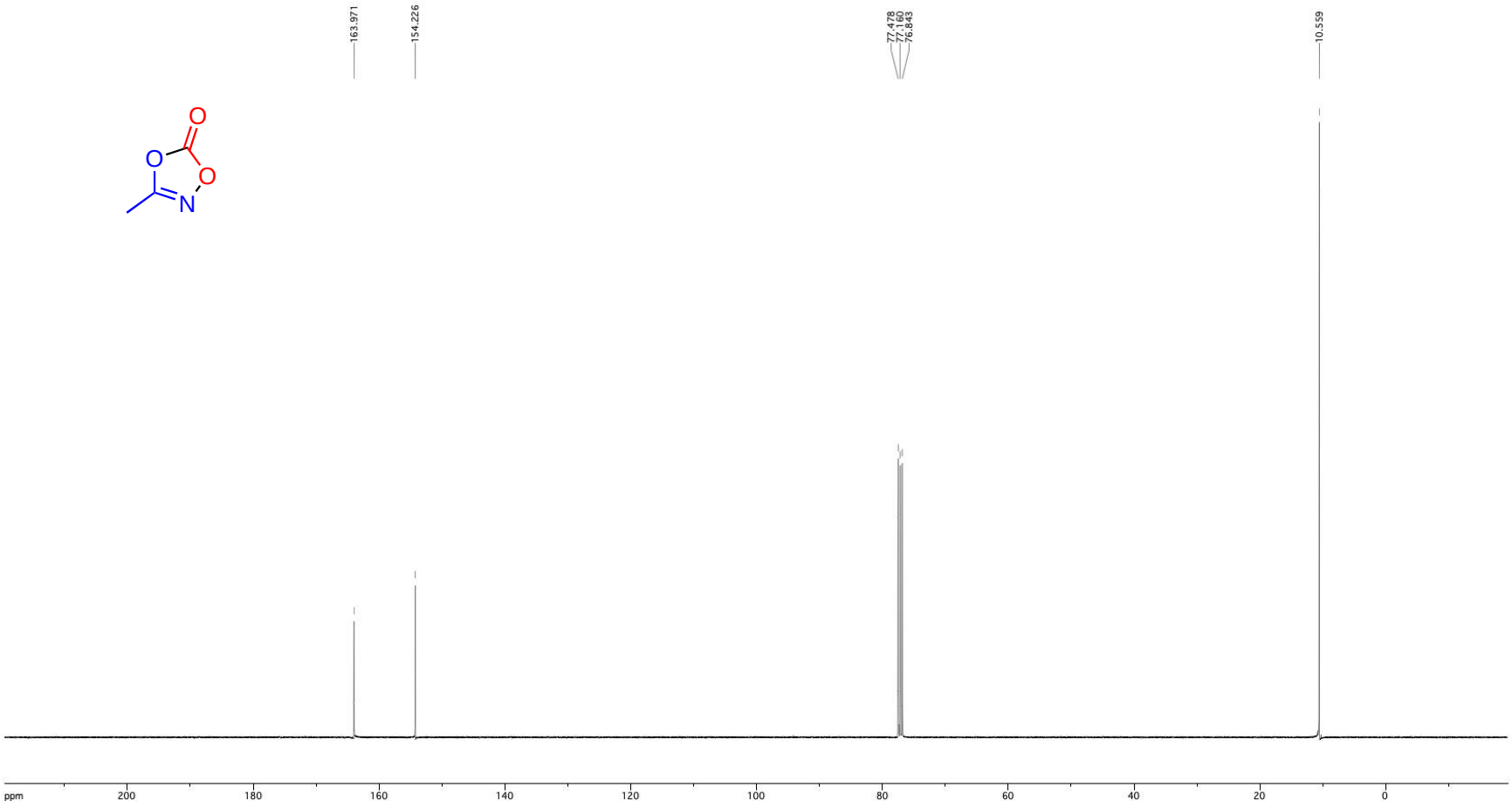
^{1}H-NMR spectrum of *N*-acetoxyacetamide **2b** obtained in $CDCl_3$ at 23 °C.



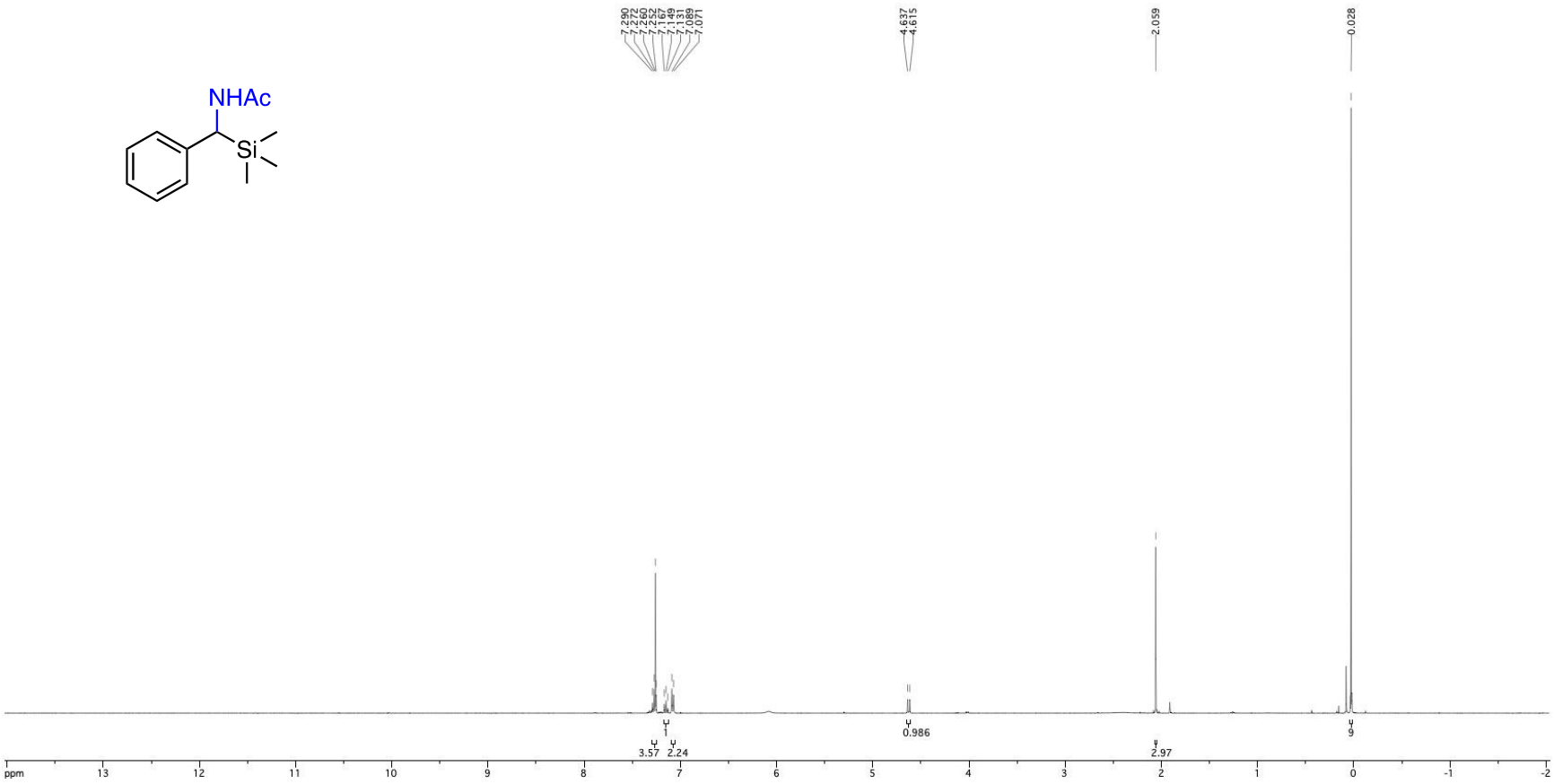
^{13}C-NMR spectrum of *N*-acetoxyacetamide **2b** obtained in $CDCl_3$ at 23 °C.



