Asymmetric synthesis of primary amines catalyzed by thermotolerant fungal reductive aminases

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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

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Section S1. General

Solvents used were of HPLC grade and, when necessary, they were further dried over molecular sieves. Column chromatography was performed on silica gel (Fluka (Buchs, Switzerland), 220-440 mesh). Spectra from ^{1}H and ^{13}C NMR runs were recorded on a Bruker Avance 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) in $CDCl_3$ or CD_3OD using residual protic solvent as an internal standard. Reported chemical shifts (δ) (in parts per million (ppm) are relative to the residual protic solvent signal ($CHCl_3$ in $CDCl_3$, ^{1}H = 7.26; $CDCl_3$, ^{13}C = 77.0; CHD_2OD in CD_3OD, ^{1}H = 3.31; CD_3OD, ^{13}C = 49.0).

High-resolution mass spectrometry (HRMS) was recorded using a Waters LCT time-of-flight mass spectrometer, connected to a Waters Alliance LC (Waters, Milford, MA, USA). Data were processed with Waters Masslynx software. Samples for IR spectroscopy were run on a Nicolet 5700 FT-IR (Thermo Electron, Madison, WI, USA) using a Smart Orbit Diamond accessory.

Chiral normal phase HPLC was performed on an Agilent system (Santa Clara, CA, USA) equipped with a G1379A degasser, G1312A binary pump, a G1367A well plate autosampler unit, a G1316A temperature controlled column compartment and a G1315C diode array detector. CHIRALCEL®OD-H Analytical (Daicel (Osaka, Japan), 250 mm length, 4.6 mm diameter, 5 µm particle size) columns was used. The typical injection volume was 10 µL and chromatograms were monitored at 265 nm. All solvent mixtures are given in (v/v) ratios.

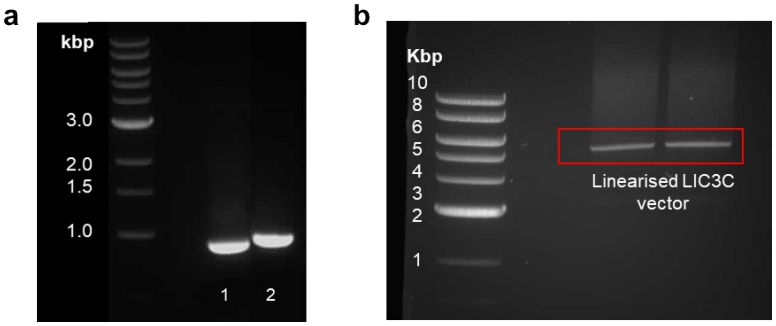
GC analysis was performed on a Agilent 6850 GC (Agilent, Santa Clara, CA, USA) with a flame ionization detector (FID) and autosampler equipped with a 25 m CP-Chirasil-DEX CB column with 0.25 mm inner diameter and 0.25 µm film thickness (Agilent, Santa Clara, CA, USA), BetaDex-325 and BetaDex-120 column (30 m x 0.25 mm x 0.25 µm) from Supelco (Bellefonte, PA, USA) and a 30 m HP-1 column with 0.32 mm inner diameter and 0.25 µm film thickness (Agilent, Santa Clara, CA, USA). Where necessary, samples were derivatised using acetic anhydride with an excess of triethylamine at room temperature prior to analysis on the GC-FID.

Section S2. Target Selection, Cloning and Expression

Based on the sequence and structure of AspRedAm, a BLAST [1] search of the databases was performed to select new RedAm targets containing residues D169, N93, Y177 and Q240 (AspRedAm numbering) that were previously shown to be important for catalysis. The sequences of the RedAm targets, from *Neosartorya fumigata* (*Nf*RedAm), and *Neosartorya fischeri (Nfis*RedAm) both have 58% overall sequence homology with *Asp*RedAm. Codon-optimized genes for the recombinant expression of each in *E. coli* were synthesized by GeneArt (Life technologies) and cloned into a locally engineered pET-YSBLIC3C vector [2] using the In-Fusion® HD Cloning kit (Clontech Laboratories, Inc.). The gene of interest was amplified by PCR reaction using relevant primers (Table S1) and purified using a QIAquick® Gel Extraction Kit. The In-Fusion reaction was performed using linearized YSBLIC3C vector and purified genes according to manufacturer's protocol. Insertion of target genes into the final constructs was verified by DNA sequencing.

Target gene	Primer ID	Base Sequence 5' to 3'
*Nfis*RedAm	fwd	TTCCAGGGACCAGCAATGAGCAGCGTTAGCATTTTTGGTCTG
	rev	CATGCTAGCCATATGTTAGGCGCTTTTCACAAAATCAATCAGGC
*Nf*RedAm	fwd	TTCCAGGGACCAGCAATGAGCAGCGTTAGCATTTTTGGTCTGG
	rev	CATGCTAGCCATATGTTAGGTGCTTTTCACAAAATCAATCAGGC

Supplementary Table S1. Primers used for Infusion Cloning of RedAm genes into pET-YSBLIC-3C.



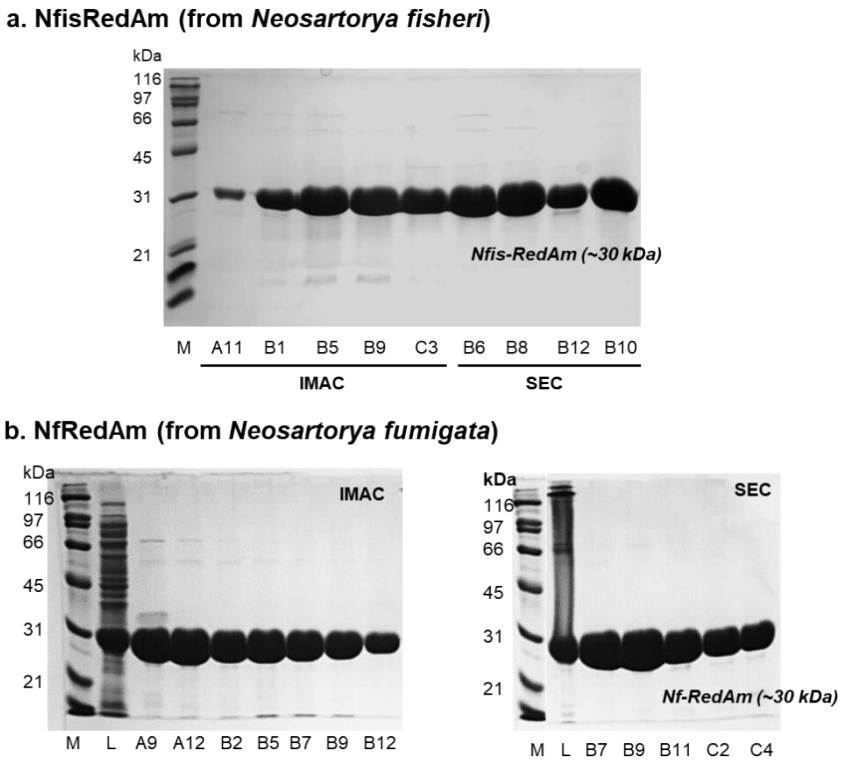
1 - Q4WDZ8_*Nf*lRED (from *Neosartorya fumigata*)
2 - A1D0J0_*Nfis*lRED (from *Neosartorya fischeri*)

Supplementary Figure S1. a. DNA gel visualization of PCR products of the corresponding genes for *Neosartorya* homologues. b. DNA visualization of linearized YSBLIC3C vector used for In-Fusion reaction

The plasmids containing the genes for target enzymes were used to transform *E. coli* BL21(DE3) competent cells for gene expression. Pre-cultures were grown in LB-medium (5 mL) containing 30 μg mL^{-1} kanamycin for 18 h at 37 °C with shaking at 180 r.p.m. 1 L

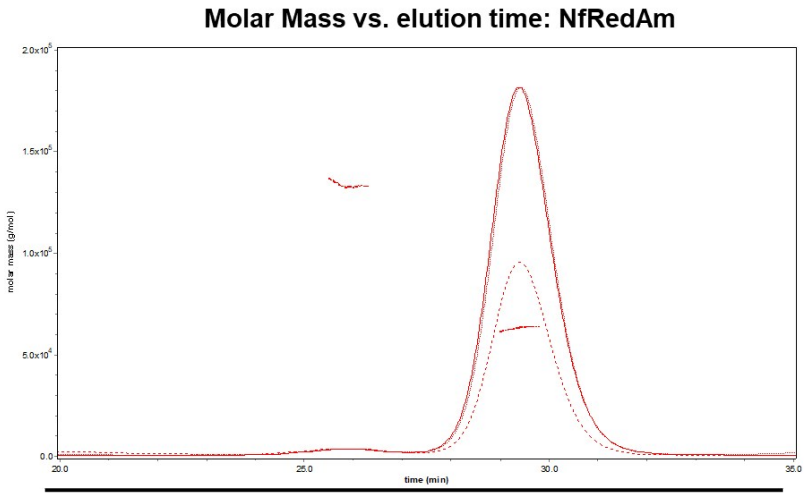
volume cultures were inoculated with the pre-culture (5 mL) and incubated at 37°C, with shaking at 180 r.p.m. until an OD_{600} of 0.6-0.8 was reached. Gene expression was induced by addition of IPTG (1 mM) and shaking was continued overnight at 18°C with shaking at 180 r.p.m. The cells were then harvested by centrifugation at 5000 g for 20 min and resuspended in 50 mM Tris-HCl buffer pH 7.5, containing 300 mM NaCl. Cells were disrupted by ultrasonication for 3 x 5 min, 30 s on, 30 s off cycles, and the suspension was centrifuged at 50,000 g for 30 min to yield a clear lysate.

The N-terminal His_6-tagged proteins were purified using immobilised-metal affinity chromatography (IMAC) using Ni-NTA column, followed by size exclusion chromatography (SEC) (Figure 3a-d). In each case, the lysate was loaded onto a pre-equilibrated Ni-NTA column, followed by washing with a load buffer (50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole pH 7.5). The bound protein was eluted using a linear gradient with buffer containing 500 mM imidazole. RedAm fractions were pooled, concentrated and loaded onto a HiLoad 16/600 Superdex 75 gel filtration column pre-equilibrated with 50 mM Tris-HCl, 300 mM NaCl pH 7.5 buffer. The concentrated protein sample after gel filtration was used for crystallization screening and biotransformation reactions.



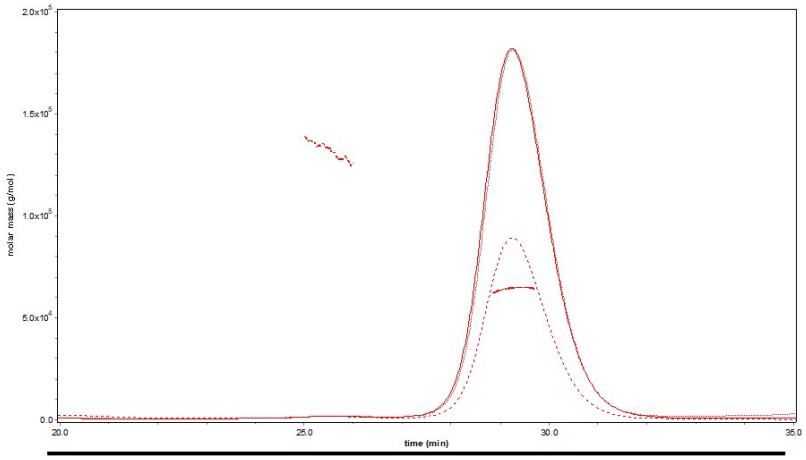
Supplementary Figure S2. Purification of *Neosartorya* RedAms. SDS-PAGE analysis of the IMAC purification using Ni-NTA column (left) and size exclusion chromatography (right) are presented.

A



Peak	from	to (min)	Amount (µg)	MW kDa
Major Peak	27.14	32.00	385	63.15
Minor Peak	24.00	26.30	9.4	133.7

B



Peak	from	to (min)	Amount (µg)	MW kDa
Major Peak	26.80	32.00	293	64.24
Minor Peak	24.00	25.80	3.7	132.0

Supplementary Figure S3. SEC-MALS analysis reveals that *Nf*RedAm and *Nfis*Redam exist as dimers in solution. UV-trace and an average molecular weight trace (red), calculated from the refractive index and light scattering signal giving mass estimation of 63 kDa which corresponds to a dimer. A minor peak corresponding to larger oligomer (tetramer) was (observed in a ratio of 40 to 1 for *Nf*RedAm and 80 to 1 for *Nfis*RedAm) when compared with the dimer.

Protein sequences of the Reductive aminases from *Neosartorya* spp.

Reductive aminase from *Neosartorya fumigata* (*Nf*RedAm)

MSSVSIFGLGAMGTALASRFLEEKYKVAVWNRSPEKASSLLGKGATLSHTAVDGINASDLIIICLLDNA
AVEATLAGALDHLHGKTIINLTNGTPDQARKLSDRFVSHGARYVHGGIMATPSMIGSPYALVLYSGSP
DAFKAAEGDLSVLAKCVFLGEDAGTASLHDLALLSGMYGLFSGFLHATALVRSSTPAVKFMDLLVPW
LGAMTEYTKGMAKQIDEGKYTSEGSNLAMQLVGIQNIIDASEAQQVSAEFIRPMKEFMQKAVAAGHG
GDDISSLIDFVKST

Reductive aminase from *Neosartorya fischeri* (*Nfis*RedAm)

MSSVSIFGLGAMGTALASRFLEEKYKVAVWNRSPEKASSLLEKGATLSYTAVDGINASDLIIICLLDNAA
VEATLAGALDHLHGKTIINLTNGTPDQARRLSDRFVSHGARYVHGGIMATPSMIGSPHALVLYSGSPD
AFKAAEADLSVLAKCVFLGEDAGSASLHDLALLSGMYGLFSGFLHATALVRSSTPAVKFVDLLVPWLG
AMTEYTKGMARQIDEGNYASEGSNLGMQLVAIQNIIDASAAQKVSADFIRPMKEFMGEAVAAGHGGD
DISSLIDFVKSA

Thermal Denaturation Studies

CD thermal unfolding measurements were performed using a Jasco J810 CD Spectrophotometer fitted with a computer-controlled Peltier temperature control unit, using protein solutions at 0.25 mg mL^{-1} in 100 mM NaPi pH7.8 containing 1 mM NADPH cofactor. The protein solution was heated through a 5 °C ramp with a 5 min relaxation time between the recording of CD spectra at different temperatures from 20-90 °C. The unfolding curves were built by using the CD signal at 220 nm to obtain melting temperature values (T_m). The results are shown in **Figure S4**. Unfolding of fungal homologues was studied by recording CD_{222} at different temperatures (20-85 ºC) as a measure of thermodynamic stability in comparison to previously reported *Asp*RedAm.

Section S3. Protein Crystallisation; Data Collection and Refinement.

Protein crystallisation

Initial screening of crystallization conditions was performed using commercially available INDEX (Hampton Research), PACT premier and CSSI/II (Molecular Dimensions) screens in 96-well sitting drop trays. Further optimization was carried out in a 24-well hanging-drop format to obtain optimised crystals for X-ray diffraction. Crystals of *Nf*RedAm-apo were grown using *Nf*RedAm at 40 mg mL^{-1} in 50 mM Tris pH 7.5, 300 mM NaCl in a drop with 1 µL protein; 2 µL mother liquor containing 3 M NaCl, 0.1 M Tris pH 8.5 and 0.5% glycerol as additive. For co-crystallization experiments, 0.2 M stock solutions of cofactor $NADP^+$ was prepared in water. 1 M stock solutions of carbonyl **2** (in DMSO) and 1 M propargylamine was prepared in 100 mM Tris pH 8.5. *Nf*RedAm- $NADP^+$ crystals were grown with *Nf*RedAm at

40 mg mL^{-1} in 50 mM Tris pH 7.5, 300 mM NaCl using a drop containing 0.15 µL protein: 0.15 µL mother liquor, the latter comprising 20% PEG (polyethylene glycol) 6000 (w/v), 0.2 M $CaCl_2.2H_2O$, 0.1 M HEPES pH 7 along with ligands 2 mM $NADP^+$, 5 mM substrate **2** and **a** each, but density for the substrates was not observed in the structure obtained.

Data collection, structure solution and refinement

Crystals of *Nf*RedAm were transferred into a cryoprotectant solution of 10% (w/v) glycerol in the mother liquor using nylon CryoLoops™ (Hampton Research) before flash-cooling with liquid nitrogen. The datasets described in this report were collected at the Diamond Light Source, Didcot, Oxfordshire, U.K. on beamlines I04-1 (*apo*-) and I03 ($NADP^+$ complex). Data were processed and integrated using XDS [3] and scaled using SCALA [4] included in the Xia2 processing system [5]. Data collection statistics are provided in **Table S2**. Crystals of *apo-Nf*RedAm were obtained in space group $P3_22_1$, with one molecule in the asymmetric unit; crystals of the $NADP^+$ complex were in space group *P*1. The solvent content in the crystals was 67% and 47%. The structure of the *apo-Nf*RedAm was solved by molecular replacement using MOLREP [6] with the monomer of *At*RedAm (PDB Code 6EOD) [7] as the model. The structure was built and refined using iterative cycles in Coot [8] and REFMAC,[9] employing local NCS restraints in the refinement cycles. The structure of *apo-Nf*RedAm was then used to solve the structure of the $NADP^+$ complex. Following building and refinement of the protein and water molecules in this complex, residual density was observed in the omit maps at the dimer interfaces, which could be clearly modelled as $NADP^+$ .The final structures exhibited % R_{cryst} / R_{free} values of 20.0/23.5 and 28.6/35.7 respectively. Refinement statistics for the structures are presented in **Table S1.** The Ramachandran plot for the *apo-Nf*RedAm showed 94.6% of residues to be situated in the most favoured regions, 4.0% in additional allowed and 1.4% residues in outlier regions. The values for the NAD complex were 86.5%, 9.7% and 3.8% respectively. The structures have been deposited in the Protein Databank (PDB) with accession codes **6SKX** (*apo-Nf*RedAm) and **6SLE** ($NADP^+$) complex**.**

	*Nf*RedAm-*apo*	*Nf*RedAm- $NADP^+$
Beamline	I04-1	I03
Wavelength (Å)	0.92819	0.97631
Resolution (Å)	98.73-2.25 (2.32-2.25)	48.28-2.77 (2.85-2.77)
Space Group	$P3_22_1$	$P1$
Unit cell (Å)	a = b = 114.0; c = 60.0	a = b = 66.4; b = 89.3; c =97.9
	α = β = 120.0°; γ = 90.0°	α = 105.6; β = 90.0°; γ = 94.00°
No. of molecules in the asymmetric unit	1	8
Unique reflections	21645 (1994)	53924 (4393)
Completeness (%)	100.0 (100.0)	98.1 (98.2)
R_{merge} (%)	0.05 (0.76)	0.14 (0.90)
$R_{p.i.m.}$	0.03 (0.37)	0.14 (0.90)
Multiplicity	9.7 (10.1)	3.4 (3.6)
$<I/\sigma(I)>$	17.4 (3.0)	5.2 (1.3)
Overall *B* factor from Wilson plot ($Å^2$)	47	36
$CC_{1/2}$	1.00 (0.96)	0.99 (0.56)
R_{cryst}/ R_{free} (%)	20.0/23.5	28.6/35.7
r.m.s.d 1-2 bonds (Å)	0.009	0.008
r.m.s.d 1-3 angles (°)	1.63	1.67
Avge main chain B ($Å^2$)	61	63
Avge side chain B ($Å^2$)	66	60
$NADP^+$ B ($Å^2$)	-	46
Avge water B ($Å^2$)	54	23

Supplementary Table S2. Data Collection and Refinement Statistics for *Nf*RedAm. Numbers in brackets refer to data for highest resolution shells.

Section S3. Determination of kinetic constants of the fungal RedAms from *Neosartorya spp*.

Kinetic assays were performed using a modified method to that previously reported in Aleku et al.[3] For the determination of kinetic constants of fungal RedAms for ketones, a typical reaction mixture contained 0.2-60 mM of the corresponding ketone, 60 or 100 mM allylamine **b** from buffer stock adjusted to pH 9, 0.4 mM NADPH, 1 % (v/v) dimethylsulfoxide and 5–100 µg of purified RedAm in a total volume of 200 µl (100 mM Tris-HCl, pH 9). Activity measurements were performed in triplicate at 340 nm (ε = 6.22 mM^{-1} cm^{-1}) or 370 nm (ε = 2.216 mM^{-1} cm^{-1}) using a Tecan infinite M200 microplate reader (Tecan Group, Switzerland). A similar set-up was used for the determination of kinetic constants of fungal RedAms for allylamine **b** where a typical reaction mixture contained 0.2-80 mM amine, 10 mM cyclohexanone **4**, 0.4 mM NADPH, 1 % (v/v) dimethylsulfoxide and 5–100 µg of purified RedAm in a total volume of 200 µL (100 mM Tris-HCl, pH 9). Kinetic constants were determined through non-linear regression based on Michaelis-Menten kinetics using Prism7 software.

RedAm	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} mM^{-1})
***Nf*RedAm**	4.13 ± 0.26	2.48 ± 0.32	0.60
***Nfis*RedAm**	0.91 ± 0.45	0.14 ± 0.02	0.15

Conditions: 0.2-60 mM cyclohexanone **3** concentration, 60 mM allylamine **b**, 0.4 mM NADPH, 1 % (v/v) dimethylsulfoxide and 5–100 µg of purified RedAm in 100 mM Tris-HCl pH 9 buffer.

Supplementary Table S3. Kinetic parameters for cyclohexanone **3** in the RedAm-catalysed reductive amination of cyclohexanone **3** and allylamine **b.**

RedAm	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} mM^{-1})
***Nf*RedAm**	1.52 ± 0.07	0.06 ± 0.04	3.9×10^{-2}
***Nfis*RedAm**	0.003 ± 0.001	0.03 ± 0.01	8.6

Conditions: 0.2-60 mM 4-phenyl-2-butanone **5** concentration, 60 mM allylamine **b**, 0.4 mM NADPH, 1 % (v/v) dimethylsulfoxide and 5–100 µg of purified RedAm in 100 mM Tris-HCl pH 9 buffer

Supplementary Table S4. Kinetic parameters for 4-phenyl-2-butanone **5** in the RedAm-catalysed reductive amination of 4-phenyl-2-butanone **5** and allylamine **b**.

Section S4. Chemicals: Synthesis of Substrates and Product Standards.

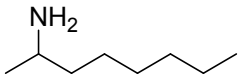
Procedure for the preparation of amine product standards and spectroscopic characterisation.

Compounds **3**-**20**, **3f**, **4f**, (*S*)-**4f**, **5f**, (*S*)-**5f**, **6f**, (*S*)-**6f**, **8f**, (*R*)-**8f**, **10f**, (*S*)-**10f**, **11f**, (*S*)-**11f**, **13f**, **14f**, (*R*)-**14f**, **16f**, (*S*)-**16f**, **17f** and (*R*)-**17f** were purchased from commercial suppliers. Preparation and spectroscopic characterisation for compounds **1a-c, 2a-b, 3a-e, 4a-e, 5a-e, 6b** and **7b** are reported in Aleku *et al.* [10]and Sharma *et al*.[7]

Synthesis of Amines 6f, 7f and 20f by Reductive Amination

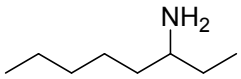
To a solution of ketone (4.0 mmol) in methanol (35 mL) were added ammonium acetate (3.08 g, 40.0 mmol) and sodium cyanoborohydride (754 mg, 12 mmol). The reaction was stirred overnight and then concentrated to 5 mL by rotary evaporation. The reaction was then quenched through the addition of 1 M aqueous NaOH (40 mL) and the crude product was extracted with EtOAc (2 x 20 mL). The combined organic layers were then acidified and extracted with 1 M aqueous HCl (3 x 15 mL) and the aqueous phase was basified to pH = 12 through the addition of 5M aqueous NaOH. The final product was then extracted with EtOAc (2 x 20 mL) before drying over anhydrous magnesium sulfate. The solvent was then removed under reduced pressure to afford the final amine product.

2-Aminooctane (**6f**)



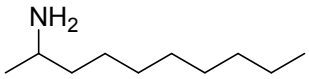
^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.93 – 2.78 (m, 1H), 1.55 (s, 2H), 1.29 (dt, *J* = 14.3, 3.9 Hz, 10H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.95 – 0.77 (m, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 47.10, 40.23, 31.99, 29.53, 26.52, 23.97, 22.75, 14.22. **HRMS** calcd. for $C_8H_{20}N^+$ 130.1551 $[M+H]^+$, found 130.1590.

3-Aminooctane (**20f**)



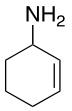
^{1}H NMR (500 MHz, Chloroform-*d*) δ 2.59 (tt, *J* = 7.2, 4.6 Hz, 1H), 1.49 – 1.32 (m, 4H), 1.30 – 1.23 (m, 7H), 0.88 (dt, *J* = 10.5, 7.3 Hz, 6H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 52.82, 37.71, 32.17, 30.81, 26.01, 22.80, 14.19, 10.53. **HRMS** calcd. for $C_8H_{20}N^+$ 130.1551 $[M+H]^+$, found 130.1590.

2-Aminodecane (**7f**)



^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.86 (h, *J* = 5.8 Hz, 1H), 1.45 (m, 2H), 1.28 (d, *J* = 11.4 Hz, 12H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.87 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 47.10, 40.36, 32.03, 29.90, 29.75, 29.43, 26.59, 24.10, 22.81, 14.24. **HRMS** calcd. for $C_{10}H_{24}N^+$ 158.1864 $[M+H]^+$, found 158.1896.

Synthesis of amine 19f via Mitsunobu Reaction



Following a procedure by Mangas-Sanchez *et al*.,[11] to a stirred solution of 2-cyclohexen-1-ol (500 mg, 5.09 mmol, 1 eq.) in anhydrous THF (25 mL) under an atmosphere of N_2 at 0 °C was added PPh_3 (1.61 g, 6.12 mmol, 1.2 eq.), phthalimide (900 mg, 6.12 mmol, 1.2 eq.) and diisopropyl azodicarboxylate (1.20 mL, 6.12 mmol, 1.2 eq.). The reaction mixture was allowed to warm to rt and stirred for 3 hours, after which a further portion of THF (25 mL) and EtOH (10 mL) were added, followed by the addition of hydrazine monohdydrate (50 wt%, 3.67 mL, 38.2 mmol, 7.5 eq.). The reaction mixture was heated to reflux for 2 h, then cooled to rt and filtered through a pad of Celite. The Celite was washed with THF (100 mL), then the filtrate was collected and concentrated *in vacuo*. The residue was taken up in 3 M HCl (25 mL) and washed with DCM (3 x 20 mL). The aqueous phase pH was then adjusted to pH 12 with 5 M NaOH, and extracted with DCM (3 x 20 mL). The combined organic extracts were washed with saturated brine solution (30 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product which was used without further purification. The signals in the ^{1}H NMR spectrum matched those previously reported in the literature.[12]

General procedure for transaminase reductive amination.

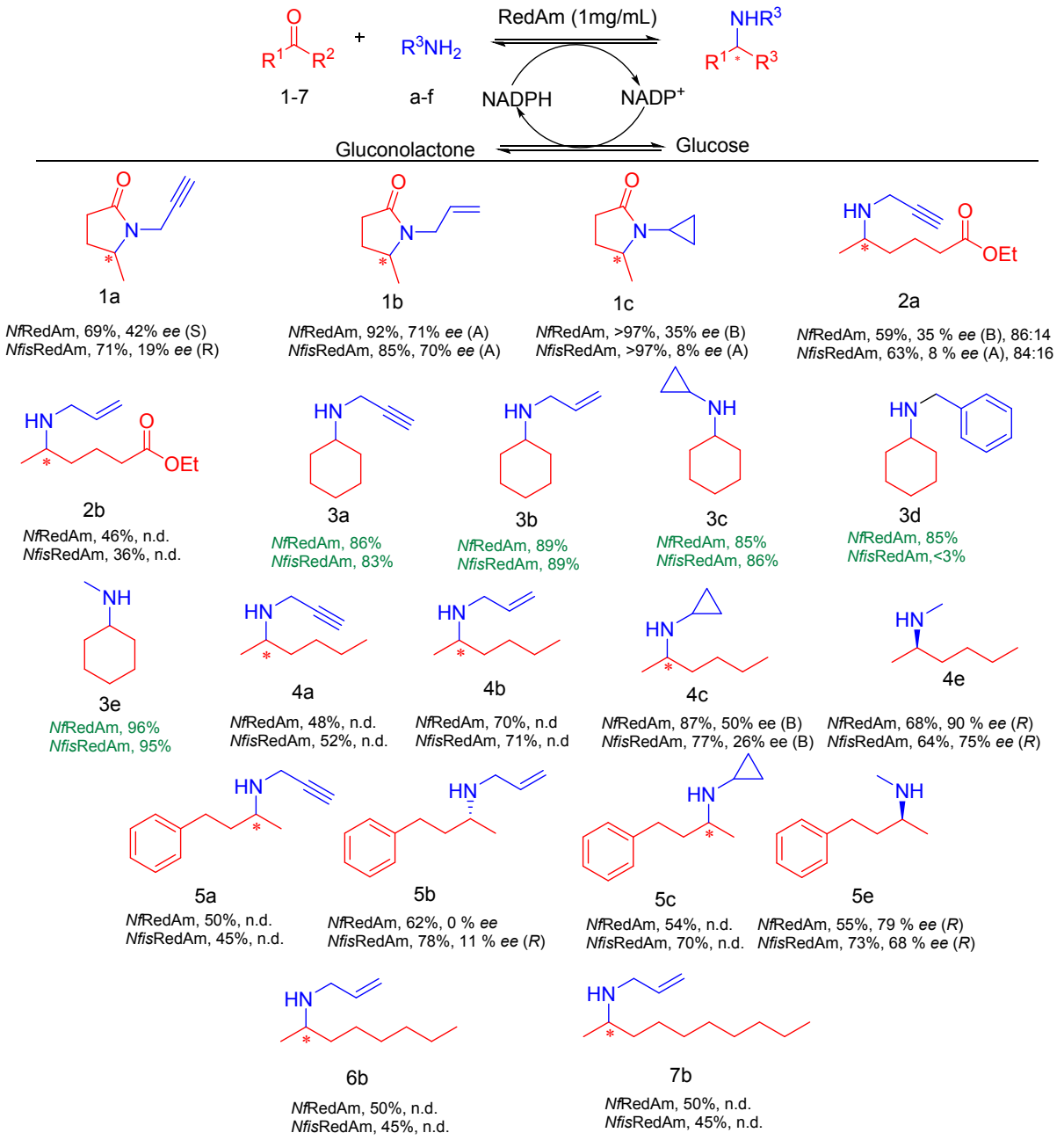
To a solution of the corresponding ketone **7** or **20** (10 mM) in 100 mM NaPi buffer pH 8 containing isopropylamine (1M) and PLP (1 mM), ATA-113 (2.5 mg mL^{-1}) was added in 500 µL total reaction volume. Reactions incubated at 30°C, 250 rpm, 24 h. Reactions basified to pH 12 with 10 M NaOH, extracted into MTBE (1 mL), dried over $MgSO_4$and analysed by GC-FID.

Section S5. Biotransformations of ketone-amine partners by RedAms.

Typical procedure for RedAm-catalysed reductive amination.

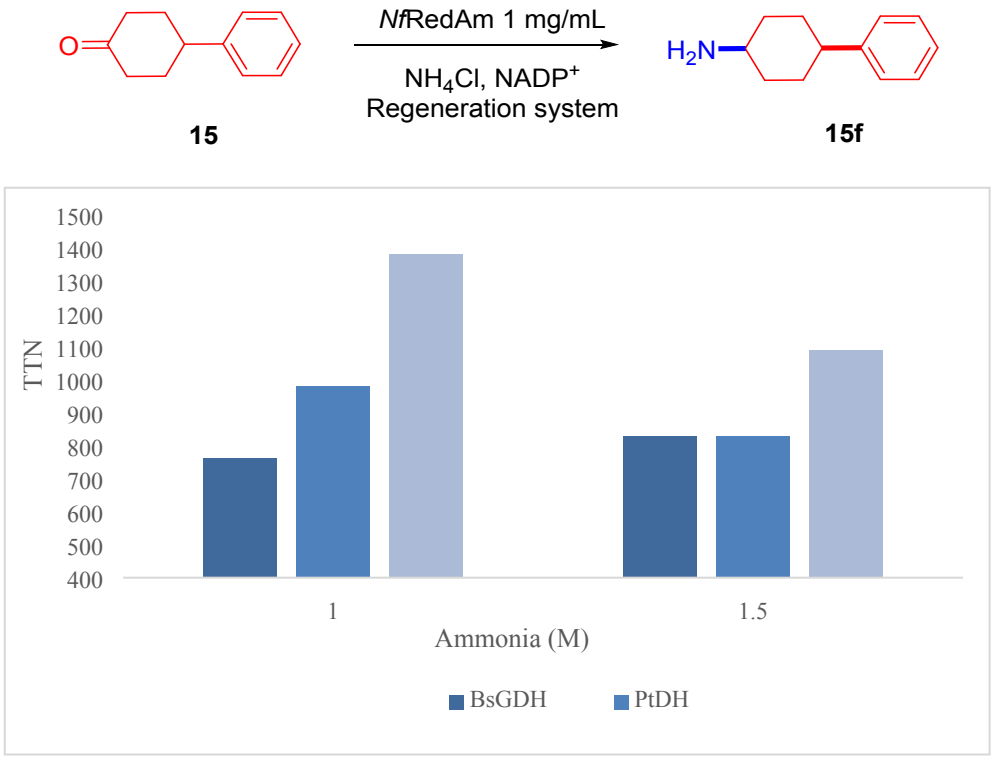
A 500 µL reaction mixture contained 20 mM D-glucose, 0.5 mg mL^{-1} GDH (Codexis, CDX-901), 0.5 mM $NADP^+$, 1 mg mL^{-1} purified RedAm, 5 mM ketone, the appropriate ratio of amine nucleophile (in Tris-HCl buffer adjusted to pH 9) and 2 % v/v DMSO. Reactions were incubated at 30 °C with 250 r.p.m. shaking for 24 h, after which they were quenched by the addition of 30 µl of 10M NaOH and extracted twice with 500 µl *tert*-butyl methyl ether. The organic fractions were combined and dried

over anhydrous $MgSO_4$ and analysed by HPLC or GC-FID on a chiral stationary phase.

