LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

LLNL-TR-841592

Compound Specific Stable Isotope Signatures of Chemical Threat Agents: Incapacitants

M. A. Singleton, S. Hok, C. A. Valdez, R. N. Leif

October 21, 2022

Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Compound Specific Stable Isotope Signatures of Chemical Threat Agents: Incapacitants

LLNL-TR-XXXXX

Michael J. Singleton, Saphon Hok, Carlos A. Valdez, Roald N. Leif

Disclaimer

Summary

In year 2 of this project (2017), our group focused on determining the causes of isotopic signatures in fentanyl. In order to better predict isotopic signatures in fentanyl, we analyzed the nitrogen isotope compositions of two key intermediates of fentanyl, NPP and ANPP. In addition, we analyzed additional fentanyl products produced using the Valdez route, along with five other alternative fentanyl routes. A new, more sensitive GC-C-IRMS method has been developed, which allows for compound specific isotope analysis of Chemical Attribution Signatures (CAS) in fentanyl.

List of Acronyms

ANPP: 1-Phenethyl-N-phenylpiperidine-4-amine
CAS: Chemical attribution signature(s)
CTA: Chemical threat agent
EA-IRMS: Elemental analysis-isotope ratio mass spectrometry
GC-C-IRMS: Gas chromatography-combustion-isotope ratio mass spectrometry
NPP: N-phenethyl-4-piperidone

Background

This project is a continuation of our efforts to develop methods and standard operating procedures (SOP) for stable isotope analysis of chemical threat agents. Stable isotope analyses of CTAs show great promise for providing signatures that can be used to distinguish between multiple sources of material collected as evidence, and in some cases may lead investigators to the source or production plant of an unknown sample (Benson et al., 2006; Singleton et al., 2009; Volpe and Singleton, 2011; Moran et al., 2011; Kreuzer et al., 2012). Our FY11-FY12 project has developed the techniques needed to analyze stable isotope signatures in real-world samples such as impure mixtures, and CTAs dissolved in a food or water matrix (Singleton et al., 2012; Singleton et al., 2013).

Many CTAs are not commercially available and must be synthesized, but CTA synthesis provides a great advantage to stable isotope signature studies beyond just making these CTAs available. Synthesis of toxic compounds reveals the persistence of precursor isotope signatures in the final CTA product. This link between product and precursor enables the prediction of stable isotope variation in CTAs (e.g., Singleton 2011), and provides a way to link precursor samples collected as evidence with CTAs used in a crime. Such information is often lacking when studying CTAs from a commercial source.

Summary of first year

We are using expertise at LLNL on synthesis procedures for incapacitants to produce products from precursors having a range of isotopic compositions. Stable isotope signatures provide valuable evidence for the association of two samples if it can be demonstrated that significant variation exists in the commercially available precursor materials. **The goal of this project** is to establish whether stable isotope signatures provide

valuable evidence for these compounds. During the first year of this project we: 1) have procured a library of fentanyl precursor chemicals, 2) measured the stable isotope variations in those precursors, 3) synthesized fentanyl products from precursors with a range of different isotopic compositions, and 4) evaluated whether the precursor stable isotope signatures can be used to associate the precursors used in synthesis to the final CTA product. Methods have been developed for stable isotope analysis of fentanyl and associated precursors.

Our data indicate that fentanyl products reflect the stable isotope signature of their precursor materials, and that **it may be possible to associate precursors** and fentanyl produced using the Valdez et al. (2014) synthesis route. A preliminary examination suggests that a similar relation may also exist for other fentanyl synthesis routes, but that the apparent nitrogen isotope fractionation between products and precursors may be route dependent.

Methods

Stable isotope methods

Stable isotope measurements are carried out in LLNL's Nuclear and Chemical Sciences Division Stable Isotope Laboratory. The Stable Isotope Laboratory is a multi-capability facility with two GV Instruments isotope ratio mass spectrometers (IRMS) and numerous online and offline sample preparation systems. Light stable isotopic compositions can be measured for H, O, C, N and S in liquids, solids, dissolved ions such as nitrate and bicarbonate, and oxygen-bearing minerals and chemical compounds. Solid and liquid samples are analyzed for C, N, and S isotopic composition and elemental composition using an Elemental Analyzer that is coupled to the IRMS (EA-IRMS). A gas chromatograph-combustion system is coupled to the IRMS (GC-C-IRMS) system to perform compound specific stable isotope analyses of carbon and nitrogen.