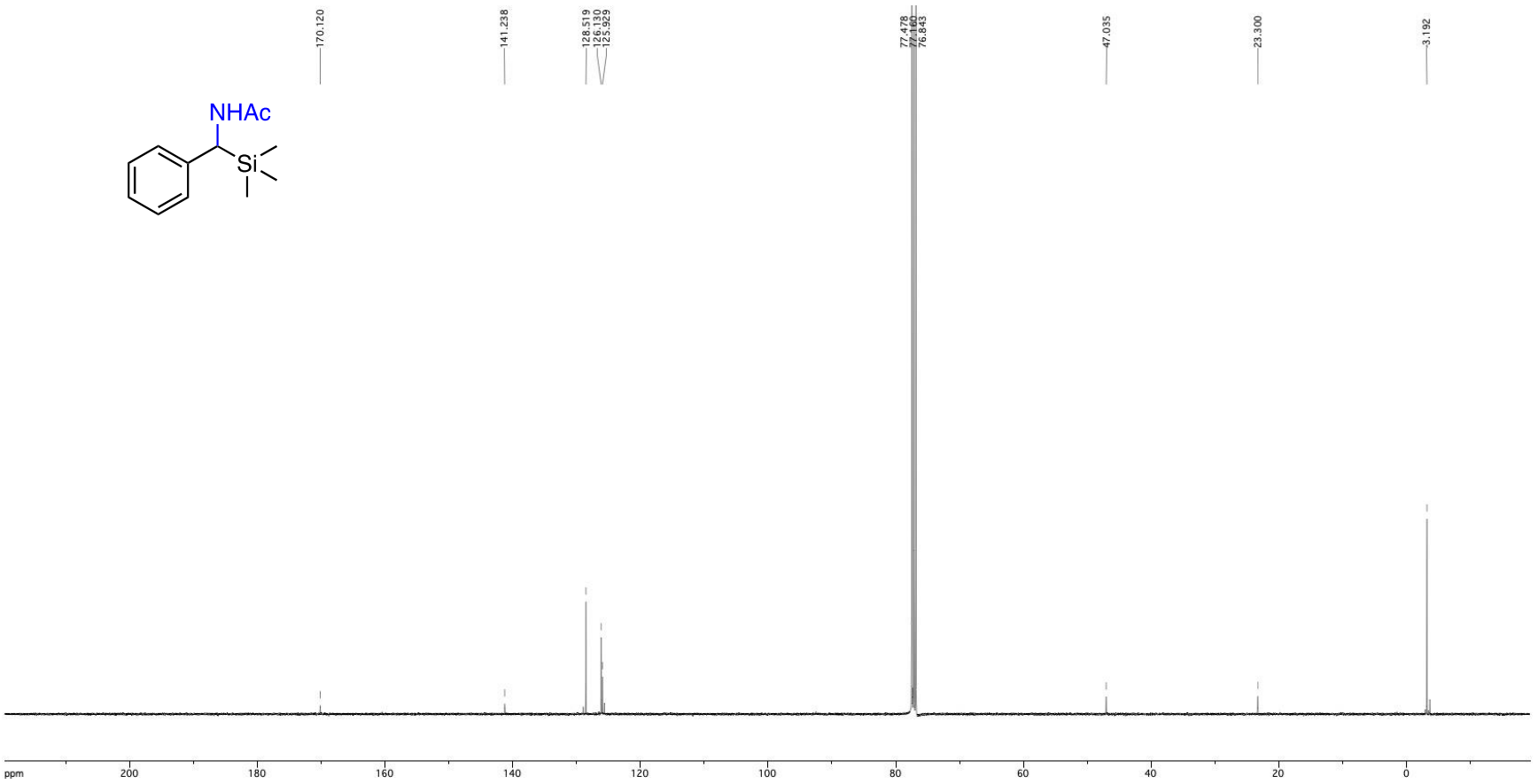
^{1}H-NMR spectrum of 3-methyl-1,4,2-dioxazol-5-one **2c** obtained in $CDCl_3$ at 23 °C.



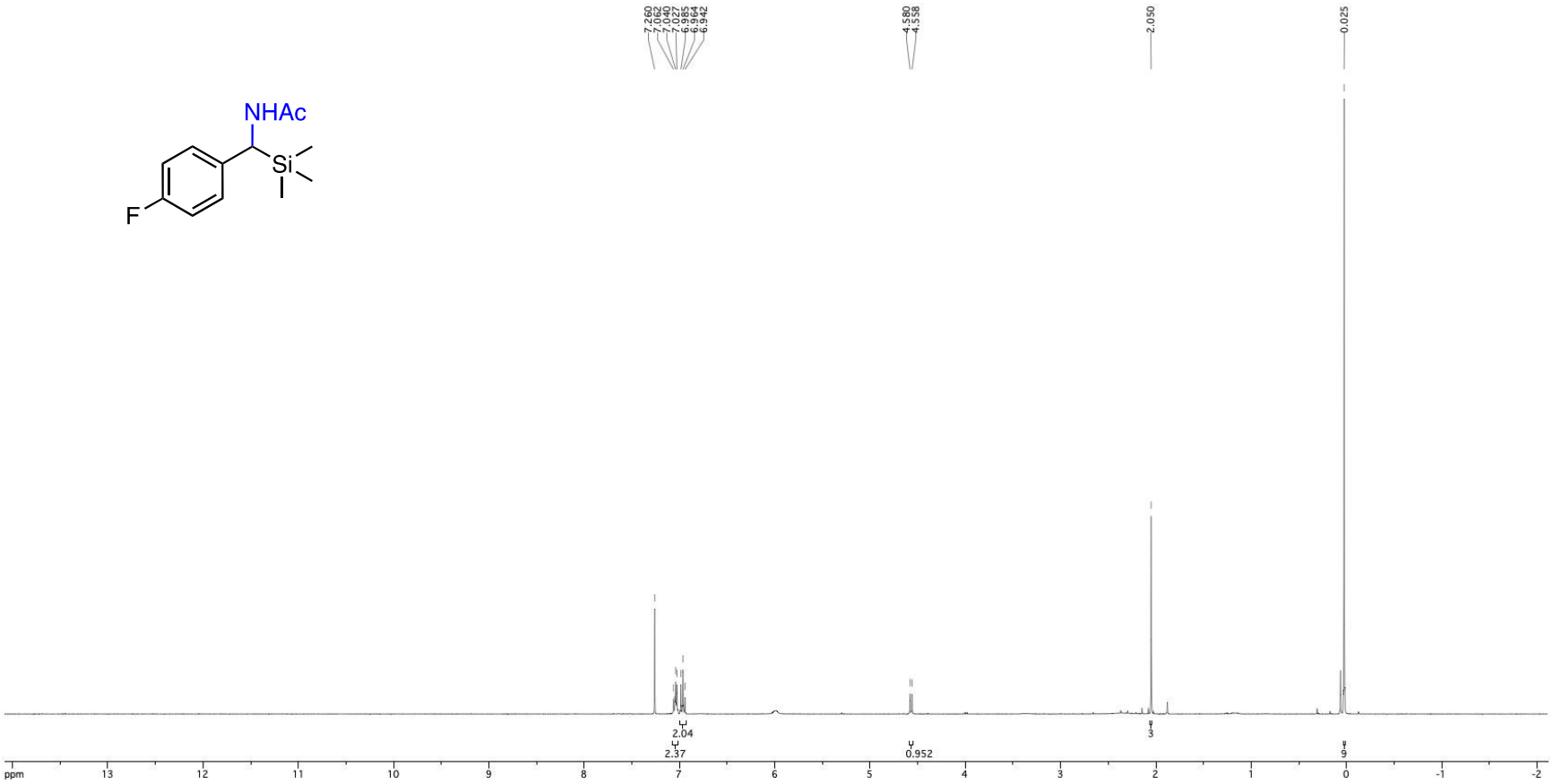
^{13}C-NMR spectrum of 3-methyl-1,4,2-dioxazol-5-one **2c** obtained in $CDCl_3$ at 23 °C.