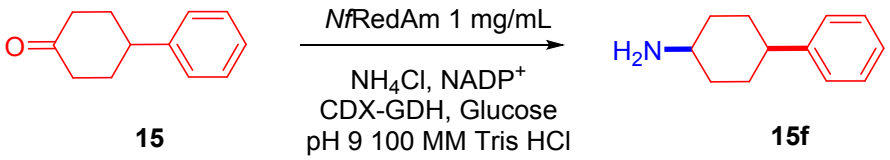


Supplementary Figure S4. GC conversions after 24h in the bioreductive amination of a series of carbonyl compounds with primary amines catalysed by the Redams from *N. fumigata* and *N. fischeri*. Conditions: RedAm 1 mg mL^{-1}, 5 mM susbtrate, 5 mM amine (green) or 25 mM amine (black) concentration in pH 7 100 mM Tris-HCl. Conversions based on the areas of substrate and product determined by GC-FID.

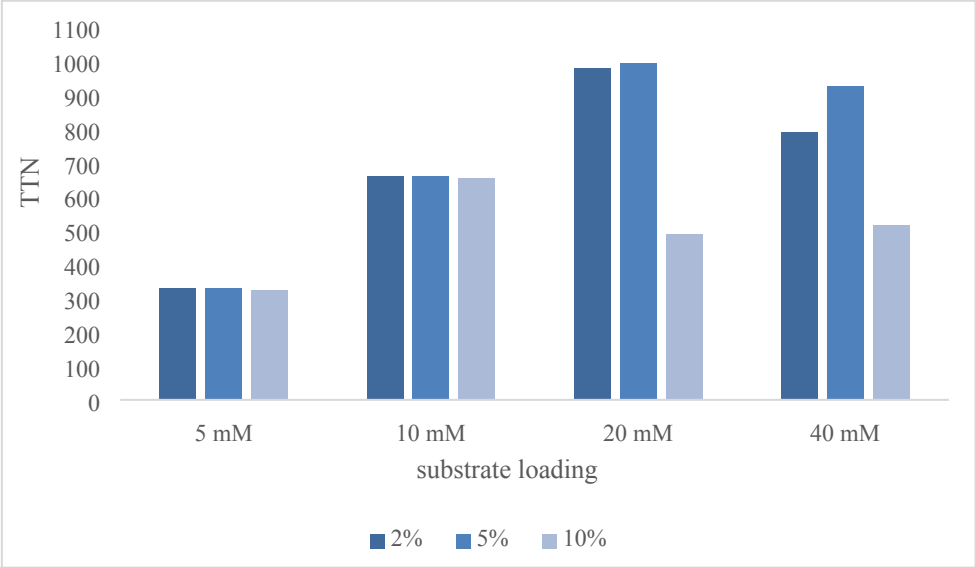
Study of different regeneration systems and DMSO concentration.



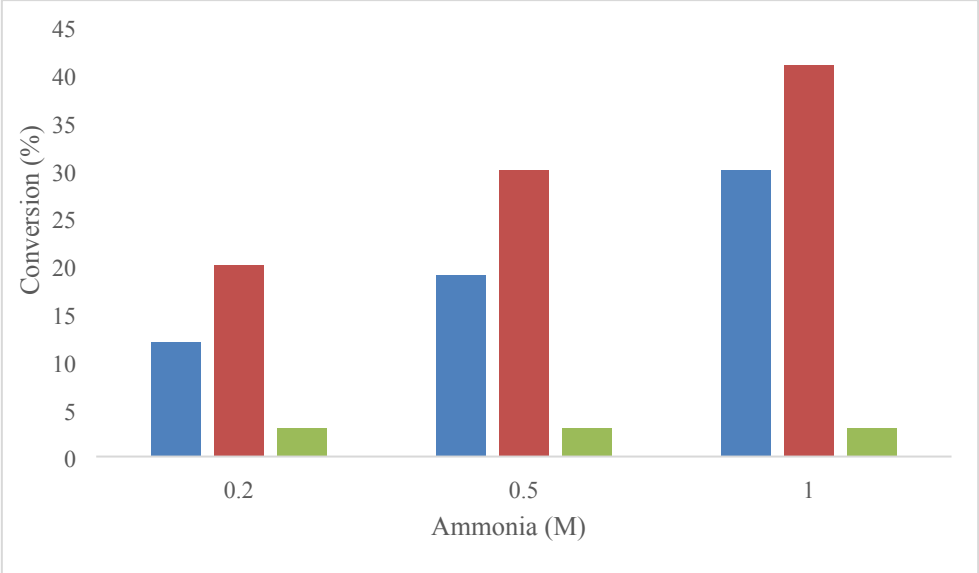
Supplementary Figure S5. Turnover numbers (TN) obtained with different regeneration systems at different ammonia concentrations in the bio-reductive amination of **15** and ammonia catalysed by *Nf*RedAm.



15 **15f**

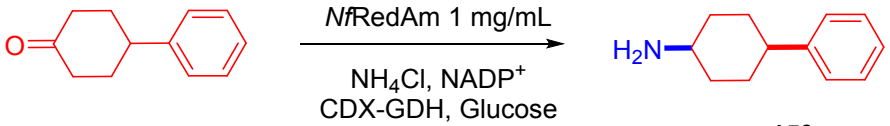


Supplementary Figure S6. Turnover numbers (TN) obtained at different DMSO and substrate concentrations in the bio-reductive amination of **15** and ammonia catalysed by *Nf*RedAm.



Supplementary Figure S7. Conversions after 24 h in the bio-reductive amination of **5** with different ammonia sources. Ammonium hydroxide (NH_4OH, blue bar), ammonium chloride (NH_4Cl, red bar) and ammonium sulphate (NH_4SO_4, green bar). Conversions based on the areas of substrate and product determined by GC-FID.

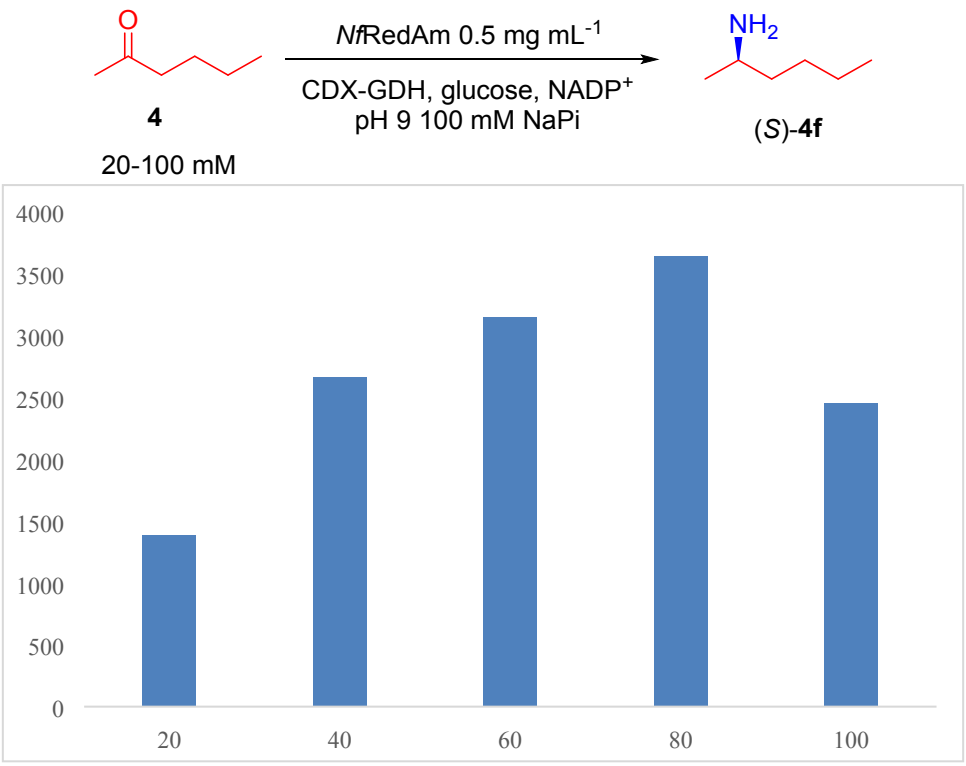
Reactions at high substrate loadings.



15 **15f**

DMSO (%)	Substrate (mM)	NH_4Cl (M)	TN	Conversion (%)
4	20	1	>577	>97
4	20	1.5	>577	>97
8	40	1	1149	97
8	40	1.5	933	80
12	60	1	1206	69
12	60	1.5	140	8

Supplementary Figure S8. Bioreductive amination of 4-phenyl-cyclohexanone **15** and ammonia **f** at high substrate loadings. Conversions after 24 h. determined by GC-FID based on the areas of substrate and product. Turnover numbers (TN) calculated using conversions after 24 h.



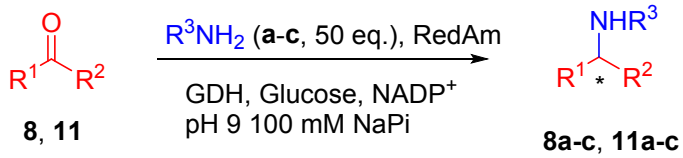
Supplementary Figure S9. Bioreductive amination of 2-hexanone (**4**) and ammonia (**f**) at high substrate loadings. Turnover numbers (TN) calculated using conversions after 24 h. determined by GC-FID based on the areas of substrate and product.

Biotransformations at high substrate loadings employing primary amines

Entry	Ketone	Ketone concentration (mM)	Amine	Amine equivalents	Conversion (%)	TTN
1	**3**	100	**b**	1	93	3255
2	**3**	100	**b**	1	90	6300
3	**3**	100	**b**	1	95	2217
4	**3**	200	**b**	1	85	5950
5	**3**	200	**b**	1	82	11480
6	**3**	200	**b**	1	88	4107
7	**3**	300	**b**	1	82	8610
8	**3**	300	**b**	1	66	13860
9	**3**	300	**b**	1	69	4830
10	**3**	100	**e**	2	83	2905
11	**4**	100	**c**	5	97	3395
12	**4**	200	**c**	2	97	6790
13	**5**	100	**c**	5	62	2170

Supplementary Table S5. Bioreductive amination of a series of carbonyl compounds with primary amines at high substrate loadings. Conversions after 24 h. determined by GC-FID based on the areas of substrate and product. Turnover numbers (TN) calculated using conversions after 24 h.

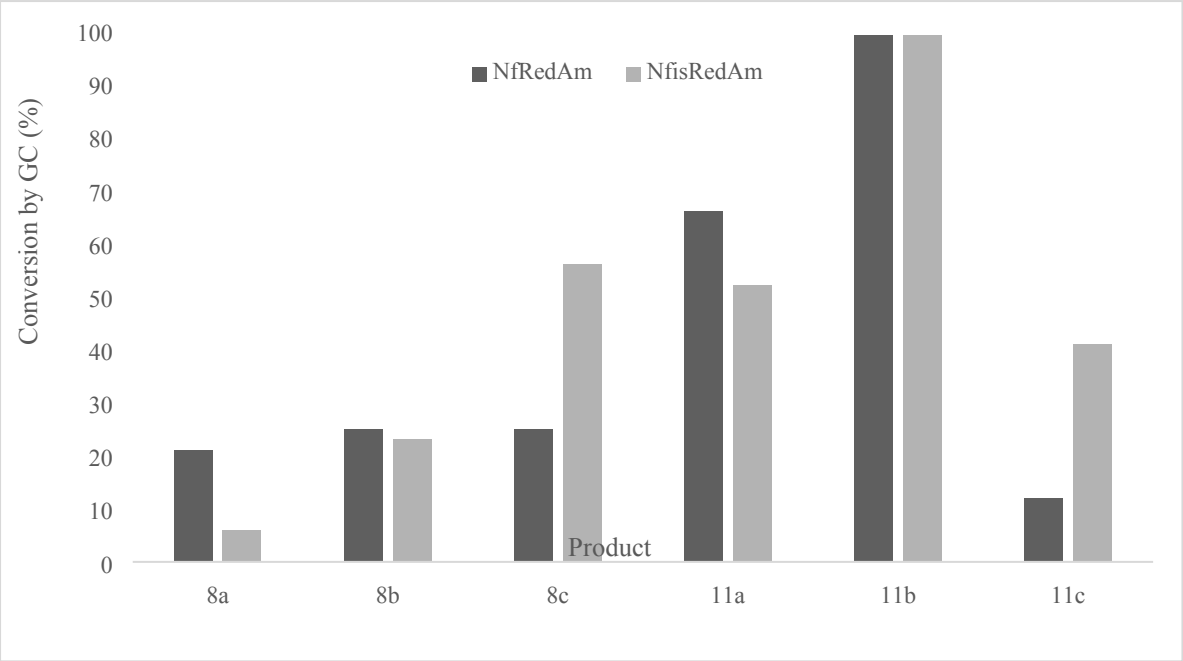
Bioreductive amination of acetophenone 8 and 4-fluorophenyl acetone 11 with different amine partners a-c using 50 amine equivalents.



8, **11** **8a-c**, **11a-c**

8: R^1= Ph; R^2= Me
11: R^1= CH_2Ph; R^2= Me

8a: R^1= Ph; R^2= Me; R^3= CH_2CCH
8b: R^1= Ph; R^2= Me; R^3= CH_2CHCH_2
8c: R^1= Ph; R^2= Me; R^3= CH_2CyPr
11a: R^1= CH_2Ph; R^2= Me; R^3= CH_2CCH
11b: R^1= CH_2Ph; R^2= Me; R^3= CH_2CHCH_2
11c: R^1= CH_2Ph; R^2= Me; R^3= CH_2CyPr



Supplementary Figure S10. Bioreductive amination of acetophenone (**8**) and 4-fluorophenyl acetone (**11**) with different amine partners **a**-**c**. Conversions based on the areas of substrate and product determined by GC-FID after 24. **8a**-**c** *ee* from 91% to >97% (absolute configuration not determined). **11a** obtained in 90% *ee* (*R*) *Nf*RedAm and >97% *ee* (*R*) *Nfis*RedAm. Optical purity for **11b**-**c** not determined.

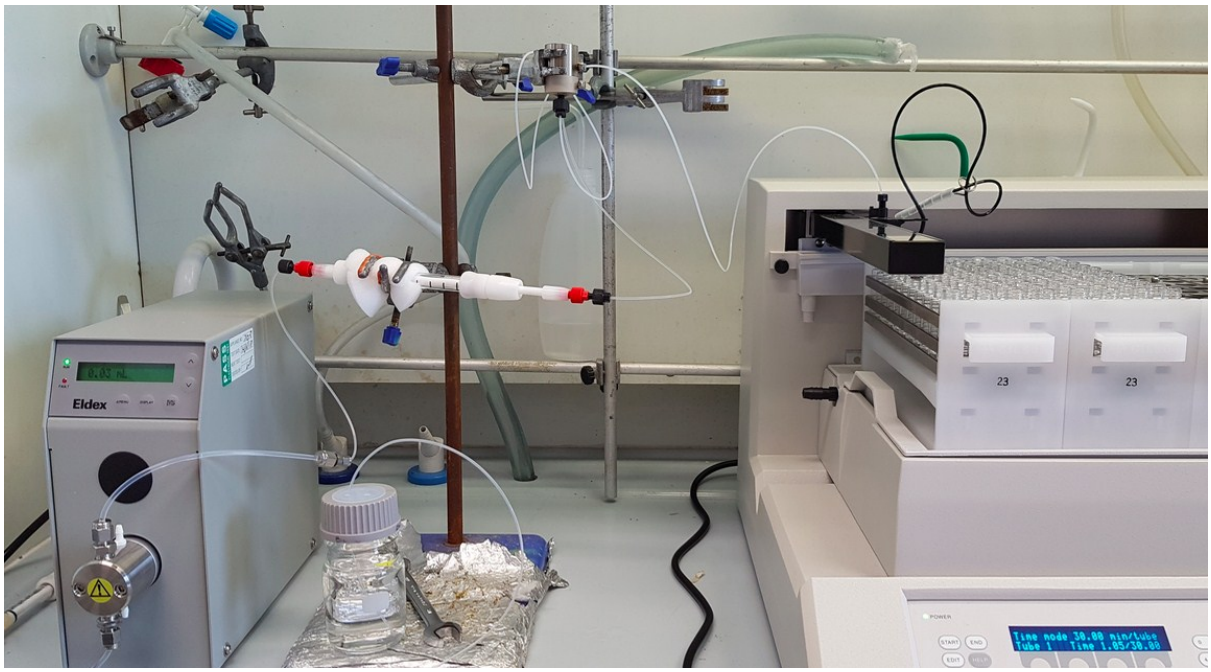
Section S6. Flow reactions

General flow experimental

Continuous flow reactions were performed using the following equipment: Eldex Optos series pumps model 2SM (flow rate 0.003-5.000 mL min^{-1}), Kinesis Omnifit glass columns (I.D 0.68 mm) and PTFE tubing (I.D 1/16″) were purchased from Cole-Parmer (Cambridge, UK). Stainless steel and PEEK fittings were purchased from Swagelok (Manchester, UK). A Gilson FC204 110/220V fraction collector was purchased from Gilson (Dunstable, UK). An SSI flow-through back-pressure regulator (5-75 psi) was purchased from Sigma-Aldrich (Gillingham, UK). EziG was purchased from EnginZyme (Stockholm, Sweden).

Flow experiments

Flow experiments were carried out using the set-up show in Supplementary Figure **S11**.



Supplementary Figure S11. Flow reactions set-up

Immobilisation procedure

EziG was weighed out to give 10 wt% with respect to the biocatalyst. It was found that EziG^2 was the best support for *Nf*RedAm. To immobilise *Nf*RedAm (25 mg), EziG^2 (225 mg) was suspended in Tris-HCl (20 mM, 300 mM NaCl, pH 8) and the biocatalyst solution was added to give a final total volume of 5 mL. This was stirred using orbital rotation at 12 rpm for 30 minutes, after which the solution was centrifuged and the supernatant biocatalyst concentration recorded using a Nanodrop. When the concentration was <0.1 mg mL^{-1}, the supernatant was discarded and the immobilised enzyme preparation washed with the same Tris-HCl buffer (x 3). This was combined with immobilised *Bs*GDH-EziG^3 (10 wt% w.r.t GDH, 75 mg total mass) and stirred for 5 minutes to ensure efficient mixing. The combined enzyme preparation was the packed into a column, and the final volume of the packed bed calculated as according to the manufacturer's instructions. The combined mass of 325 mg typically gave a column volume of ~1 mL.

Flow Reactions

The column was attached to the pump, and after the column a back-pressure regulator was placed and calibrated to 60 psi. A total of 10 column volumes of Tris-HCl buffer (100 mM, pH 8) was passed through the reactor at 500 μL min^{-1} and discarded, after which the column was primed with reaction buffer (see table), which also contained substrate, co-factor and glucose. After being primed, the reactions were slowed to the appropriate time to give a 30 minute residence time. For example, a column with a volume of 1 mL would require a 33 μL min^{-1} flow rate. The effluent was collected using a fraction collector, and the fractions were analysed individually by gas chromatography.

Entry[a]	RedAm	EziG[b]	Buffer	Conc	RV conv loss[c]
1	*Nf*RedAm	3	NH_4Cl	1 M	10 (23%)
2	*Nf*RedAm	3	NH_4Cl	0.5 M	20 (77%)
3	*Nf*RedAm	3	NH_4OH	0.5 M	20 (70%)
4	*Nf*RedAm	3	NH_4CO_2H	0.5 M	15 (52%)
5	*Nf*RedAm	2	NH_4Cl	0.5 M	25 (68%)
6	*Nf*RedAm	2[d]	NH_4Cl	0.5 M	25 (77%)
7	*Ad*RedAm	3	NH_4Cl	0.5 M	26 (68%)
8	*Nf*RedAm	2	NH_4Cl	0.25 M	20 (92%)[e]

Supplementary Figure S12. Flow reactions. [a]Conditions: 2:1 RedAm:*Bs*GDH (10 wt% Enzyme), 2-hexanone (40 mM), $NADP^+$ (1 mM), glucose (200 mM), DMSO (5%), t_{res}: 0.5 h, 35 °C; [b]*Bs*GDH always immobilised on EziG[3]; [c]First reactor volume where conversion is not >95%; [d] Soluble CDX GDH-901 used instead of immobilised *Bs*GDH; [e] Conversion had dropped to 26% by RV30;

Section S7. Analytical Procedures

GC analysis

Analytical procedures for compounds **1a-c, 2a-b, 3a-e, 4a-e, 5a-e, 6b** and **7b** are reported in Aleku *et al.* [10]and Sharma *et al*.[7]

Entry	Ketone	Ketone retention time (min)	Amine	Amine retention time (min)
1	**3**	7.7[a]	**3f**	6.8[a]
2	**4**	3.9[b]	**4f**	3.8
3	**6**	13.4[a]	**6f**	13.2[a]
4	**9**	21.0[a]	**9f**	21.9[a]
5	**13**	14.0[a]	**13f**	15.1[a]
6	**15**	22.7[a]	**15f**	22.4 and 22.5[a]
7	**16**	17.1[a]	**16f**	16.7[a]
8	**18**	13.3[a]	**18f**	13.1[a]
9	**19**	8.7[b]	**19f**	7.1[b]

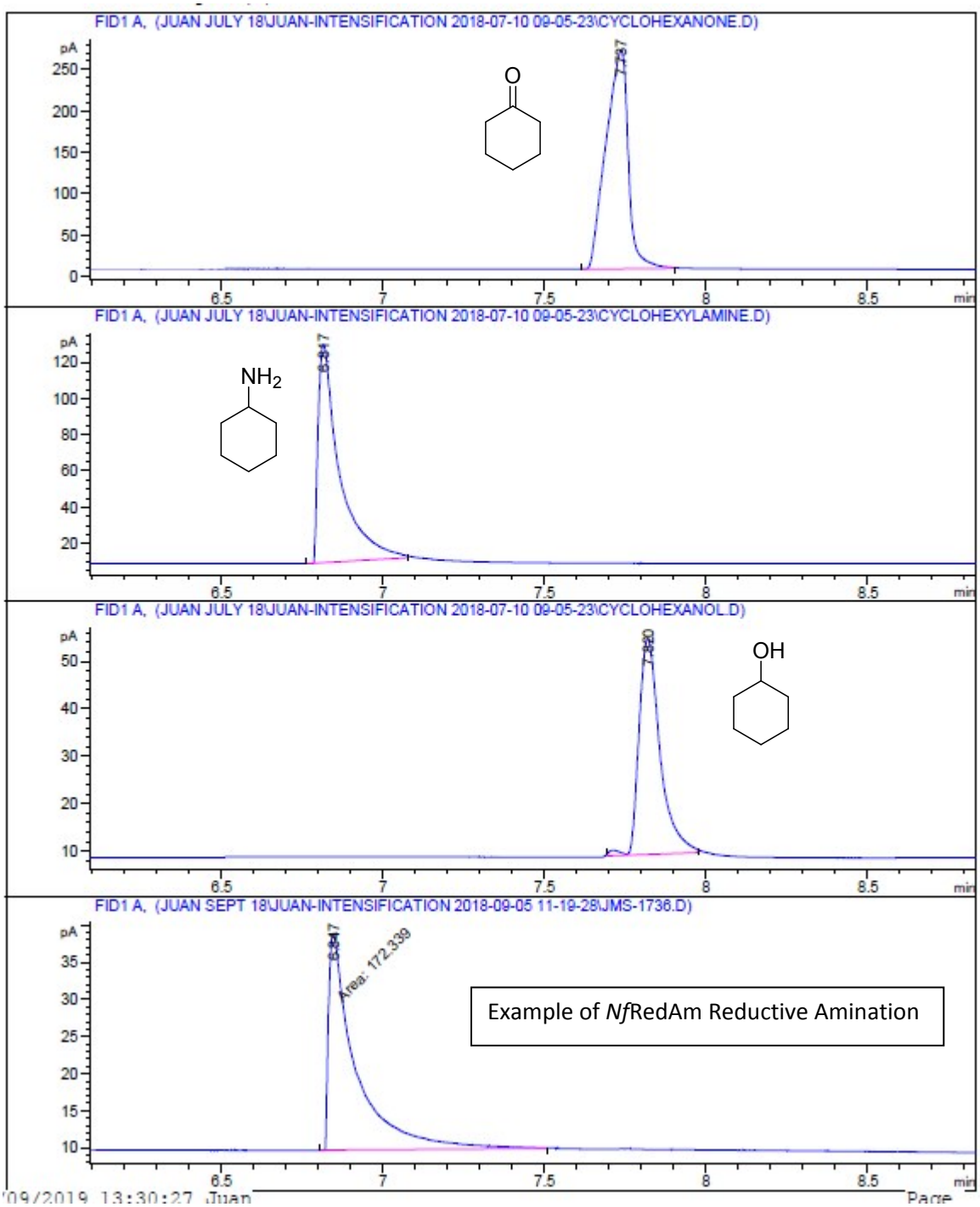
Supplementary table S6. GC analysis: methods and retention times of ketone substrates and amine products from biotransformations. Column: HP-1, [a] temperature program: 50 °C for 0 min then 5 °C min^{-1} until 200 °C, then hold for 2 min. Injector temperature 200 °C, detector temperature 250 °C, helium flow: 1.2 mL min^{-1}. [b]. Column: HP-1 temperature program: 40 °C for 1 min then 3 °C min^{-1}

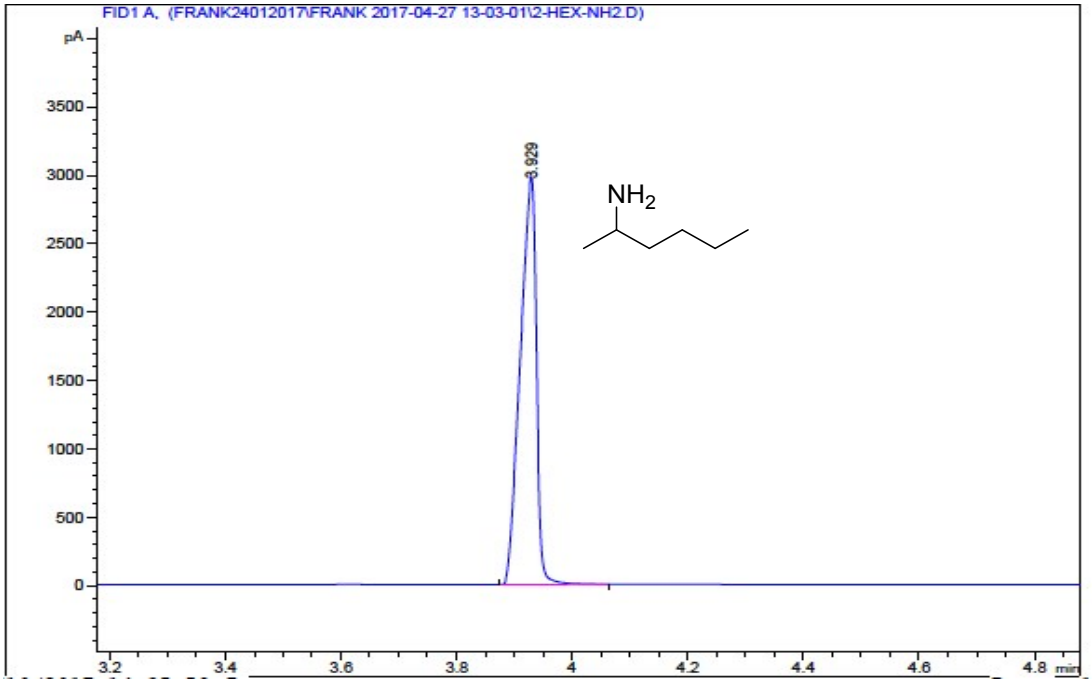
until 120 °C, then 3 °C min^{-1} until 200 °C and then hold for 2 min. Injector temperature 200 °C, detector temperature 250 °C, helium flow: 1.2 mL min^{-1}.

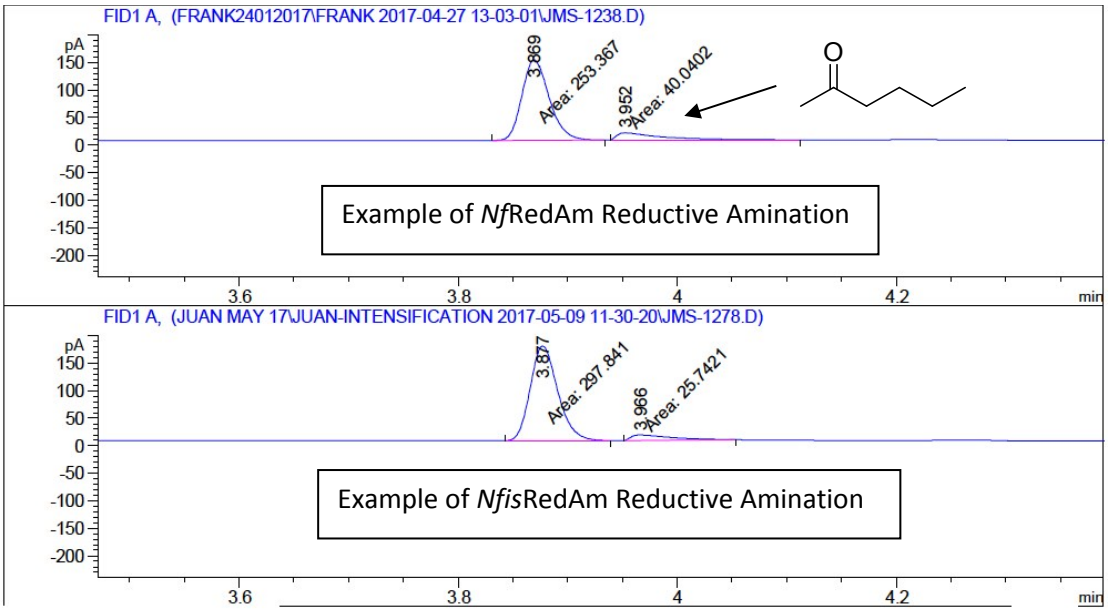
Entry	Ketone	Ketone retention time (min)	Amine	Amine retention time (min)	
1	**4**	---	**4f**	15.2 (*S*)	15.8 (*R*)
2	**5**	16.7	**5f**	25.7 (*S*)	25.9 (*R*)
3	**7**	14.2	**7f**	23.4 (R)	23.6 (S)
4	**8**	12.2	**8f**	21.8 (*S*)	22.4 (*R*)
5	**10**	20.1	**10f**	28.0 (*S*)	28.4 (*R*)
6	**11**	15.0	**11f**	22.8 (*S*)	23.1 (*R*)
7	**14**	17.9	**14f**	26.0 (*S*)	26.4 (*R*)
8	**17**	12.0	**17f**	21.0 (*S*)	21.5 (*R*)
9	**20**	6.8	**20f**	18.3 (*R*)	18.6 (*S*)

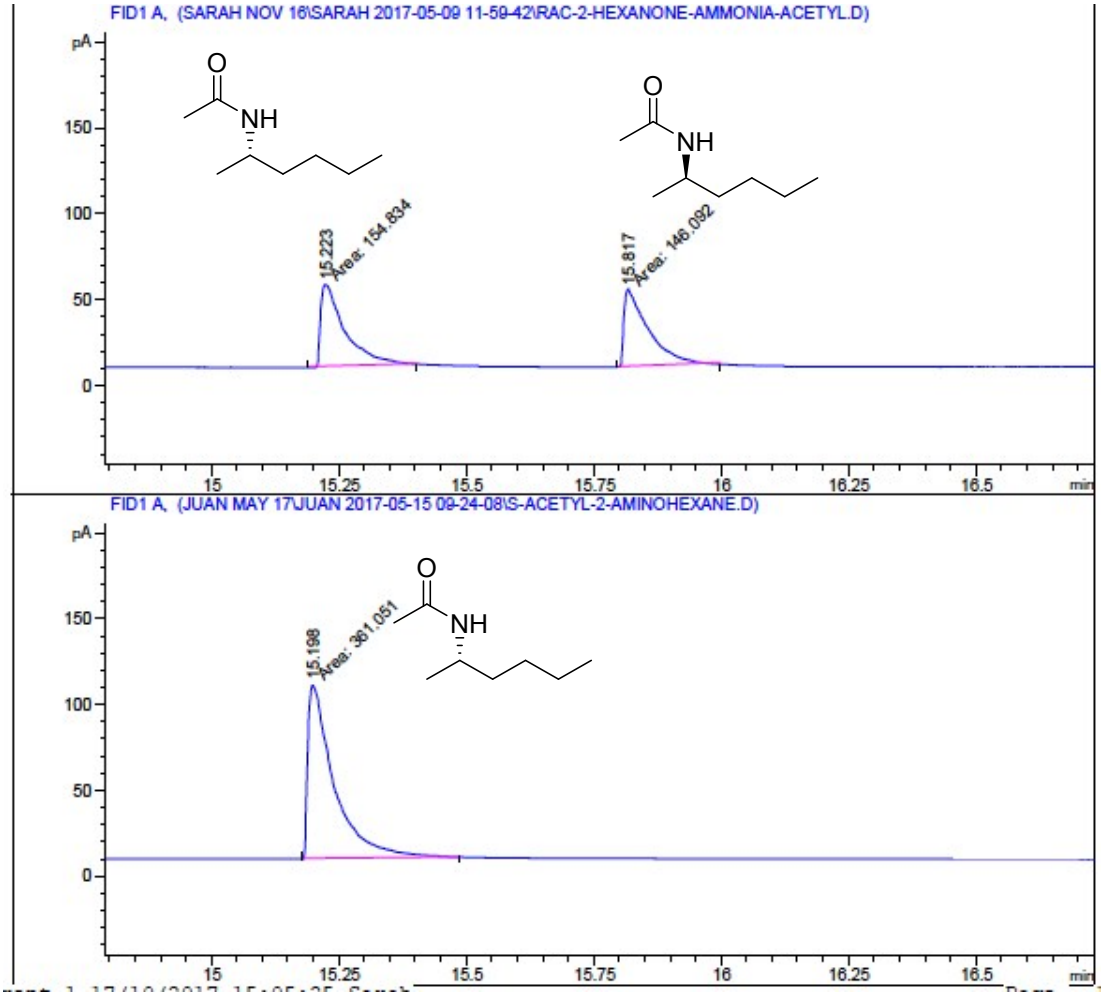
Supplementary table S7. GC analysis: methods and retention times of ketone substrates and amine products from biotransformations. Column: CP-ChiralSil-DEX CB, temperature program: 50 °C for 0 min then 5 °C min^{-1} until 200 °C, then hold for 2 min. Injector temperature 200 °C, detector temperature 250 °C, helium flow: 1.2 mL min^{-1}. Reactions were acetylated using acetic anhydride and 4-(dimethylamino)pyridine prior to analysis. Absolute configurations were determined either via commercial standards or by comparison with ATA-113 transamination.