Isotopic ratios are reported as delta (δ) values, which represent the normalized difference of a sample relative to an internationally accepted reference (atmospheric "air" N_2 for nitrogen, Vienna Pee Dee Belemite (VPDB) for carbon). The delta values are expressed in parts per thousand, or per mil (‰), and are defined by:

$$\delta^{13}C = \frac{\left[\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{VPDB}\right]}{\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}} \quad (1)$$

$$\delta^{15}N = \frac{\left[\left(\frac{^{15}N}{^{14}N}\right)_{sample} - \left(\frac{^{15}N}{^{14}N}\right)_{AIR}\right]}{\left(\frac{^{15}N}{^{14}N}\right)_{AIR}} \quad (2)$$

Stocks of high purity (>99%) caffeine (CAF-1) and adamantane (AD-1) were selected for use in solutions as QA/QC standards for GC-C-IRMS analysis. The carbon and nitrogen isotope compositions of CAF-1 and AD-1 were determined by EA-IRMS and were calibrated against international standard reference materials USGS-25, IAEA-N1, USGS-40, USGS-41, ANU-Sucrose, and NBS-21 (Table 1). Six replicate analyses of CAF-1 were run by EA-IRMS with resulting values of $\delta^{15}N$ = -16.4 +/- 0.3, and $\delta^{13}C$ = -35.9 +/- 0.2 ‰. Four replicates of AD-1 gave a $\delta^{13}C$ value of -26.4 +/- 0.1 ‰.

Table 1. Standard reference materials used to calibrate GC-C-IRMS laboratory standards CAF-1 and AD-1.

Name	NIST RM #	Reference	Formula	$\delta^{13}C$	+/-	$\delta^{15}N$	+/-
IAEA-N1	8547	5	$(NH_4)_2SO_4$			0.43	0.07
USGS-25	8550	5	$(NH_4)_2SO_4$			-30.41	0.27
USGS-40	8573	1,2,7	$C_5H_9NO_4$	-26.39	0.09	-4.52	0.12
USGS-41	8574	1,2,7	$C_5H_9NO_4$	+37.63	0.1	+47.57	0.22
ANU Sucrose	8542	3	$C_{12}H_{22}O_{11}$	-10.45	0.07		
NBS-21		8	C (graphite)	-28.20			

Determinations of sulfur, nitrogen and carbon elemental abundance in powdered solid samples are carried out using an automated elemental analyzer (EA). Samples are loaded into tin capsules, with a target weight of approximately 80 micrograms of the element of interest. The tin capsules are loaded onto a rotary autosampler that is integrated into the EA. Elemental compositions are determined using a Elementar Vario PyroCube EA. Samples dropped into the EA are combusted at 1020 °C over tungsten oxide in a continuous stream of helium carrier gas to produce SO_2, N_2 and CO_2 from any sulfur, nitrogen and carbon present in the sample. The resulting gases then pass through a reduced copper reactor at 650 °C to reduce NOx to N_2, reduce SO_3 to SO_2, and trap any volatile halogen compounds on silver wool. Following water removal using a Sicapent adsorption tube, the N_2 and CO_2 analyte gases are separated and purified using purge-trap columns. The purified gases are then carried through a thermal conductivity detector (TCD). The TCD signal is passed to computer software that calculates elemental abundances based on

integrated TCD peak areas. The samples gases then pass through the IRMS open split, and are analyzed for isotopic compositions.

Compound-specific isotope analysis of solutes and mixtures are carried out on the gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) system. Using this technique, it is possible to analyze the individual stable isotope signatures of various substances in a single sample. The GC-C-IRMS at LLNL consists of an Agilent 7890A gas chromatograph that is coupled with an IsoPrime Ltd. GC-5 combustion interface and an IsoPrime100 isotope ratio mass spectrometer. A heart-split valve is used to select whether the output from the GC column is sent to either the FID or the combustion-IRMS interface. The combustion-IRMS interface is coupled directly to the GC. The interface is heated to 300 °C and the combustion tube is heated to 850 °C for carbon analyses and 1060 °C for nitrogen analyses. The outlet of the furnace tube serves as an open split connection to the IRMS. A fraction of the furnace outlet gas is pumped into the IRMS after passing through a water or water/CO_2 trap, while the remainder of the combustion gases is exhausted through a small capillary.

Carbon and nitrogen stable isotope compositions were analyzed using a GC method consisting of a 1 minute isothermal step at 40 °C, followed by a 15 degrees/min increase up to 300 °C, where the temperature is held for 5 minutes. The method uses a split inlet flow for higher concentration solutions, and a splitless inlet for low concentration analyses of CAS. Stable isotope compositions were measured on 10,000 ppm solutions of fentanyl and its intermediates in DCM.

Results

Fentanyl Synthesis Routes

In year 1, **we synthesized 6 Fentanyl products** following the open literature route published by Valdez and co-workers (Valdez et al, 2014), but **without any purification** steps. A scheme showing the overall route is presented in Figure 1. Intermediate products were collected after steps I and II. Syntheses were carried out in duplicate, while varying the commercial source of the two nitrogen-containing precursors, 4,4-piperidinediol hydrochloride and aniline, used for its production (Figure 1). The precursors were selected from our library in order to provide a range of starting isotope compositions. The isotope compositions of precursors and products can then be compared to determine whether the isotopic signatures of the two can be linked as discussed below.