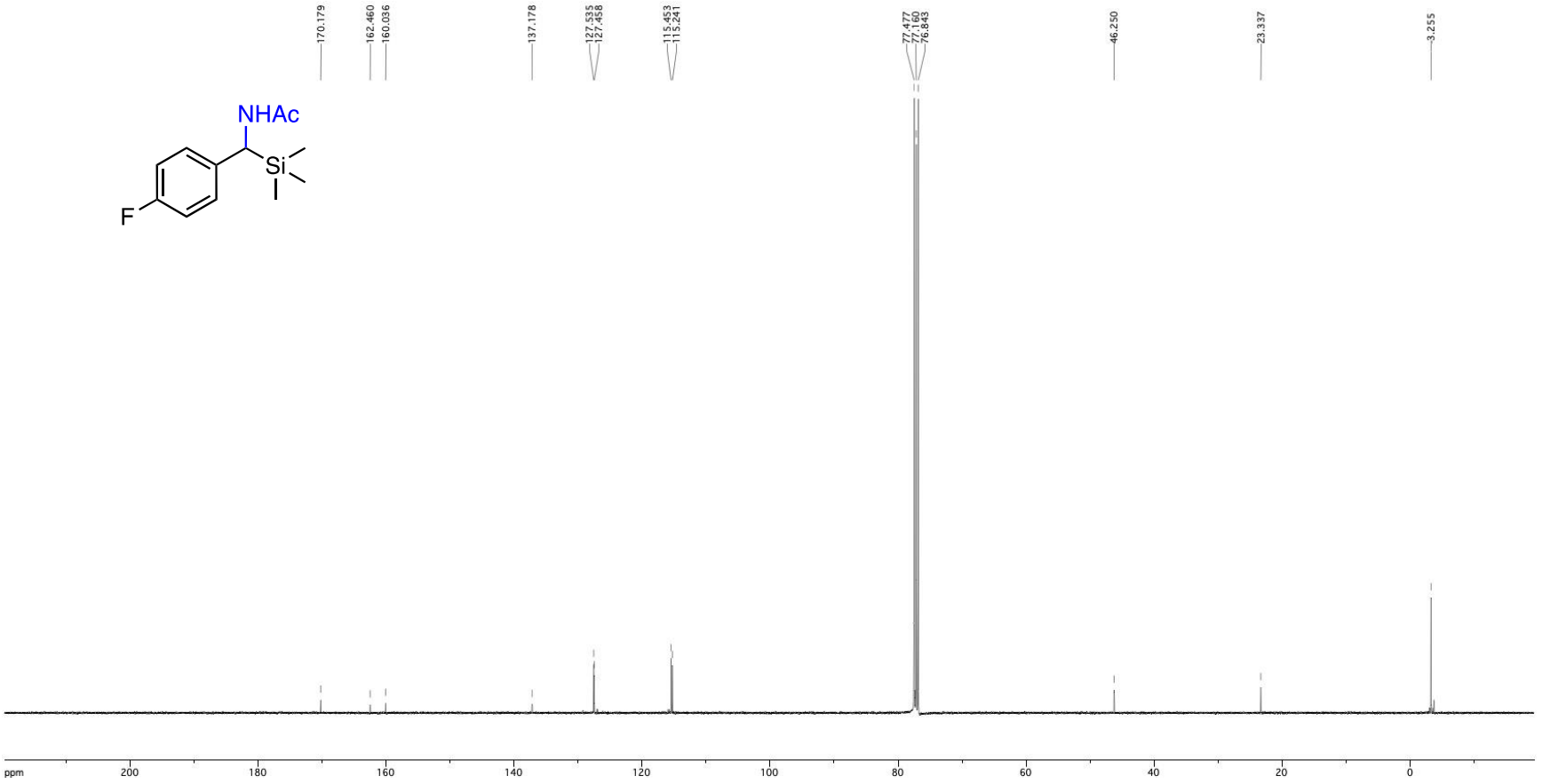
^{1}H-NMR spectrum of *N*-(phenyl(trimethylsilyl)methyl)acetamide **3a** obtained in $CDCl_3$ at 23 °C.



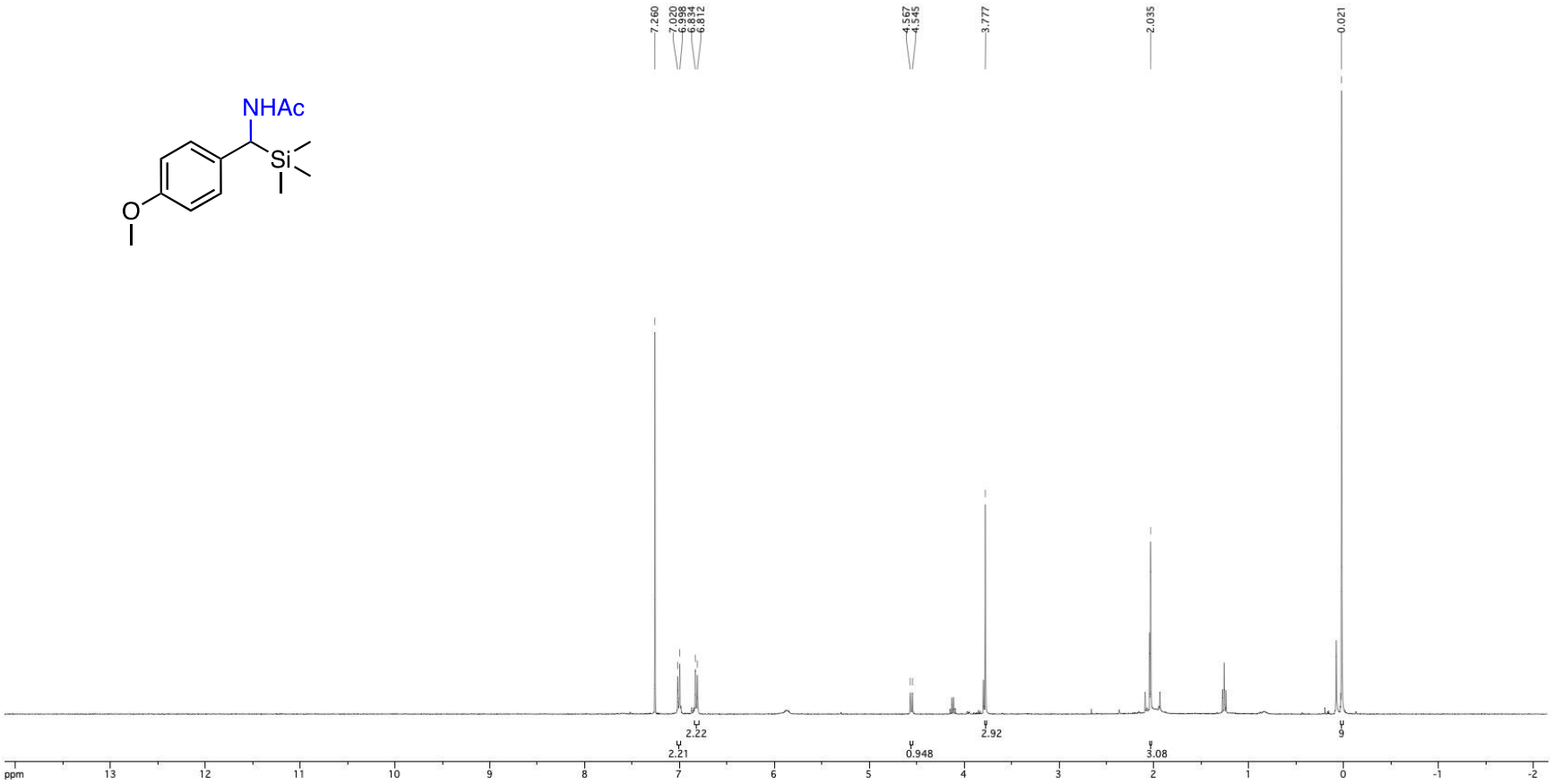
^{13}C-NMR spectrum of *N*-(phenyl(trimethylsilyl)methyl)acetamide **3a** obtained in $CDCl_3$ at 23 °C.