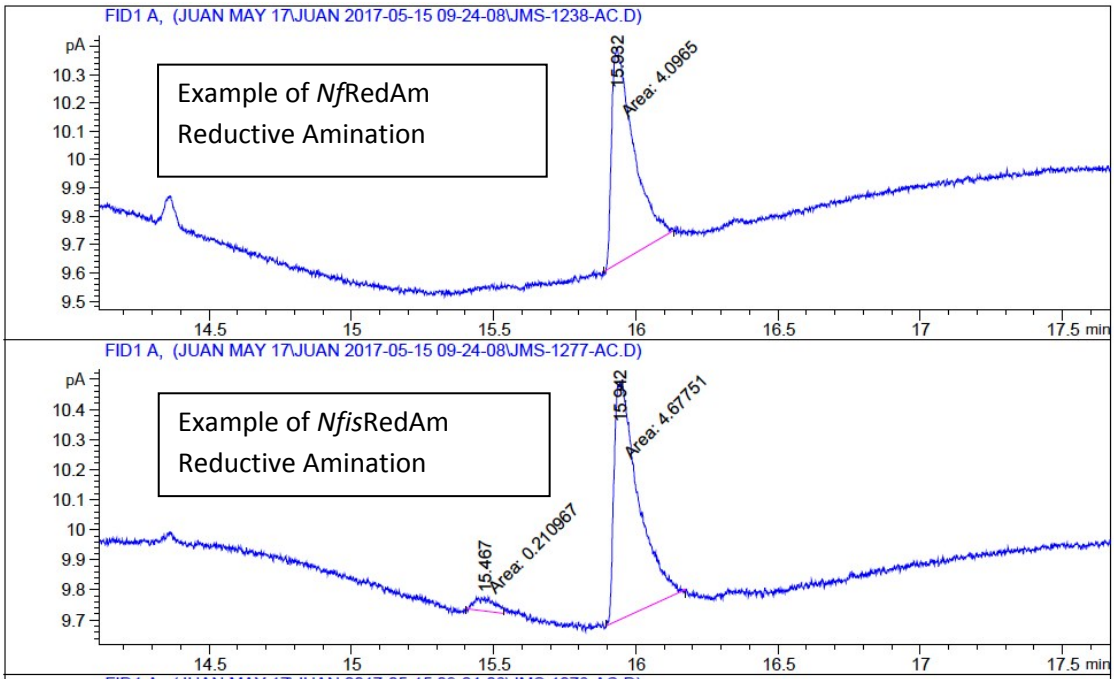
Section S8. Selected Chromatograms

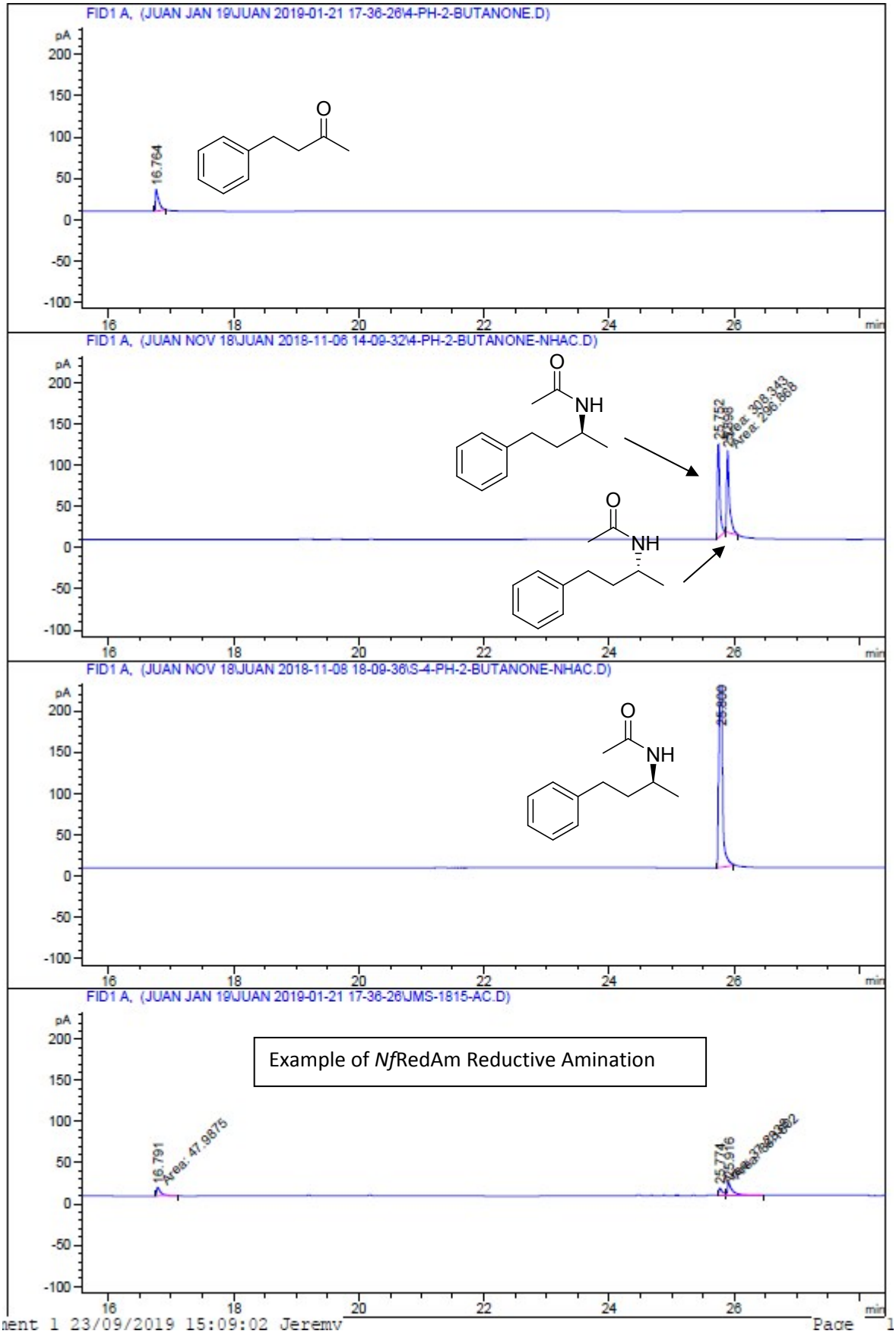


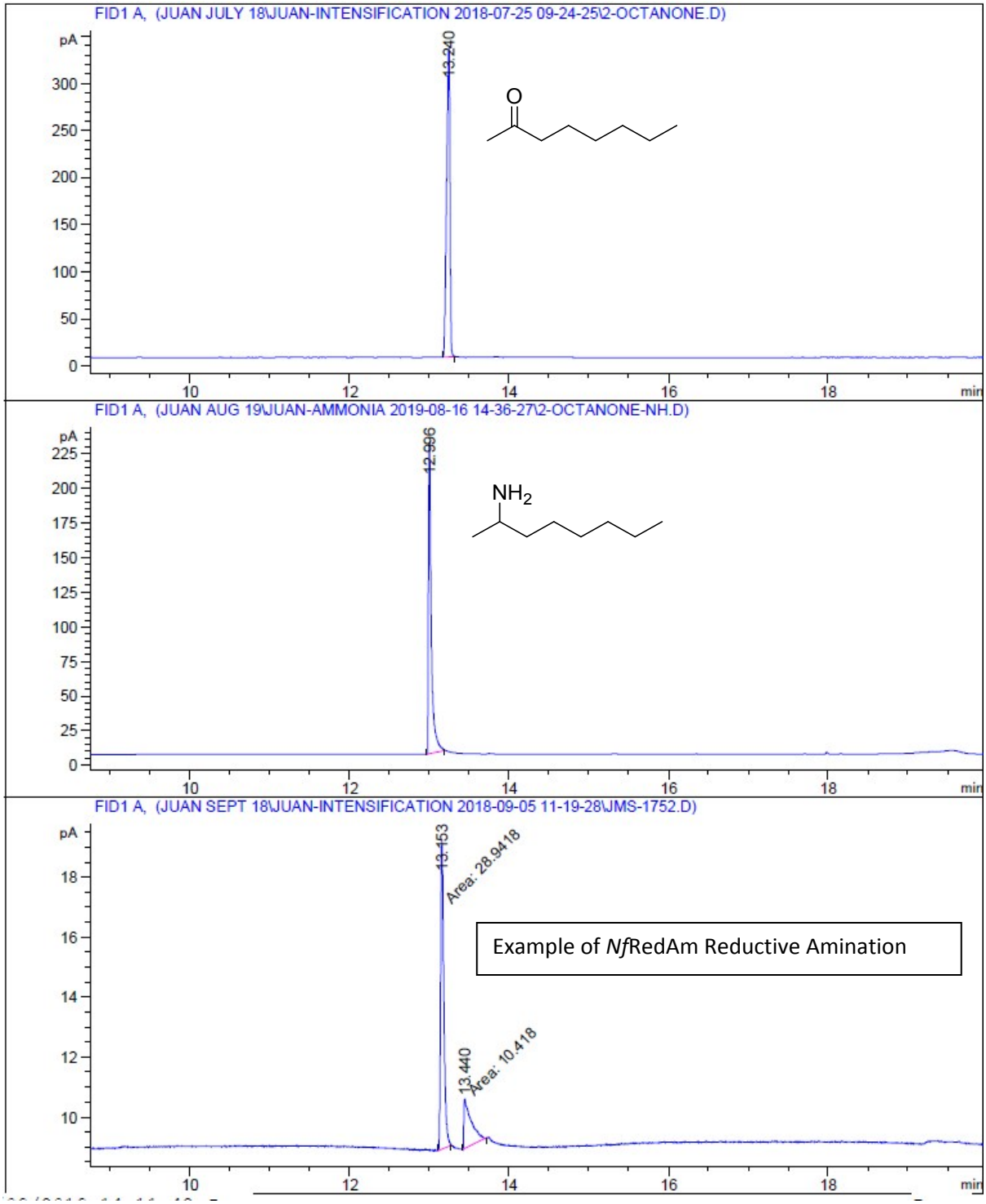

FID1 A, (FRANK24012017\FRANK 2017-04-27 13-03-01\2-HEX-NH2.D)
pA
3500
3000
2500
2000
1500
1000
500
0
3.929
NH2
3.2
3.4
3.6
3.8
4
4.2
4.4
4.6
4.8
min

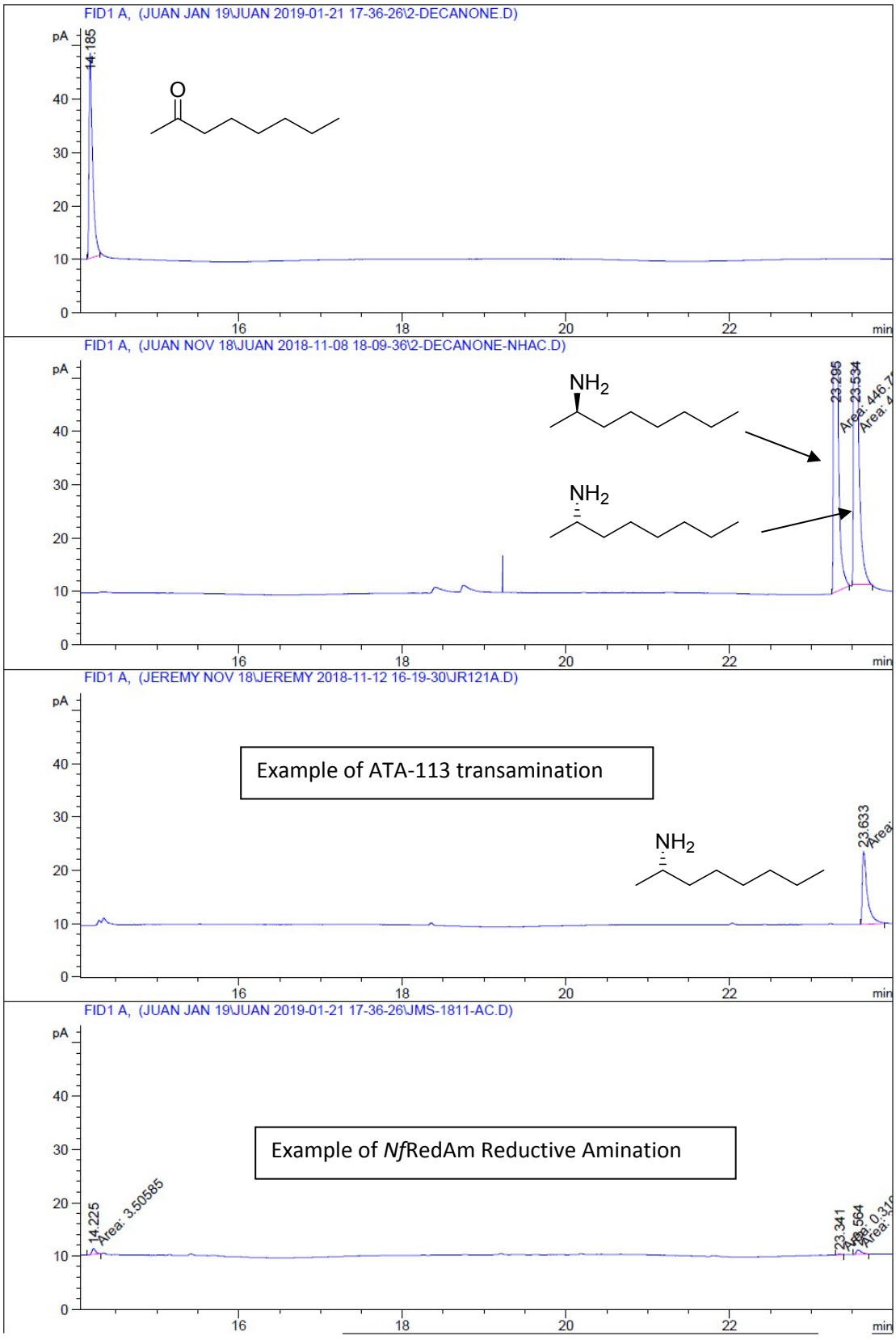

FID1 A, (FRANK24012017\FRANK 2017-04-27 13-03-01\JMS-1238.D)
pA
150
100
50
0
-50
-100
-150
-200
3.869
Area: 253.367
3.952
Area: 40.0402
O
Example of *Nf*RedAm Reductive Amination
3.6
3.8
4
4.2
min
FID1 A, (JUAN MAY 17\JUAN-INTENSIFICATION 2017-05-09 11-30-20\JMS-1278.D)
pA
150
100
50
0
-50
-100
-150
-200
3.877
Area: 297.841
3.966
Area: 25.7421
Example of *Nfis*RedAm Reductive Amination
3.6
3.8
4
4.2
min

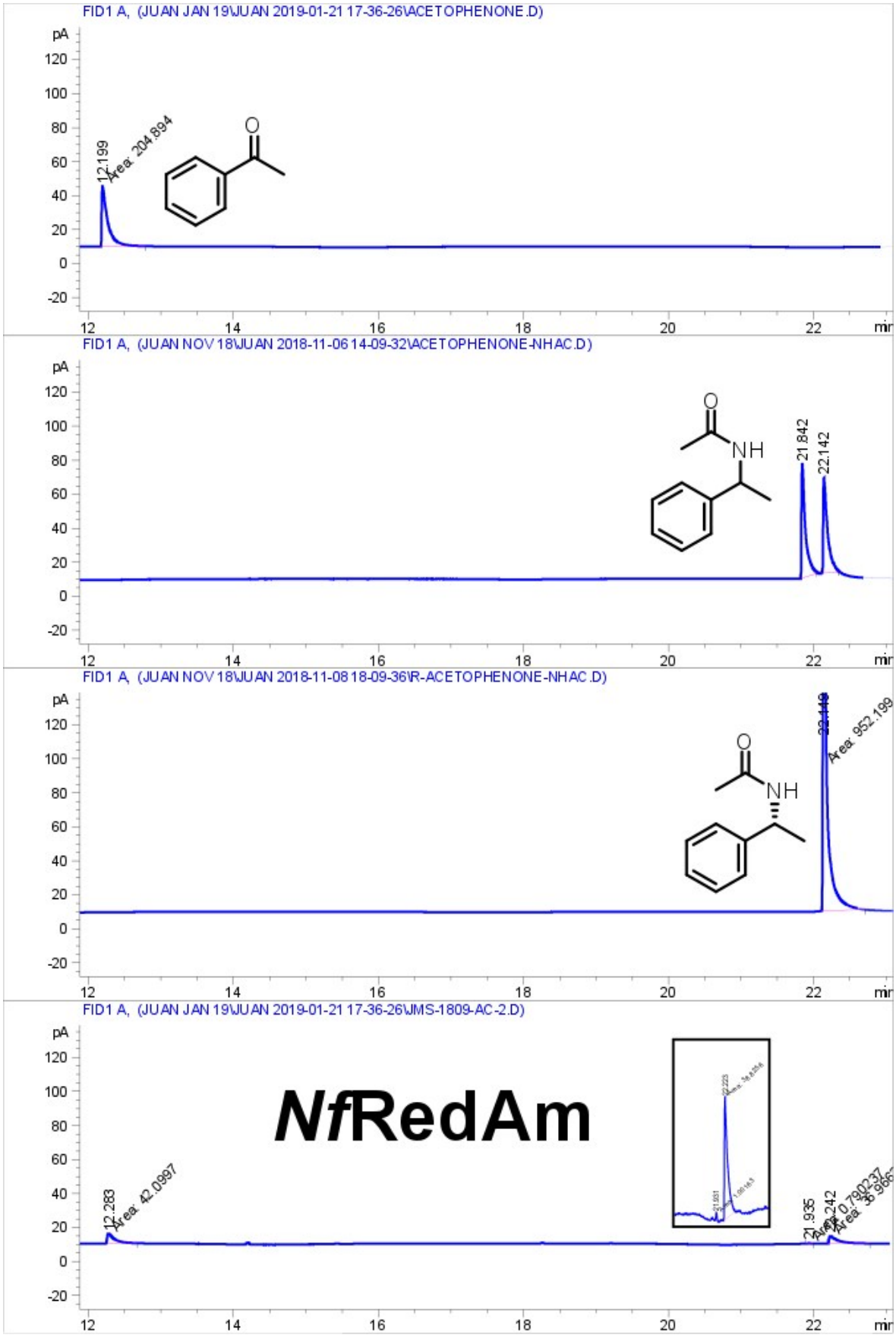

FID1 A, (SARAH NOV 16\SARAH 2017-05-09 11-59-42\RAC-2-HEXANONE-AMMONIA-ACETYL.D)
pA
150
100
50
0
O
NH
15.223
Area: 154.834
15.817
Area: 146.092
15
15.25
15.5
15.75
16
16.25
16.5
min
FID1 A, (JUAN MAY 17\JUAN 2017-05-15 09-24-08\S-ACETYL-2-AMINOHEXANE.D)
pA
150
100
50
0
O
NH
15.198
Area: 361.051
15
15.25
15.5
15.75
16
16.25
16.5
min

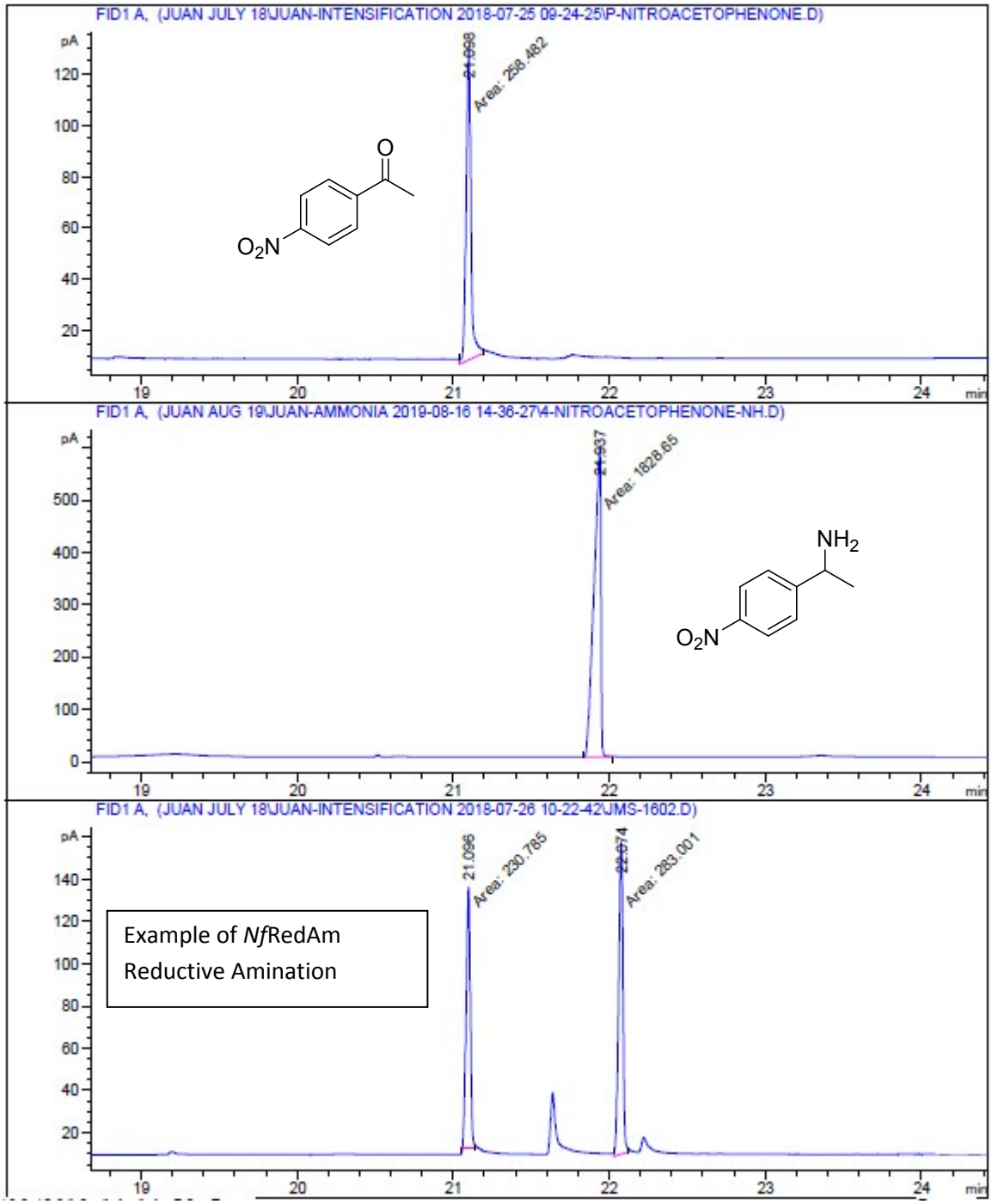

FID1 A, (JUAN MAY 17\JUAN 2017-05-15 09-24-08\JMS-1238-AC.D)
pA
10.3
10.2
10.1
10
9.9
9.8
9.7
9.6
9.5
Example of *Nf*RedAm
Reductive Amination
15.992
Area: 4.0965
14.5
15
15.5
16
16.5
17
17.5 min
FID1 A, (JUAN MAY 17\JUAN 2017-05-15 09-24-08\JMS-1277-AC.D)
pA
10.4
10.3
10.2
10.1
10
9.9
9.8
9.7
Example of *Nfis*RedAm
Reductive Amination
15.942
Area: 4.67751
15.467
Area: 0.210967
14.5
15
15.5
16
16.5
17
17.5 min

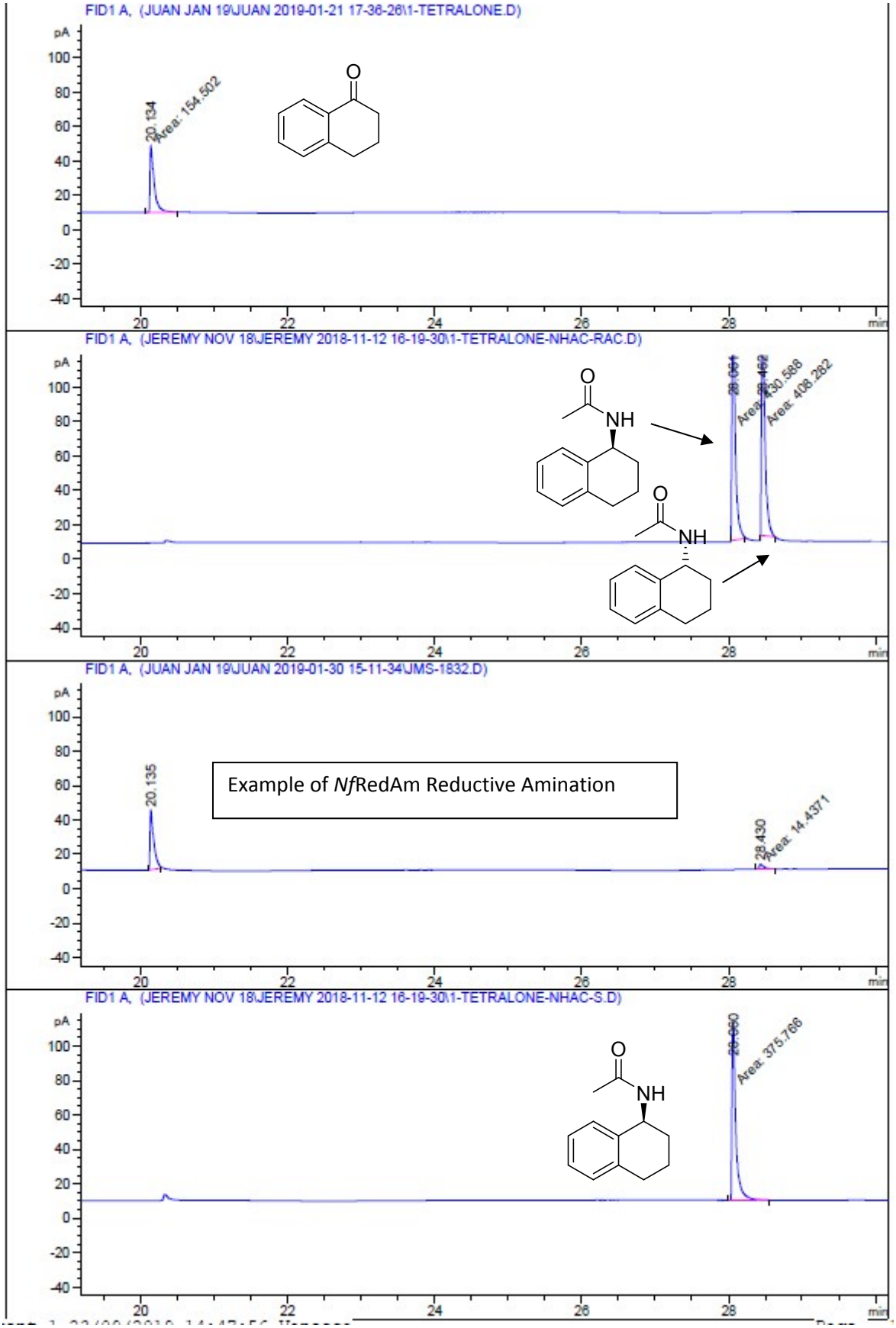

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\4-PH-2-BUTANONE.D)
16.764
FID1 A, (JUAN NOV 18\JUAN 2018-11-08 14-09-32\4-PH-2-BUTANONE-NHAC.D)
FID1 A, (JUAN NOV 18\JUAN 2018-11-08 18-09-36\S-4-PH-2-BUTANONE-NHAC.D)
FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\JMS-1815-AC.D)
16.791
Area: 47.9875
Example of *Nf*RedAm Reductive Amination
ment 1 23/09/2019 15:09:02 Jeremy
Page 1

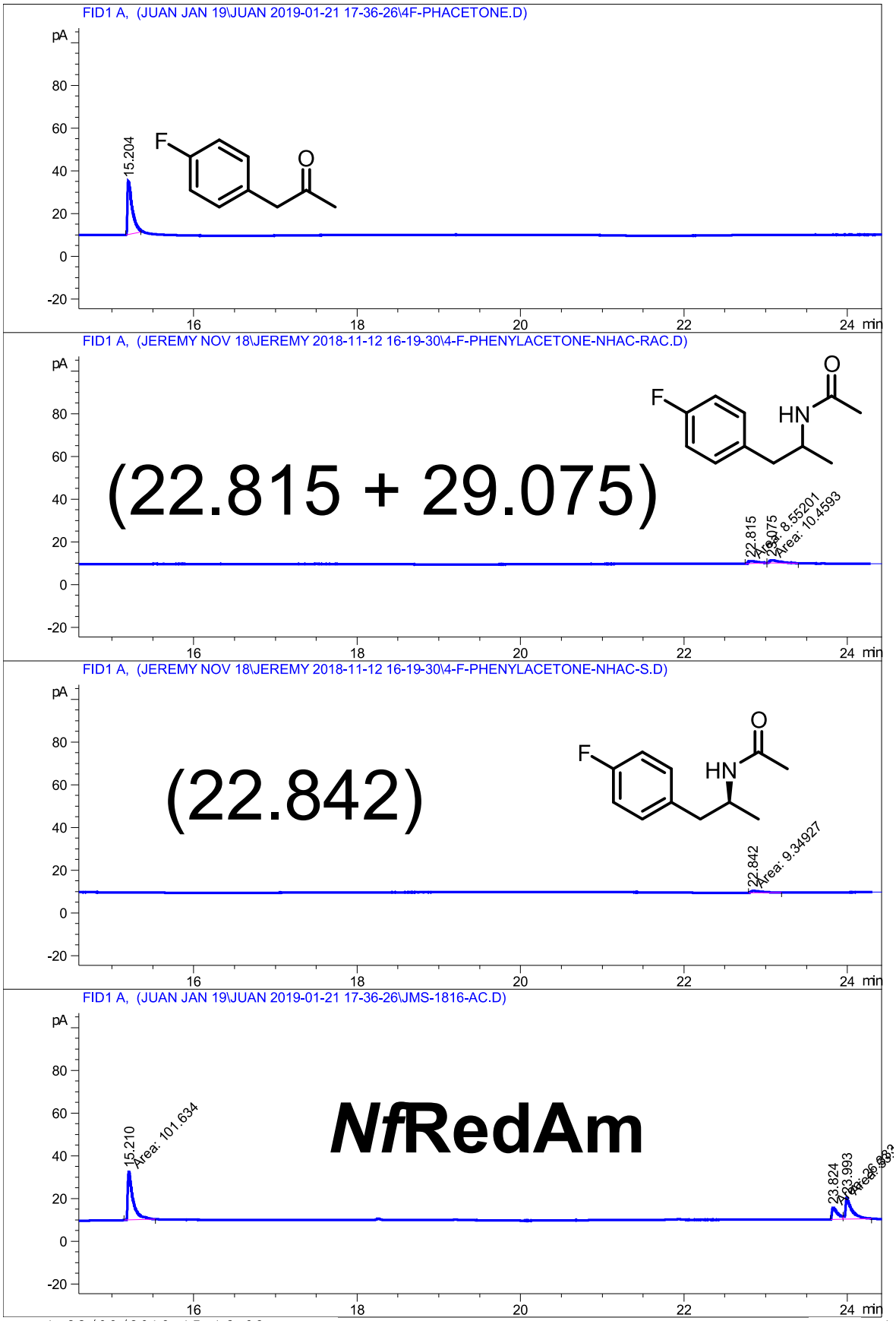

FID1 A, (JUAN JULY 18\JUAN-INTENSIFICATION 2018-07-25 09-24-25\2-OCTANONE.D)
pA
13.240
O
FID1 A, (JUAN AUG 19\JUAN-AMMONIA 2019-08-16 14-36-27\2-OCTANONE-NH.D)
12.996
NH2
FID1 A, (JUAN SEPT 18\JUAN-INTENSIFICATION 2018-09-05 11-19-28\JMS-1752.D)
13.153
Area: 28.9418
13.440
Area: 10.418
Example of *Nf*RedAm Reductive Amination
min