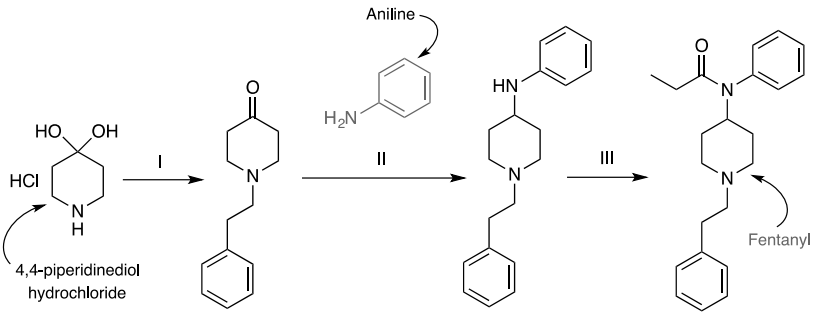


Figure 1. Synthetic route to fentanyl synthesis route used for this study. The two sources of nitrogen for fentanyl are the starting 4,4-piperidinediol hydrochloride and the aniline introduced in Step II.

In order to investigate the stable isotope signatures from alternate synthesis routes, we are leveraging previous syntheses done at LLNL as part of the Mayer and Williams Fentanyl CAS project for DHS (Mayer et al., 2016). From this work, we have samples of fentanyl that were synthesized in triplicate using six different routes. We will use these samples to investigate whether isotopic fractionation may be route dependent. In addition, we have samples of intermediates (NPP and ANPP) that were collected during Year 1 synthesis for the current project. We have analyzed the isotopic compositions of these intermediate compounds in order to develop a better understanding of the isotopic fractionation that was observed in Year 1.

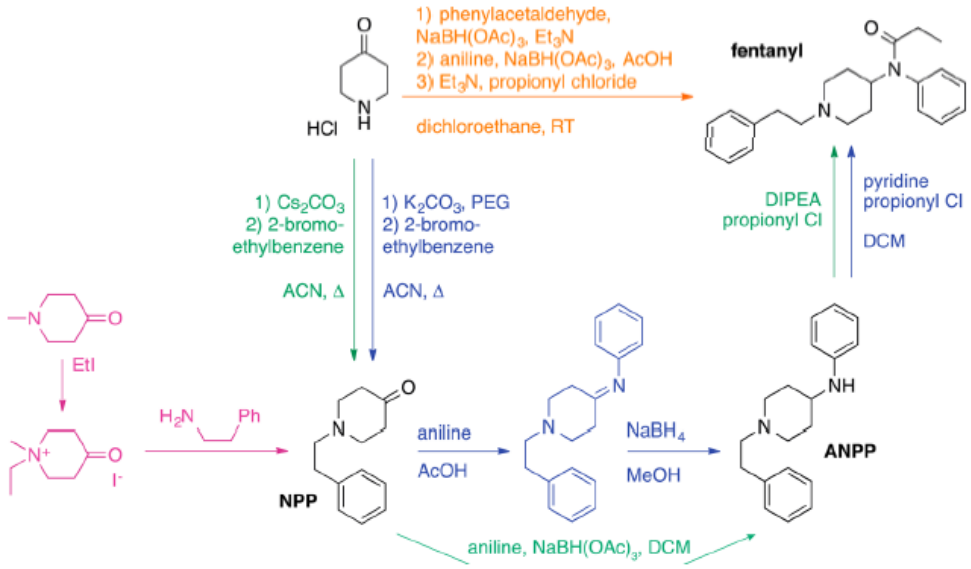


Figure 2. Alternate fentanyl synthesis routes from Mayer et al., 2016. Compounds in black are those common to the majority of the synthetic routes. Compounds for the Valdez method used in Year 1 of this study and also in Mayer et al., 2016 are shown in green. Specific compounds for the One Pot Synthesis are shown in orange, and compounds involved in the Siegfried Method are shown in blue. An alternate synthesis that uses N-methylpiperidone rather than 4,4-piperidinediol as the precursor to NPP is shown in purple. Additional methods were hybrid composed of the Valdez and Siegfried routes. ACN = acetonitrile, AcOH = acetic acid, Et_3N = triethylamine, EtI = ethyl iodide, MeOH = methanol.

