

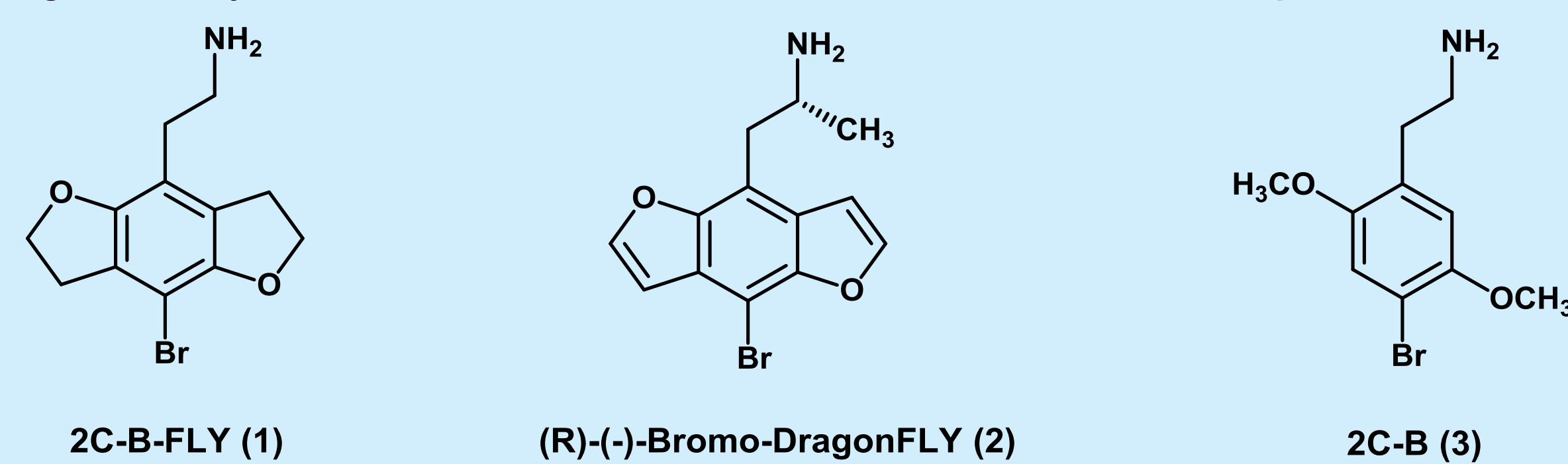
# Design and Synthesis of Labeled 2C-B-FLY and Bromo-DragonFLY for Internal Standards Used in Forensic/Clinical Toxicology Analysis

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## Background

2C-B-FLY (**1**) and Bromo-DragonFLY (**2**), are recreational designer drugs based on phenethylamines such as 2C-B (**3**). 2C-B-FLY and Bromo-DragonFLY exhibit potent and long lasting psychedelic and hallucinogenic properties.<sup>1-3</sup> Compounds **1** and **2** were synthesized from literature methods,<sup>1-3</sup> and formulated into certified reference materials (CRM's) for forensic and clinical toxicology testing by LC/MS and/or GC/MS.

Stable labeled internal standards (IS's) are required for accurate quantitation by mass spectrometry (MS). Labeling can be accomplished through the incorporation of deuterium, carbon-13 and/or nitrogen-15. The design and synthesis of the desired labeled materials will be presented.