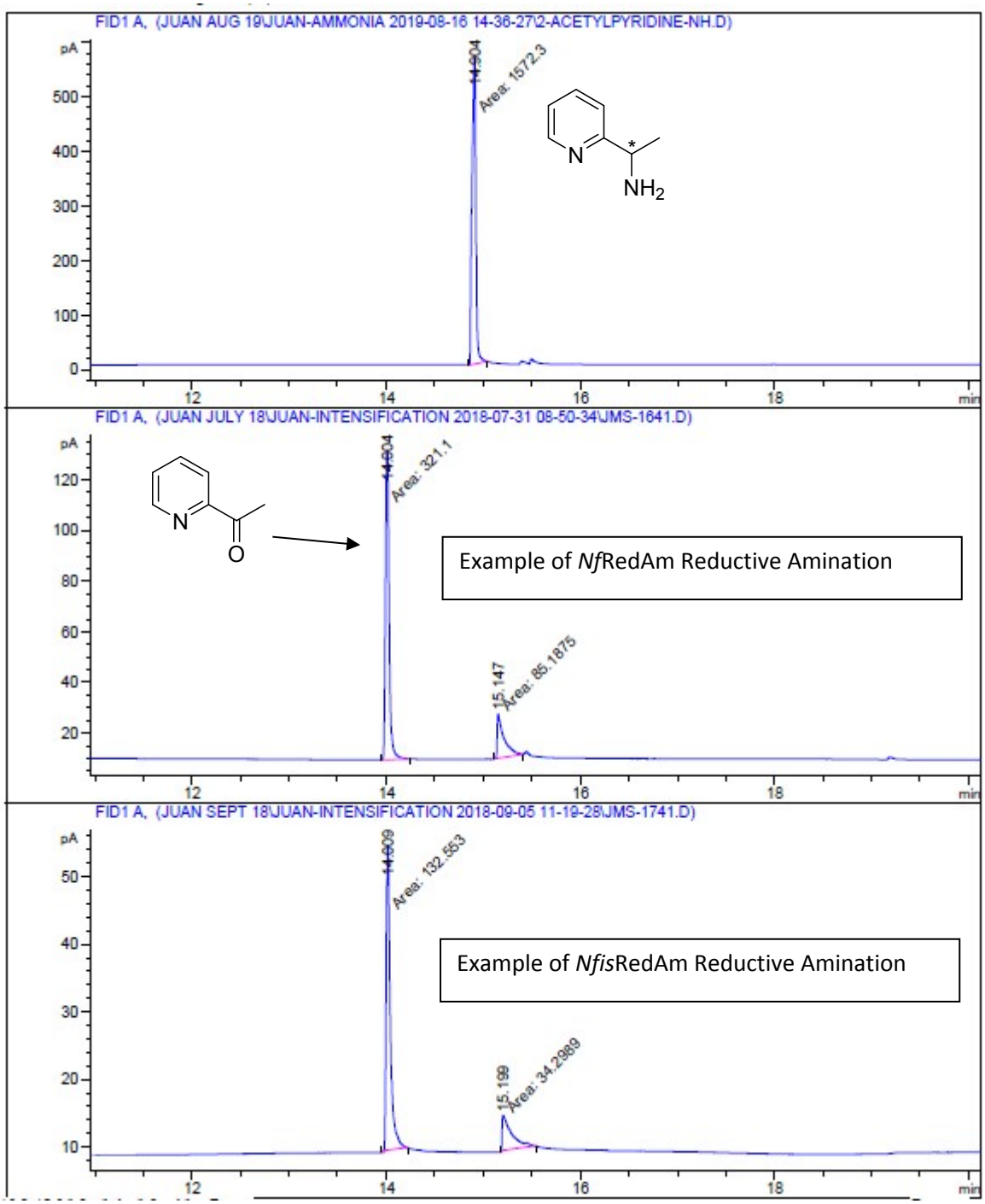

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\2-DECANONE.D)
pA
14.185
O
FID1 A, (JUAN NOV 18\JUAN 2018-11-08 18-09-36\2-DECANONE-NHAC.D)
NH2
NH2
23.295
23.534
FID1 A, (JEREMY NOV 18\JEREMY 2018-11-12 16-19-30\JR121A.D)
Example of ATA-113 transamination
NH2
23.633
FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\JMS-1811-AC.D)
Example of NfRedAm Reductive Amination
14.225
Area: 3.50585
23.341
23.564
16
18
20
22
min

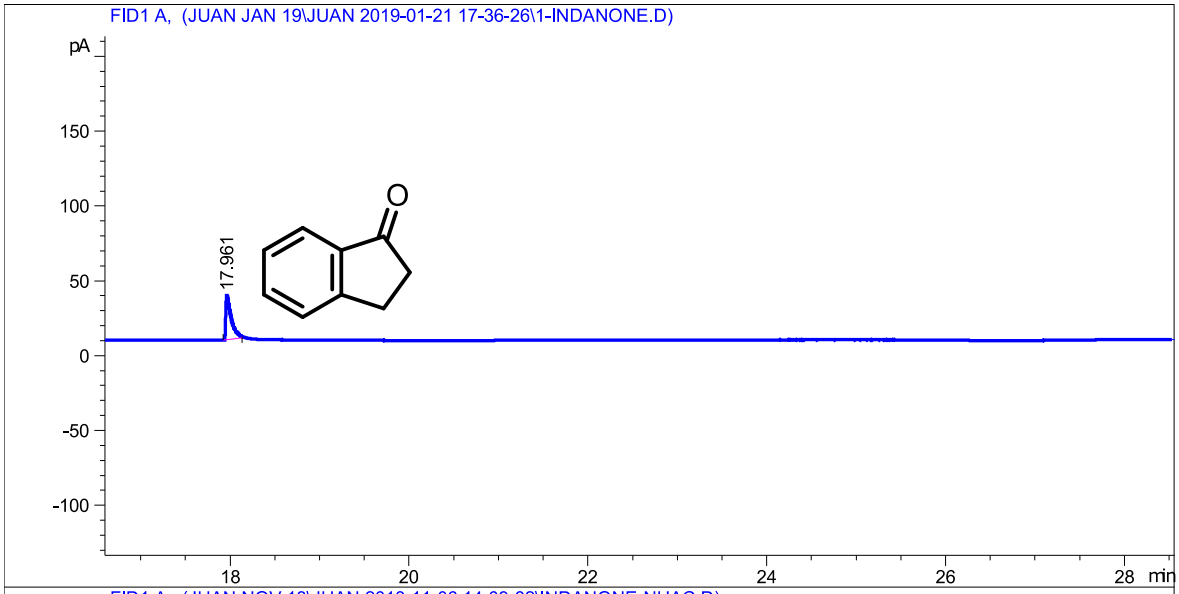

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\ACETOPHENONE.D)
pA
120
100
80
60
40
20
0
-20
12.199
Area: 204.894
O
12
14
16
18
20
22
min
FID1 A, (JUAN NOV 18\JUAN 2018-11-06 14-09-32\ACETOPHENONE-NHAC.D)
O
NH
21.842
22.142
FID1 A, (JUAN NOV 18\JUAN 2018-11-08 18-09-36\R-ACETOPHENONE-NHAC.D)
Area: 952.199
FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\JMS-1809-AC-2.D)
NfRedAm
12.283
Area: 42.0997
21.935
22.242
Area: 0.790037

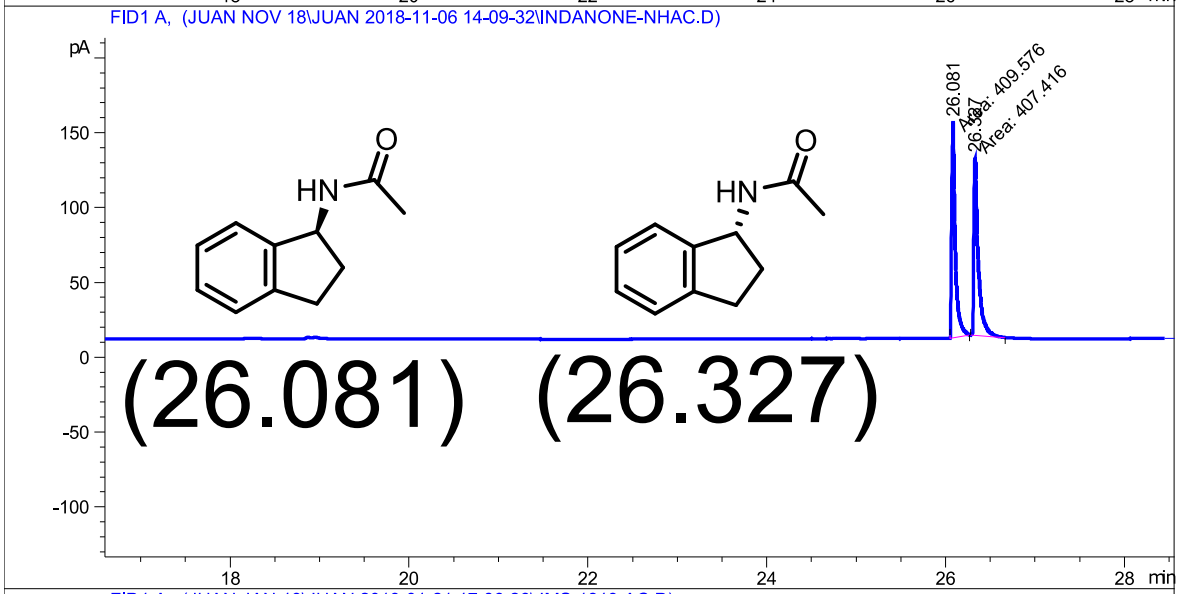

FID1 A, (JUAN JULY 18\JUAN-INTENSIFICATION 2018-07-25 09-24-25\P-NITROACETOPHENONE.D)
pA
21.098
Area: 258.482
O
O2N
FID1 A, (JUAN AUG 19\JUAN-AMMONIA 2019-08-16 14-36-27\4-NITROACETOPHENONE-NH.D)
21.937
Area: 1828.65
NH2
O2N
FID1 A, (JUAN JULY 18\JUAN-INTENSIFICATION 2018-07-26 10-22-42\JMS-1602.D)
21.096
Area: 230.785
22.074
Area: 283.001
Example of NfRedAm
Reductive Amination
min

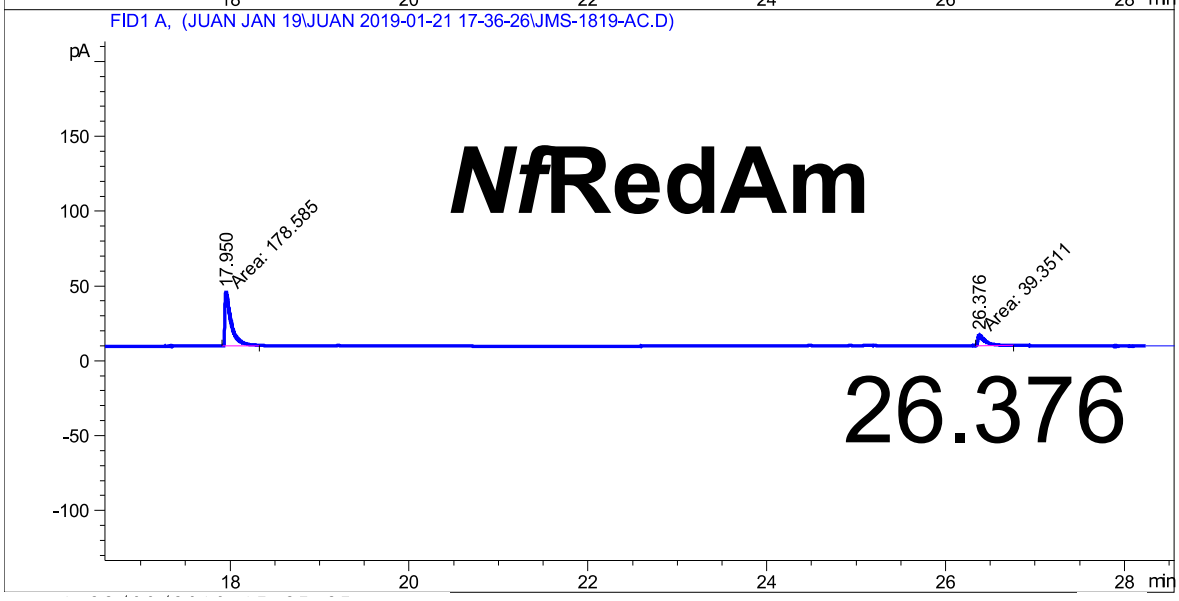

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\1-TETRALONE.D)
pA
20.134
Area: 154.502
O
FID1 A, (JEREMY NOV 18\JEREMY 2018-11-12 16-19-30\1-TETRALONE-NHAC-RAC.D)
O
NH
Area: 408.282
FID1 A, (JUAN JAN 19\JUAN 2019-01-30 15-11-34\JMS-1832.D)
20.135
Example of *Nf*RedAm Reductive Amination
28.430
Area: 14.4371
FID1 A, (JEREMY NOV 18\JEREMY 2018-11-12 16-19-30\1-TETRALONE-NHAC-S.D)
Area: 375.766
min

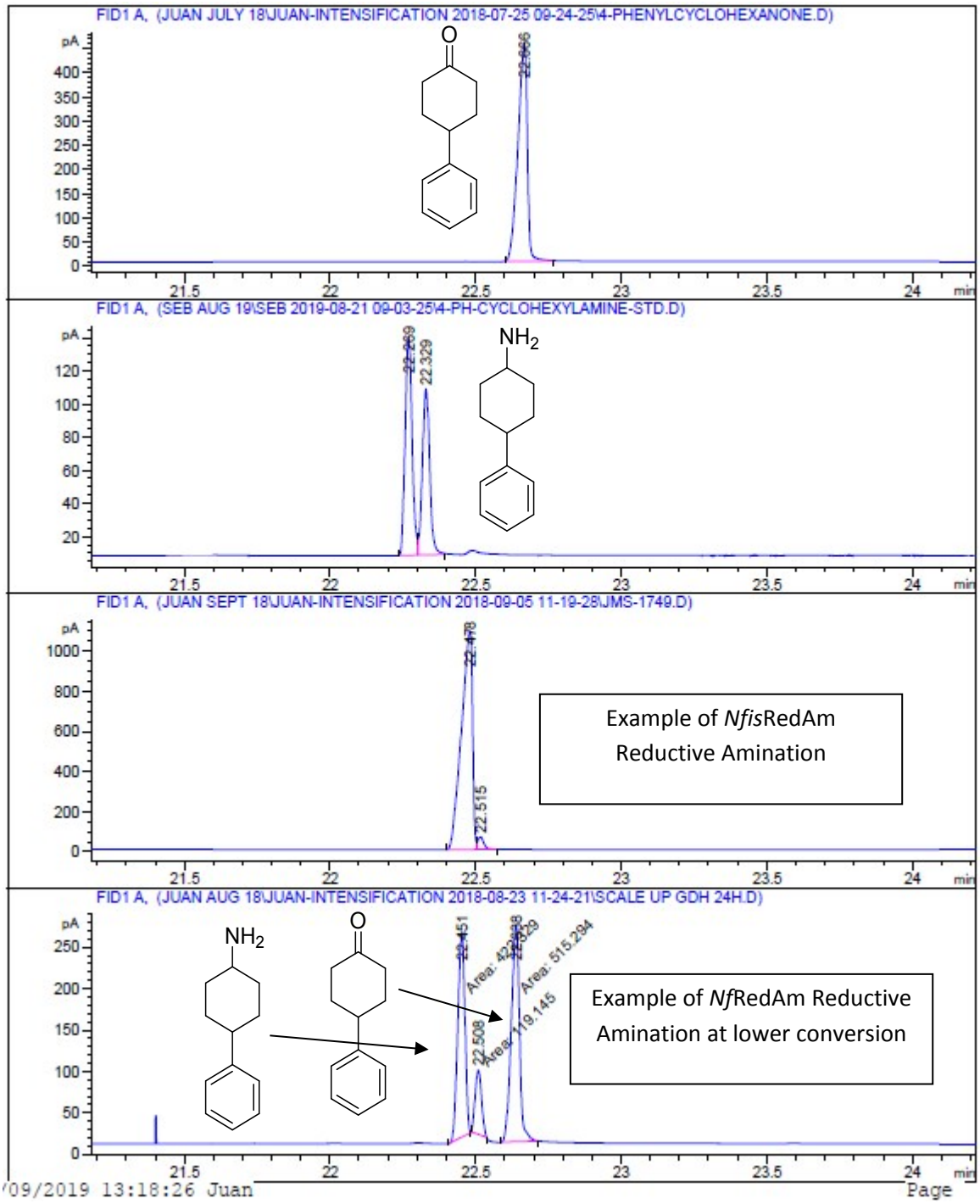

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\4F-PHACETONE.D)
pA
15.204
F
O
FID1 A, (JEREMY NOV 18\JEREMY 2018-11-12 16-19-30\4-F-PHENYLACETONE-NHAC-RAC.D)
(22.815 + 29.075)
F
HN
O
22.815
Area: 8.55201
29.075
Area: 10.4593
FID1 A, (JEREMY NOV 18\JEREMY 2018-11-12 16-19-30\4-F-PHENYLACETONE-NHAC-S.D)
(22.842)
F
HN
O
22.842
Area: 9.34927
FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\JMS-1816-AC.D)
NfRedAm
15.210
Area: 101.634
23.824
23.993
min

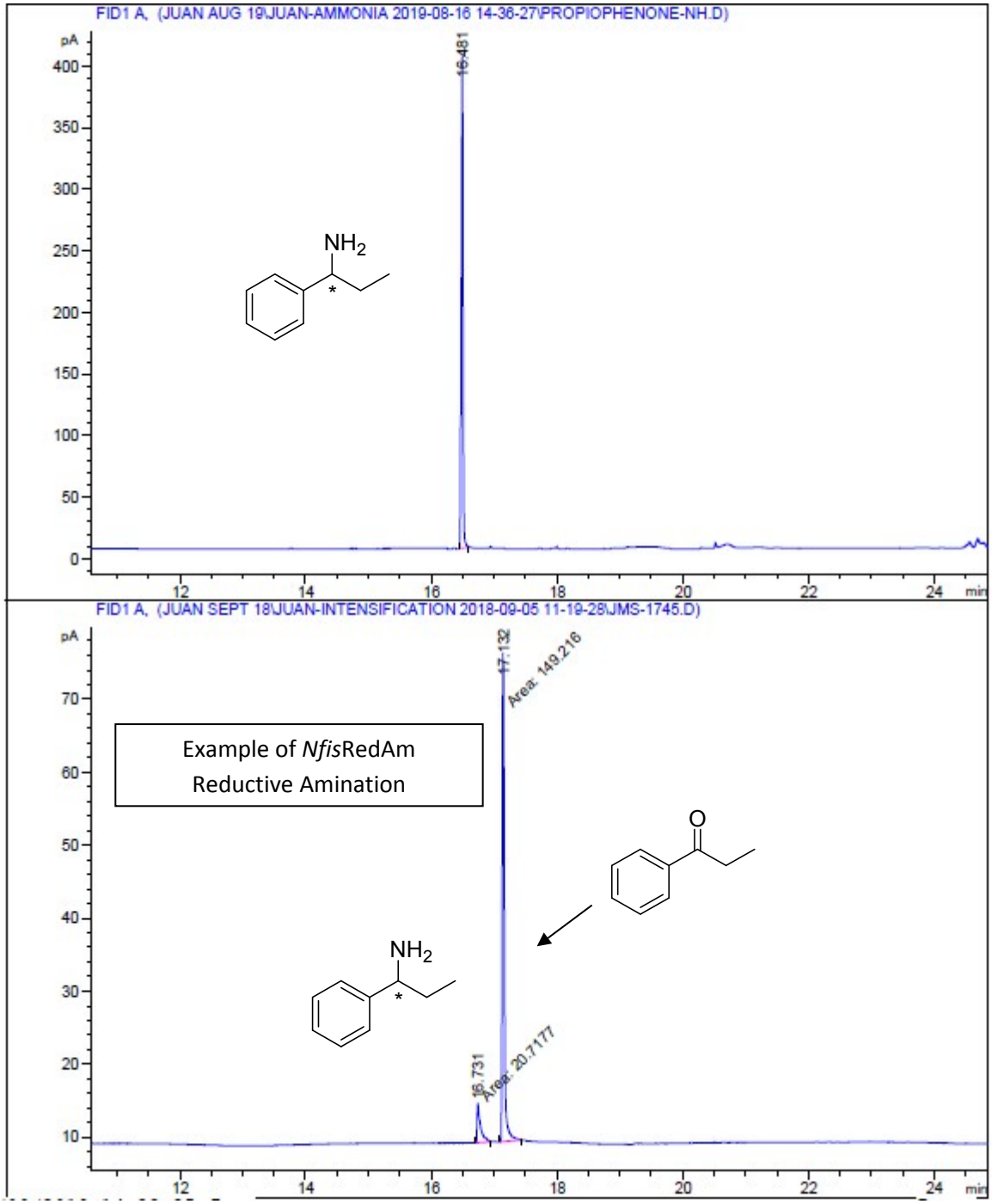

FID1 A, (JUAN AUG 19\JUAN-AMMONIA 2019-08-16 14-36-27\2-ACETYLPYRIDINE-NH.D)
pA
14.904
Area: 1572.3
N
*
NH2
FID1 A, (JUAN JULY 18\JUAN-INTENSIFICATION 2018-07-31 08-50-34\JMS-1641.D)
14.904
Area: 321.1
N
O
Example of NfRedAm Reductive Amination
15.147
Area: 85.1875
FID1 A, (JUAN SEPT 18\JUAN-INTENSIFICATION 2018-09-05 11-19-28\JMS-1741.D)
14.909
Area: 132.553
Example of NfisRedAm Reductive Amination
15.199
Area: 34.2989
min

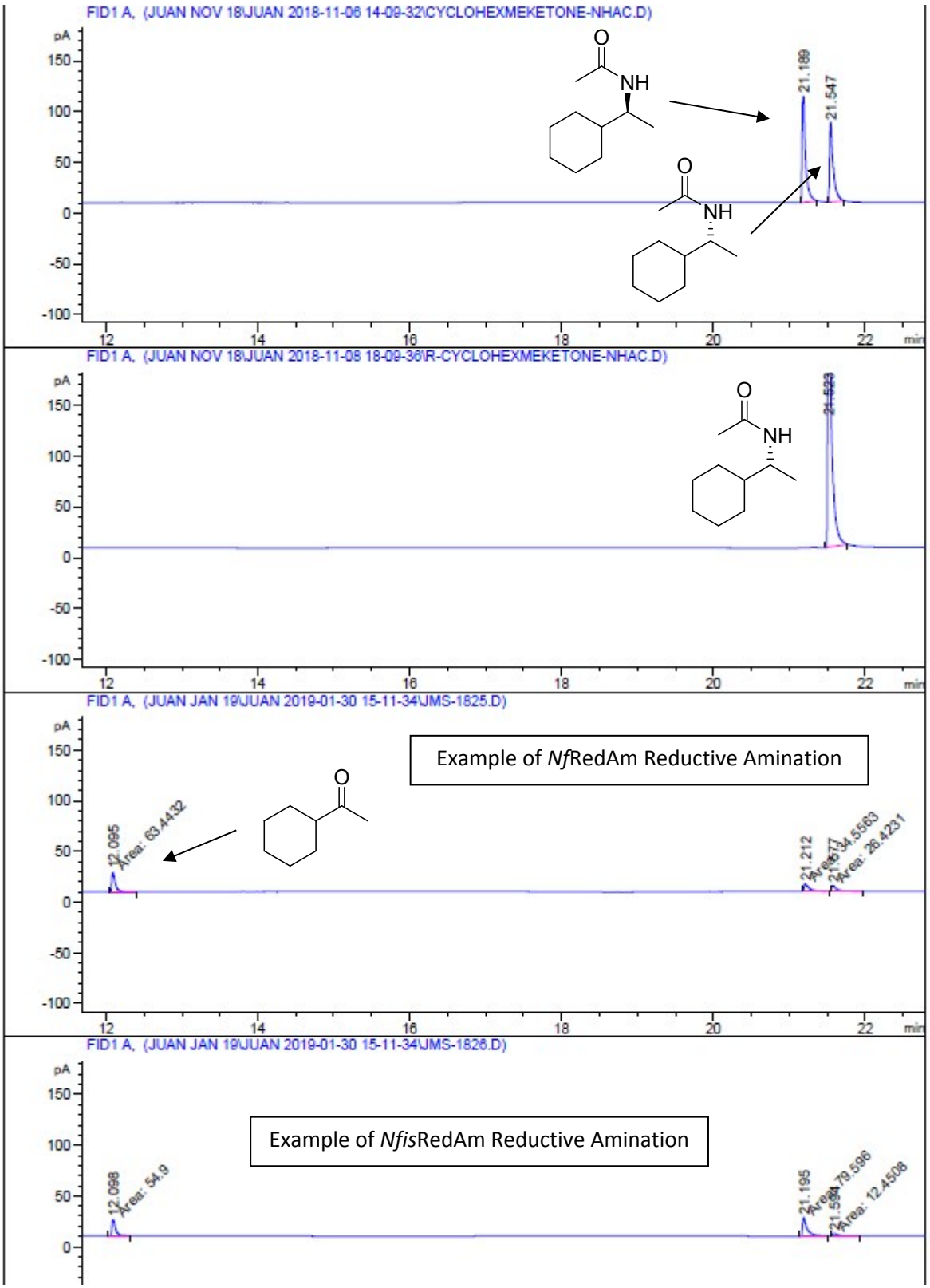

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\1-INDANONE.D)
pA
150
100
50
0
-50
-100
17.961
O
18
20
22
24
26
28
min

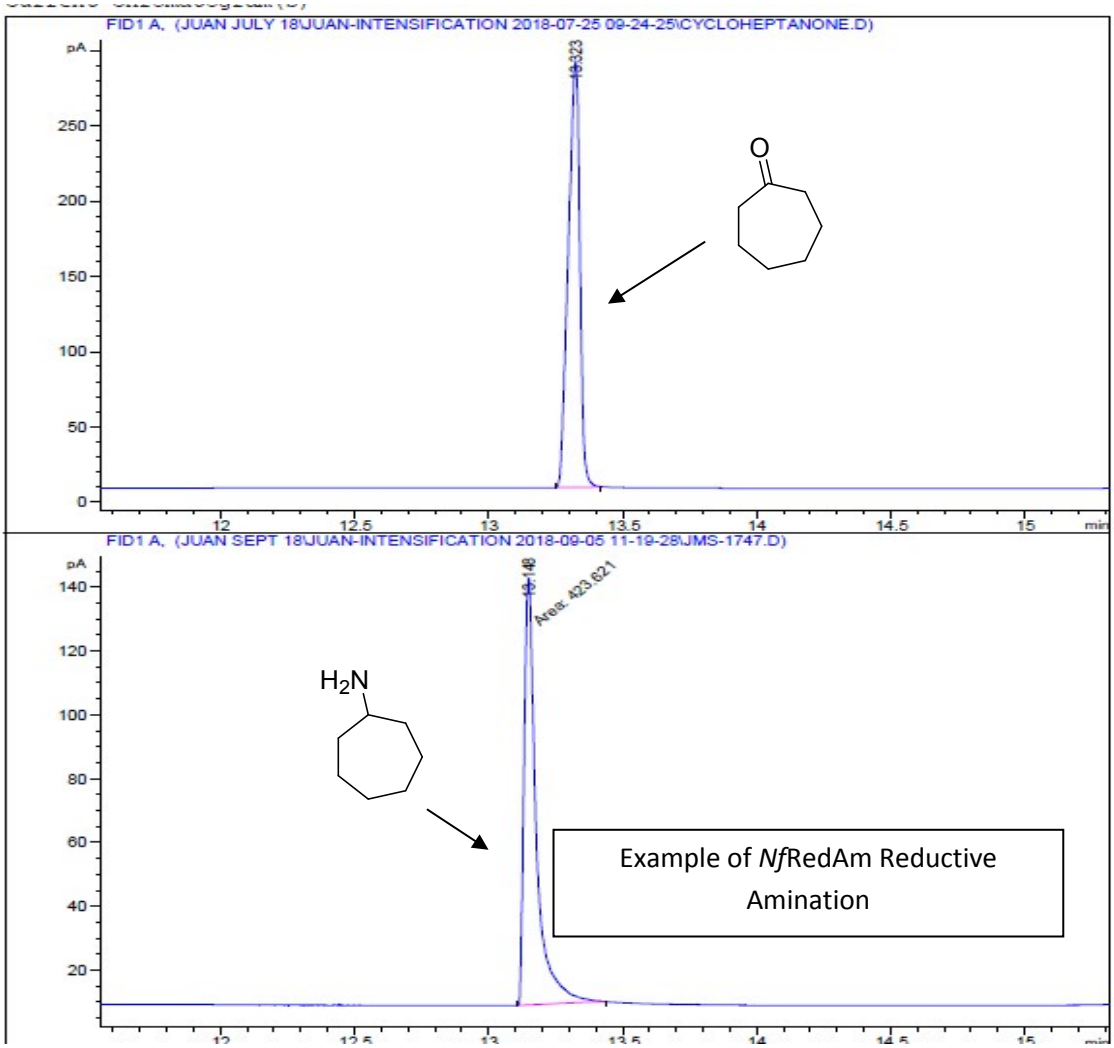

FID1 A, (JUAN NOV 18\JUAN 2018-11-06 14-09-32\INDANONE-NHAC.D)
pA
150
100
50
0
-50
-100
HN
O
HN
O
26.081
Area: 409.576
Area: 407.416
(26.081)
(26.327)
18
20
22
24
26
28
min

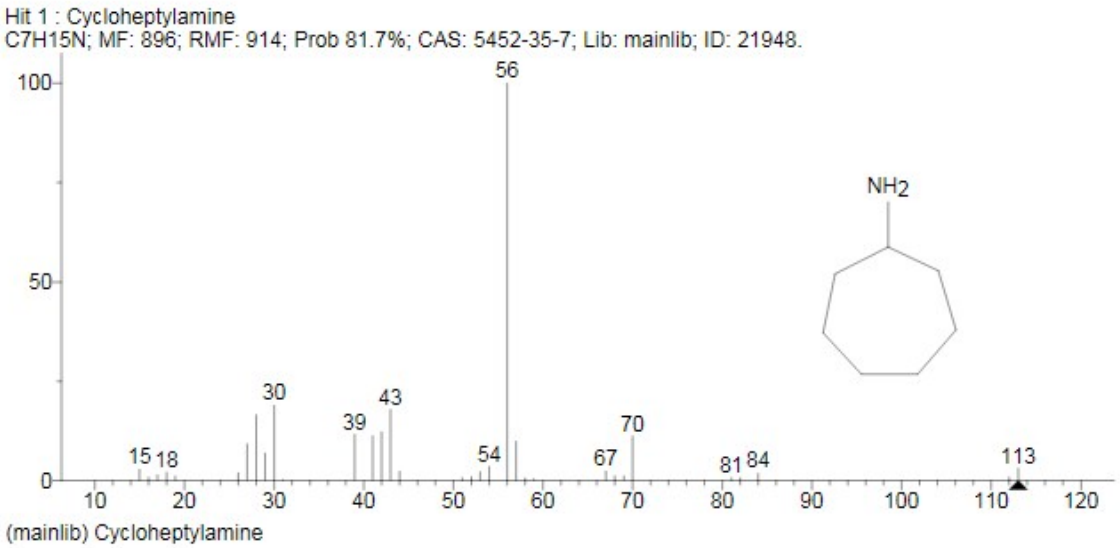

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\JMS-1819-AC.D)
pA
150
100
50
0
-50
-100
NfRedAm
17.950
Area: 178.585
26.376
Area: 39.3511
26.376
18
20
22
24
26
28
min

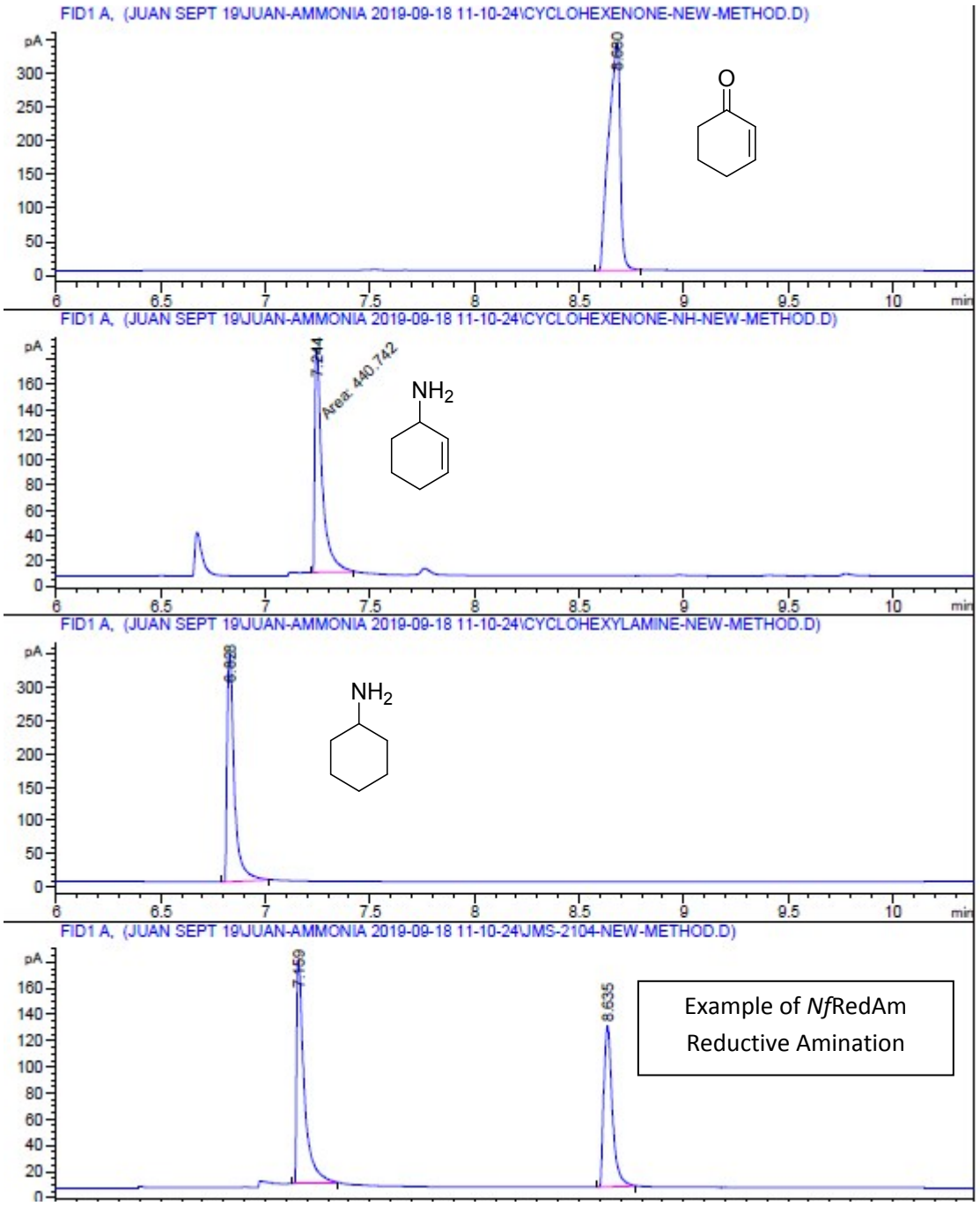

FID1 A, (JUAN JULY 18\JUAN-INTENSIFICATION 2018-07-25 09-24-25\4-PHENYLCYCLOHEXANONE.D)
O
FID1 A, (SEB AUG 19\SEB 2019-08-21 09-03-25\4-PH-CYCLOHEXYLAMINE-STD.D)
NH2
22.329
FID1 A, (JUAN SEPT 18\JUAN-INTENSIFICATION 2018-09-05 11-19-28\JMS-1749.D)
22.515
Example of NfisRedAm Reductive Amination
FID1 A, (JUAN AUG 18\JUAN-INTENSIFICATION 2018-08-23 11-24-21\SCALE UP GDH 24H.D)
NH2
O
Area: 515.294
Area: 119.145
22.508
Example of NfRedAm Reductive Amination at lower conversion
pA
min
21.5
22
22.5
23
23.5
24
09/2019 13:18:26 Juan
Page