Precursor Library

The nitrogens in fentanyl is derived from 4,4-piperidinediol hydrochloride and aniline, which are common building blocks to most synthesis routes. In Year 1, we collected and analyzed a variety of these precursors in order to determine whether there is significant variation in the carbon and nitrogen isotope compositions of commercially available precursors. Chemicalbook.com lists 230 sources for 4,4-piperidinediol hydrochloride, and 152 suppliers for aniline worldwide. We selected a subset of 18 aniline sources and 17 of the 4,4- piperidinediol hydrochloride sources from suppliers that provide small quantities of these chemicals. Due to lack of inventory and/or difficulties in the procurement process, 9 of the aniline suppliers and 7 of the 4,4-piperidinediol hydrochloride suppliers were not able to provide products. We gathered three distinct sources of aniline and two sources of 4,4-piperidinediol hydrochloride that were already in the LLNL inventory prior to this project, including the precursors used for syntheses in the Mayer et al. (2016) study. In total, we collected 12 sources of aniline and 12 sources of 4,4-piperidinediol hydrochloride where the manufacturer or lot number was unique (Table 2).

Table 2. Fentanyl precursor library.

Compound	CAS	MW	MF	ID	Supplier	Country	Purity	Lot Number
Aniline	62-53-3	93.13	$C_6H_5NH_2$	A1	Sigma Aldrich	USA	99%	SHBF0478V
				A2	Acros Organics	USA	99.5	A0349831
				A3	TCI America	USA	>98%	SI5WEHC
				A4	Santa Cruz Biotech	USA	>98%	G2715
				A5	Alfa Aesar	USA	99+%	10183788
				A6	Accustandard	USA	98%	25568
				A7	Apollo Scientific	UK	99+%	AS459030
				A8	Vickers Laboratories	UK	98.5	301274
				A9	Chiron	Norway	>99.5	3923
				A10	Alfa Aesar		99%	10174748
				A11	Sigma Aldrich			58896LMV
				A12	Sigma Aldrich			12025KR
4,4-piperidinediol hydrochloride	40064-34-4	153.6	$C_5H_{12}ClNO_2$	P1	Sigma Aldrich	France	98%	STBD0380V
				P2	Alfa Aesar	UK	98%	10129664
				P3	VWR / TCI America	Japan	98%	CYRRA-BM
				P4	VWR/Chem Impex International	USA	99%	10017718
				P5	EMD Millipore	France	98%	S4123672 303
				P6	Matrix Scientific	USA	95+%	T12N
				P7	Toronto Research Chemicals	Canada	98%	1-MLM-151-1
				P8	Apollo Scientific	UK	97%	AS445503
				P9	Manchester Organics	UK	97%	G18527(4)
				P10	Apin Chemicals	UK		215229
				P11	Sigma Aldrich			CD5003223
				P12	Sigma Aldrich			STBD5288V

C and N Isotope Compositions of Fentanyl Precursors

The 12 sources of 4,4-piperidinediol hydrochloride and 12 sources of aniline have been analyzed for carbon and nitrogen isotope compositions using the EA-IRMS technique, (Figure 3,
Table 3). In both cases, the carbon isotope signatures of these compounds show a small range of variability (2 per mil for aniline and 5 per mil for 4,4-piperidinediol hydrochloride), with several of the sources having overlapping carbon isotope compositions. However, for 4,4-piperidinediol hydrochloride there is a 12 per mil range in $\delta^{15}N$ values, and most samples can be distinguished based on their nitrogen isotopic signature. Aniline has a total range in $\delta^{15}N$ values of 7 per mil, but most samples fall within

a smaller range from -0.6 to 1.2 per mil. These results indicate that in most cases, both nitrogen-bearing precursors used in fentanyl production can be distinguished based on their nitrogen isotope compositions. It also may be possible to distinguish sources of 4,4-piperidinediol hydrochloride based on carbon isotope compositions.

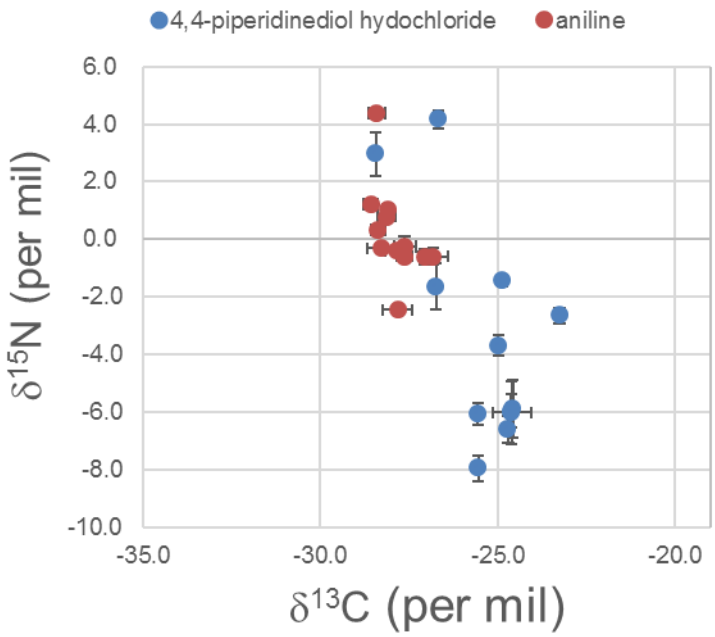


Figure 3. Carbon and nitrogen isotope compositions of the two nitrogen–bearing fentanyl precursors.