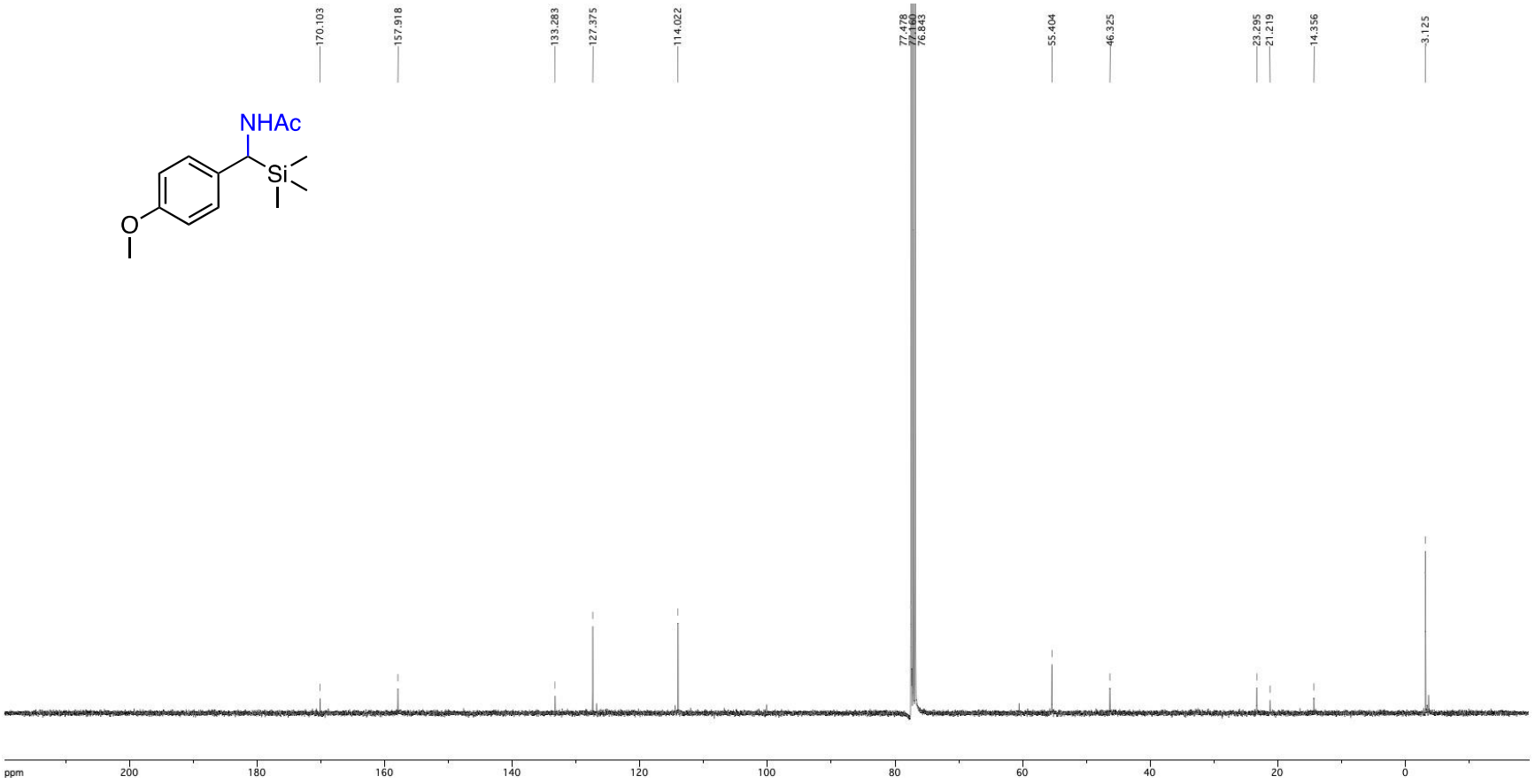
^{1}H-NMR spectrum of *N*-((4-fluorophenyl)(trimethylsilyl)methyl)acetamide **3b** obtained in $CDCl_3$ at 23 °C.



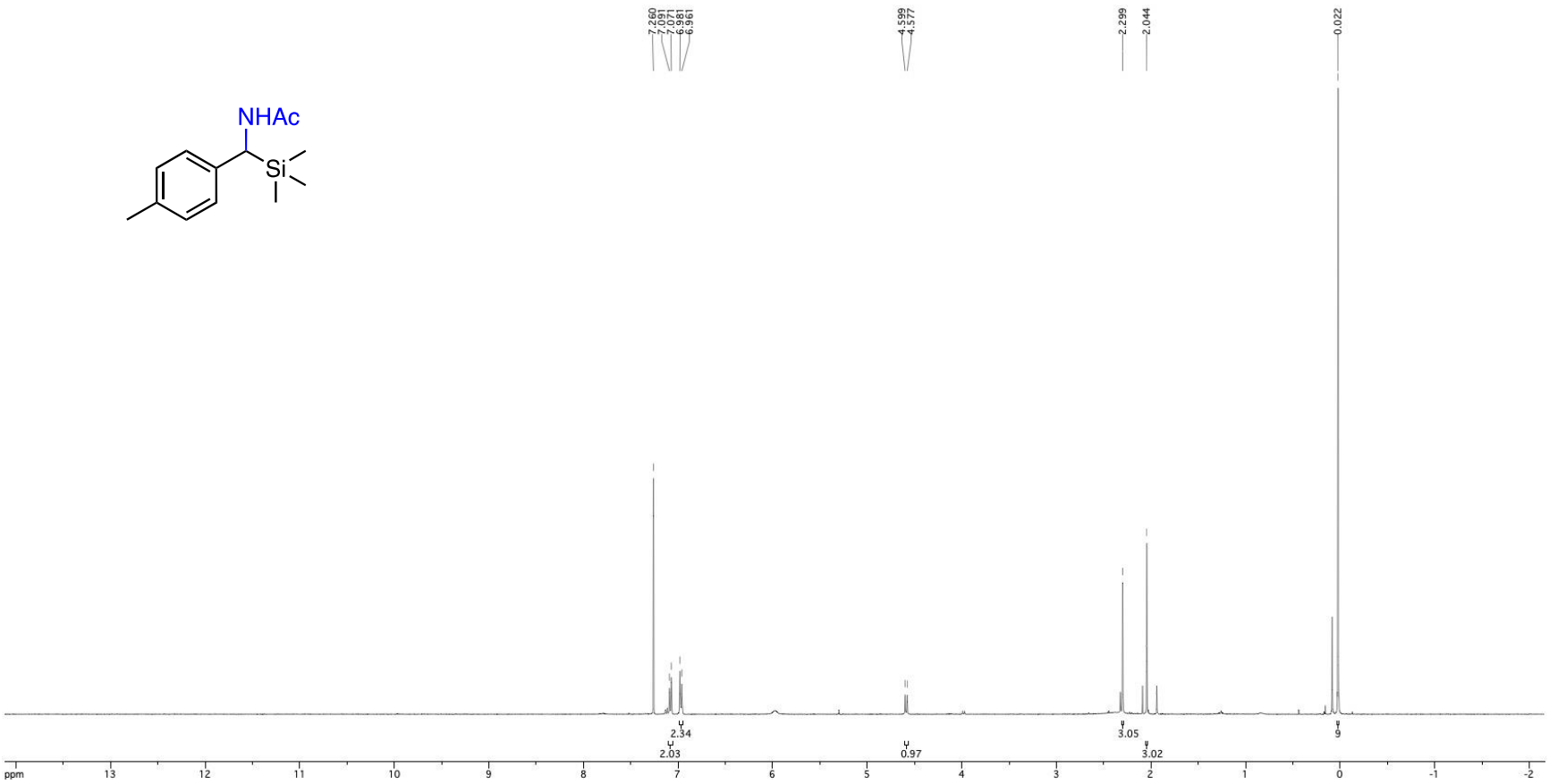
^{13}C-NMR spectrum of *N*-((4-fluorophenyl)(trimethylsilyl)methyl)acetamide **3b** obtained in $CDCl_3$ at 23 °C.