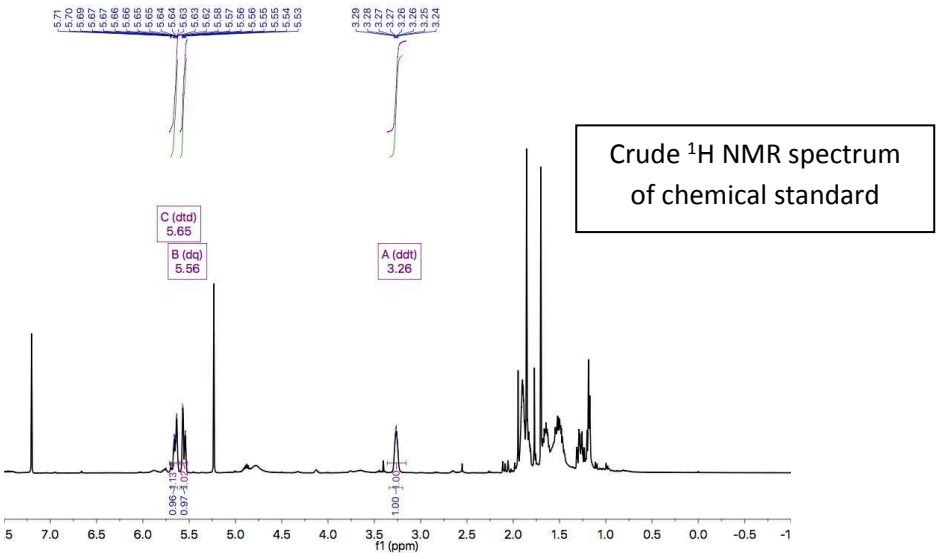

FID1 A, (JUAN AUG 19\JUAN-AMMONIA 2019-08-16 14-36-27\PROPIOPHENONE-NH.D)
pA
16.481
NH2
*
FID1 A, (JUAN SEPT 18\JUAN-INTENSIFICATION 2018-09-05 11-19-28\JMS-1745.D)
17.132
Area: 149.216
Example of NfisRedAm
Reductive Amination
O
NH2
*
16.731
Area: 20.7177
min

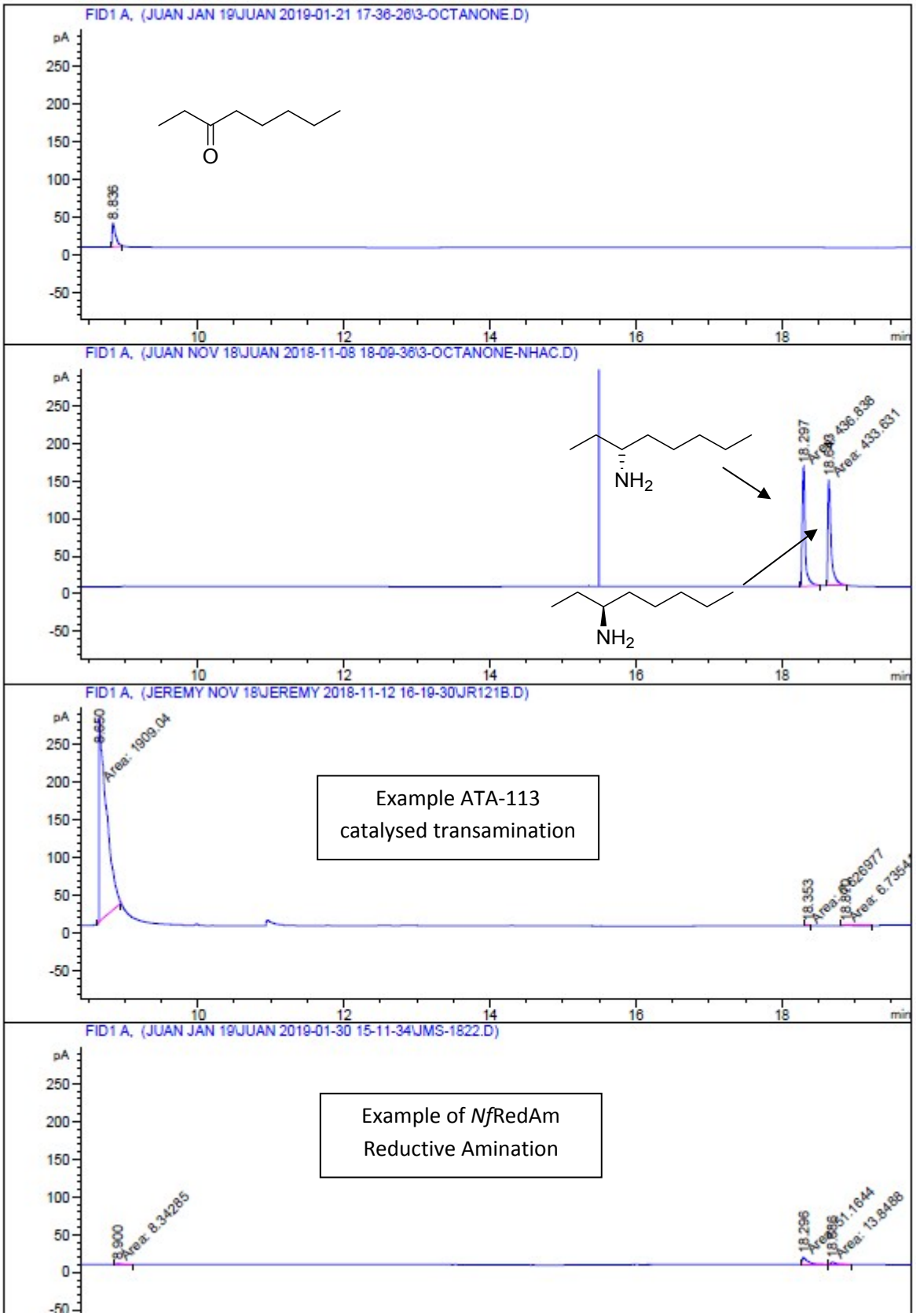

FID1 A, (JUAN NOV 18\JUAN 2018-11-06 14-09-32\CYCLOHEXMEKETONE-NHAC.D)
pA
150
100
50
0
-50
-100
12
14
16
18
20
22
min
O
NH
21.189
21.547
O
NH
FID1 A, (JUAN NOV 18\JUAN 2018-11-08 18-09-36\R-CYCLOHEXMEKETONE-NHAC.D)
pA
150
100
50
0
-50
-100
12
14
16
18
20
22
min
O
NH
21.523
FID1 A, (JUAN JAN 19\JUAN 2019-01-30 15-11-34\JMS-1825.D)
pA
150
100
50
0
-50
-100
12
14
16
18
20
22
min
Example of *Nf*RedAm Reductive Amination
O
12.095
Area: 63.4432
21.212
Area: 34.5563
Area: 26.4231
FID1 A, (JUAN JAN 19\JUAN 2019-01-30 15-11-34\JMS-1826.D)
pA
150
100
50
0
Example of *Nfis*RedAm Reductive Amination
12.098
Area: 54.9
21.195
Area: 79.596
Area: 12.4508

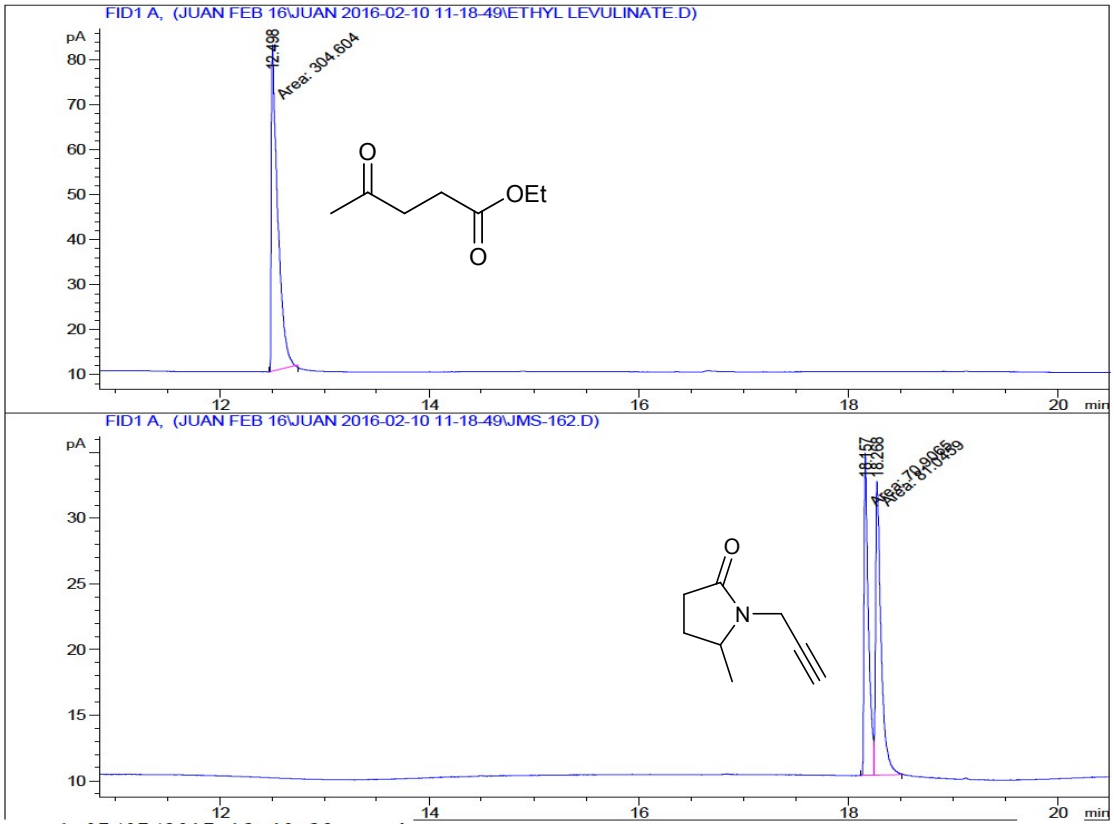

FID1 A, (JUAN JULY 18\JUAN-INTENSIFICATION 2018-07-25 09-24-25\CYCLOHEPTANONE.D)
pA
250
200
150
100
50
0
12
12.5
13
13.5
14
14.5
15
min
O
FID1 A, (JUAN SEPT 18\JUAN-INTENSIFICATION 2018-09-05 11-19-28\JMS-1747.D)
pA
140
120
100
80
60
40
20
Area: 423.621
H_2N
Example of *Nf*RedAm Reductive Amination

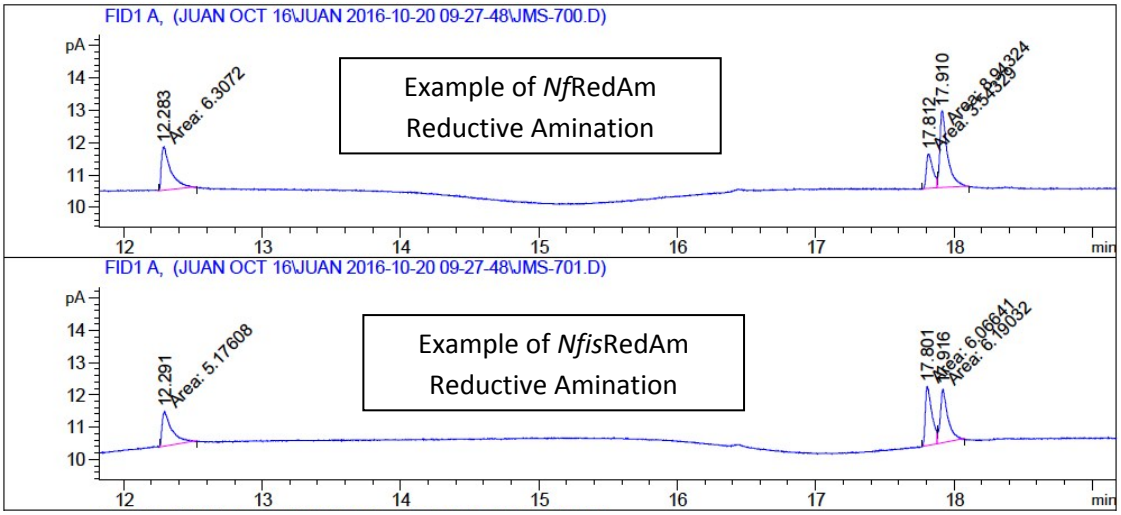

Hit 1 : Cycloheptylamine
C7H15N; MF: 896; RMF: 914; Prob 81.7%; CAS: 5452-35-7; Lib: mainlib; ID: 21948.
100
50
0
56
NH_2
30
43
39
70
15
18
54
67
81
84
113
10
20
30
40
50
60
70
80
90
100
110
120
(mainlib) Cycloheptylamine

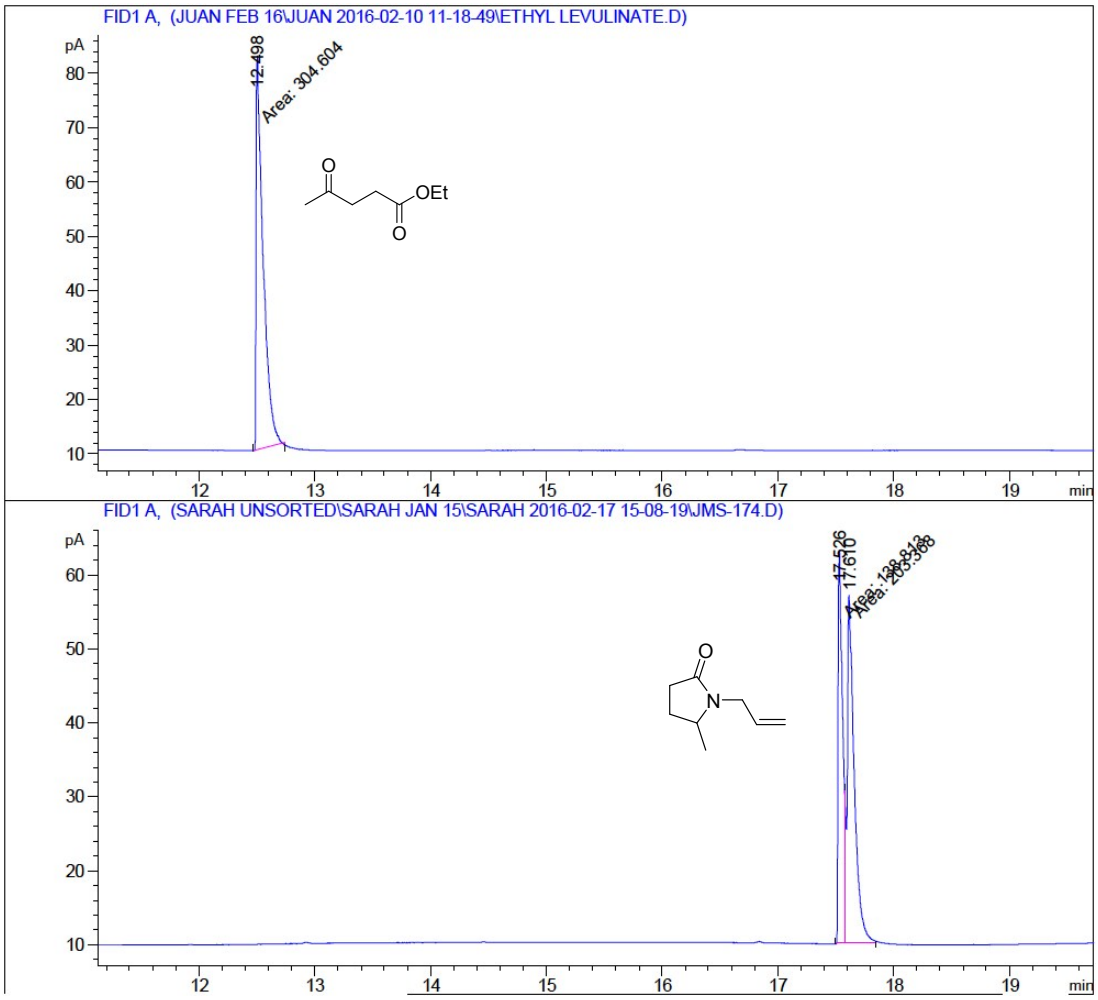

FID1 A, (JUAN SEPT 19\JUAN-AMMONIA 2019-09-18 11-10-24\CYCLOHEXENONE-NEW-METHOD.D)
pA
O
min
FID1 A, (JUAN SEPT 19\JUAN-AMMONIA 2019-09-18 11-10-24\CYCLOHEXENONE-NH-NEW-METHOD.D)
7.244
Area: 440.742
NH2
FID1 A, (JUAN SEPT 19\JUAN-AMMONIA 2019-09-18 11-10-24\CYCLOHEXYLAMINE-NEW-METHOD.D)
NH2
FID1 A, (JUAN SEPT 19\JUAN-AMMONIA 2019-09-18 11-10-24\JMS-2104-NEW-METHOD.D)
7.159
8.635
Example of NfRedAm
Reductive Amination

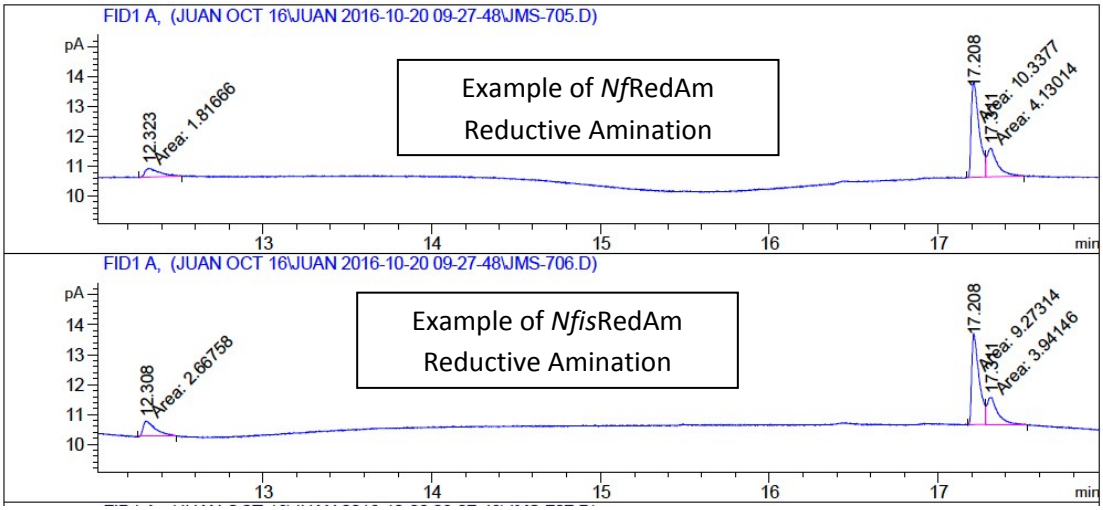

C (dtd)
5.65
B (dq)
5.56
A (ddt)
3.26
f1 (ppm)
Crude 1H NMR spectrum
of chemical standard

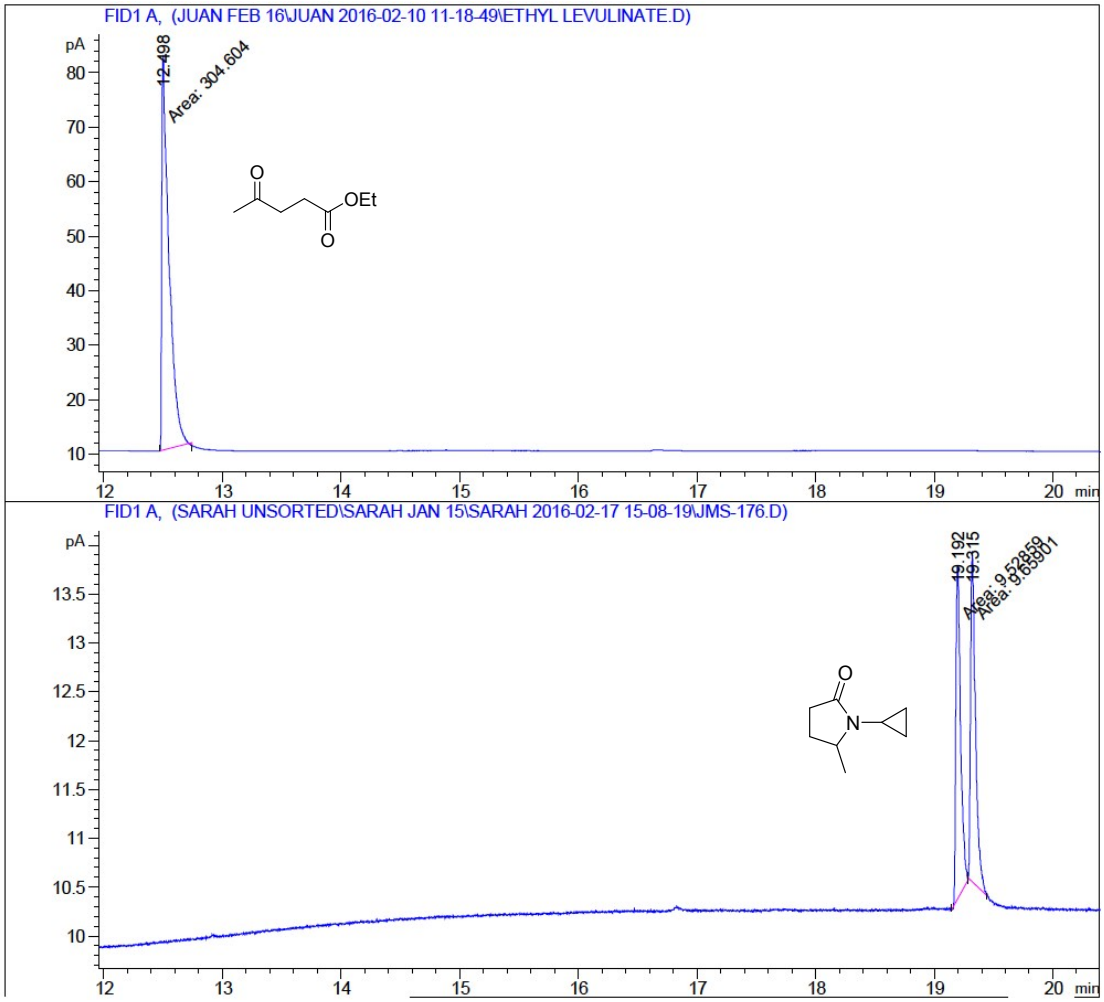

FID1 A, (JUAN JAN 19\JUAN 2019-01-21 17-36-26\3-OCTANONE.D)
pA
8.836
O
min
FID1 A, (JUAN NOV 18\JUAN 2018-11-08 18-09-36\3-OCTANONE-NHAC.D)
NH2
18.297
Area: 436.838
Area: 433.631
NH2
min
FID1 A, (JEREMY NOV 18\JEREMY 2018-11-12 16-19-30\JR121B.D)
8.650
Area: 1909.04
Example ATA-113
catalysed transamination
18.353
Area: 6.73547
min
FID1 A, (JUAN JAN 19\JUAN 2019-01-30 15-11-34\JMS-1822.D)
8.900
Area: 8.34285
Example of *Nf*RedAm
Reductive Amination
18.296
Area: 13.8488
min

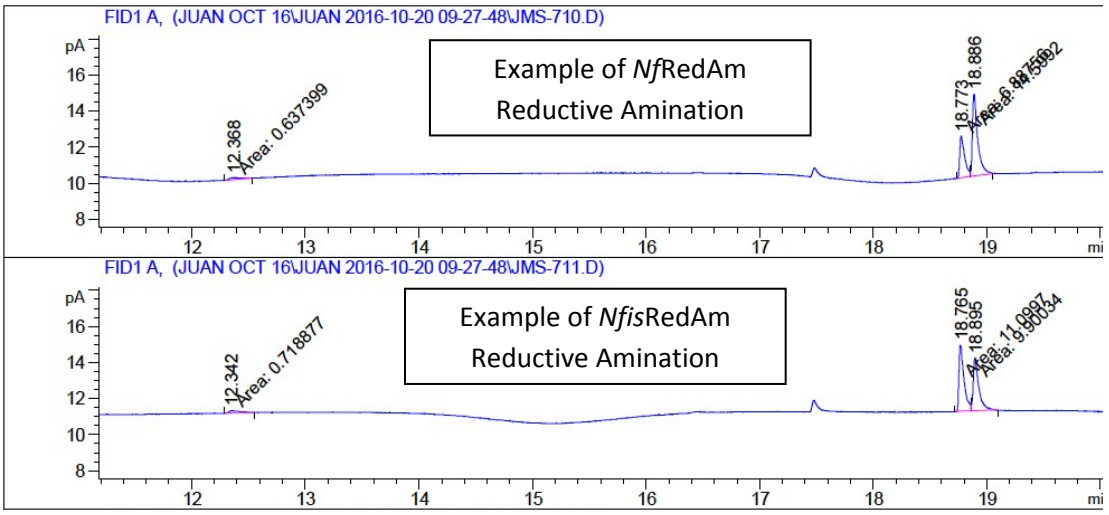

FID1 A, (JUAN FEB 16\JUAN 2016-02-10 11-18-49\ETHYL LEVULINATE.D)
pA
12.498
Area: 304.604
O
OEt
O
min
FID1 A, (JUAN FEB 16\JUAN 2016-02-10 11-18-49\JMS-162.D)
pA
18.157
18.288
O
N
min

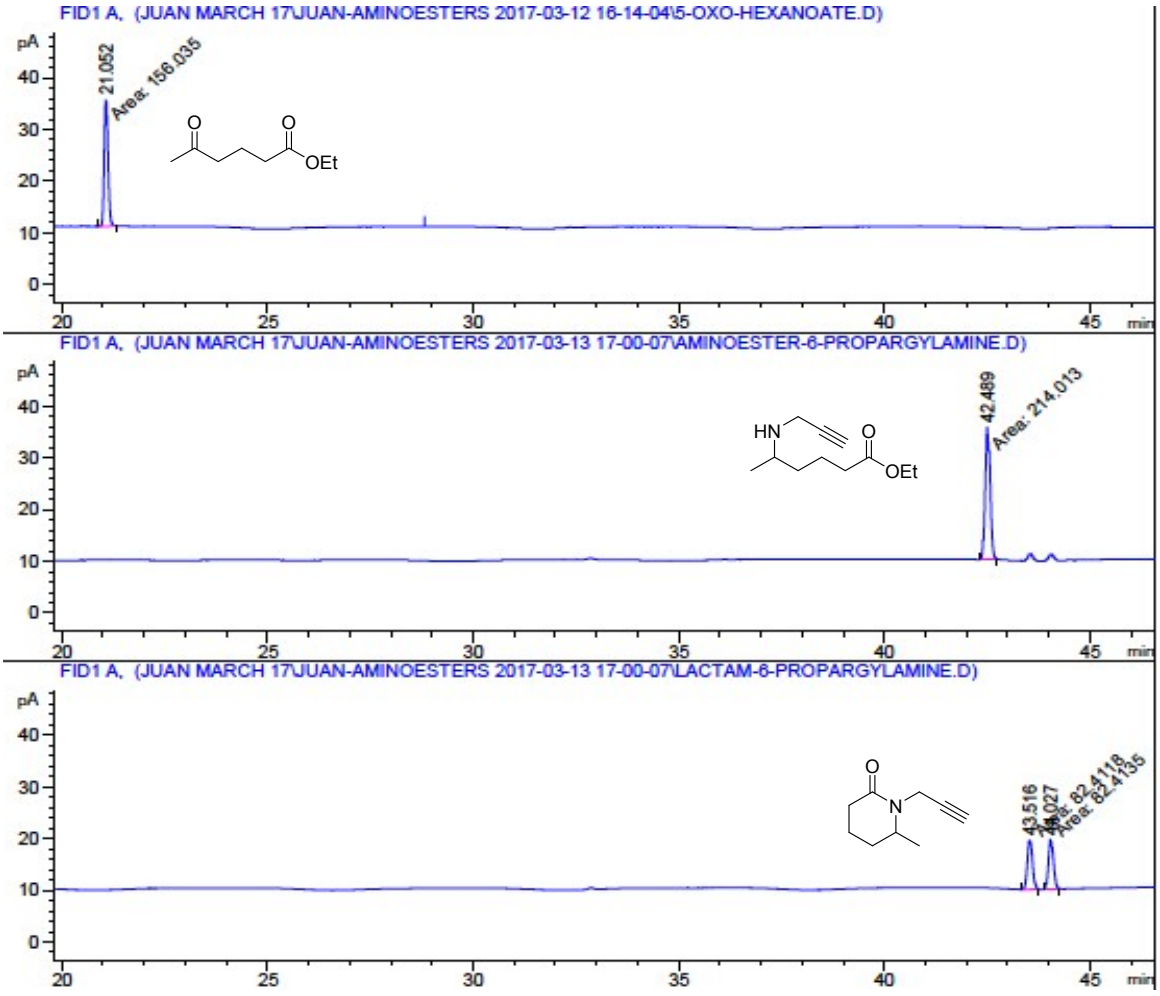

FID1 A, (JUAN OCT 16\JUAN 2016-10-20 09-27-48\JMS-700.D)
pA
12.283
Area: 6.3072
Example of *Nf*RedAm
Reductive Amination
17.812
17.910
min
FID1 A, (JUAN OCT 16\JUAN 2016-10-20 09-27-48\JMS-701.D)
pA
12.291
Area: 5.17608
Example of *Nfis*RedAm
Reductive Amination
17.801
17.916
min

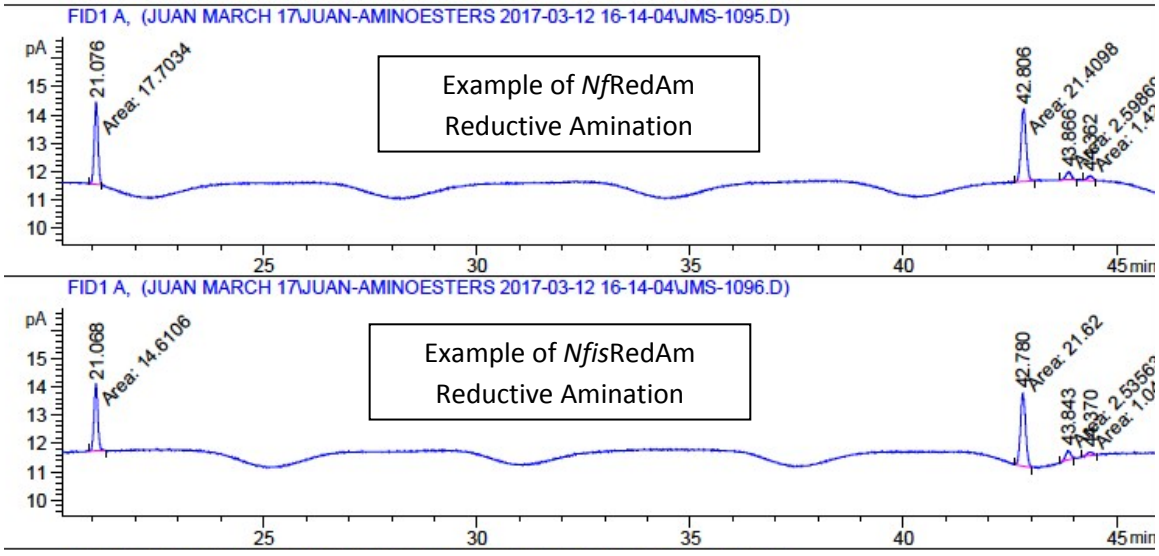

FID1 A, (JUAN FEB 16\JUAN 2016-02-10 11-18-49\ETHYL LEVULINATE.D)
pA
12.498
Area: 304.604
OEt
O
O
min
FID1 A, (SARAH UNSORTED\SARAH JAN 15\SARAH 2016-02-17 15-08-19\JMS-174.D)
pA
17.526
17.610
N
O
min