In Year 1, the $\delta^{15}N$ results for aniline that was analyzed using the GC-C-IRMS method gave poor precision, with standard deviations for triplicate analyses averaging 1.1 per mil. After numerous attempts to improve the GC-C-IRMS analysis of aniline, we have decided instead to use the EA-IRMS method to analyze this compound. The EA-IRMS method directly combusts the aniline precursors without any separation by gas chromatography. Since the purchased anilines are of high purity, the bulk nitrogen isotope values provided by EA-IRMS analysis should not be affected by any contaminant compounds. Since aniline is a volatile liquid, it is loaded into smooth tin capsules that are sealed with a cold weld under a flow of helium gas. This loading method prevents contamination by atmospheric N_2, while also preventing loss of the aniline due to volatilization prior to analysis.

We have rerun all of the aniline precursors using EA-IRMS and provide the updated values in
Table 3 below. Precision is now improved from an average of +/- 1.1 per mil to an average of +/- 0.15 per mil. The previously reported values obtained by GC-C-IRMS were mis-calibrated due to an error in the method used for the Caf-1 standard. We have re-analyzed the anilines by GC-C-IRMS, and have confirmed that the compound specific method now produces the correct values. However, we will use the EA-IRMS values below, since we are able to achieve better precision with this method.

Table 3. Fentanyl precursor stable isotope compositions analyzed using EA-IRMS.

Precursor	$\delta^{13}C$	SD	$\delta^{15}N$	SD
Aniline				
A1	-28.3	0.4	-0.3	0.1
A2	-28.4	0.2	0.3	0.1
A3	-27.1	0.1	-0.6	0.3
A4	-27.8	0.4	-2.4	0.1
A5	-27.9	0.3	-0.4	0.1
A6	-27.6	0.3	-0.3	0.3
A7	-27.6	0.2	-0.6	0.1
A8	-28.1	0.1	1.0	0.2
A9	-28.4	0.2	4.4	0.1
A10	-28.6	0.2	1.2	0.1
A11	-28.1	0.3	0.8	0.2
A12	-26.8	0.4	-0.6	0.3
4,4-piperidinediol hydrochloride				
P1	-24.6	0.1	-6.0	0.6
P2	-23.3	0.1	-2.6	0.3
P3	-25.0	0.1	-3.7	0.3
P4	-26.7	0.1	-1.6	0.8
P5	-26.7	0.01	4.2	0.3
P6	-24.6	0.04	-5.9	1.0
P7	-24.9	0.02	-1.5	0.2
P8	-24.7	0.1	-6.6	0.5
P9	-25.6	0.04	-6.1	0.4
P10	-28.4	0.05	3.0	0.8
P11	-25.6	0.1	-8.0	0.4
P12	-24.6	0.6	-6.0	1.1

Stable Isotope Compositions of Fentanyl Intermediates

In Year 1, it was discovered that fentanyl synthesis results in a product that has systematically higher $\delta^{15}N$ values than the precursors used in the synthesis. In year 2, we have tracked these effects through each step of the reaction by analyzing the isotope compositions of the two intermediates NPP and ANPP (Figure 4).

In theory, the nitrogen isotope composition of fentanyl should reflect the average value of the $\delta^{15}N$ values of the two nitrogen-bearing precursors used in production (Figure 4). In

order to quantify the isotopic fractionation that occurs during synthesis, we have calculated the difference between the precursors and product nitrogen isotope compositions, defined as:

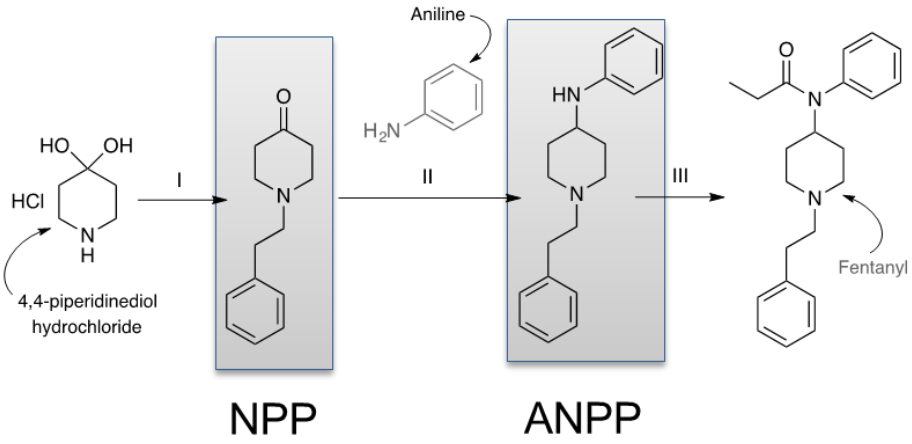
$\Delta^{15}N_{products\text{-}precursors} = \delta^{15}N_{fentanyl} - \delta^{15}N_{precursors\_average}$