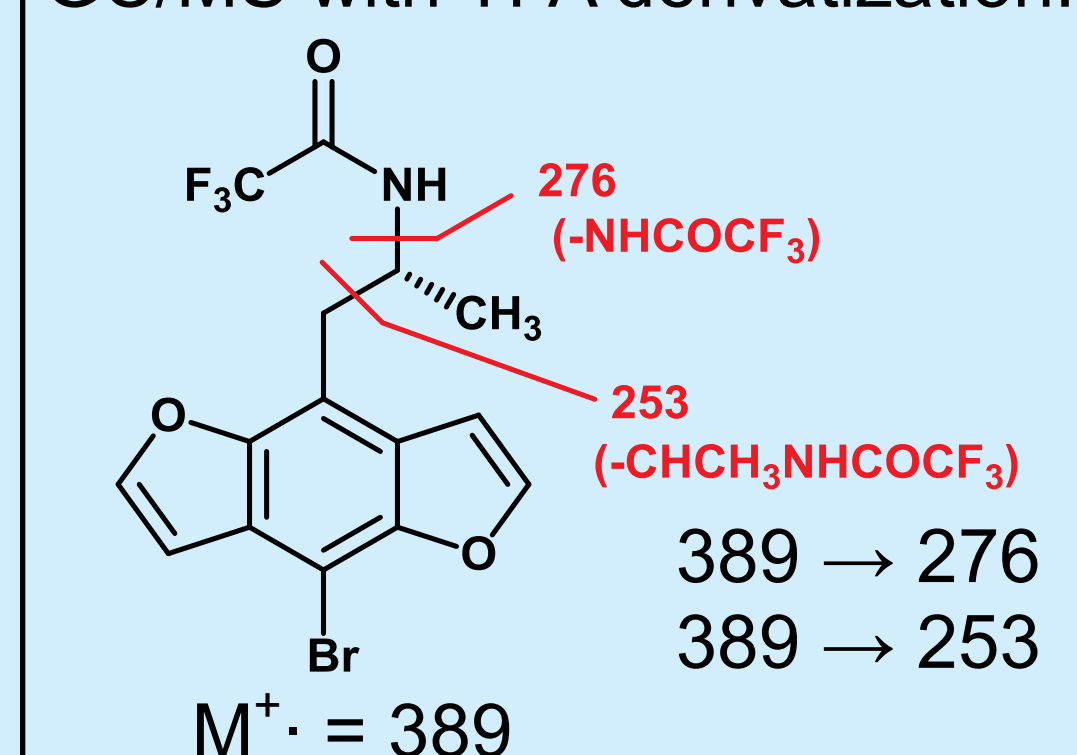


## Synthetic and Product Design

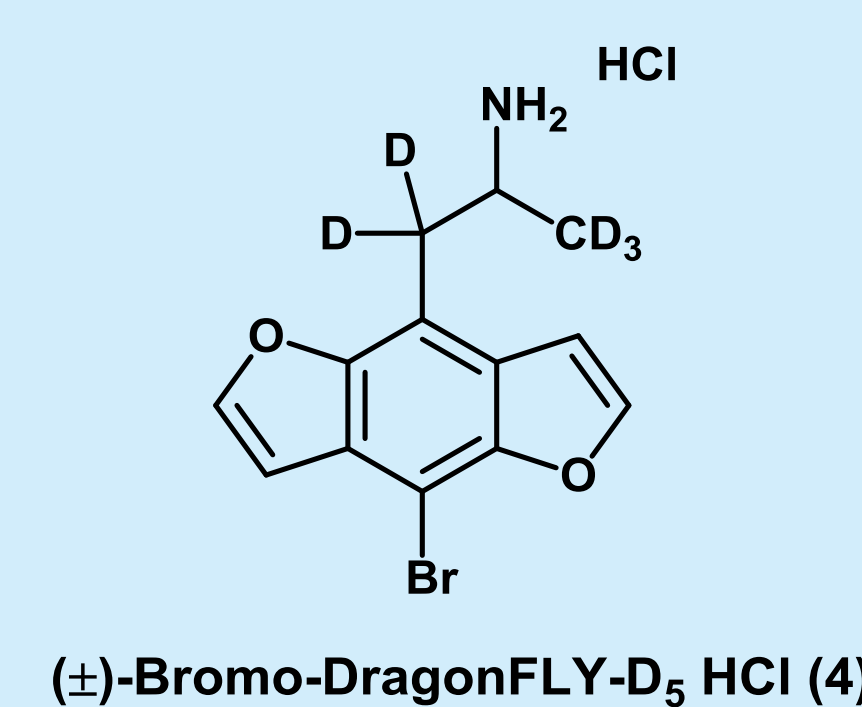
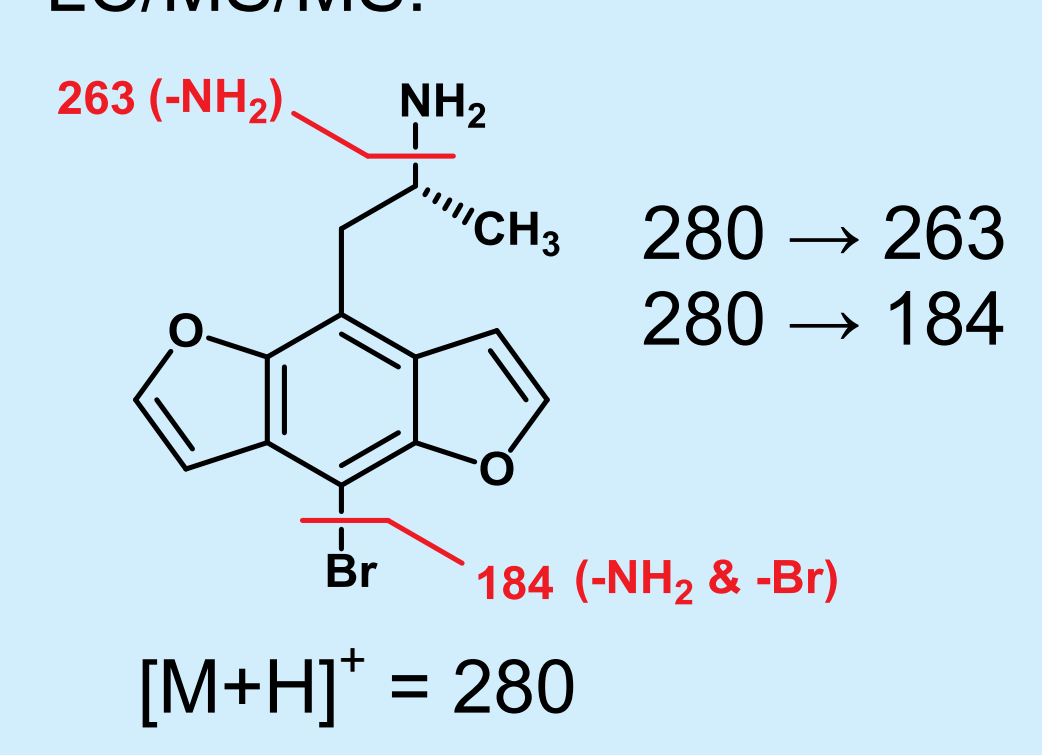
In order to provide an effective IS, the fragments being monitored by MS during testing should retain the labels incorporated during synthesis. Published LC/MS/MS and GC/MS fragmentation patterns of compounds **1** and **2** were evaluated to identify the optimal location for deuterium incorporation.<sup>4</sup> Based on this information and synthetic feasibility, the ethyl side-chain was targeted for labeling and a synthetic scheme was developed to synthesize compounds **4** and **5**.