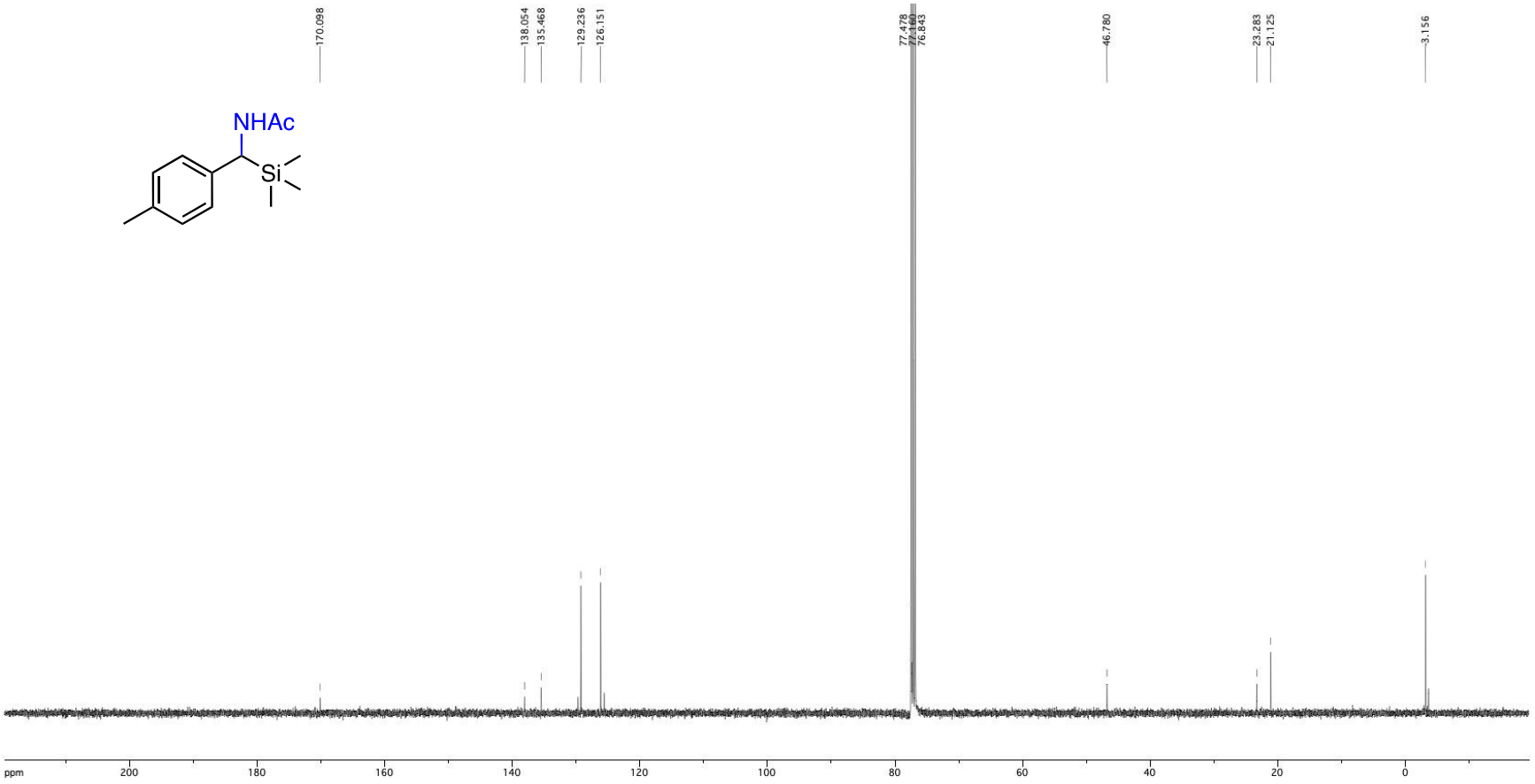
^{1}H-NMR spectrum of *N*-((4-methoxyphenyl)(trimethylsilyl)methyl)acetamide **3c** obtained in $CDCl_3$ at 23 °C.



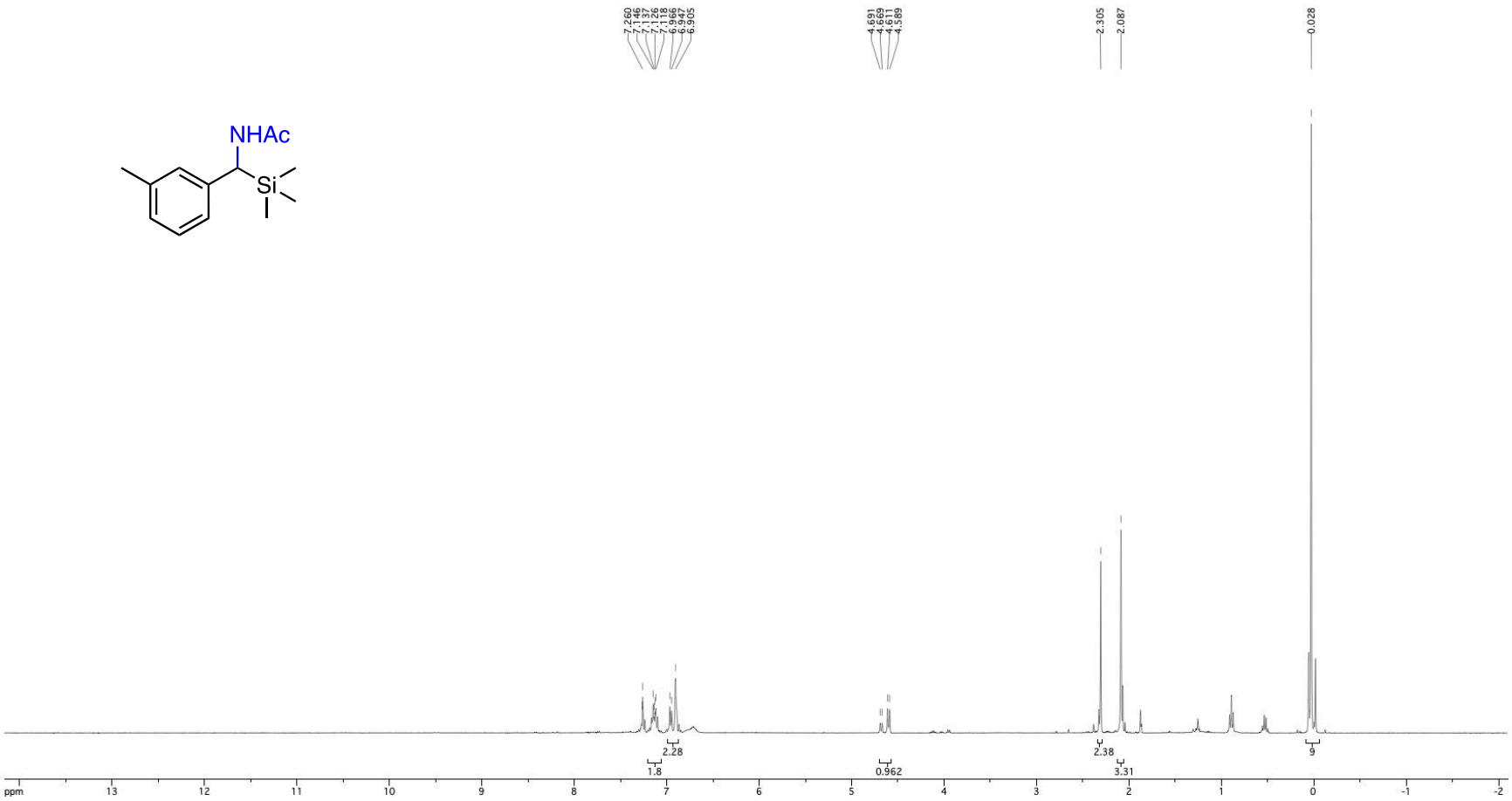
^{13}C-NMR spectrum of *N*-((4-methoxyphenyl)(trimethylsilyl)methyl)acetamide **3c** obtained in $CDCl_3$ at 23 °C.