Our results show that fentanyl synthesized using the Valdez route (Valdez et al, 2014), consistently reflects the nitrogen isotope compositions of its nitrogen-bearing precursors, and that there is good agreement between duplicate batches using the same route and precursors. However, there is consistent offset of 9.6 +/- 0.4 per mil between the fentanyl produced and the predicted $\delta^{15}N$ value based on the average $\delta^{15}N$ values of the 4,4-piperidinediol hydrochloride and aniline precursors. ($\Delta^{15}N_{products\text{-}precursors}$ in Table 4). This offset was consistent for the 6 batches of fentanyl that were synthesized.

Table 4. Stable isotope compositions of precursors and fentanyl from six batches synthesized using the Valdez route. Nitrogen isotope fractionation is calculated by calculating the difference between the final fentanyl product and the average value of the two nitrogen containing precursors.

	Aniline					*4,4-piperidinediol hydrochloride*					Fentanyl				$\Delta_{products\text{-}precursors}$	
Batch#	**ID**	**$\delta^{13}C$**	**+/-**	**$\delta^{15}N$**	**+/-**	**ID**	**$\delta^{13}C$**	**+/-**	**$\delta^{15}N$**	**+/-**	**$\delta^{13}C$**	**+/-**	**$\delta^{15}N$**	**+/-**	**$\Delta^{15}N$**	**+/-**
1	A3	-27.1	0.1	-0.6	0.3	P8	-24.7	0.1	-6.6	0.5	-28.6	0.1	5.6	0.1	9.2	0.6
2	A3	-27.1	0.1	-0.6	0.3	P8	-24.7	0.1	-6.6	0.5	-28.7	0.1	6.3	0.1	9.9	0.6
3	A8	-28.1	0.1	1.0	0.2	P8	-24.7	0.1	-6.6	0.5	-28.9	0.1	7.4	0.1	10.2	0.5
4	A8	-28.1	0.1	1.0	0.2	P8	-24.7	0.1	-6.6	0.5	-29.0	0.1	7.0	0.3	9.8	0.6
5	A3	-27.1	0.1	-0.6	0.3	P5	-26.7	0.0	4.2	0.3	-29.4	0.01	10.9	0.2	9.1	0.5
6	A3	-27.1	0.1	-0.6	0.3	P5	-26.7	0.0	4.2	0.3	-29.1	0.1	11.1	1.1	9.4	1.2

In order to better understand the causes of fractionation during fentanyl synthesis, we have analyzed the nitrogen isotope compositions of two key intermediates, NPP and ANPP (Figure 4). These analyses were carried out to develop an understanding of the observed N isotope fractionation that is common to all the fentanyl routes that use NPP as an intermediate. Our results indicate that there is a small nitrogen isotope fractionation (0.9 +/- 0.4 per mil) during the first step of the reaction going from 4,4-piperidinediol HCl to NPP. N-isotope analysis of ANPP shows that most of the fractionation (5.5 +/- 0.7 per mil) occurs during the reductive amination step that involves addition of aniline. An additional fractionation (3.6 +/- 0.7 per mil) occurs during the transformation of ANPP to fentanyl.



Step	I (NPP)	II (ANPP)	III (fentanyl)
$\Delta^{15}N$ measured	**0.9 +/- 0.4**	**5.5 +/- 0.7**	**3.6 +/- 0.7**

Figure 4. Intermediates produced in three steps of fentanyl synthesis and their measured nitrogen isotope fractionation.

Stable Isotope Compositions of Alternate Fentanyl Synthesis Routes

We have analyzed the nitrogen isotope compositions of fentanyl produced using the Valdez route, plus 5 alternative routes (Figure 2, Table 5) by the previous study of Mayer et al. (2016). This study also produced fentanyl using the same Valdez method that was used in Year 1 of this study. The Mayer study fentanyl syntheses were carried out by different chemists relative to this project. By comparing results of both sets of fentanyl syntheses, we can gain an understanding of the variability amongst fentanyl's between routes and between chemists.

Table 5. Stable isotope compositions of precursors and fentanyl from six batches synthesized using various routes as discussed in Mayer et al (2016). Nitrogen isotope fractionation is calculated by calculating the difference between the final fentanyl product and the average value of the two nitrogen containing precursors.