### Bromo-DragonFLY:

GC/MS with TFA derivatization:



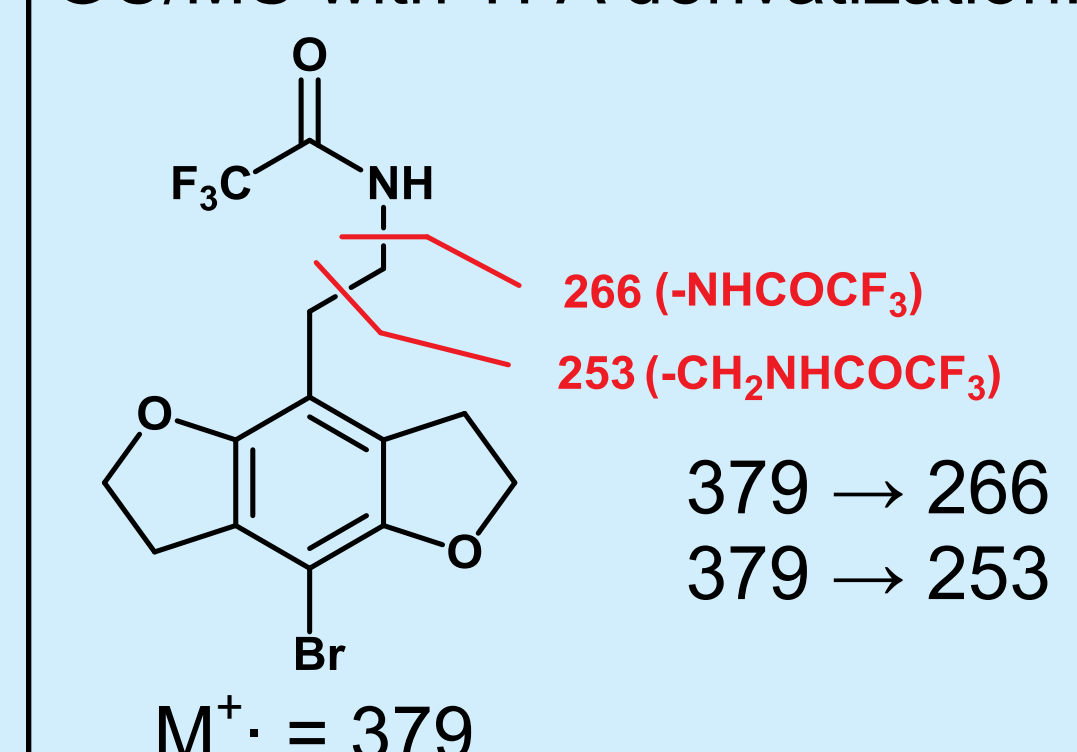
LC/MS/MS:



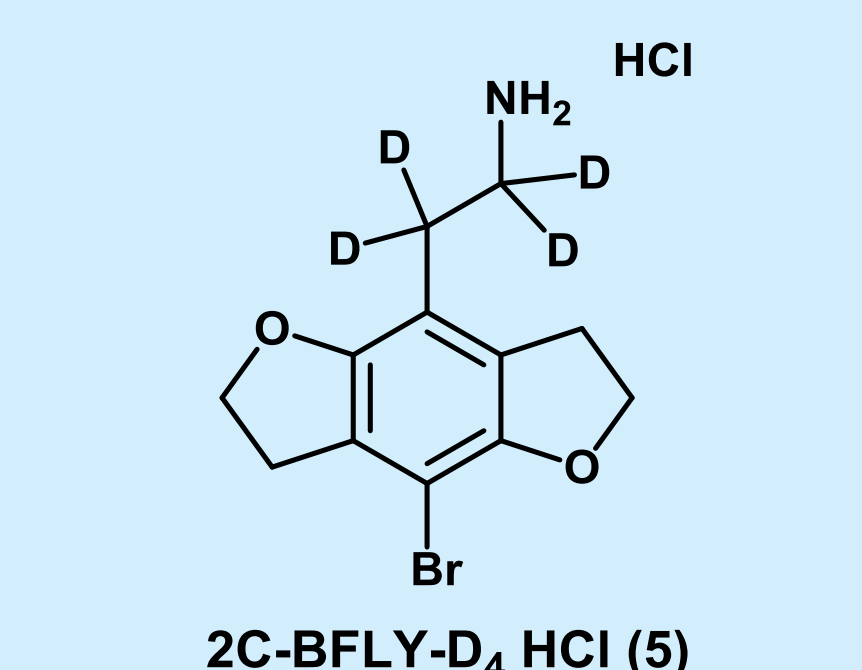
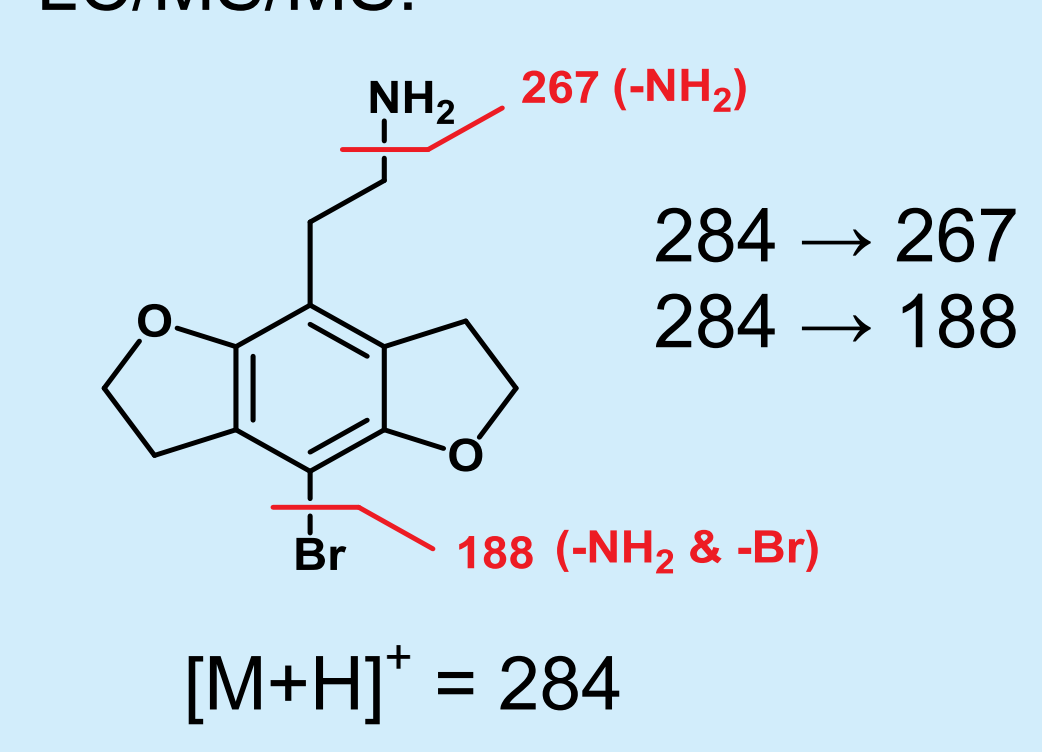
Proposed labeling on Bromo-DragonFLY and 2-C-B-FLY based on GC/MS and LC/MS/MS

### 2-C-B-FLY:

GC/MS with TFA derivatization:



LC/MS/MS:



Stability of a compound in solution is an important factor in product design. Accelerated solution stability studies of compounds **1** and **2** were performed and indicate the ampouled solution standards are stable long-term over a range of storage conditions based on HPLC purities.

