

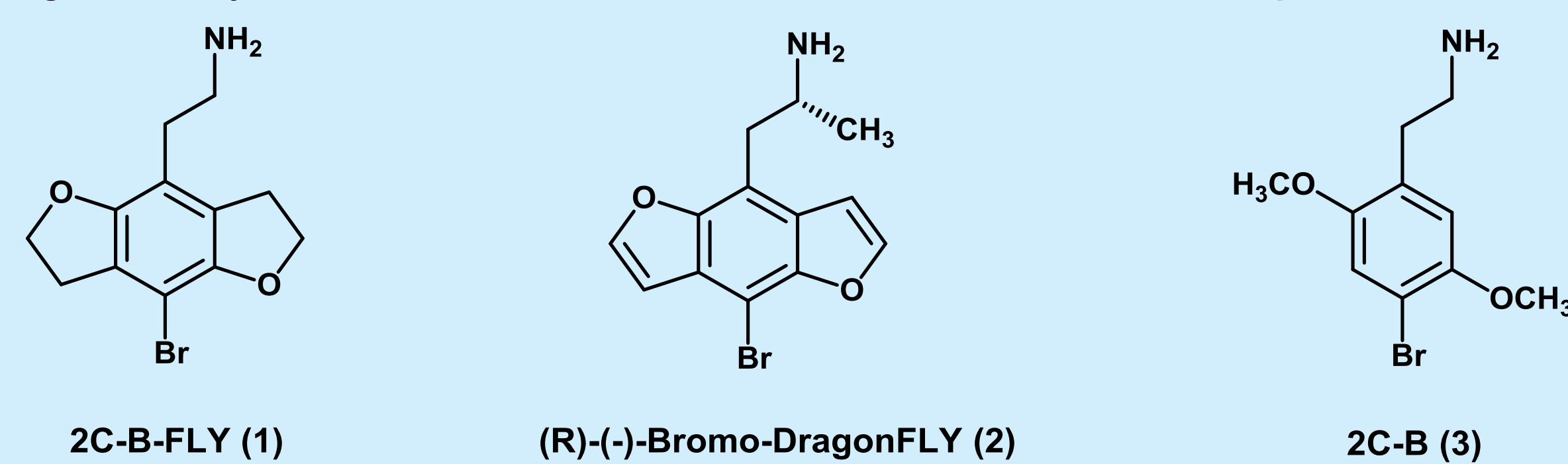
Design and Synthesis of Labeled 2C-B-FLY and Bromo-DragonFLY for Internal Standards Used in Forensic/Clinical Toxicology Analysis

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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.¹⁻³ Compounds **1** and **2** were synthesized from literature methods,¹⁻³ 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.