	Aniline					*4,4-piperidinediol hydrochloride*					Fentanyl				$\Delta_{products\text{-}precursors}$	
Route	**ID**	**$\delta^{13}C$**	**+/-**	**$\delta^{15}N$**	**+/-**	**ID**	**$\delta^{13}C$**	**+/-**	**$\delta^{15}N$**	**+/-**	**$\delta^{13}C$**	**+/-**	**$\delta^{15}N$**	**+/-**	**$\Delta^{15}N$**	**+/-**
One Pot	A11	-28.1	0.3	0.8	0.2	P11	-25.6	0.1	-8.0	0.4	-28.7	0.2	8.3	1.0	11.9	0.5
Siegfried	A12	-26.8	0.4	-0.6	0.3	P11	-25.6	0.1	-8.0	0.4	-28.0	0.2	5.2	1.0	9.5	0.5
Valdez	A11	-28.1	0.3	0.8	0.2	P11	-25.6	0.1	-8.0	0.4	-30.4	0.2	6.9	1.0	10.5	0.5
Valdez-->Siegfried	A11	-28.1	0.3	0.8	0.2	P11	-25.6	0.1	-8.0	0.4	-29.3	0.2	6.7	1.0	10.3	0.5
Siegfried-->Valdez	A11	-28.1	0.3	0.8	0.2	P11	-25.6	0.1	-8.0	0.4	-29.1	0.2	7.0	1.0	10.6	0.5
Alt NPP-->Siegfried	A11	-28.1	0.3	0.8	0.2						-28.3	0.2	11.2	1.0		

As shown in Figure 5, the nitrogen isotope compositions of fentanyl from most of these routes follow a similar relation to the precursor nitrogen isotope values, with the fentanyl $\delta^{15}N$ values being an average of 10.6 +/- 0.9 per mil higher than the average precursor values. The one pot method shows a larger difference between the fentanyl and precursor $\delta^{15}N$ values than the other routes. We also measured the isotope composition of the alternate NPP $\rightarrow$ Sigfried method, which had a significantly higher $\delta^{15}N$ value (11.2 per mil), but is not included on this plot since it uses N-methylpiperidone rather than 4,4-piperidinediol as the precursor to NPP.