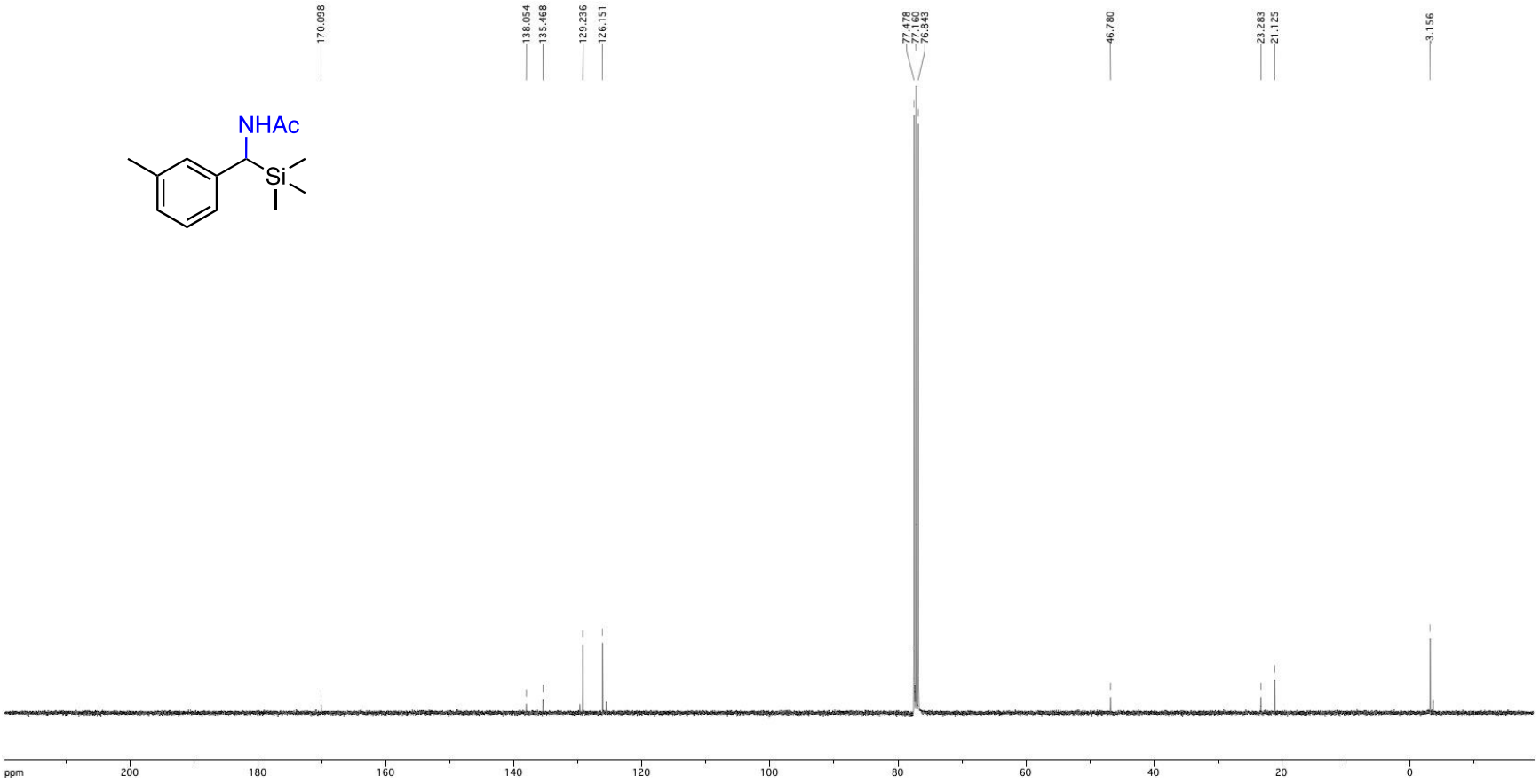
^{1}H-NMR spectrum of *N*-(*p*-tolyl(trimethylsilyl)methyl)acetamide **3d** obtained in $CDCl_3$ at 23 °C.



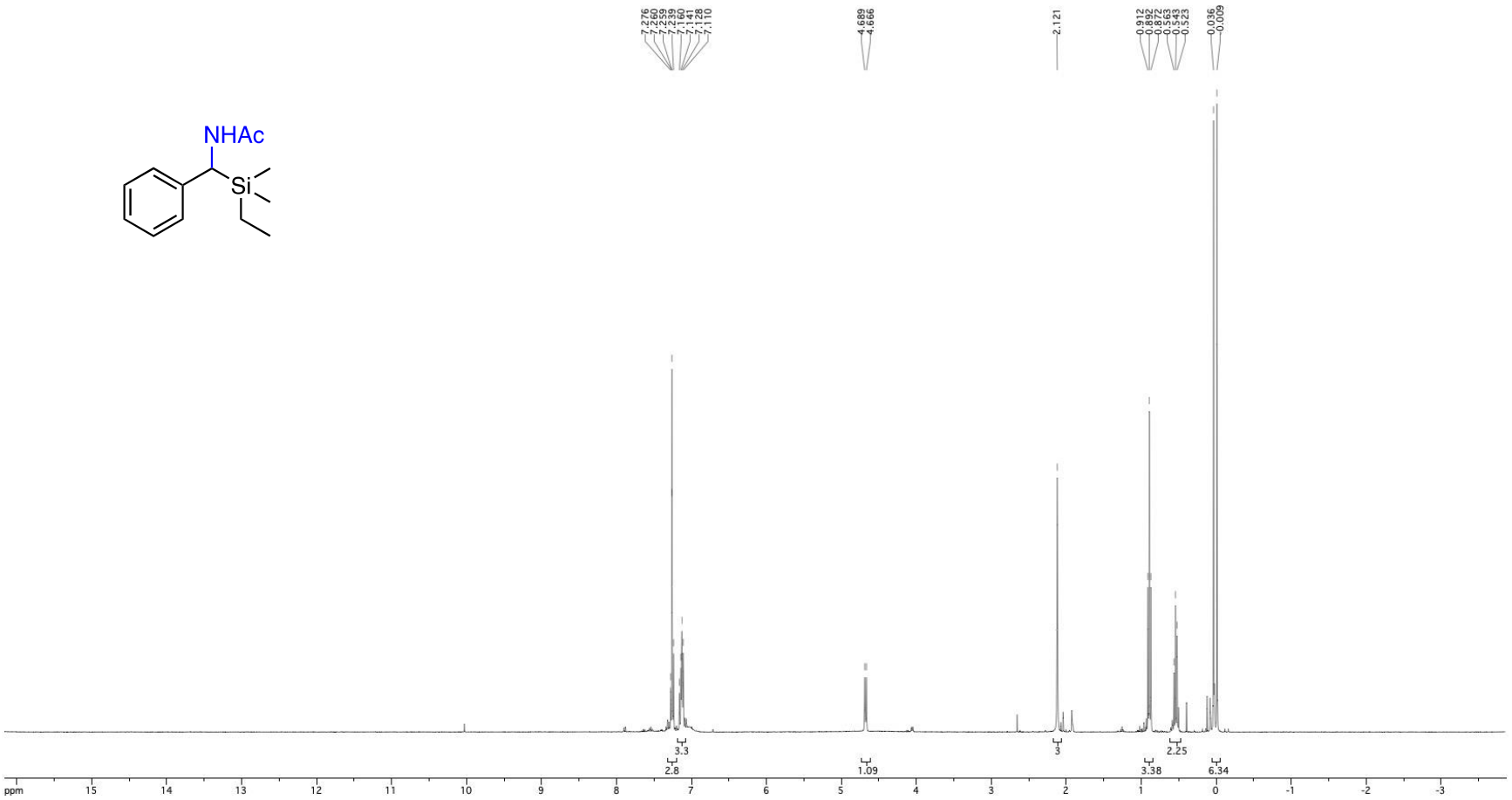
^{13}C-NMR spectrum of *N*-(*p*-tolyl(trimethylsilyl)methyl)acetamide **3d** obtained in $CDCl_3$ at 23 °C.