### Solution stability data for (R)-(-)-Bromo-DragonFLY HCl

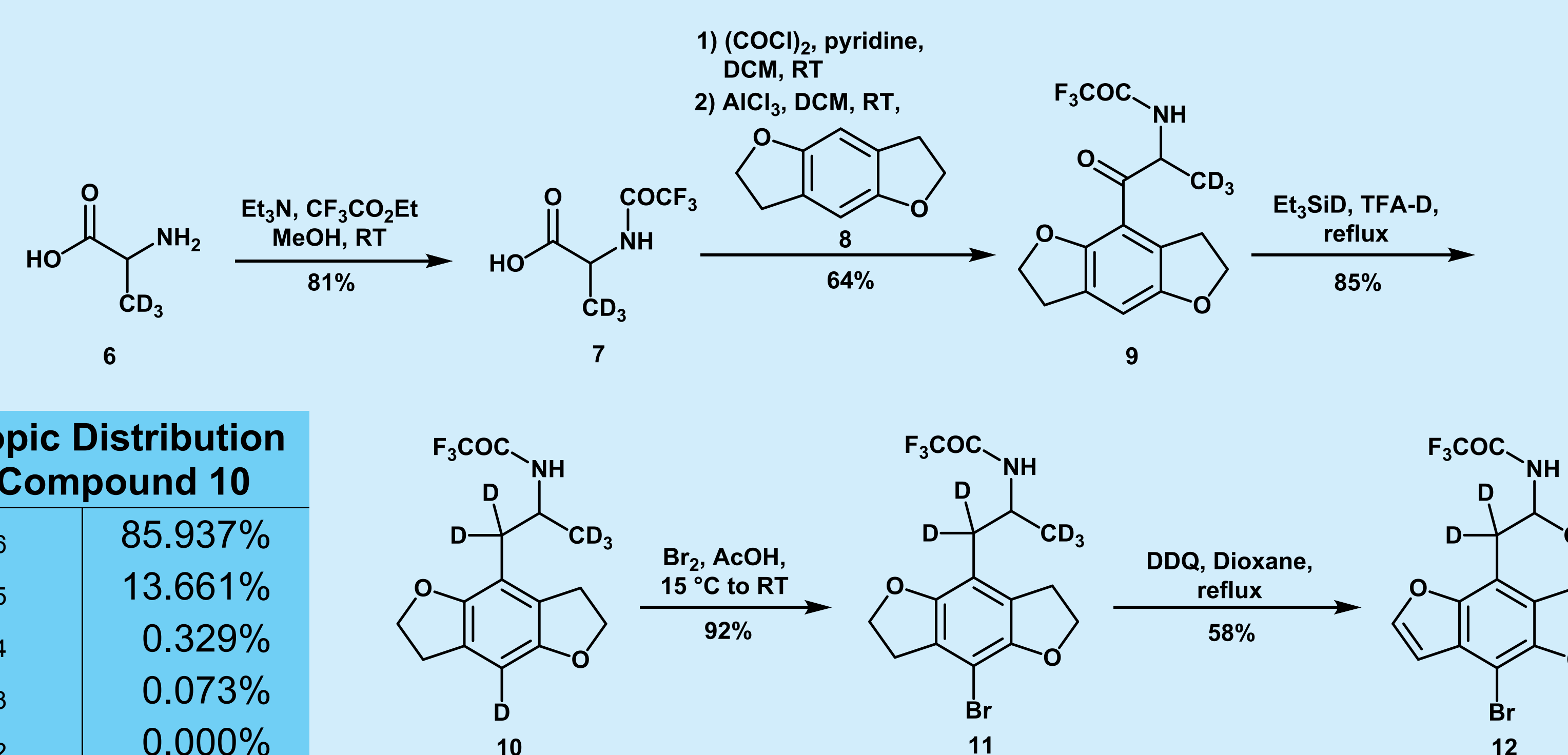
Temp	t=1 week	t=1 year
Freezer	99.87%	99.86%
Refrigerator	99.87%	99.84%
Room temp	99.79%	99.88%
40 °C	99.87%	99.91%

### Solution stability data for 2-C-B-FLY HCl

Temp	t=1 week	t=1 year
Freezer	98.82%	98.77%
Refrigerator	98.92%	98.87%
Room temp	98.75%	98.68%
40 °C	98.81%	98.82%

## Synthesis of (±)-Bromo-DragonFLY-D<sub>5</sub> HCl

The synthesis of (±)-Bromo-DragonFLY-D<sub>5</sub> HCl (**4**) was based on the literature method to prepare (R)-(-)-Bromo-DragonFLY (**1**).<sup>2</sup> DL-alanine-3,3,3-D<sub>3</sub> (**6**) was chosen as a starting point for deuterium incorporation due to reagent availability and cost. The reduction of intermediate **9** with triethylsilane-D and trifluoroacetic acid-D to give compound **10** was a critical step in the synthesis; therefore, isotopic purity and distribution at this step was carefully monitored. It was found that the level of deuterium incorporation at the benzylic position was greater than 95%. In addition, almost complete deuterium exchange had occurred on the phenyl ring as determined by <sup>1</sup>H-NMR and LC/MS-SIM. This was not problematic since the next step involved bromination at that position to give **11**. Oxidation with DDQ provided benzodifuran **12**, which upon deprotection and treatment with acidic ethanol provided the target compound **4** in six linear steps and greater than 99% chromatographic purity.