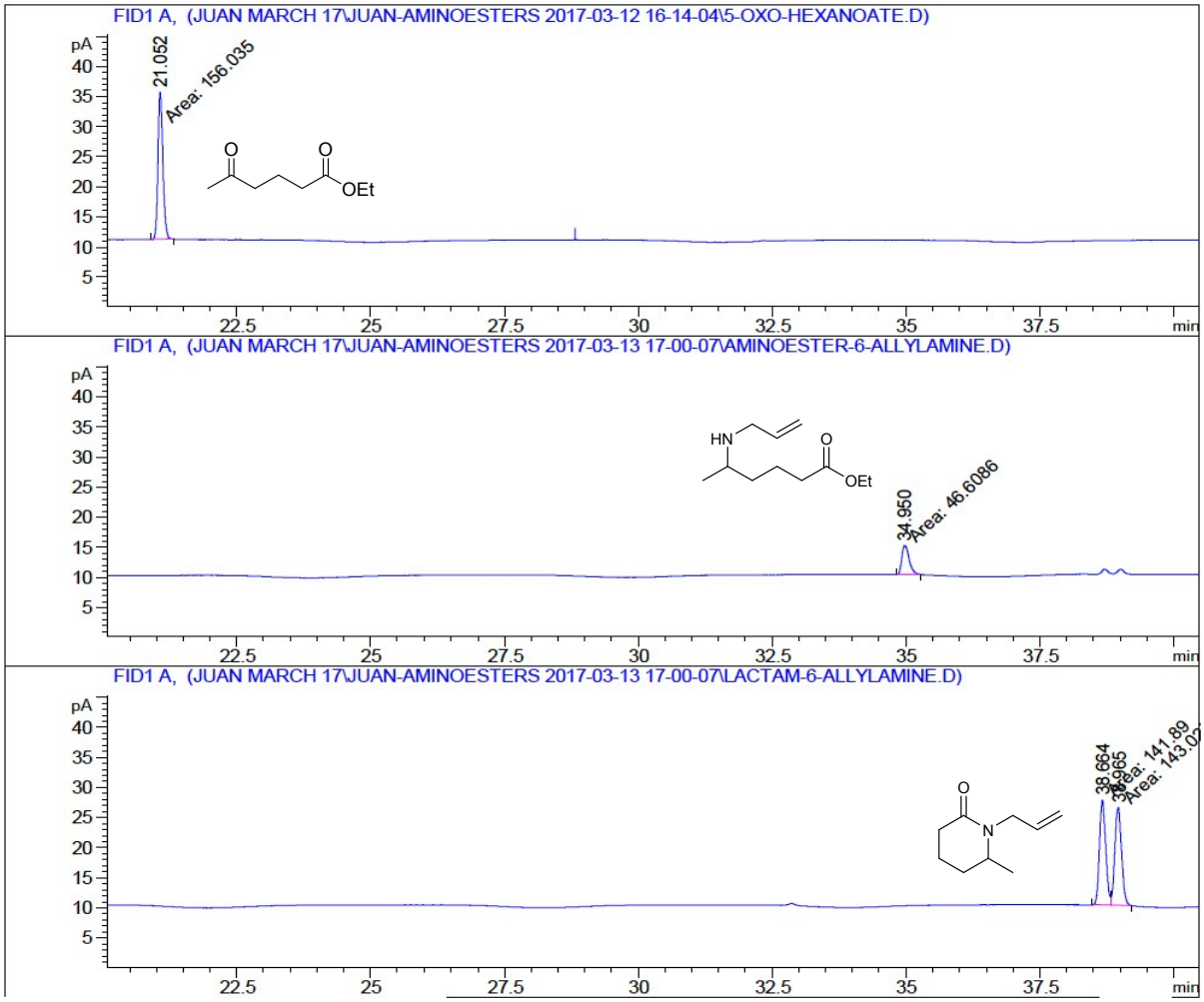

FID1 A, (JUAN OCT 16\JUAN 2016-10-20 09-27-48\JMS-705.D)
pA
12.323
Area: 1.81666
Example of *Nf*RedAm
Reductive Amination
17.208
Area: 10.3377
Area: 4.13014
min
FID1 A, (JUAN OCT 16\JUAN 2016-10-20 09-27-48\JMS-706.D)
pA
12.308
Area: 2.66758
Example of *Nfis*RedAm
Reductive Amination
17.208
Area: 9.27314
Area: 3.94146
min

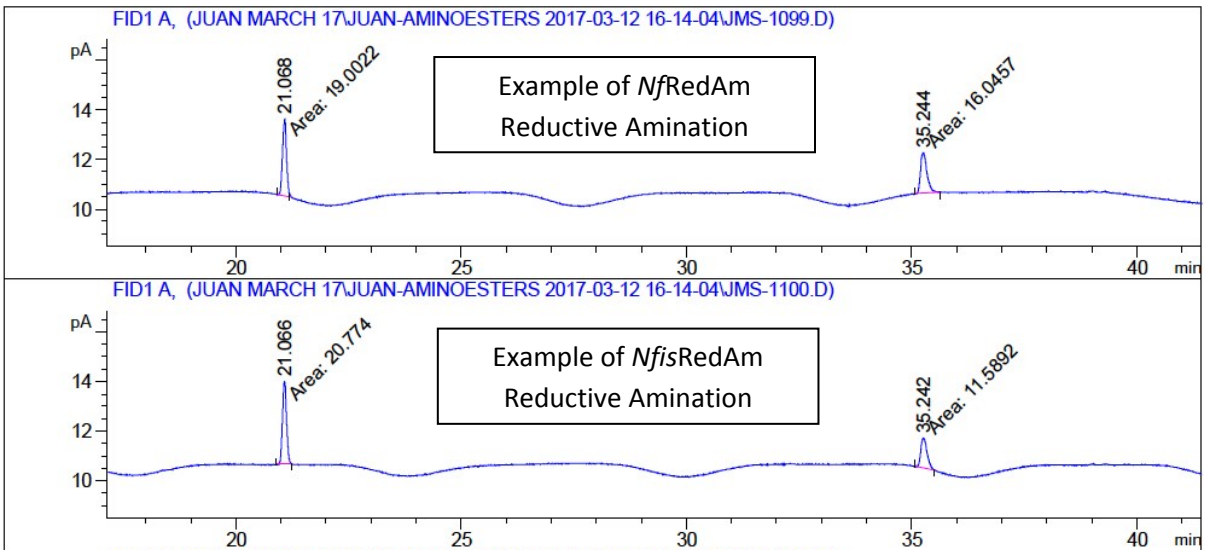

FID1 A, (JUAN FEB 16\JUAN 2016-02-10 11-18-49\ETHYL LEVULINATE.D)
pA
12.498
Area: 304.604
O
OEt
O
min
FID1 A, (SARAH UNSORTED\SARAH JAN 15\SARAH 2016-02-17 15-08-19\JMS-176.D)
pA
19.192
19.315
O
N
min

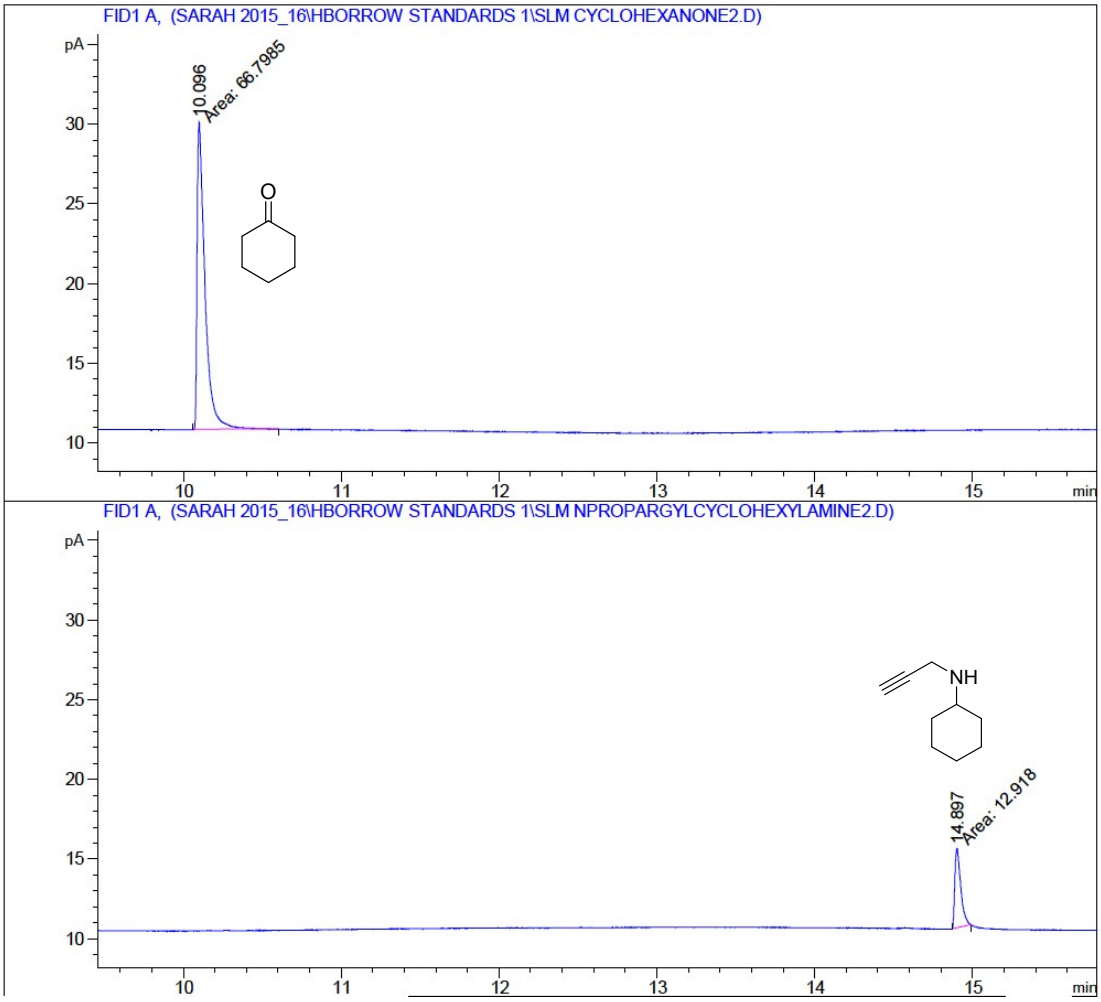

FID1 A, (JUAN OCT 16\JUAN 2016-10-20 09-27-48\JMS-710.D)
pA
12.368
Area: 0.637399
Example of *Nf*RedAm
Reductive Amination
18.773
18.886
mi
FID1 A, (JUAN OCT 16\JUAN 2016-10-20 09-27-48\JMS-711.D)
pA
12.342
Area: 0.718877
Example of *Nfis*RedAm
Reductive Amination
18.765
18.895
mi

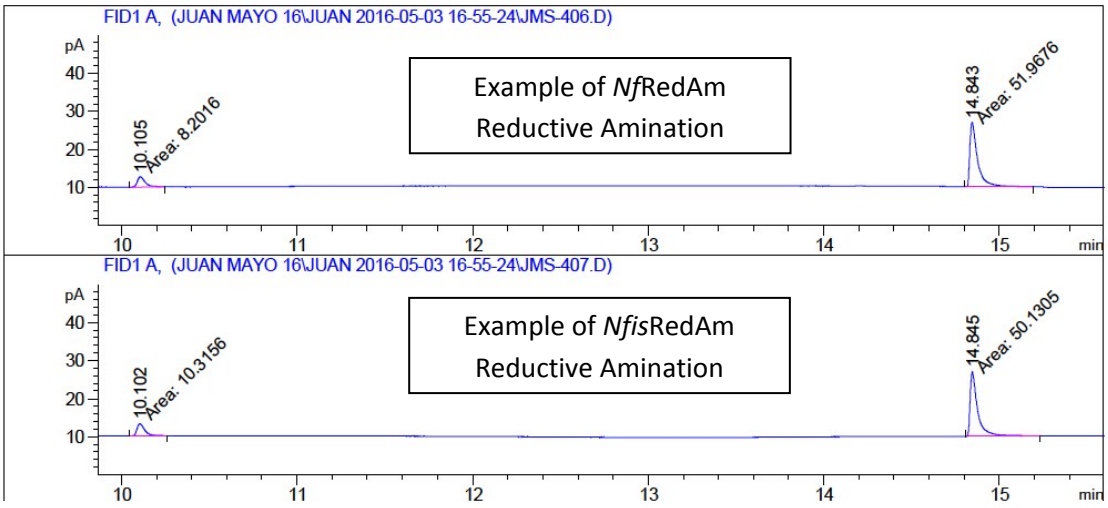

FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-12 16-14-04\5-OXO-HEXANOATE.D)
21.052
Area: 156.035
OEt
FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-13 17-00-07\AMINOESTER-6-PROPARGYLAMINE.D)
42.489
Area: 214.013
HN
OEt
FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-13 17-00-07\LACTAM-6-PROPARGYLAMINE.D)
43.516
Area: 82.4135
pA
min

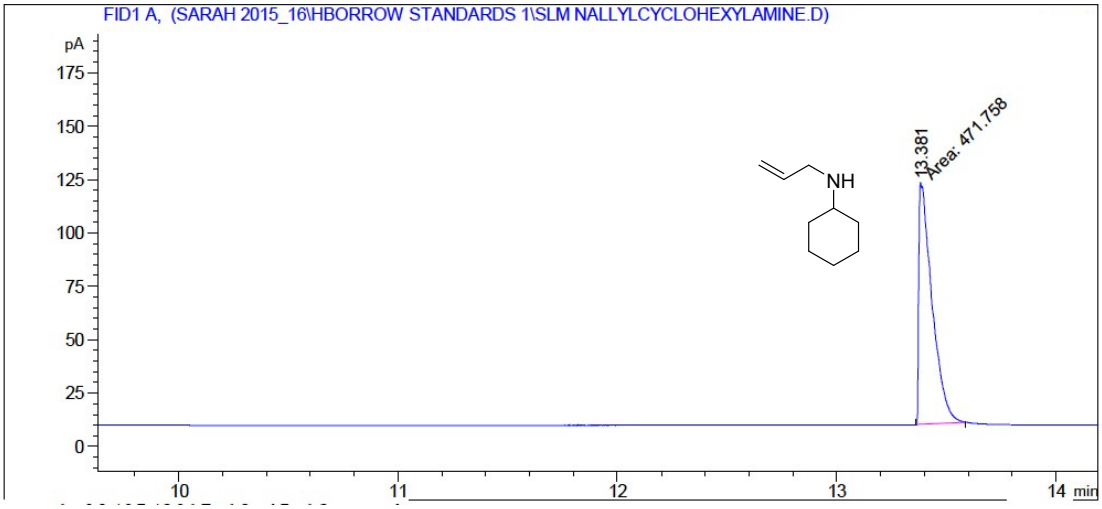

FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-12 16-14-04\JMS-1095.D)
21.076
Area: 17.7034
Example of *Nf*RedAm
Reductive Amination
42.806
Area: 21.4098
FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-12 16-14-04\JMS-1096.D)
21.068
Area: 14.6106
Example of *Nfis*RedAm
Reductive Amination
42.780
Area: 21.62
pA
min

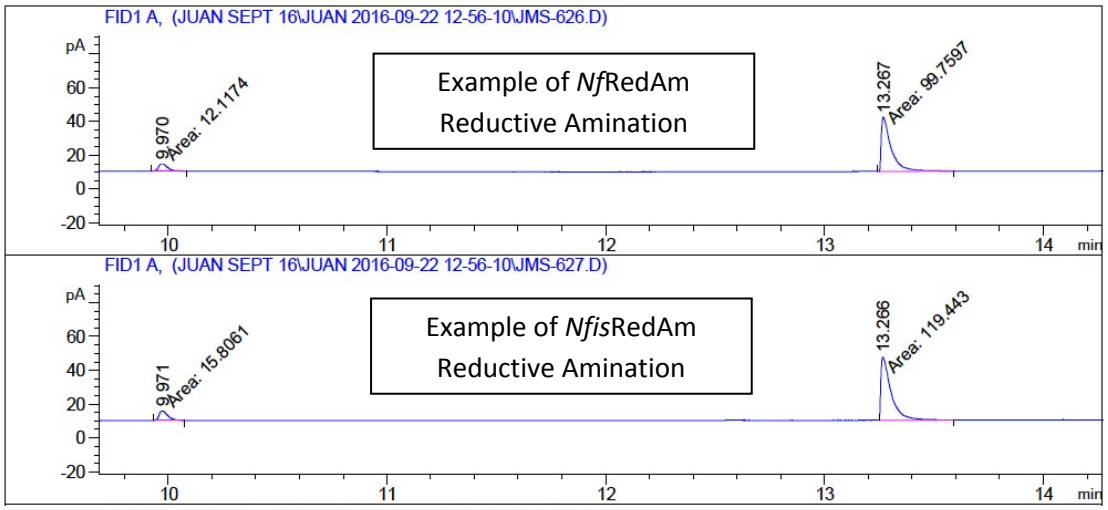

FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-12 16-14-04\5-OXO-HEXANOATE.D)
21.052
Area: 156.035
OEt
FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-13 17-00-07\AMINOESTER-6-ALLYLAMINE.D)
HN
OEt
24.950
Area: 46.6086
FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-13 17-00-07\LACTAM-6-ALLYLAMINE.D)
38.664
Area: 141.89
pA
min

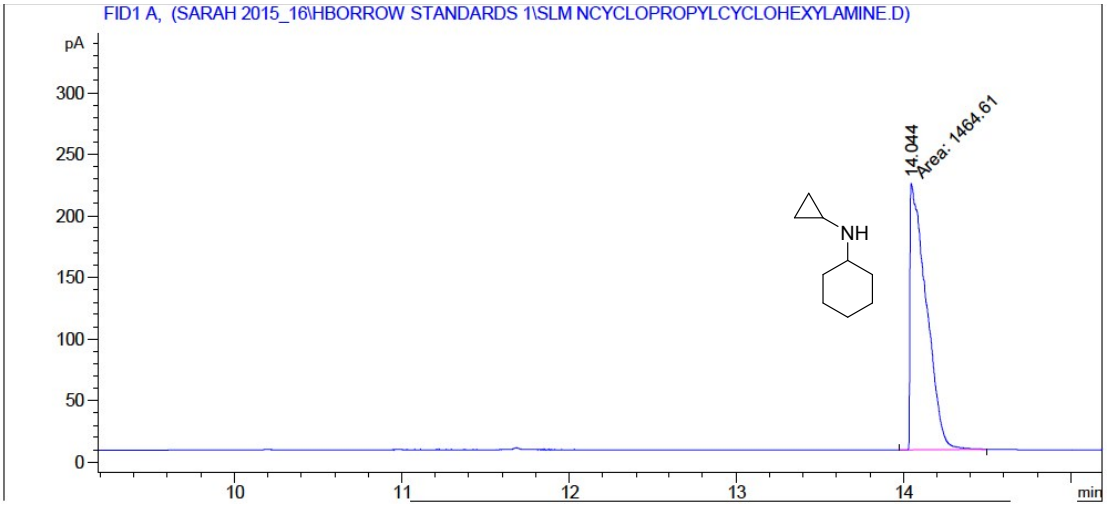

FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-12 16-14-04\JMS-1099.D)
21.068
Area: 19.0022
Example of *Nf*RedAm
Reductive Amination
35.244
Area: 16.0457
FID1 A, (JUAN MARCH 17\JUAN-AMINOESTERS 2017-03-12 16-14-04\JMS-1100.D)
21.066
Area: 20.774
Example of *Nfis*RedAm
Reductive Amination
35.242
Area: 11.5892
pA
min

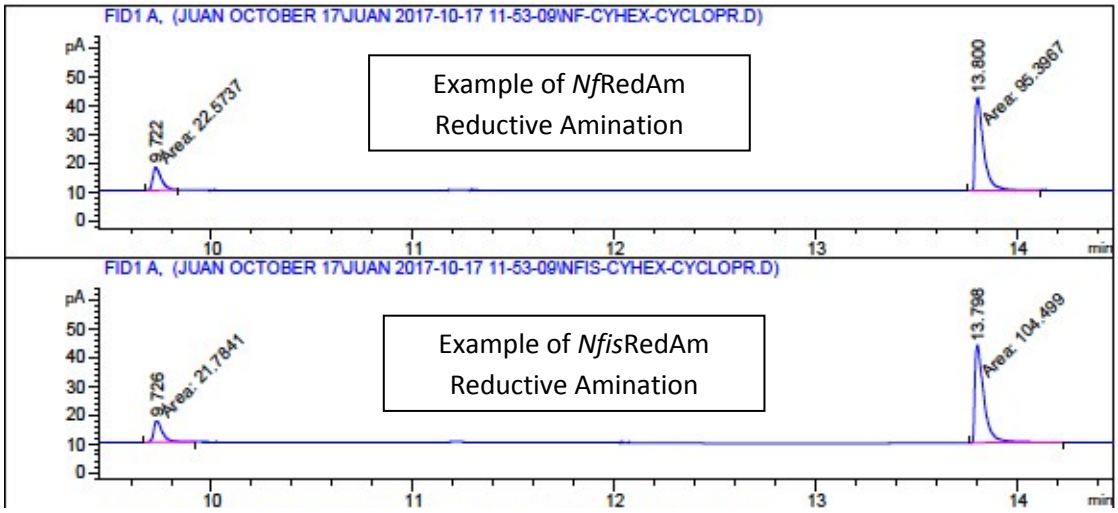

FID1 A, (SARAH 2015_16\HBORROW STANDARDS 1\SLM CYCLOHEXANONE2.D)
pA
10.096
Area: 66.7985
O
FID1 A, (SARAH 2015_16\HBORROW STANDARDS 1\SLM NPROPARGYLCYCLOHEXYLAMINE2.D)
NH
14.897
Area: 12.918
min

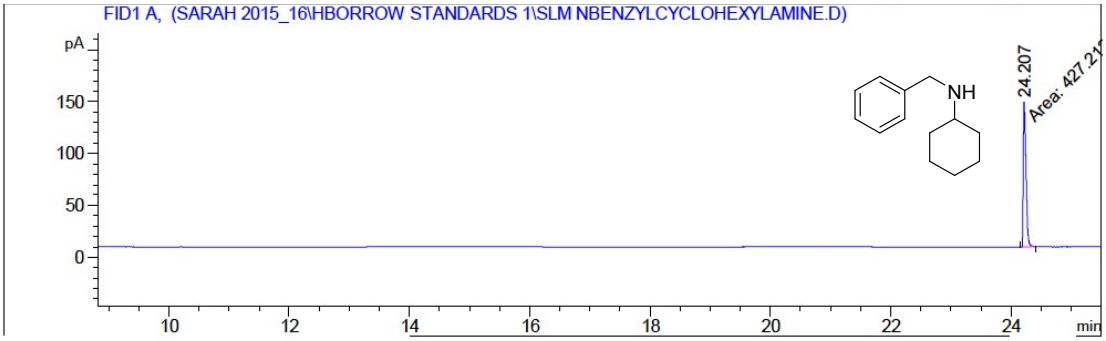

FID1 A, (JUAN MAYO 16\JUAN 2016-05-03 16-55-24\JMS-406.D)
pA
10.105
Area: 8.2016
Example of *Nf*RedAm
Reductive Amination
14.843
Area: 51.9676
min
FID1 A, (JUAN MAYO 16\JUAN 2016-05-03 16-55-24\JMS-407.D)
10.102
Area: 10.3156
Example of *Nfis*RedAm
Reductive Amination
14.845
Area: 50.1305

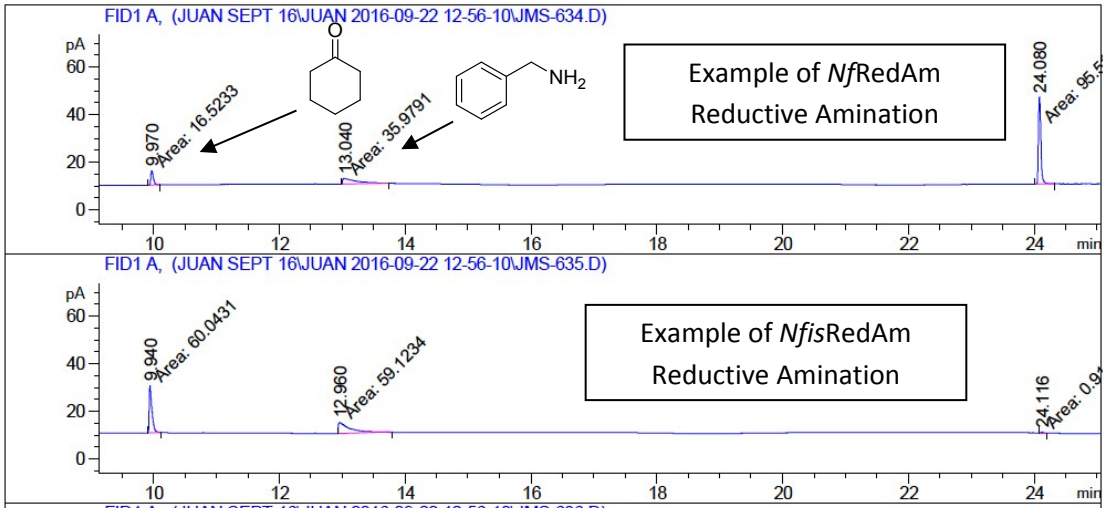

FID1 A, (SARAH 2015_16\HBORROW STANDARDS 1\SLM NALLYLCYCLOHEXYLAMINE.D)
pA
175
150
125
100
75
50
25
0
NH
13.381
Area: 471.758
10
11
12
13
14 min


FID1 A, (JUAN SEPT 16\JUAN 2016-09-22 12-56-10\JMS-626.D)
pA
60
40
20
0
-20
9.970
Area: 12.1174
Example of *Nf*RedAm
Reductive Amination
13.267
Area: 99.7597
10
11
12
13
14 min
FID1 A, (JUAN SEPT 16\JUAN 2016-09-22 12-56-10\JMS-627.D)
pA
60
40
20
0
-20
9.971
Area: 15.8061
Example of *Nfis*RedAm
Reductive Amination
13.266
Area: 119.443
10
11
12
13
14 min

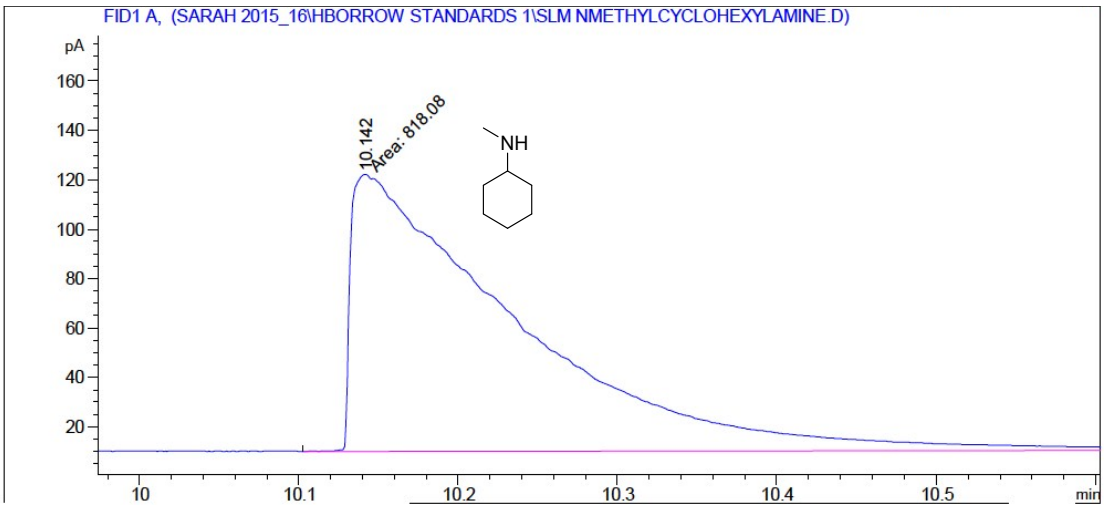

FID1 A, (SARAH 2015_16\HBORROW STANDARDS 1\SLM NCYCLOPROPYLCYCLOHEXYLAMINE.D)
pA
300
250
200
150
100
50
0
14.044
Area: 1464.61
NH
10
11
12
13
14
min

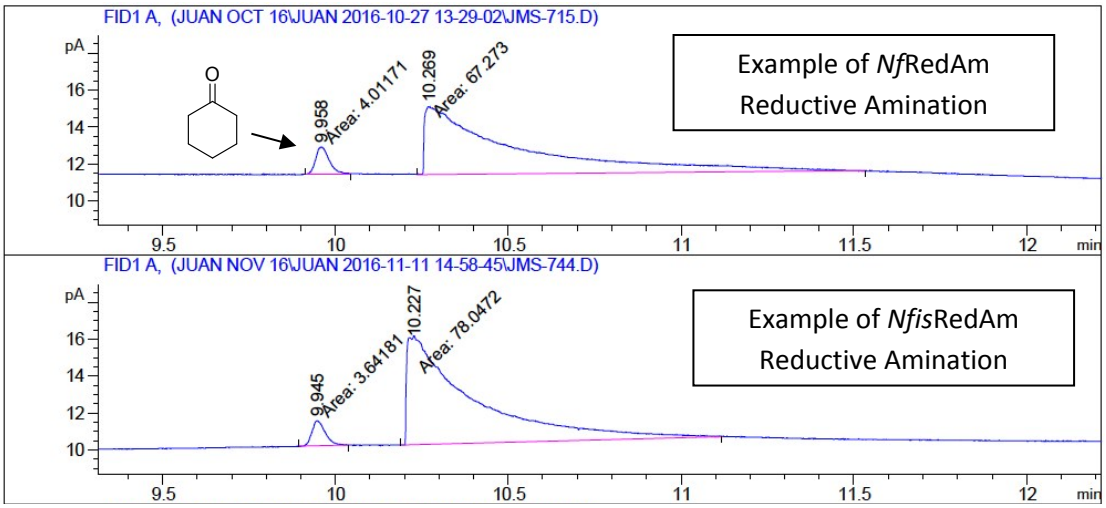

FID1 A, (JUAN OCTOBER 17\JUAN 2017-10-17 11-53-09\NF-CYHEX-CYCLOPR.D)
pA
50
40
30
20
10
0
9.722
Area: 22.5737
Example of *Nf*RedAm
Reductive Amination
13.800
Area: 95.3967
10
11
12
13
14
min
FID1 A, (JUAN OCTOBER 17\JUAN 2017-10-17 11-53-09\NFIS-CYHEX-CYCLOPR.D)
pA
50
40
30
20
10
0
9.726
Area: 21.7841
Example of *Nfis*RedAm
Reductive Amination
13.798
Area: 104.499
10
11
12
13
14
min


FID1 A, (SARAH 2015_16\HBORROW STANDARDS 1\SLM NBENZYLCYCLOHEXYLAMINE.D)
pA
150
100
50
0
NH
24.207
Area: 427.21
10
12
14
16
18
20
22
24
min

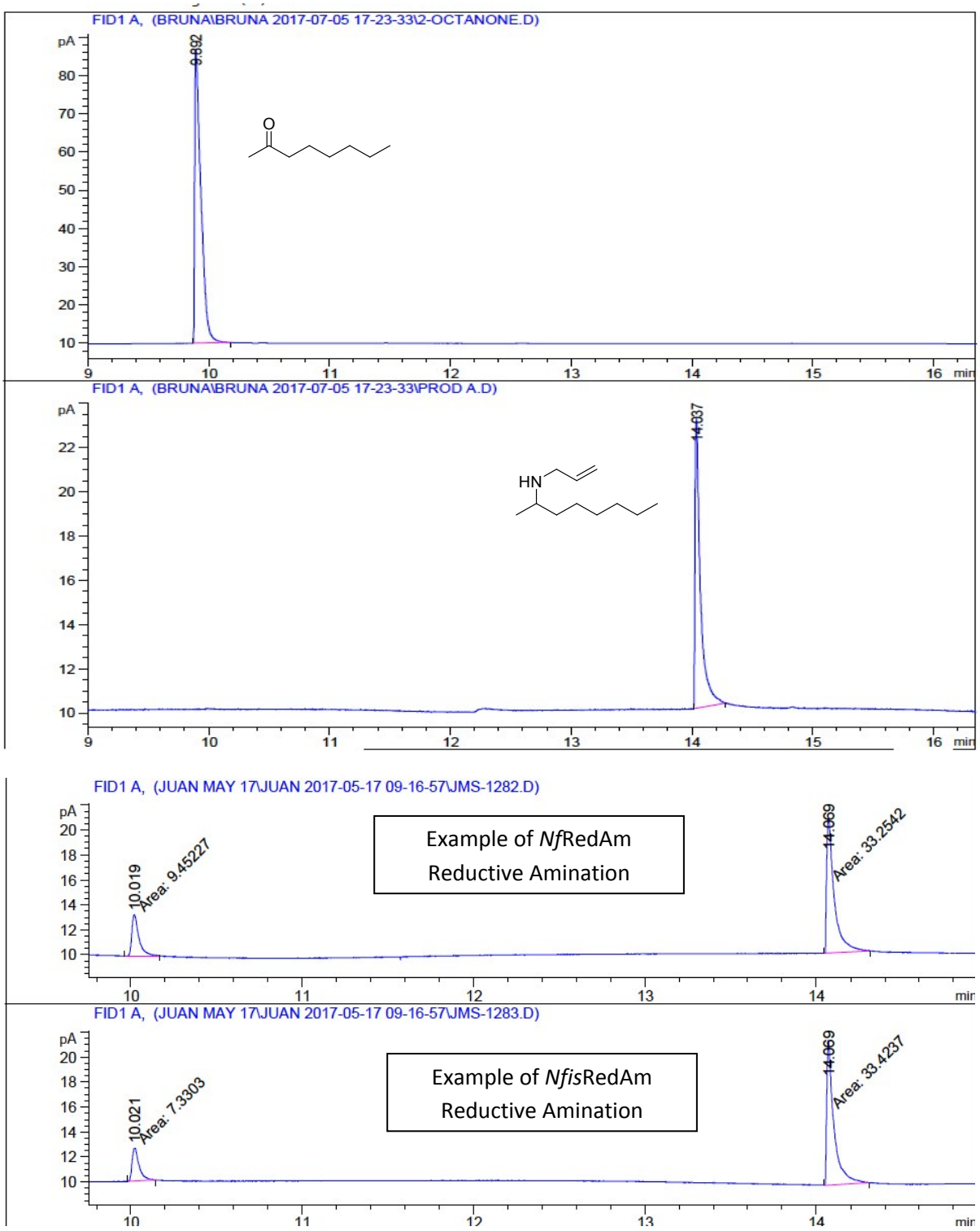

FID1 A, (JUAN SEPT 16\JUAN 2016-09-22 12-56-10\JMS-634.D)
pA
60
40
20
0
O
NH2
9.970
Area: 16.5233
13.040
Area: 35.9791
Example of *Nf*RedAm
Reductive Amination
24.080
Area: 95.5
10
12
14
16
18
20
22
24
min