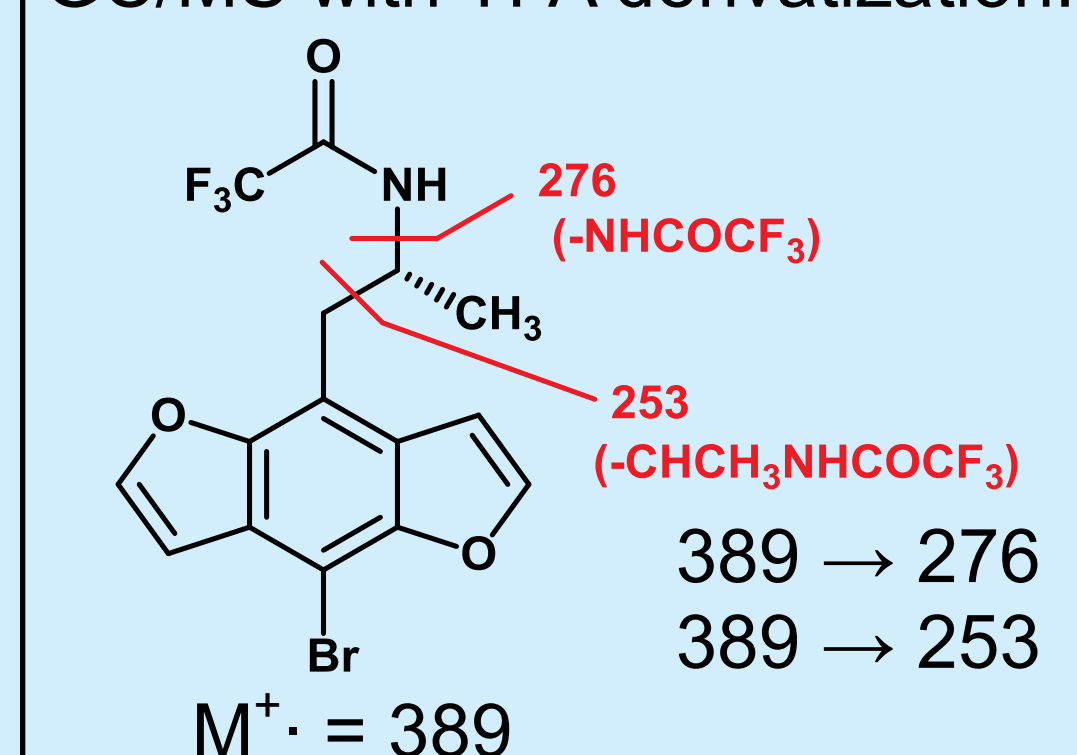


Synthetic and Product Design

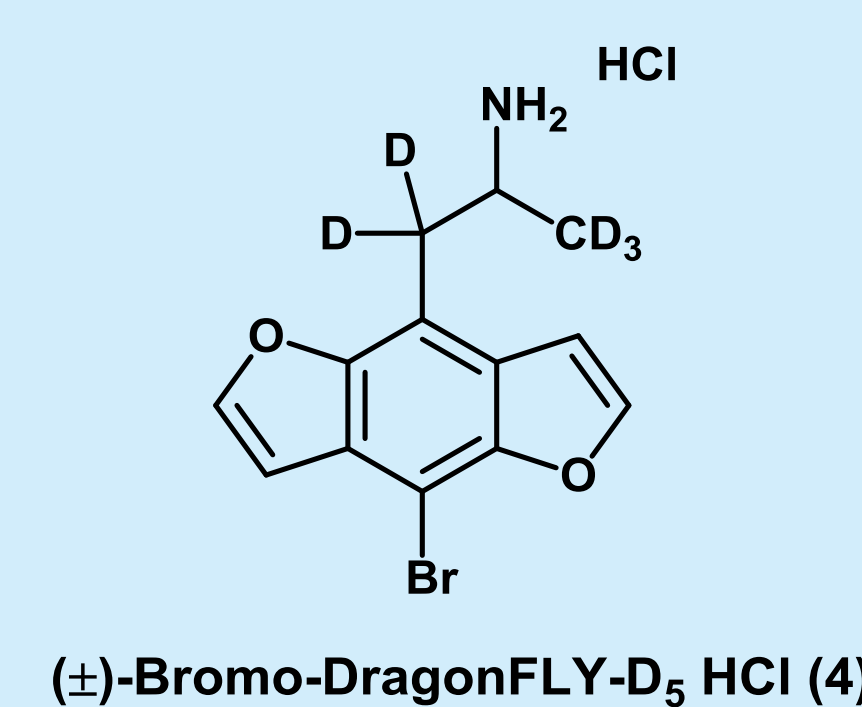
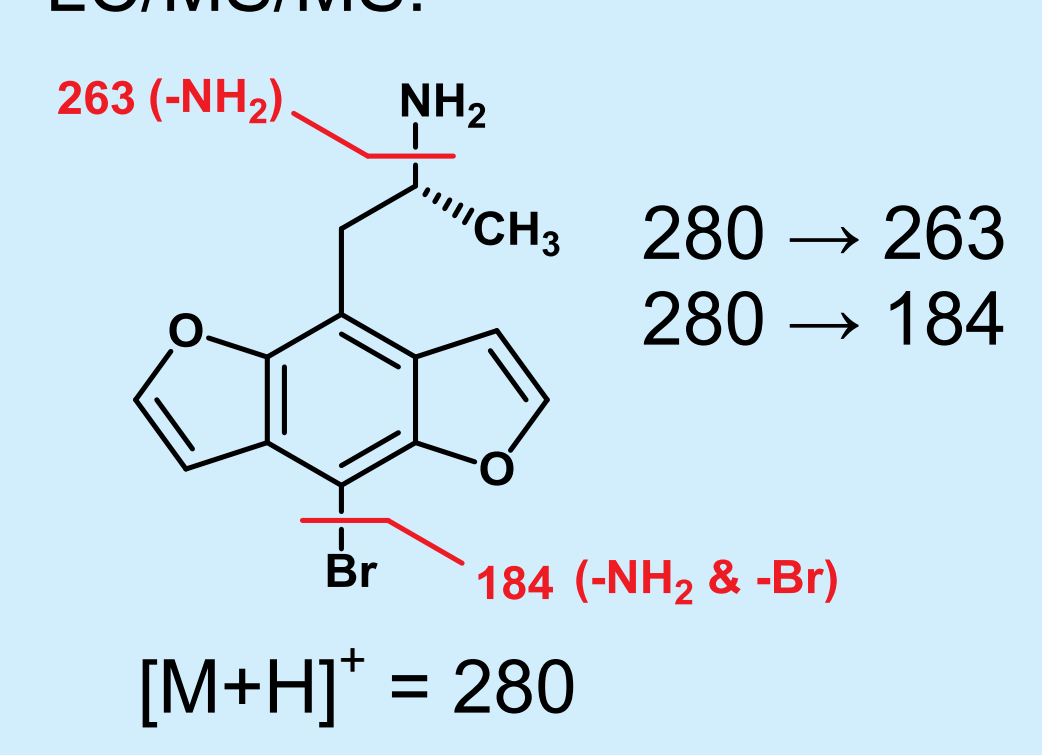
In order to provide an effective IS, the fragments being monitored by MS during testing should retain the labels incorporated during synthesis. Published LC/MS/MS and GC/MS fragmentation patterns of compounds **1** and **2** were evaluated to identify the optimal location for deuterium incorporation.⁴ 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:



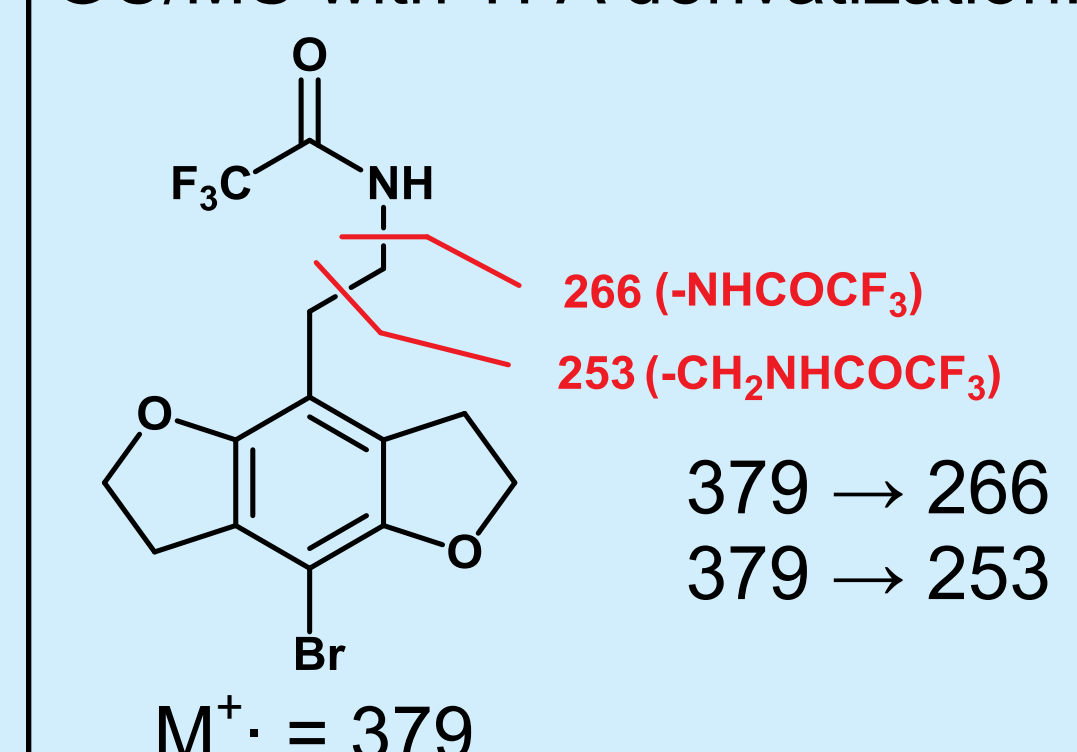
LC/MS/MS:



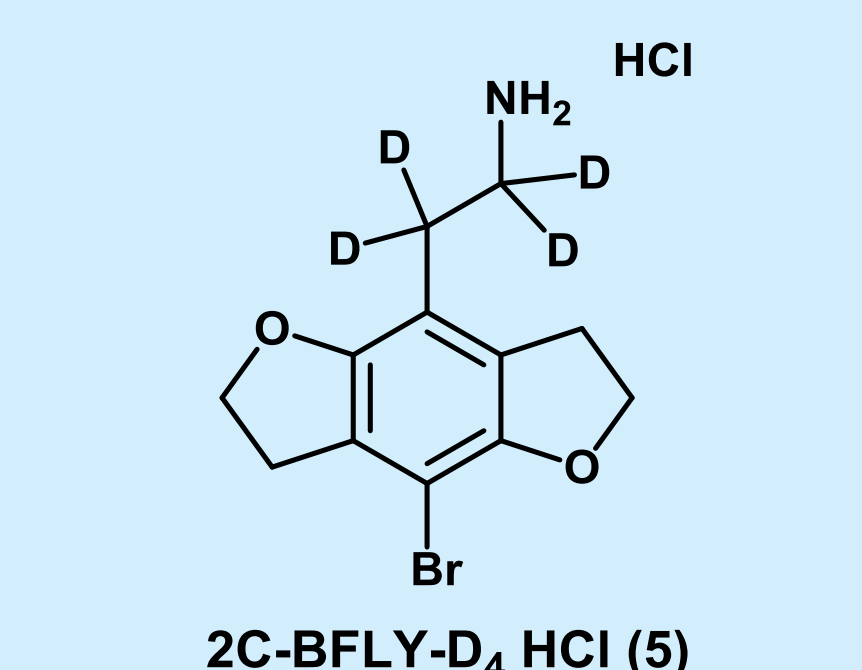
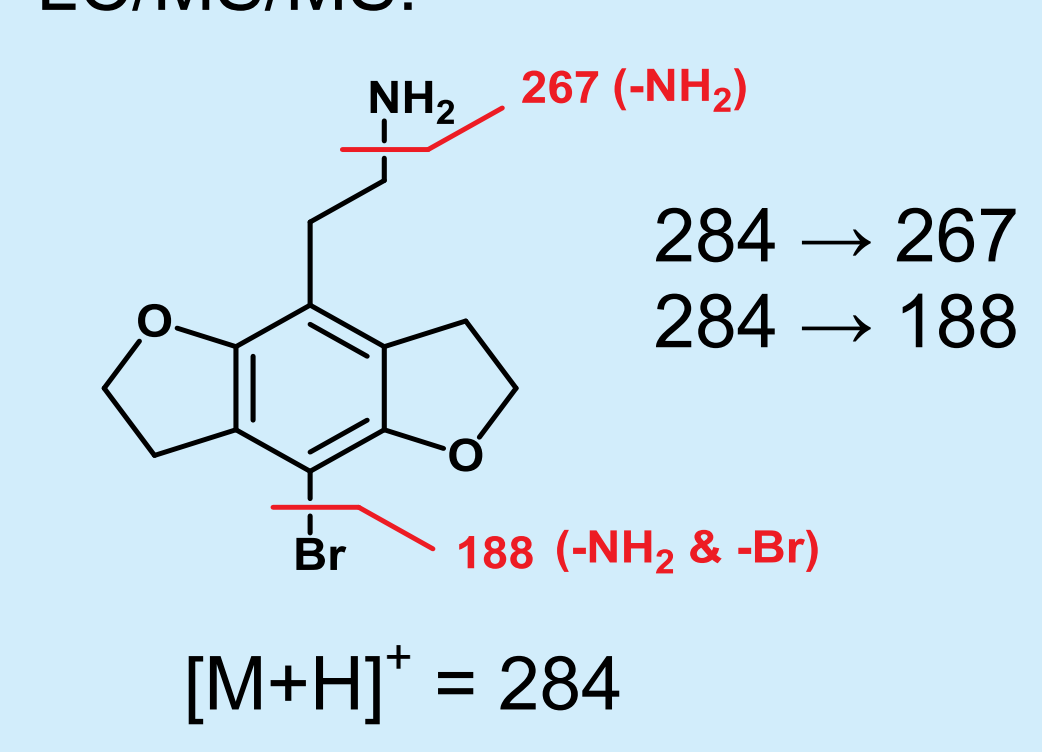
Proposed labeling on Bromo-DragonFLY and 2-C-B-FLY based on GC/MS and LC/MS/MS