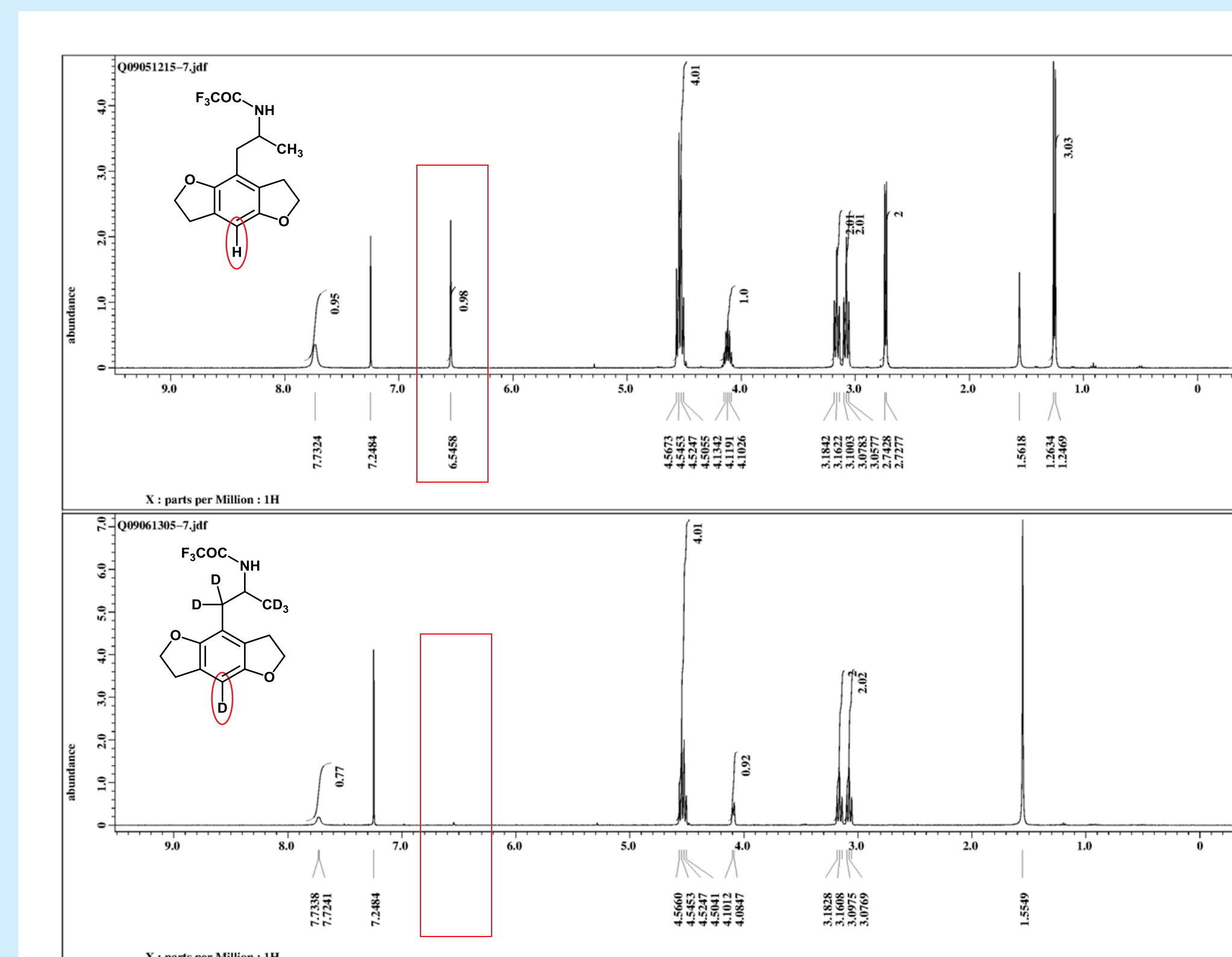


### Isotopic Distribution of Compound 10

D <sub>6</sub>	85.937%
D <sub>5</sub>	13.661%
D <sub>4</sub>	0.329%
D <sub>3</sub>	0.073%
D <sub>2</sub>	0.000%
D <sub>1</sub>	0.000%
D <sub>0</sub>	0.000%

Extra deuterium incorporation on the phenyl ring of intermediate **10** resulted from the Et<sub>3</sub>Sid reduction of ketone **9** to give a D<sub>6</sub> labeled product. Extent of deuterium incorporation on the ring was ~86%.