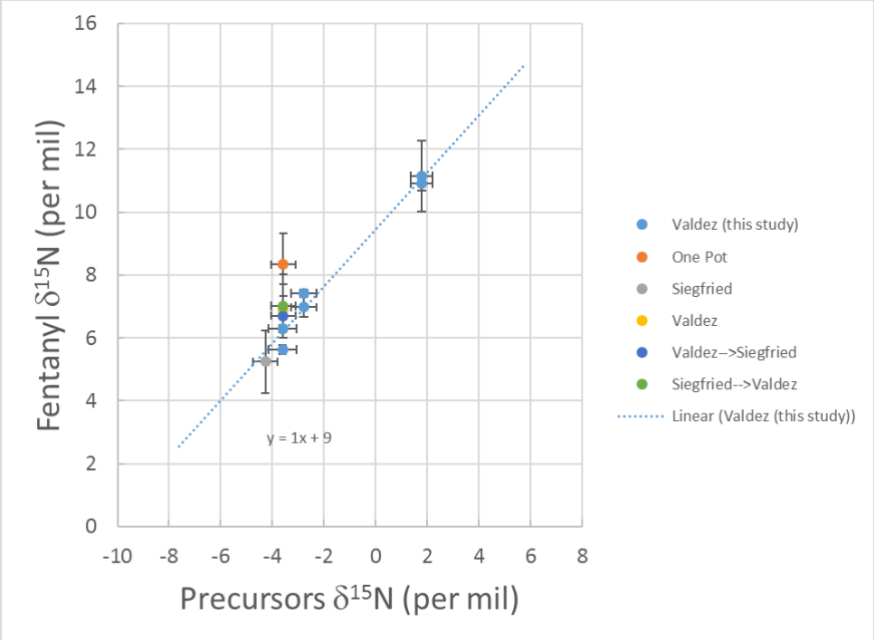
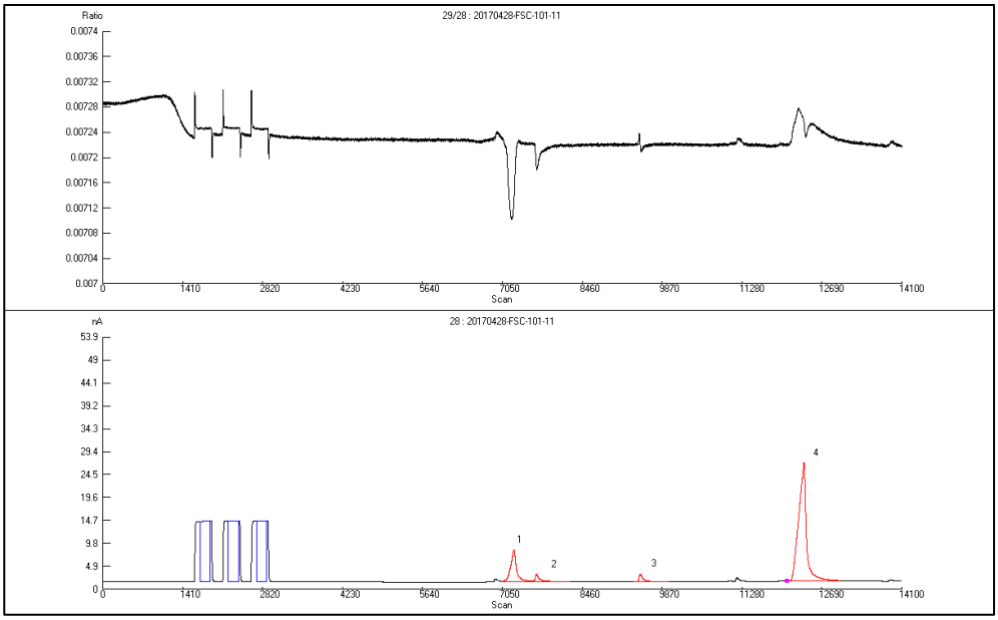
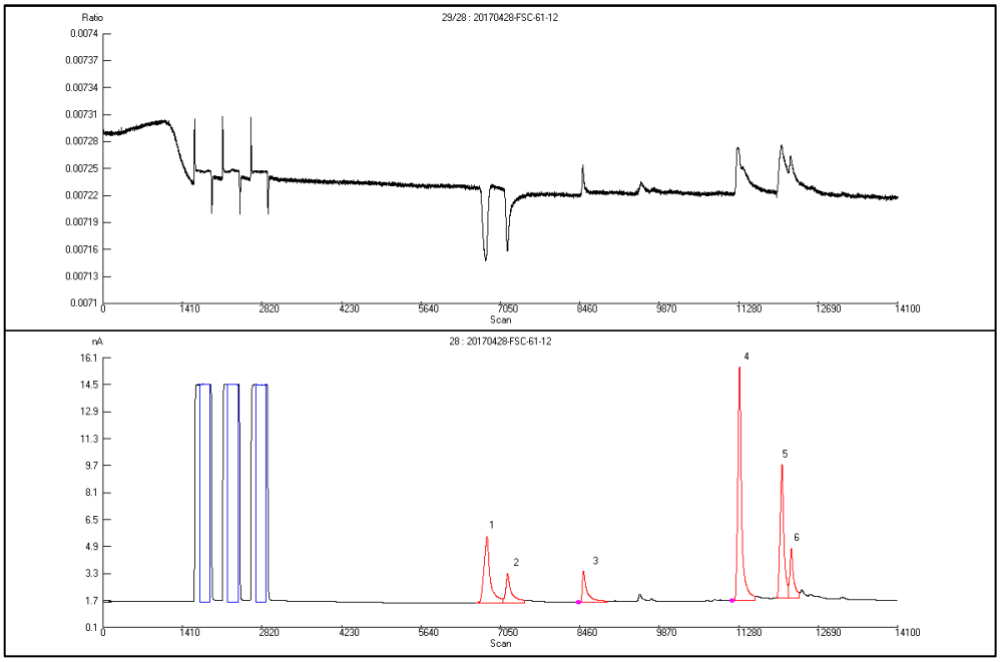


Figure 5. Nitrogen isotope compositions of fentanyl synthesized using six routes. The precursors $\delta^{15}N$ values is the average $\delta^{15}N$ value of 4,4- piperidinediol and aniline used in the synthesis.

We analyzed a subset of the alternate route fentanyls using a much more sensitive GC-C-IRMS method. The new method was developed to provided nitrogen isotope compositions on very dilute samples but is also useful for studying chemical attribution signatures in tangent with stable isotope signatures. As shown in Figure 6, the abundance of nitrogen containing compounds is consistent with the CAS discovered by Mayer et al. (2016). Using the more sensitive GC-C-IRMS technique, we also obtain nitrogen isotope compositions from these CAS compounds. The combination of CAS and isotope compositions provides an exciting new dimension to signatures analysis.



A)



B)

Figure 6. GC-C-IRMS chromatograms showing chemical attribution signatures and nitrogen isotope compositions for fentanyl produced using the Valdez method (A) and the one pot method (B). The abundance of nitrogen containing compounds are shown in red, and the nitrogen isotope ratio is shown in black. Tentative peak ID's are A) 4=fentanyl, B) 4= ANPP, 5=acetylfentanyl, 6=fentanyl.