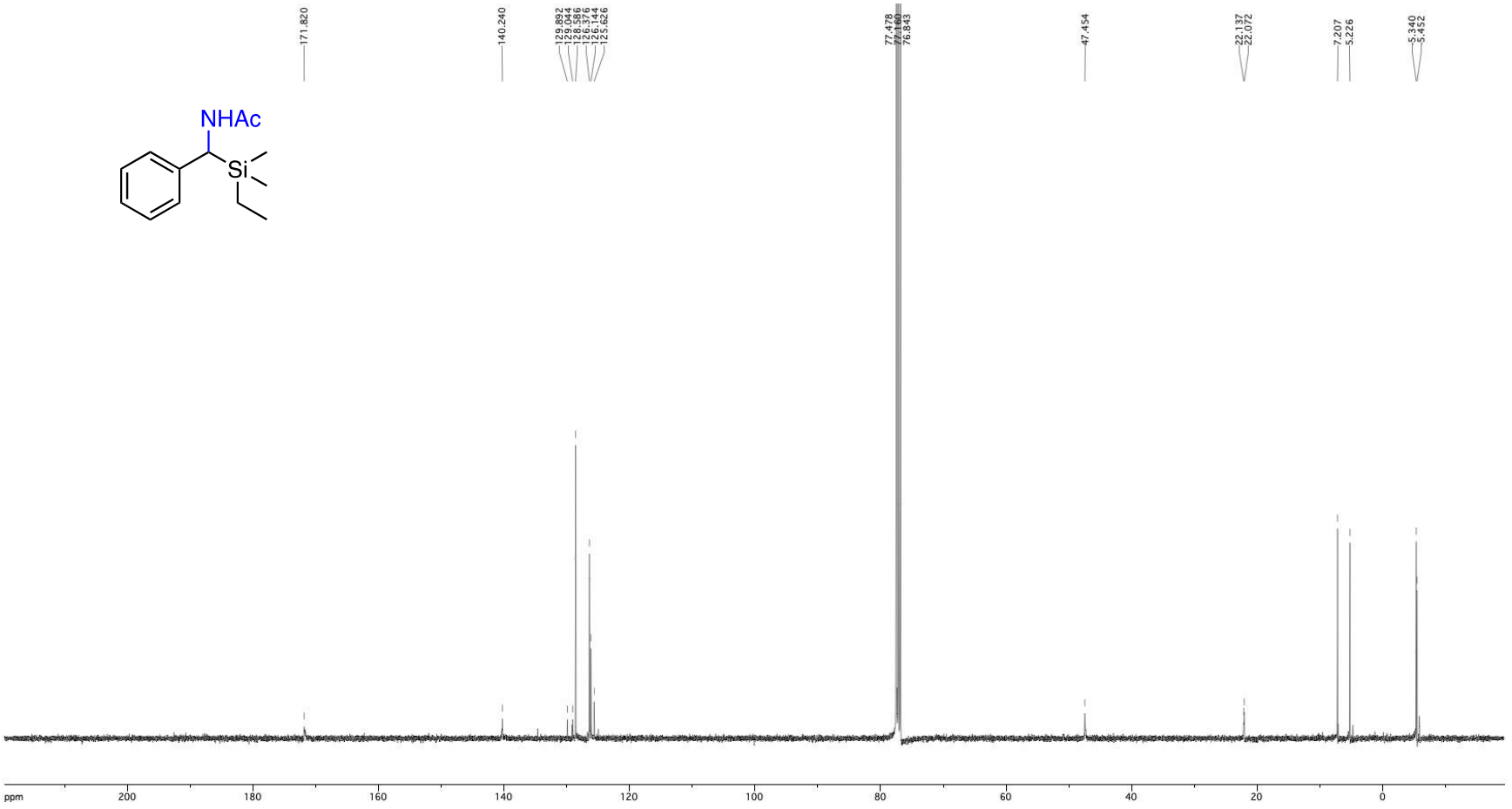
^{1}H-NMR spectrum of *N*-(*m*-tolyl(trimethylsilyl)methyl)acetamide **3e** obtained in $CDCl_3$ at 23 °C.



^{13}C-NMR spectrum of *N*-(*m*-tolyl(trimethylsilyl)methyl)acetamide **3e** obtained in $CDCl_3$ at 23 °C.



^{1}H-NMR spectrum of *N*-((ethyldimethylsilyl)(phenyl)methyl)acetamide **3g** obtained in $CDCl_3$ at 23 °C.



^{13}C-NMR spectrum of *N*-((ethyldimethylsilyl)(phenyl)methyl)acetamide **3g** obtained in $CDCl_3$ at 23 °C.

7. X-ray Crystallography

Low-temperature diffraction data (ϕ-and ω-scans) were collected on a Bruker AXS D8 VENTURE KAPPA diffractometer coupled to a PHOTON II CPAD detector with Cu K_α radiation (λ = 1.54178 Å) from an IμS micro-source for the structure of compound V23124. The structure was solved by direct methods using SHELXS[13] and refined against F^2 on all data by full-matrix least squares with SHELXL-2019[14] using established refinement techniques.[15] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included into the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U value of the atoms they are linked to (1.5 times for methyl groups).

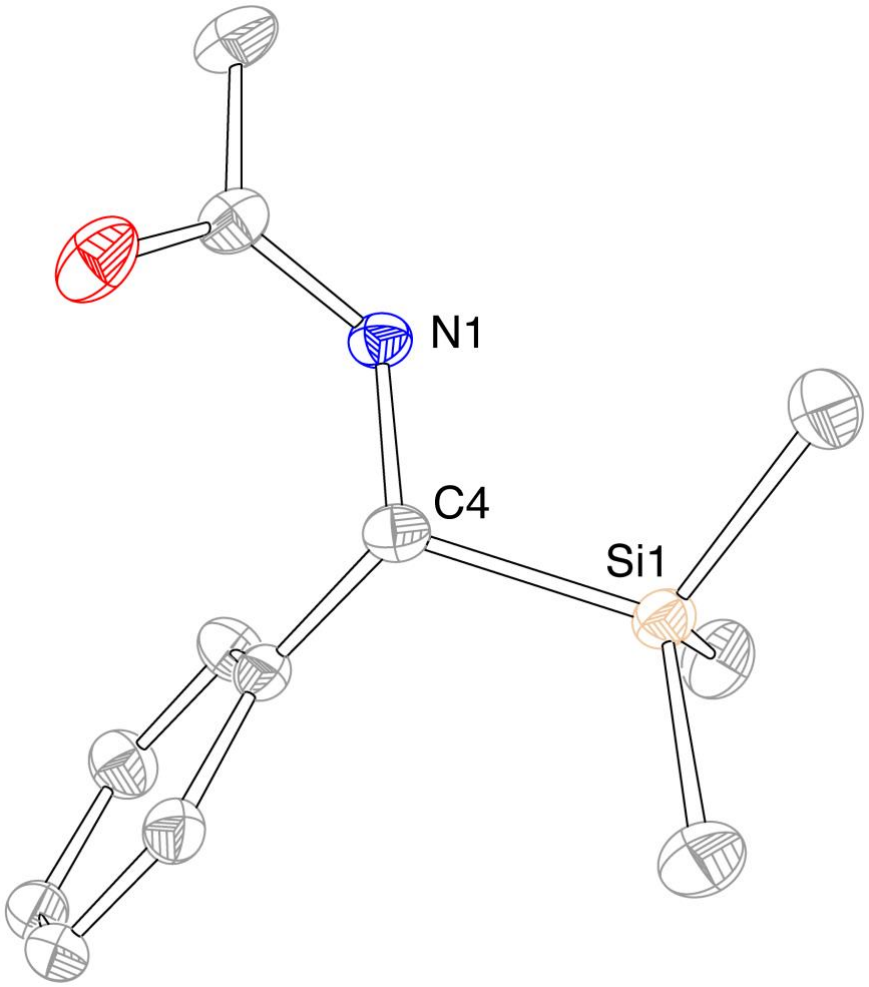


Figure S15. Displacement ellipsoid plot for **3a** obtained from enzymatic reaction, plotted at 50% probability. Single crystals of **3a** were obtained by layering a CH_2Cl_2 solution with hexanes and then storing at 4 °C. It crystallizes in the orthorhombic space group $P2_12_12_1$ with two molecules in the asymmetric unit. The coordinates for the hydrogen atoms bound to N1 and N2 were located in the difference Fourier synthesis and refined semi-freely with the help of a restraint on the N-H distance (0.88(4) Å).