FID1 A, (JUAN SEPT 16\JUAN 2016-09-22 12-56-10\JMS-635.D)
pA
60
40
20
0
9.940
Area: 60.0431
12.960
Area: 59.1234
Example of *Nfis*RedAm
Reductive Amination
24.116
10
12
14
16
18
20
22
24
min

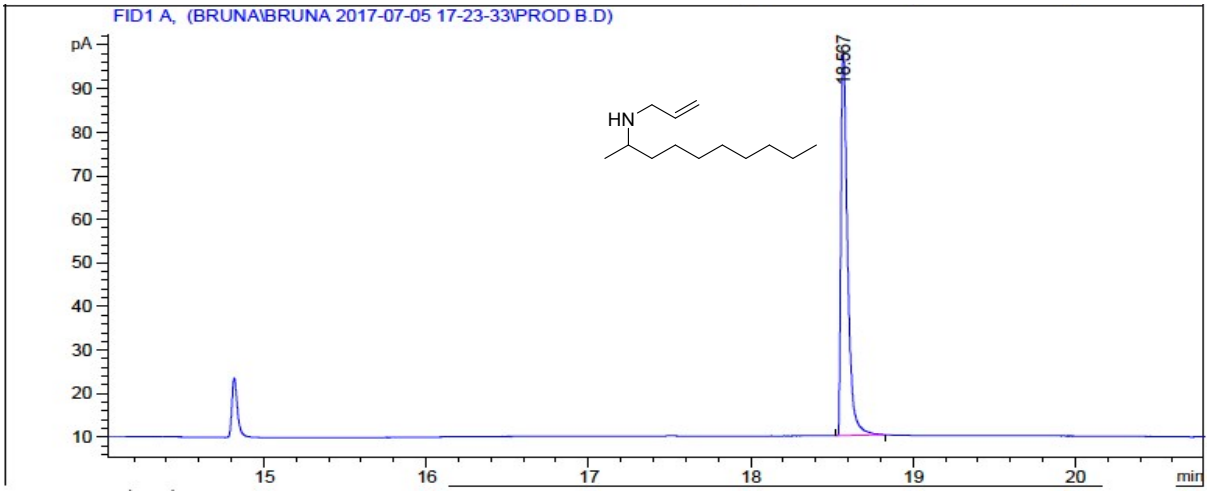

FID1 A, (SARAH 2015_16\HBORROW STANDARDS 1\SLM NMETHYLCYCLOHEXYLAMINE.D)
pA
160
140
120
100
80
60
40
20
10.142
Area: 818.08
NH
10
10.1
10.2
10.3
10.4
10.5
min

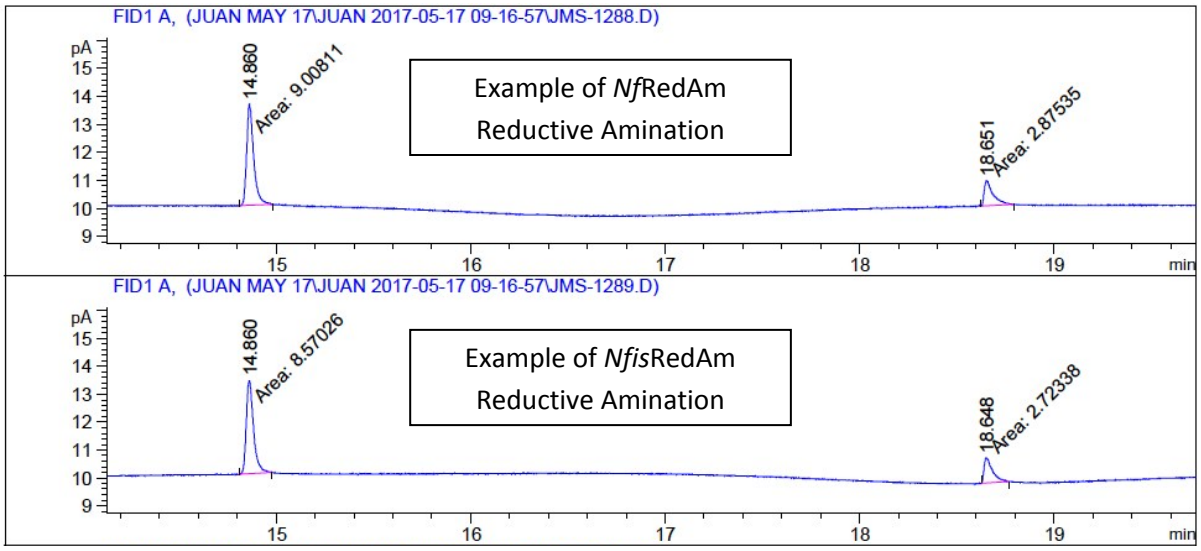

FID1 A, (JUAN OCT 16\JUAN 2016-10-27 13-29-02\JMS-715.D)
pA
16
14
12
10
O
9.958
Area: 4.01171
10.269
Area: 67.273
Example of *Nf*RedAm
Reductive Amination
9.5
10
10.5
11
11.5
12
min

FID1 A, (JUAN NOV 16\JUAN 2016-11-11 14-58-45\JMS-744.D)
pA
16
14
12
10
9.945
Area: 3.64181
10.227
Area: 78.0472
Example of *Nfis*RedAm
Reductive Amination
9.5
10
10.5
11
11.5
12
min

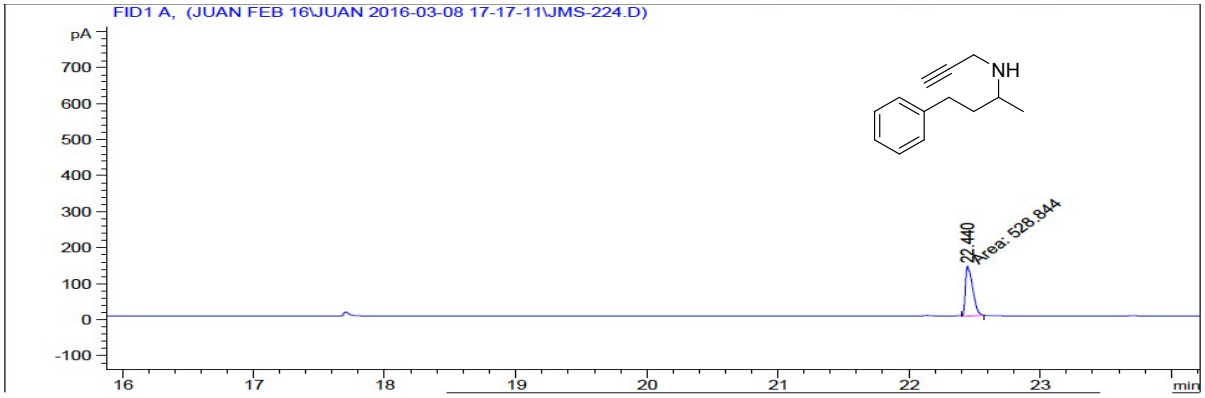


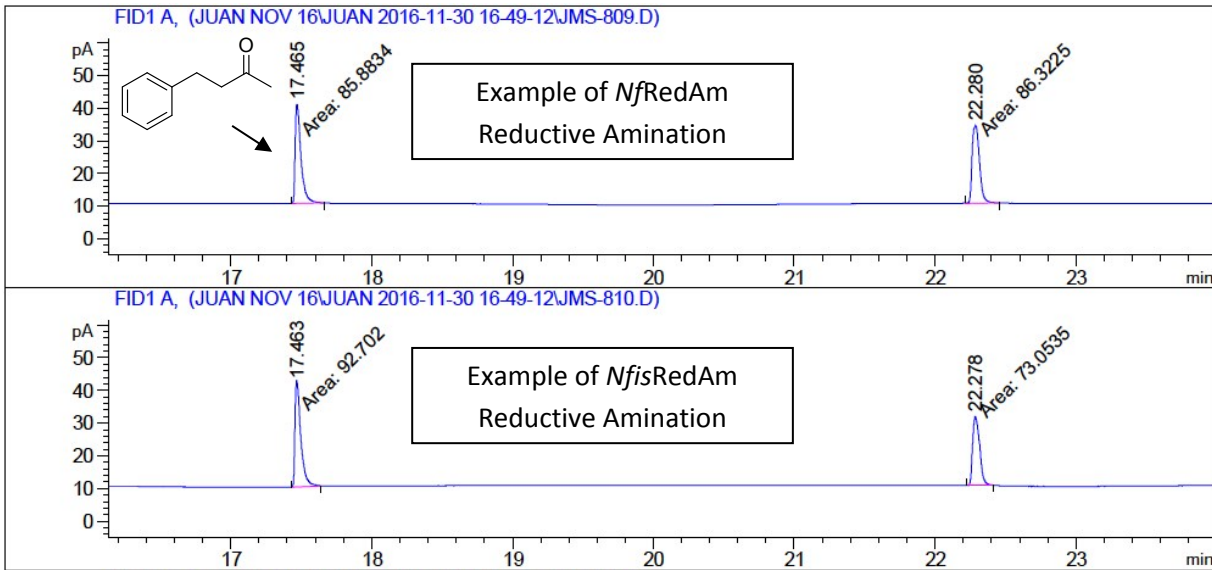
Supplementary Figure S33. GC-FID analysis: RedAm-catalysed reductive amination of 2-octanone **5** with allylamine **b** (5 equivalents) showing *Nf*RedAm (top) and *Nfis*RedAm (bottom).

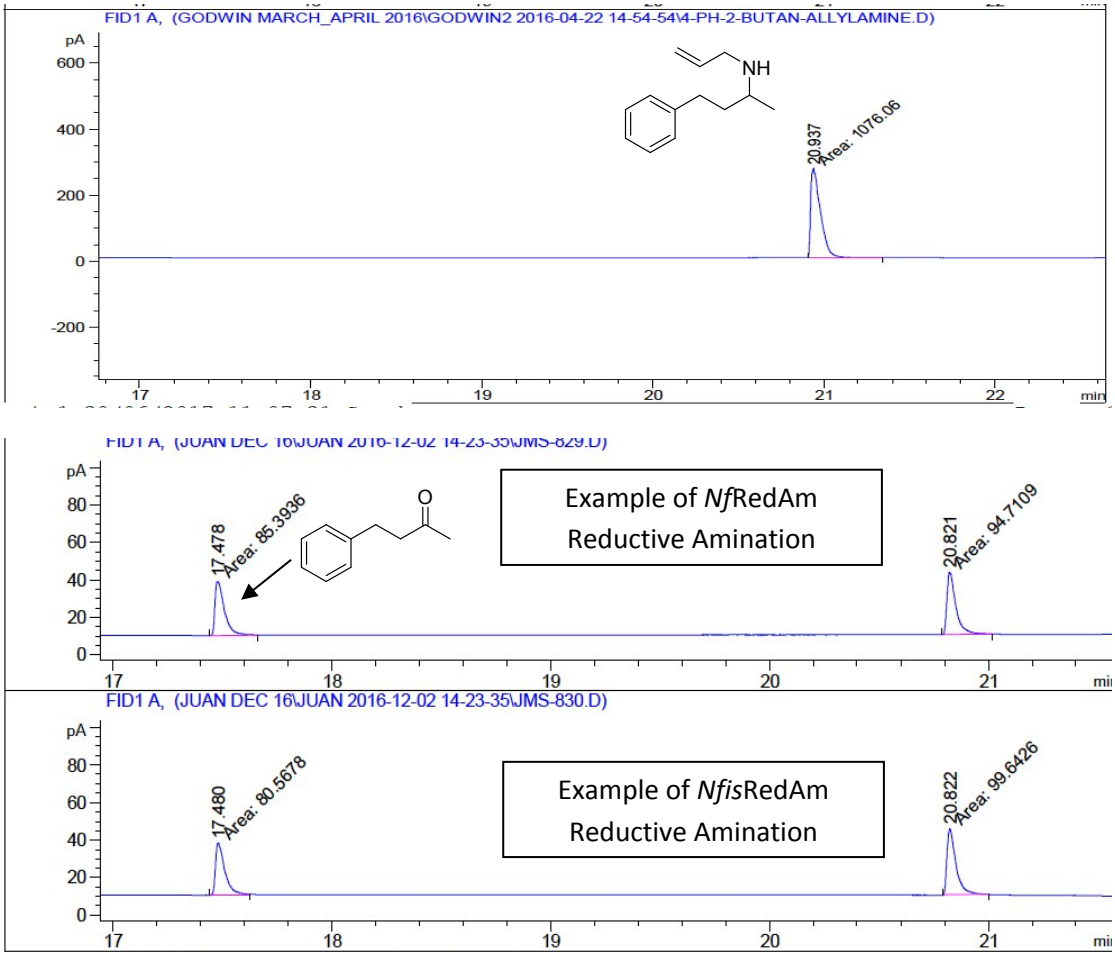

FID1 A, (BRUNA\BRUNA 2017-07-05 17-23-33\PROD B.D)
pA
18.567
HN
min

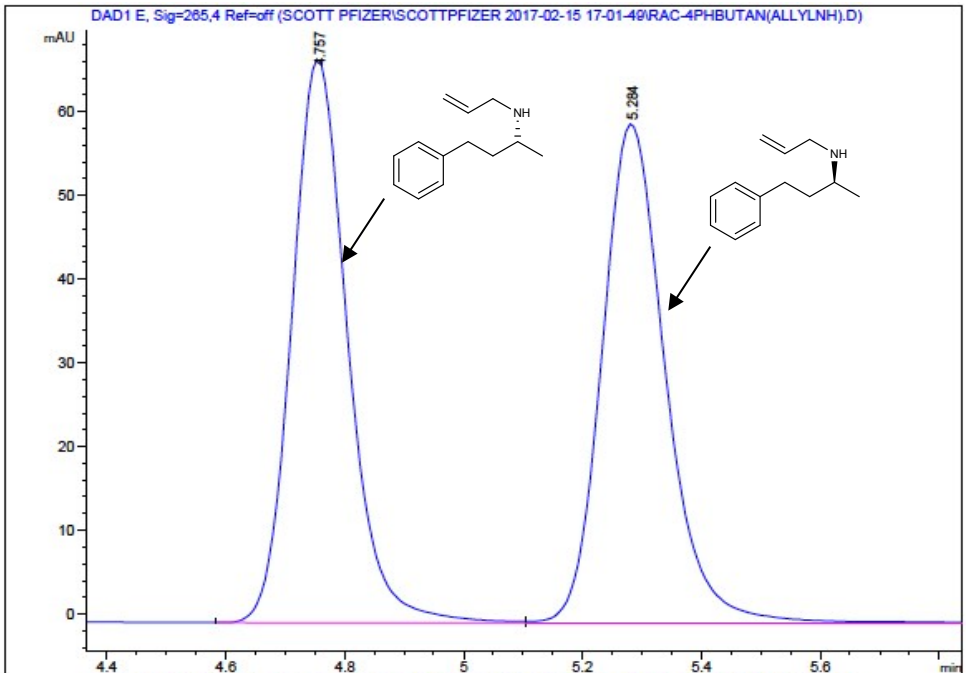

FID1 A, (JUAN MAY 17\JUAN 2017-05-17 09-16-57\JMS-1288.D)
pA
14.860
Area: 9.00811
Example of *Nf*RedAm
Reductive Amination
18.651
Area: 2.87535
min

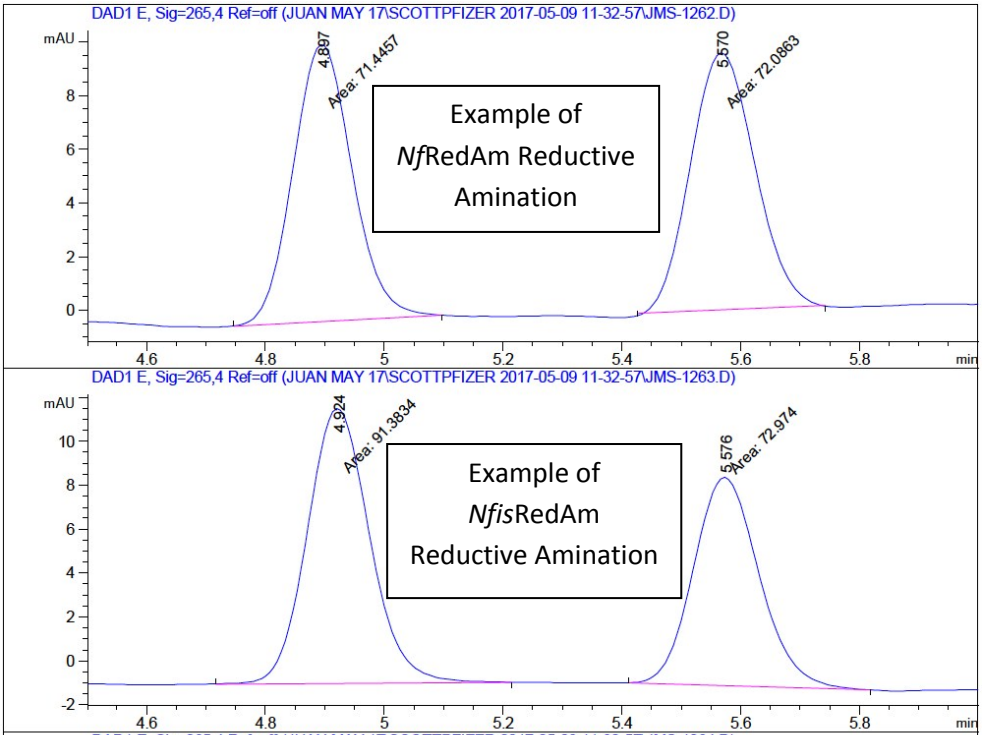
FID1 A, (JUAN MAY 17\JUAN 2017-05-17 09-16-57\JMS-1289.D)
pA
14.860
Area: 8.57026
Example of *Nfis*RedAm
Reductive Amination
18.648
Area: 2.72338
min

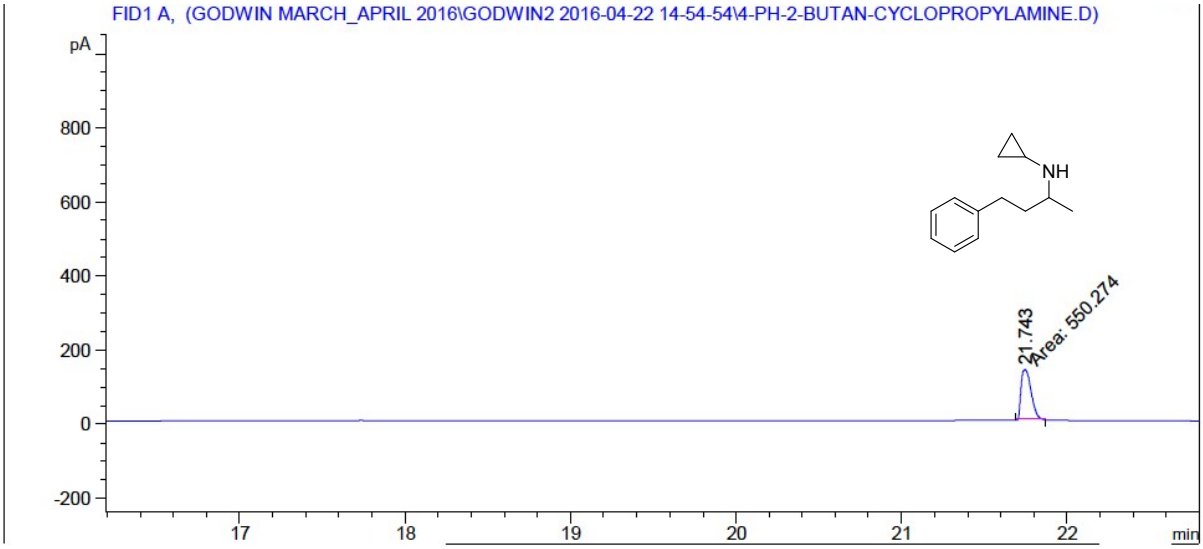

FID1 A, (JUAN FEB 16\JUAN 2016-03-08 17-17-11\JMS-224.D)
pA
700
600
500
400
300
200
100
0
-100
NH
22.440
Area: 528.844
16
17
18
19
20
21
22
23
min

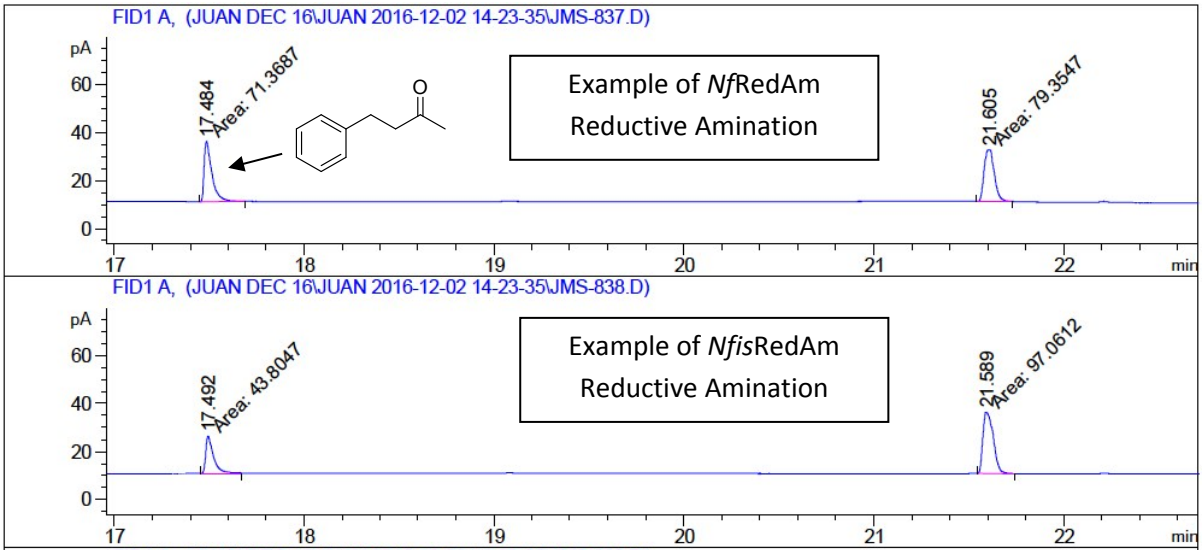

FID1 A, (JUAN NOV 16\JUAN 2016-11-30 16-49-12\JMS-809.D)
pA
50
40
30
20
10
0
O
17.465
Area: 85.8834
Example of *Nf*RedAm
Reductive Amination
22.280
Area: 86.3225
17
18
19
20
21
22
23
min
FID1 A, (JUAN NOV 16\JUAN 2016-11-30 16-49-12\JMS-810.D)
pA
50
40
30
20
10
0
17.463
Area: 92.702
Example of *Nfis*RedAm
Reductive Amination
22.278
Area: 73.0535
17
18
19
20
21
22
23
min

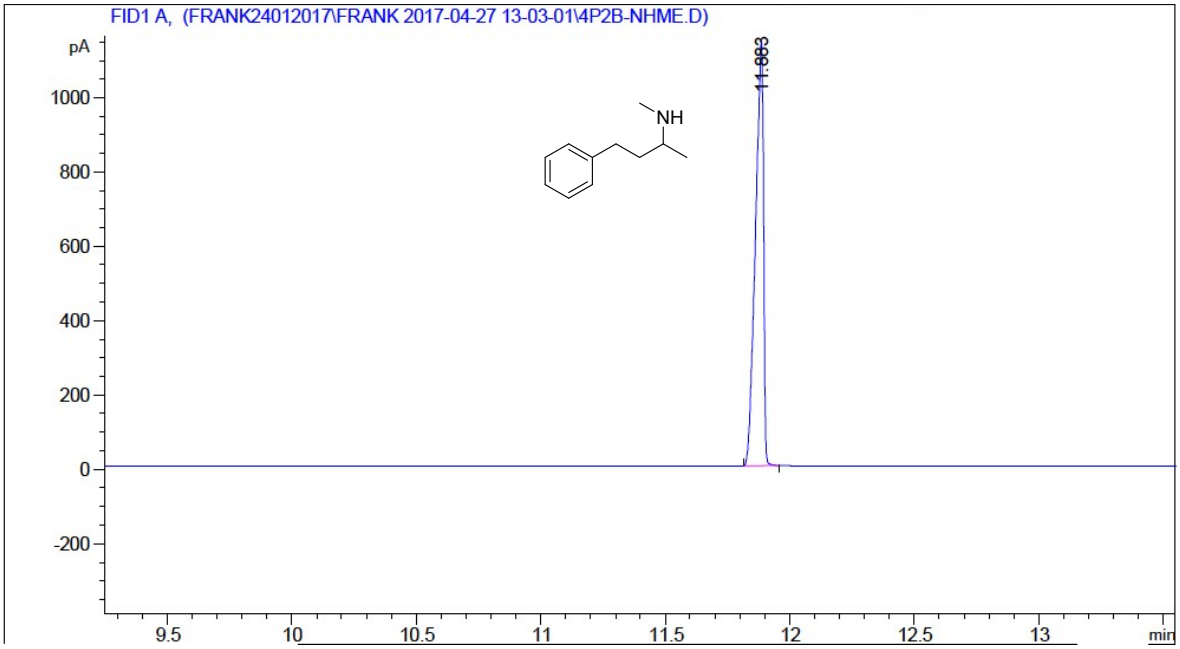

FID1 A, (GODWIN MARCH_APRIL 2016\GODWIN2 2016-04-22 14-54-54\4-PH-2-BUTAN-ALLYLAMINE.D)
pA
600
400
200
0
-200
NH
20.937
Area: 1076.06
17
18
19
20
21
22
min
FID1 A, (JUAN DEC 16\JUAN 2016-12-02 14-23-35\JMS-829.D)
pA
80
60
40
20
0
17.478
Area: 85.3936
O
Example of *Nf*RedAm
Reductive Amination
20.821
Area: 94.7109
17
18
19
20
21
FID1 A, (JUAN DEC 16\JUAN 2016-12-02 14-23-35\JMS-830.D)
pA
80
60
40
20
0
17.480
Area: 80.5678
Example of *Nfis*RedAm
Reductive Amination
20.822
Area: 99.6426
17
18
19
20
21

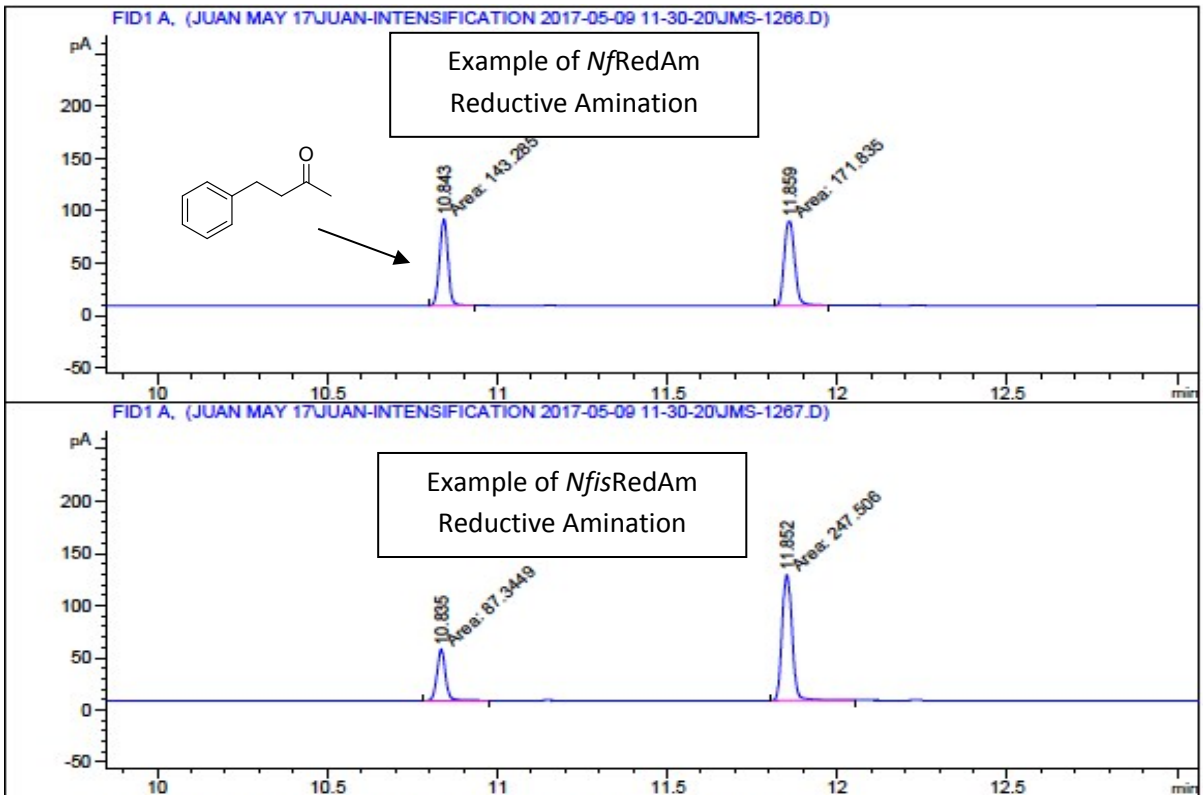

DAD1 E, Sig=265,4 Ref=off (SCOTT PFIZER\SCOTTPFIZER 2017-02-15 17-01-49\RAC-4PHBUTAN(ALLYLNH).D)
mAU
4.757
5.284
NH
NH
min

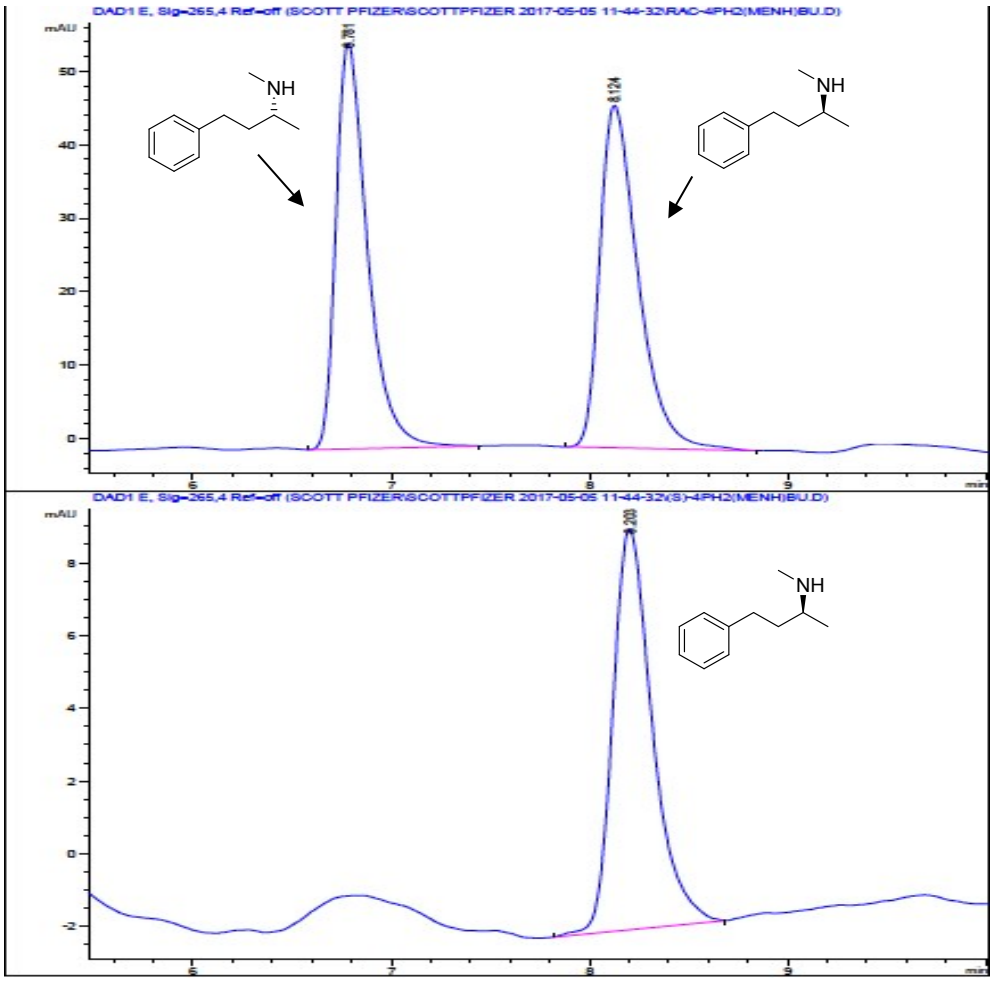

DAD1 E, Sig=265,4 Ref=off (JUAN MAY 17\SCOTTPFIZER 2017-05-09 11-32-57\JMS-1262.D)
mAU
4.897
Area: 71.4457
5.570
Area: 72.0863
Example of
*Nf*RedAm Reductive
Amination
min
DAD1 E, Sig=265,4 Ref=off (JUAN MAY 17\SCOTTPFIZER 2017-05-09 11-32-57\JMS-1263.D)
mAU
4.924
Area: 91.3834
5.576
Area: 72.974
Example of
*Nfis*RedAm
Reductive Amination
min