2-C-B-FLY:

GC/MS with TFA derivatization:



LC/MS/MS:



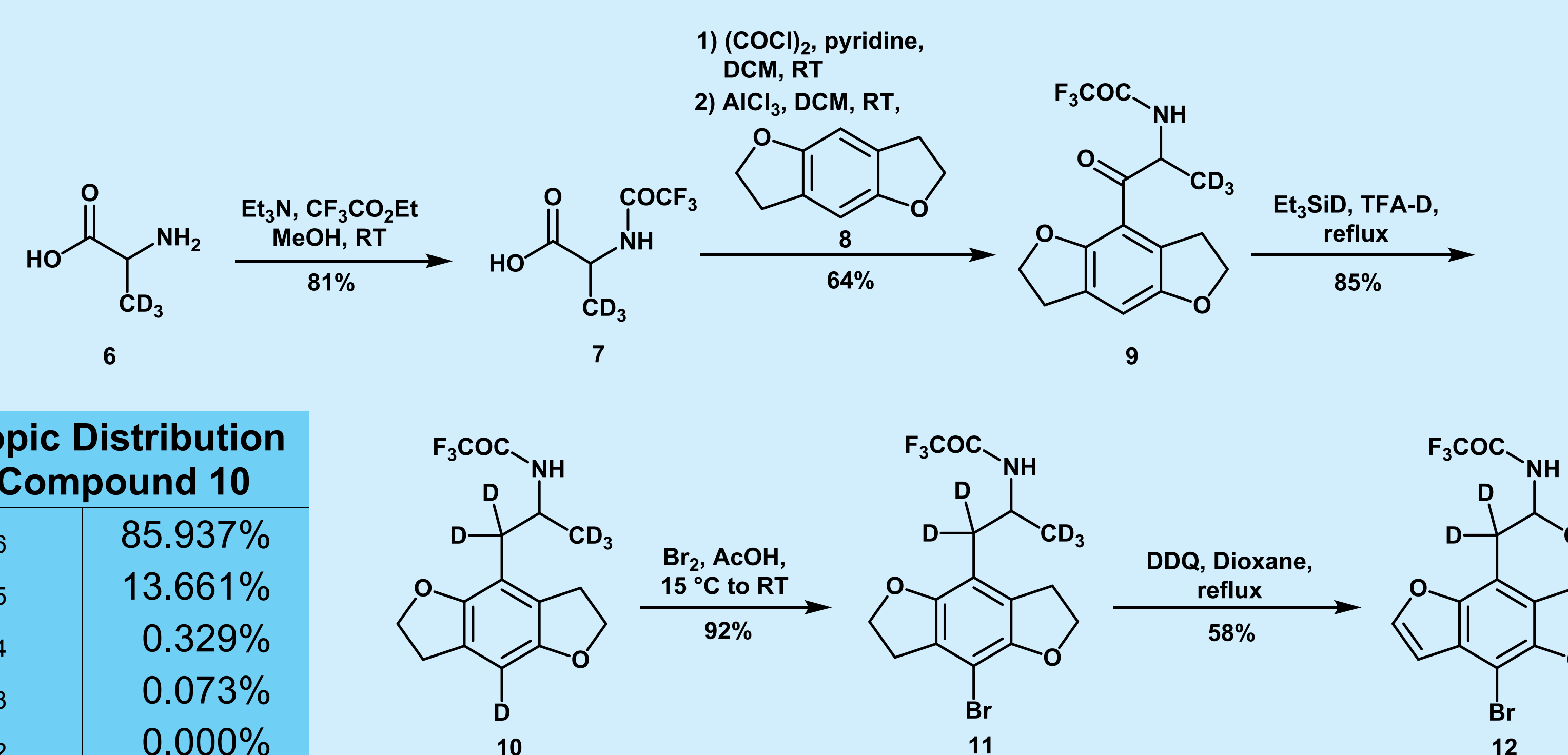
Stability of a compound in solution is an important factor in product design. Accelerated solution stability studies of compounds **1** and **2** were performed and indicate the ampouled solution standards are stable long-term over a range of storage conditions based on HPLC purities.

Solution stability data for (R)-(-)-Bromo-DragonFLY HCl 1.0 mg/mL in MeOH (as free base)		
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 1.0 mg/mL in ACN with 10% H ₂ O (as free base)		
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-D₅ HCl

The synthesis of (±)-Bromo-DragonFLY-D₅ HCl (**4**) was based on the literature method to prepare (R)-(-)-Bromo-DragonFLY (**1**).² DL-alanine-3,3,3-D₃ (**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 ¹H-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.