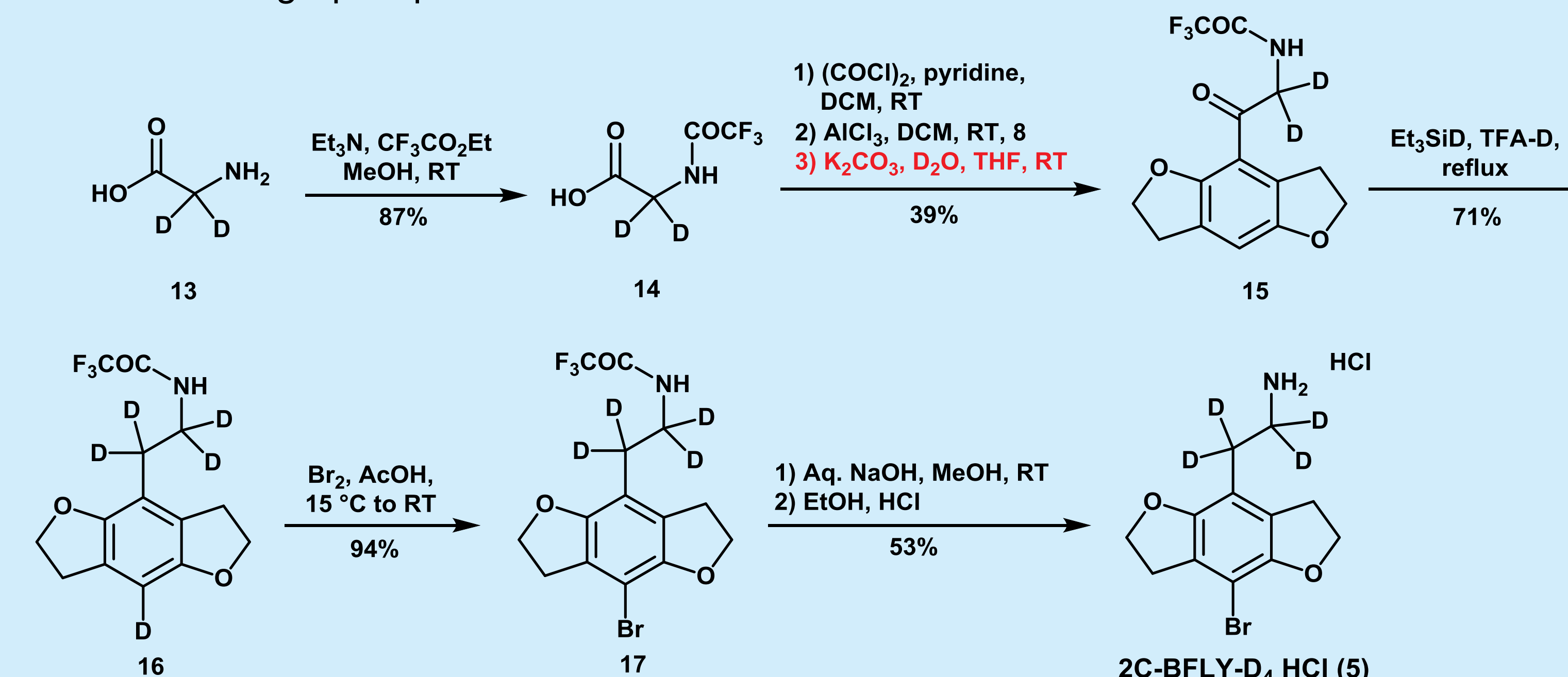
<sup>1</sup>H-NMR of compound **10** in CDCl<sub>3</sub>