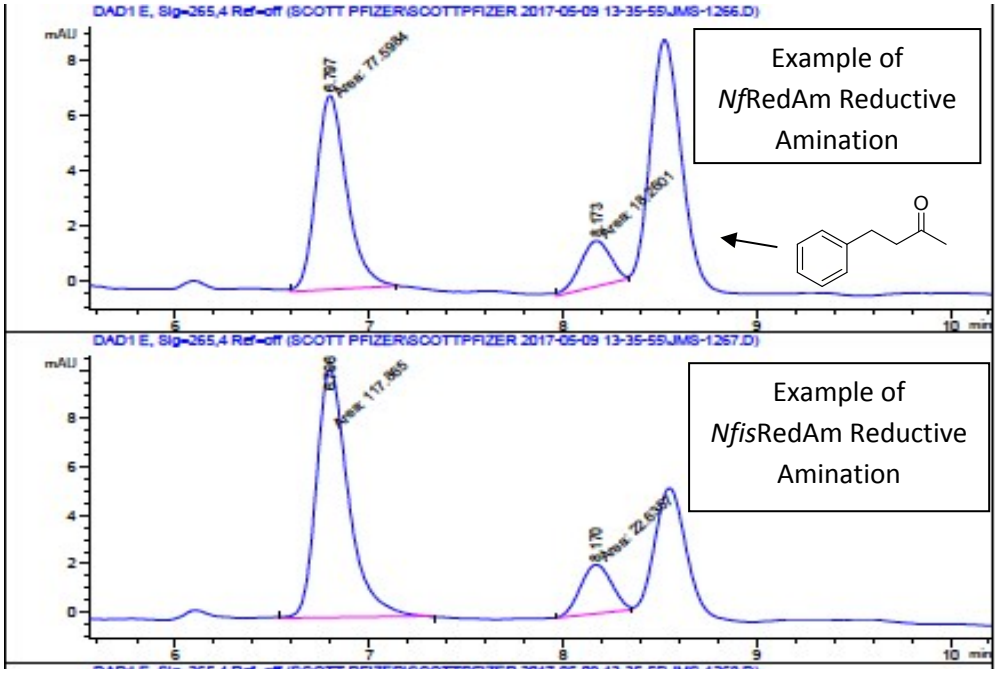

FID1 A, (GODWIN MARCH_APRIL 2016\GODWIN2 2016-04-22 14-54-54\4-PH-2-BUTAN-CYCLOPROPYLAMINE.D)
pA
800
600
400
200
0
-200
17
18
19
20
21
22
min
NH
21.743
Area: 550.274

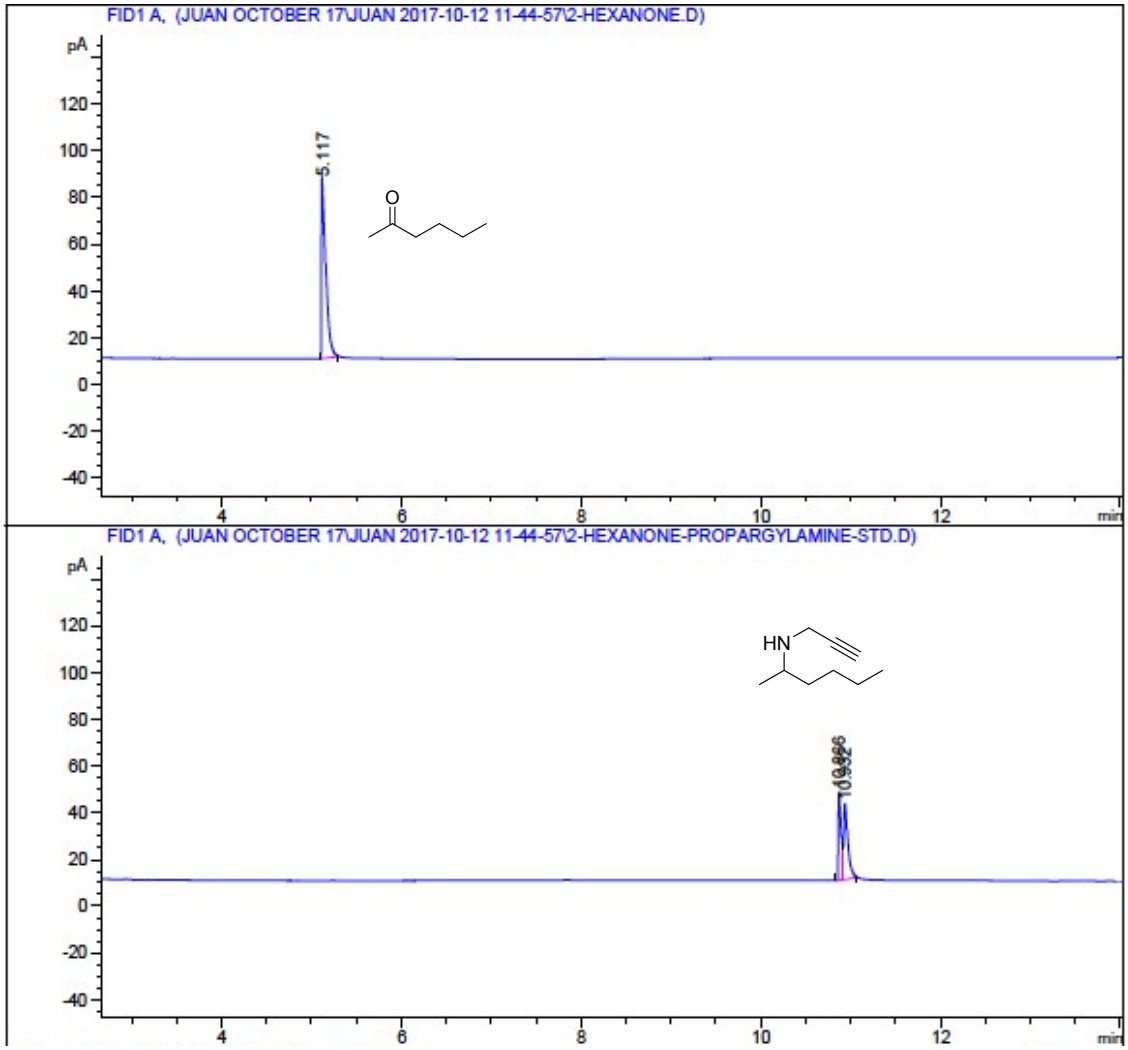

FID1 A, (JUAN DEC 16\JUAN 2016-12-02 14-23-35\JMS-837.D)
pA
60
40
20
0
17.484
Area: 71.3687
O
Example of *Nf*RedAm
Reductive Amination
21.605
Area: 79.3547
17
18
19
20
21
22
min
FID1 A, (JUAN DEC 16\JUAN 2016-12-02 14-23-35\JMS-838.D)
pA
60
40
20
0
17.492
Area: 43.8047
Example of *Nfis*RedAm
Reductive Amination
21.589
Area: 97.0612
17
18
19
20
21
22
min

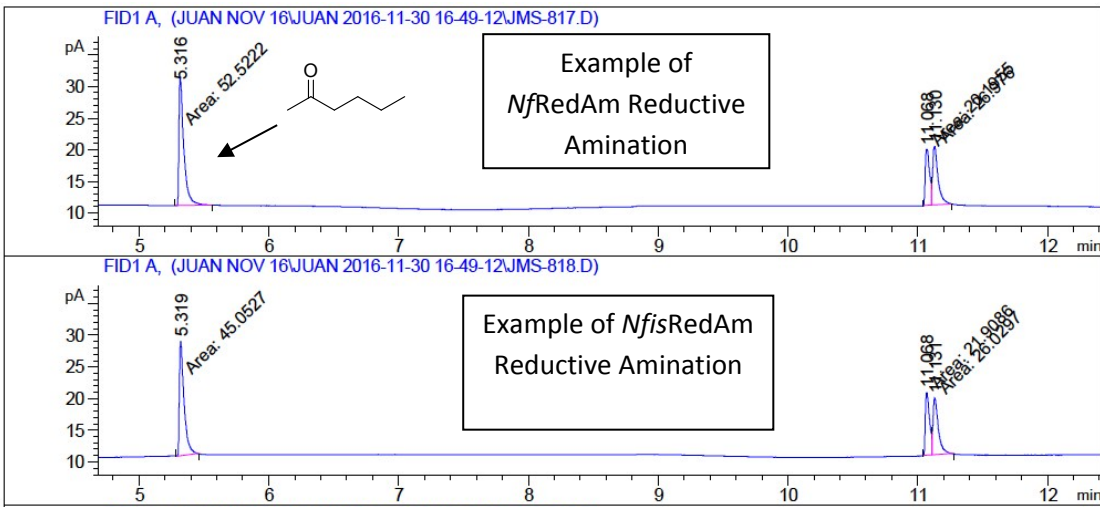

FID1 A, (FRANK24012017\FRANK 2017-04-27 13-03-01\4P2B-NHME.D)
pA
1000
800
600
400
200
0
-200
11.883
NH
9.5
10
10.5
11
11.5
12
12.5
13
min

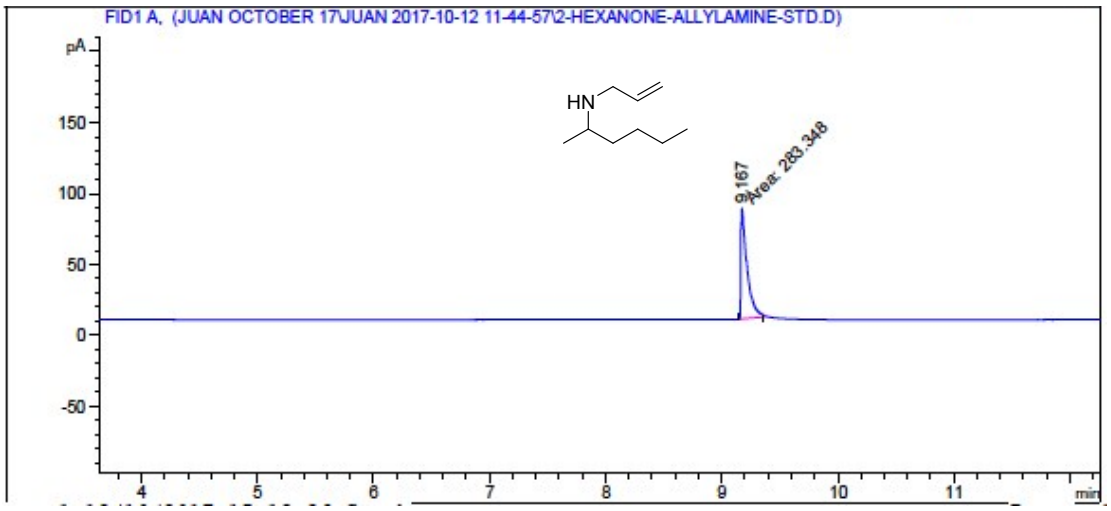

FID1 A, (JUAN MAY 17\JUAN-INTENSIFICATION 2017-05-09 11-30-20\JMS-1266.D)
Example of *Nf*RedAm
Reductive Amination
pA
200
150
100
50
0
-50
O
10.843
Area: 143.285
11.859
Area: 171.835
10
10.5
11
11.5
12
12.5
min
FID1 A, (JUAN MAY 17\JUAN-INTENSIFICATION 2017-05-09 11-30-20\JMS-1267.D)
Example of *Nfis*RedAm
Reductive Amination
pA
200
150
100
50
0
-50
10.835
Area: 87.3449
11.852
Area: 247.506
10
10.5
11
11.5
12
12.5
min

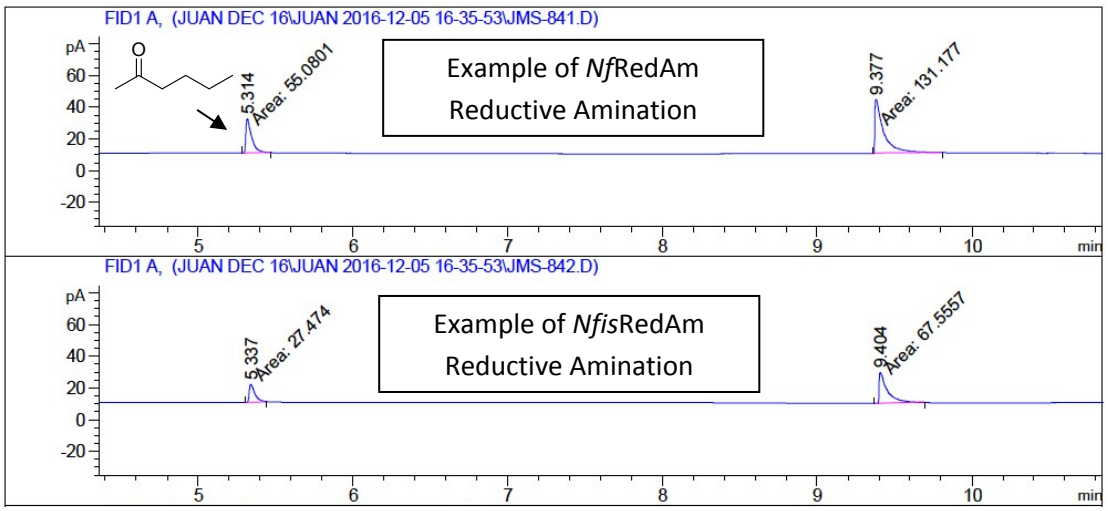

DAD1 E, Sig=265,4 Ref=off (SCOTT PFIZER\SCOTTPFIZER 2017-05-05 11-44-32\RAC-4PH2(MENH)BU.D)
mAU
NH
NH
DAD1 E, Sig=265,4 Ref=off (SCOTT PFIZER\SCOTTPFIZER 2017-05-05 11-44-32\(S)-4PH2(MENH)BU.D)
mAU
NH

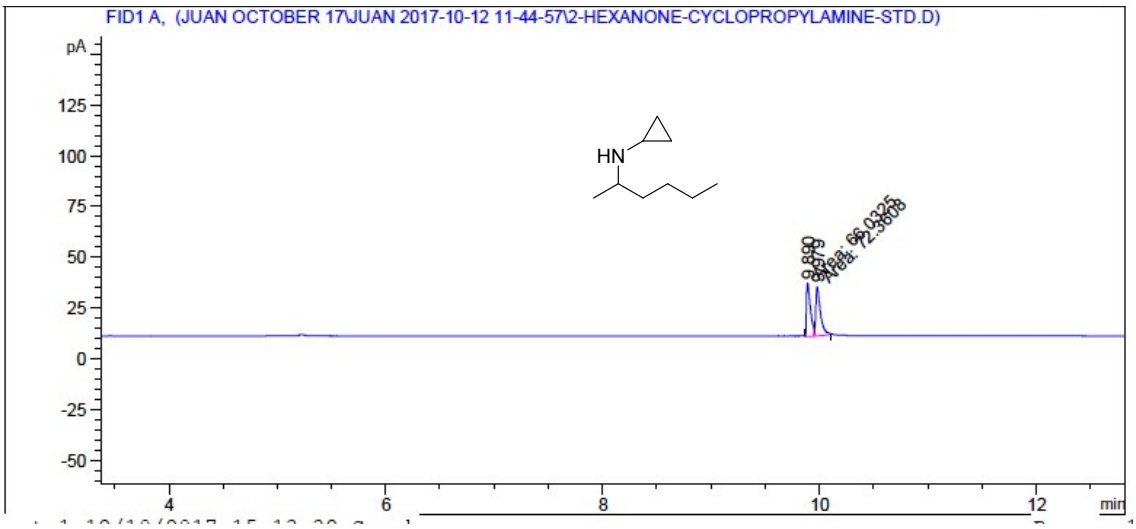

DAD1 E, Sig=265,4 Ref=off (SCOTT PFIZER\SCOTTPFIZER 2017-05-09 13-35-55\JMS-1266.D)
mAU
Example of *Nf*RedAm Reductive Amination
O
DAD1 E, Sig=265,4 Ref=off (SCOTT PFIZER\SCOTTPFIZER 2017-05-09 13-35-55\JMS-1267.D)
mAU
Example of *Nfis*RedAm Reductive Amination

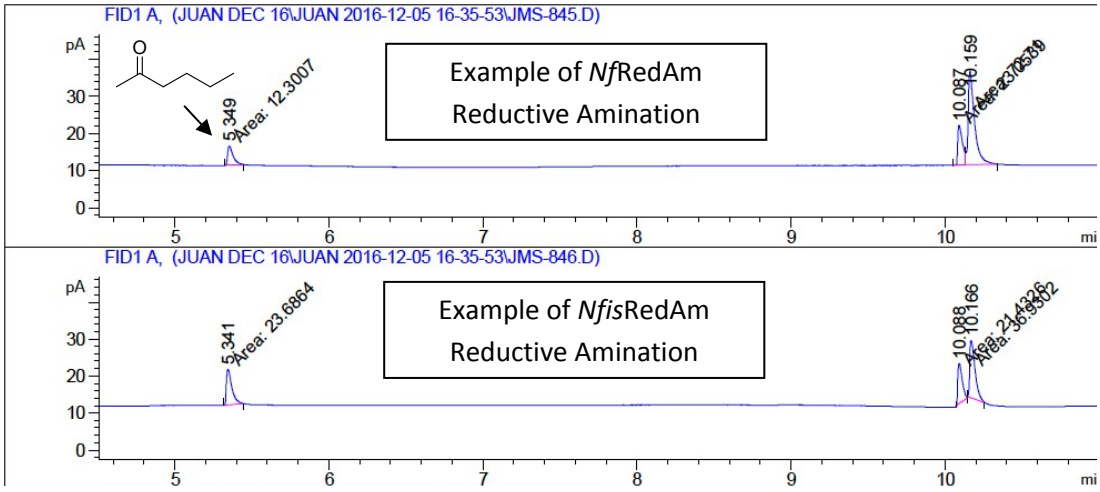

FID1 A, (JUAN OCTOBER 17\JUAN 2017-10-12 11-44-57\2-HEXANONE.D)
pA
5.117
O
FID1 A, (JUAN OCTOBER 17\JUAN 2017-10-12 11-44-57\2-HEXANONE-PROPARGYLAMINE-STD.D)
HN
min

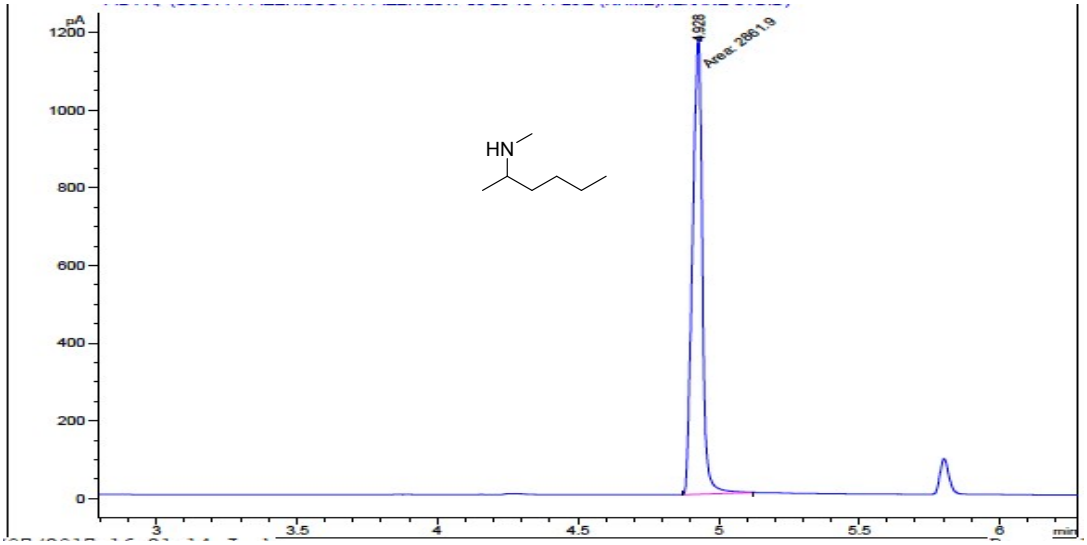

FID1 A, (JUAN NOV 16\JUAN 2016-11-30 16-49-12\JMS-817.D)
5.316
Area: 52.5222
O
Example of
*Nf*RedAm Reductive
Amination
FID1 A, (JUAN NOV 16\JUAN 2016-11-30 16-49-12\JMS-818.D)
5.319
Area: 45.0527
Example of *Nfis*RedAm
Reductive Amination
min

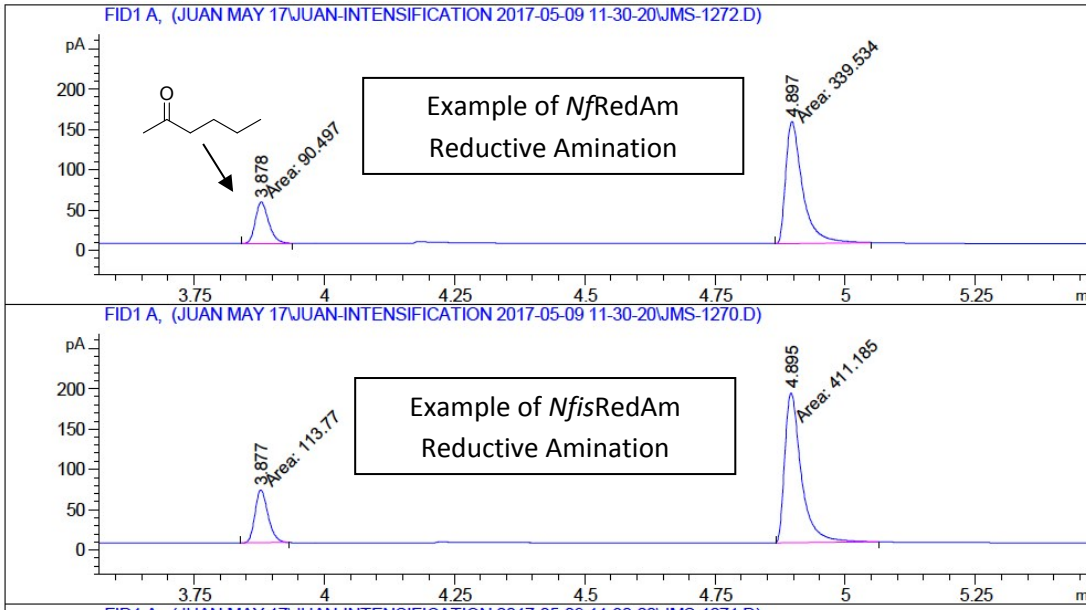

FID1 A, (JUAN OCTOBER 17\JUAN 2017-10-12 11-44-57\2-HEXANONE-ALLYLAMINE-STD.D)
pA
HN
9.167
Area: 283.348
min

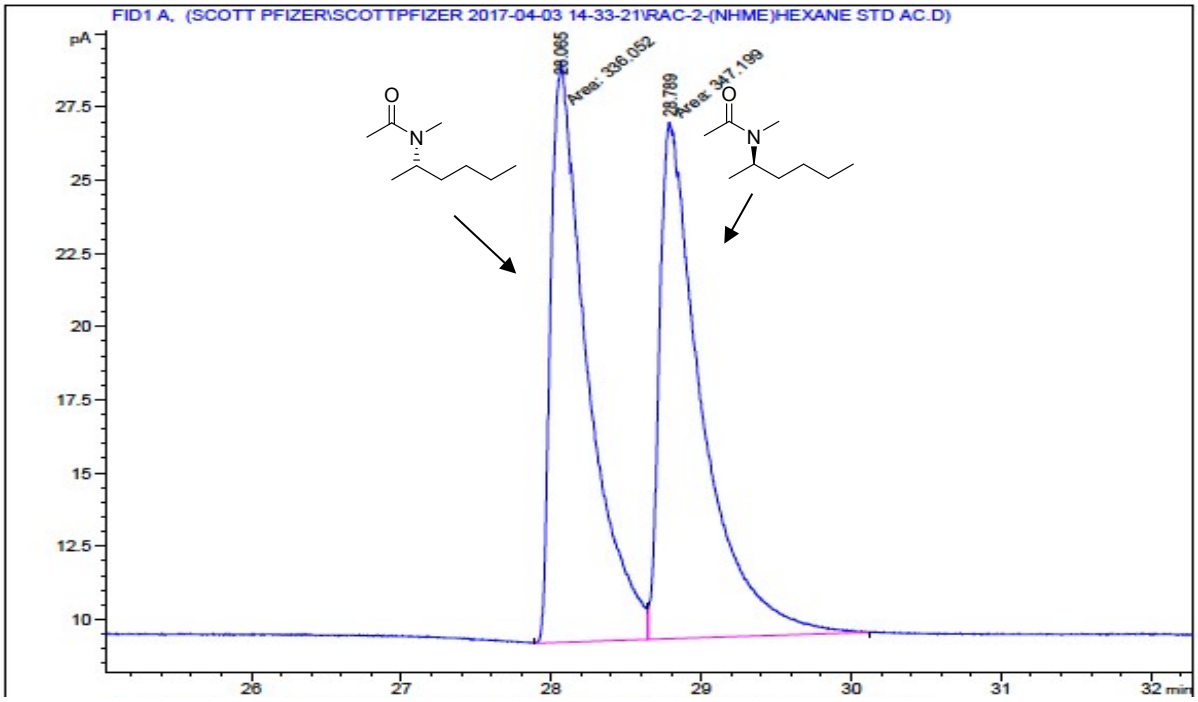

FID1 A, (JUAN DEC 16\JUAN 2016-12-05 16-35-53\JMS-841.D)
pA
O
5.314
Area: 55.0801
Example of *Nf*RedAm
Reductive Amination
9.377
Area: 131.177
min
FID1 A, (JUAN DEC 16\JUAN 2016-12-05 16-35-53\JMS-842.D)
pA
5.337
Area: 27.474
Example of *Nfis*RedAm
Reductive Amination
9.404
Area: 67.5557
min

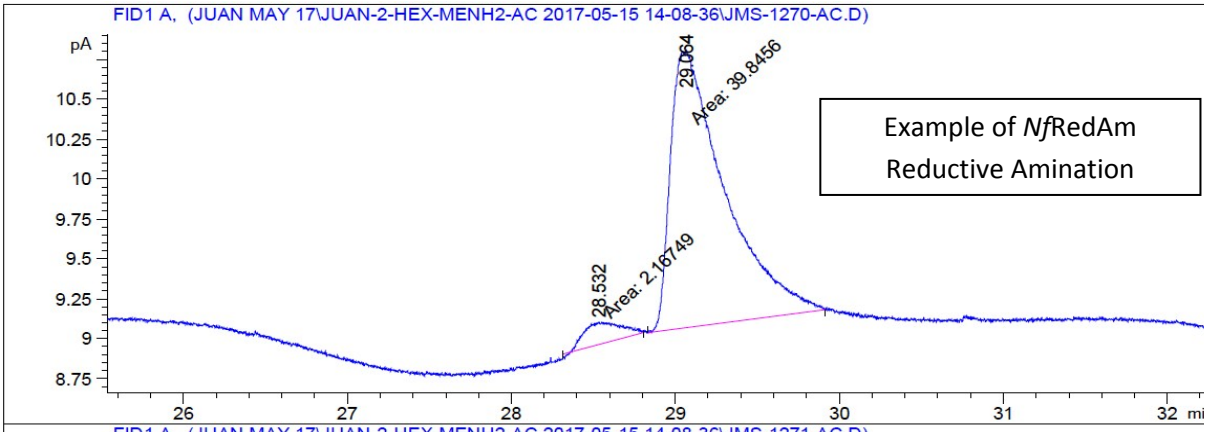

FID1 A, (JUAN OCTOBER 17\JUAN 2017-10-12 11-44-57\2-HEXANONE-CYCLOPROPYLAMINE-STD.D)
pA
HN
min

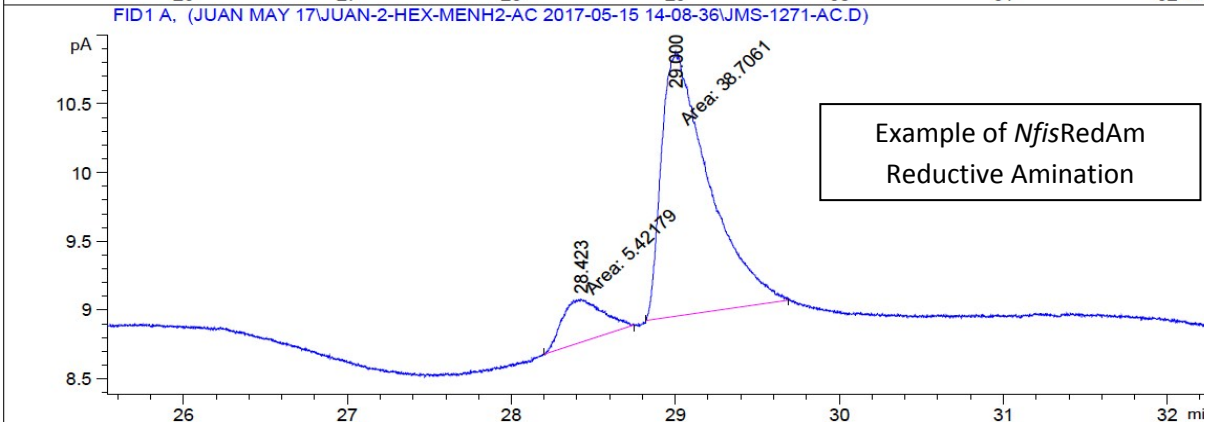

FID1 A, (JUAN DEC 16\JUAN 2016-12-05 16-35-53\JMS-845.D)
pA
Example of *Nf*RedAm
Reductive Amination
5.349
Area: 12.3007
10.087
10.159
FID1 A, (JUAN DEC 16\JUAN 2016-12-05 16-35-53\JMS-846.D)
pA
Example of *Nfis*RedAm
Reductive Amination
5.341
Area: 23.6864
10.088
10.166


pA
1200
1000
800
600
400
200
0
4.928
Area: 28619
HN
3
3.5
4
4.5
5
5.5
6
min


FID1 A, (JUAN MAY 17\JUAN-INTENSIFICATION 2017-05-09 11-30-20\JMS-1272.D)
pA
200
150
100
50
0
O
3.878
Area: 90.497
Example of *Nf*RedAm
Reductive Amination
4.897
Area: 339.534
3.75
4
4.25
4.5
4.75
5
5.25
FID1 A, (JUAN MAY 17\JUAN-INTENSIFICATION 2017-05-09 11-30-20\JMS-1270.D)
pA
200
150
100
50
0
3.877
Area: 113.77
Example of *Nfis*RedAm
Reductive Amination
4.895
Area: 411.185
3.75
4
4.25
4.5
4.75
5
5.25


FID1 A, (SCOTT PFIZER\SCOTTPFIZER 2017-04-03 14-33-21\RAC-2-(NHME)HEXANE STD AC.D)
pA
27.5
25
22.5
20
17.5
15
12.5
10
26
27
28
29
30
31
32 min
28.065
Area: 336.052
28.789
Area: 347.199
O
N
O
N


FID1 A, (JUAN MAY 17\JUAN-2-HEX-MENH2-AC 2017-05-15 14-08-36\JMS-1270-AC.D)
pA
10.5
10.25
10
9.75
9.5
9.25
9
8.75
26
27
28
29
30
31
32 mi
29.064
Area: 39.8456
28.532
Area: 2.16749
Example of *Nf*RedAm
Reductive Amination


FID1 A, (JUAN MAY 17\JUAN-2-HEX-MENH2-AC 2017-05-15 14-08-36\JMS-1271-AC.D)
pA
10.5
10
9.5
9
8.5
26
27
28
29
30
31
32 mi
29.000
Area: 38.7061
28.423
Area: 5.42179
Example of *Nfis*RedAm
Reductive Amination

Section S9. References

1 S. F. Altschul, W. Gish, W. Miller, E. W. Myers and D. J. Lipman, *J. Mol. Biol.*, 1990, **215**, 403–410.

2 M. J. Fogg and A. J. Wilkinson, *Biochem. Soc. Trans.*, 2008, **36**, 771–775.

3 W. Kabsch, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2010, **66**, 125–132.

4 P. Evans, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2006, **62**, 72–82.

5 G. Winter, *J. Appl. Crystallogr.*, 2010, **43**, 186–190.

6 A. Vagin and A. Teplyakov, *J. Appl. Crystallogr.*, 1997, **30**, 1022–1025.

7 M. Sharma, J. Mangas-Sanchez, S. P. France, G. A. Aleku, S. L. Montgomery, J. I. Ramsden, N. J. Turner and G. Grogan, *ACS Catal.*, 2018, **8**, 11534–11541.

8 P. Emsley and K. Cowtan, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2004, **60**, 2126–2132.

9 G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 1997, **53**, 240–255.

10 G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan and N. J. Turner, *Nat. Chem.*, 2017, **9**, 961–969.

11 J. Mangas-Sánchez, M. Rodríguez-Mata, E. Busto, V. Gotor-Fernández and V. Gotor, *J. Org. Chem.*, 2009, **74**, 5304–5310.

12 W. Adam, A. Pastor and T. Wirth, *Org. Lett.*, 2000, **2**, 1295–1297.