Table S2. X-ray experimental details of **3a** (CCDC **2288511**) obtained from enzymatic reaction.

Crystal data	
Chemical formula	$C_{12}H_{19}NOSi$
M_r	221.37
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	100(2)
a, *b*, *c* (Å)	12.0782 (13), 13.4109 (13), 16.437 (2)
α, β, γ (°)	90
V (Å^3)	2662.5 (5)
Z	8
Radiation type	Cu *K*α
μ (mm^{-1})	1.36
Crystal size (mm)	0.50 × 0.20 × 0.20
Data collection	
Diffractometer	Bruker D8 VENTURE Kappa Duo PHOTON II CPAD
Absorption correction	Multi-scan *SADABS2016*/2 (Sheldrick, 2014)
T_{min}, T_{max}	0.472, 0.754
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	40422, 5451, 5349
R_{int}	0.051
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.625
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.029, 0.078, 1.03
No. of reflections	5451
No. of parameters	285
No. of restraints	2
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
Γ_{max}, Γ_{min} (e Å^{-3})	0.24, -0.28
Absolute structure	Flack x determined using 2314 quotients [(I+)-(I-)]/[(I+)+(I-)] (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
Absolute structure parameter	-0.002 (8)

8. Miscellaneous Experiments

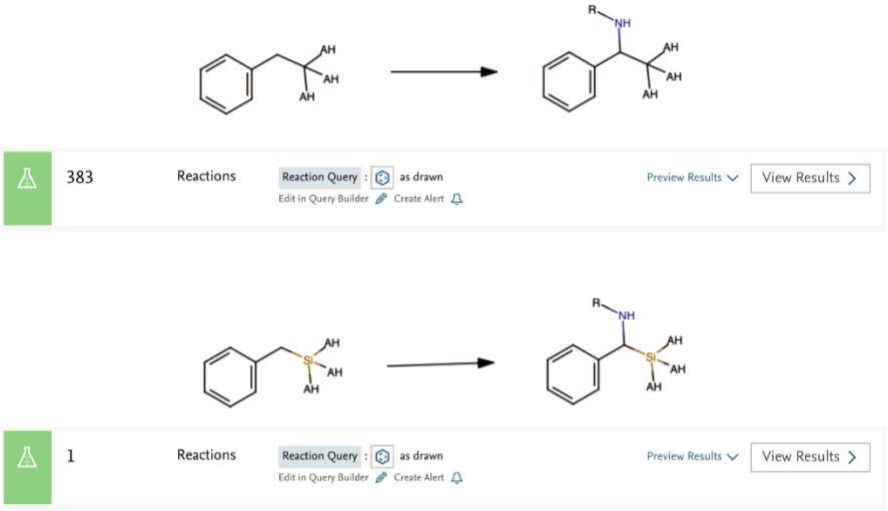


Figure S16. Reaxys quick search shows the number of reactions developed for benzylic C–C–H amination versus the number of reactions developed for benzylic Si–C–H amination.[16]

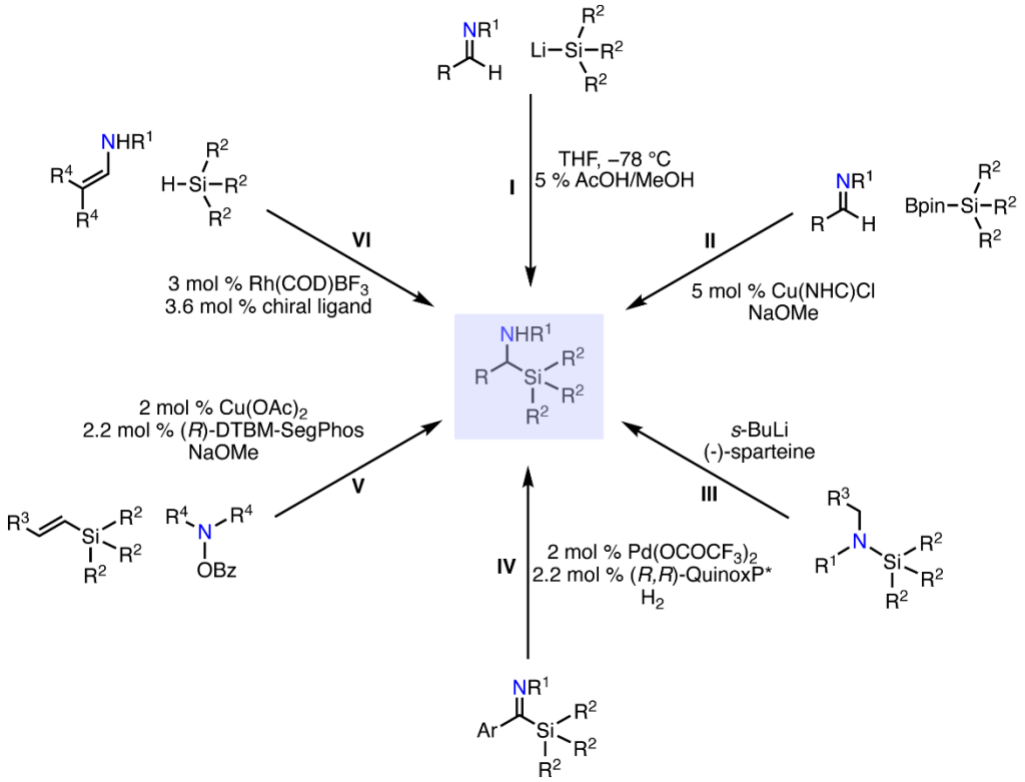


Figure S17. Synthetic methods that have been developed for accessing α-aminosilanes. These methods typically employ air-sensitive reagents, precious metal catalysts or pre-functionalized substrates.[17–23] Direct installation of amine functionality to Si–C–H bonds is underdeveloped.

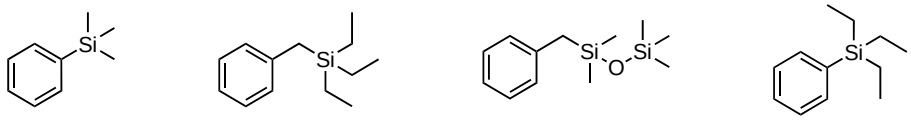


Figure S18. The substrates drawn above have no activity for the enzymatic reaction.

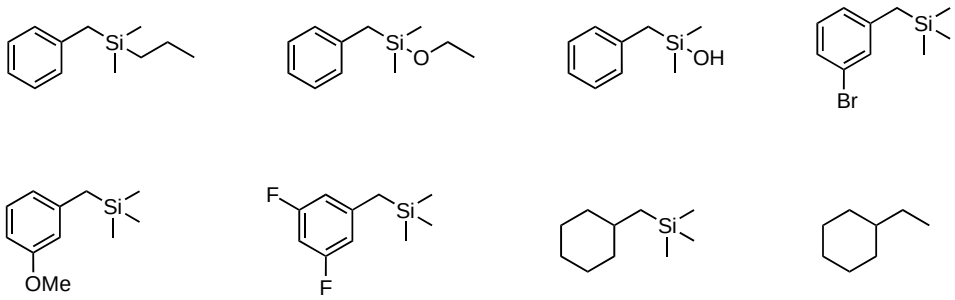


Figure S19. The substrates shown above display initial activity for the enzymatic Si–C–H amination reaction. [Note: Ethylcyclohexane was the model substrate for *uAmD5-5117* during the evolution for unactivated C–H amination. This activity is still retained and even improved in the *P411-SIA-5291* variant.]

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