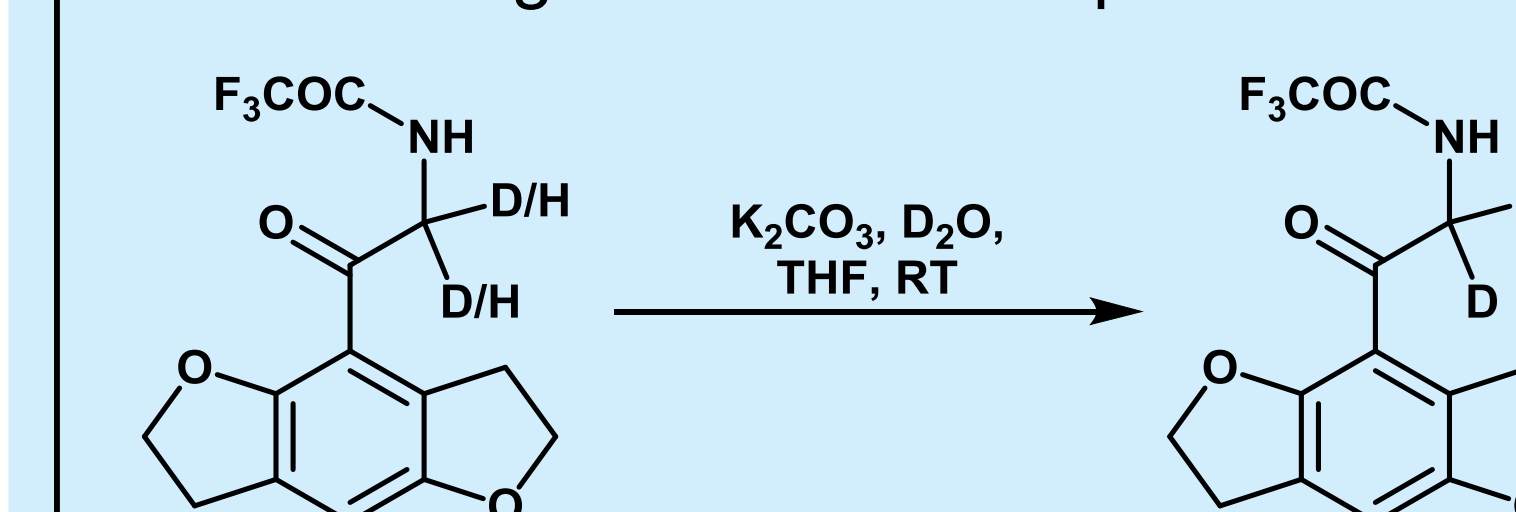
All final isotopic distributions were calculated by HRMS using a Waters Xevo G2 QTOF. Values are adjusted for natural abundance of isotopes (e.g. <sup>13</sup>C, <sup>15</sup>N, etc).

## Synthesis of 2C-B-FLY-D<sub>4</sub> HCl

2C-B-FLY-D<sub>4</sub> HCl (**5**) was prepared in an analogous fashion to (±)-Bromo-DragonFLY-D<sub>5</sub> HCl (**4**) by utilizing glycine-2,2-D<sub>2</sub> (**13**) as the starting labeled amino acid. While no H/D exchange was observed during the TFA protection in step one, the Friedel-Crafts reaction in step two resulted in significant levels of exchange at the position alpha to the carbonyl based on LC/MS-SIM values. The crude intermediate **15** was successfully treated with base in D<sub>2</sub>O to improve the isotopic distribution. Ultimately, the desired compound **5** was prepared in five linear steps and with acceptable isotopic and chromatographic purities.



H/D Exchange reaction in Step 2:



### Isotopic Distribution

	Before	After
D <sub>2</sub>	60.635%	94.223%
D <sub>1</sub>	31.513%	5.308%
D <sub>0</sub>	7.851%	0.291%

### Isotopic Distribution of 2C-B-FLY-D<sub>4</sub>

D <sub>4</sub>	95.854%
D <sub>3</sub>	3.829%
D <sub>2</sub>	0.338%
D <sub>1</sub>	0.009%
D <sub>0</sub>	0.000%

Acylation of **14** resulted in significant H/D exchange at the alpha carbon. An exchange reaction was performed to restore the alpha deuteriums. Final isotopic purity of **5** was determined to be ~96% D<sub>4</sub> with D<sub>0</sub>/D<sub>4</sub> = 0%. Chromatographic purity by GC/FID and HPLC was > 99%.

## Conclusion

- MS fragmentation patterns were used as a guide in designing the syntheses of (±)-Bromo-DragonFLY-D<sub>5</sub> HCl (**4**) and 2C-B-FLY-D<sub>4</sub> HCl (**5**) with the intent of producing stable IS's.
- Accelerated stability studies of **1** and **2** have shown that these phenethylamines are stable in solution over time. IS's should exhibit similar solution stability with no expected decrease in isotopic purity (ie. exchange or scrambling).
- A key step in preparing compounds **4** and **5** involved a triethylsilane-D reduction which allowed for incorporation of deuterium in the benzylic position of both molecules.
- Both target compounds were successfully synthesized at acceptable chromatographic and isotopic purities to be used as CRM's for quantitation in forensic and clinical toxicology testing.
- Ampouled solution standards of **4** and **5** were prepared based on the solubility and solution stability data gathered on related compounds **1** and **2**.

## References

- J. Med. Chem.* **1996**, 39, 2953-2961.
- J. Med. Chem.* **2001**, 44, 1003-1010.
- J. Med. Chem.* **1998**, 41, 5148-5149.
- Forensic Toxicol.* **2010**, 28, 9-18.