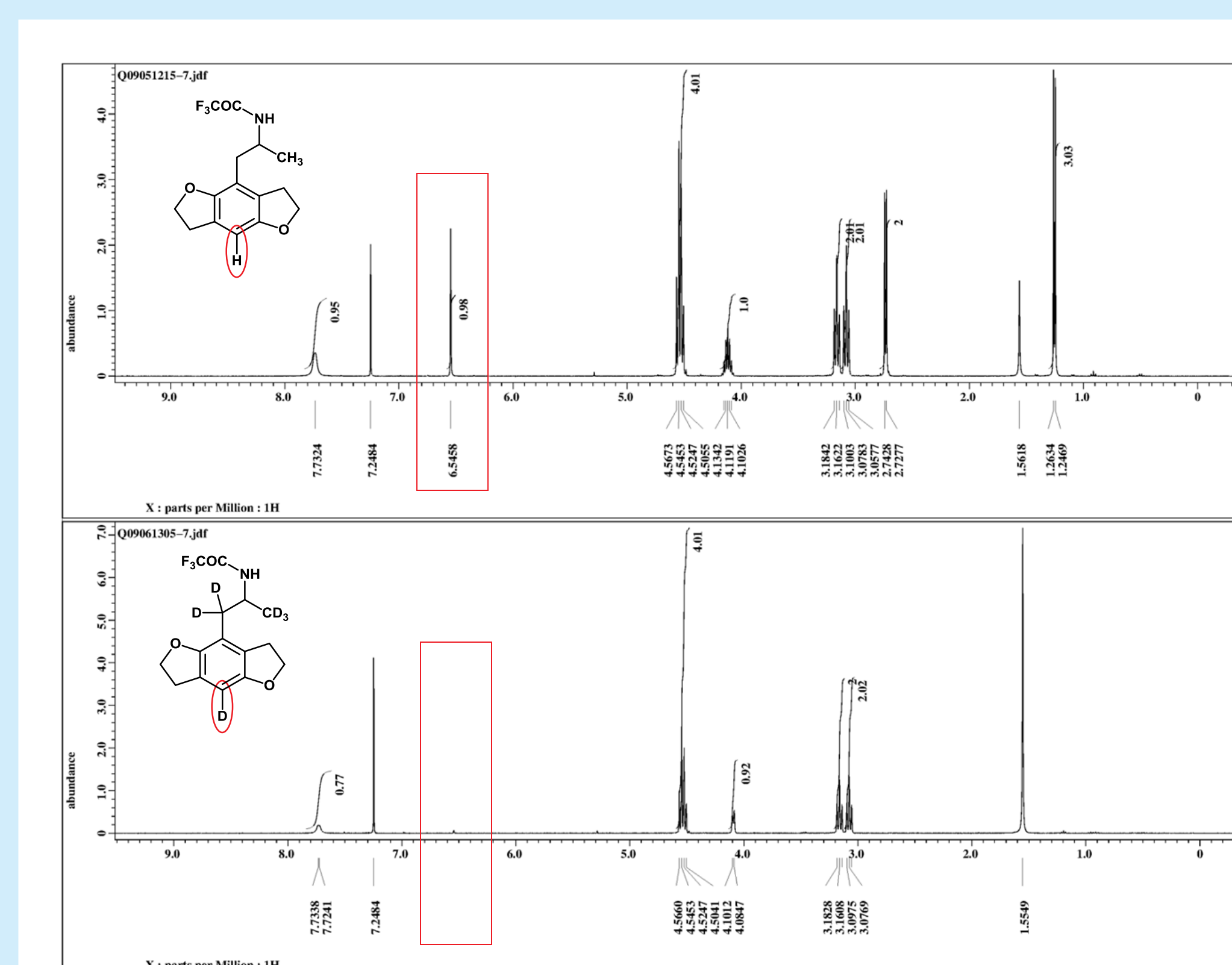


Isotopic Distribution of Compound 10

D ₆	85.937%
D ₅	13.661%
D ₄	0.329%
D ₃	0.073%
D ₂	0.000%
D ₁	0.000%
D ₀	0.000%

Extra deuterium incorporation on the phenyl ring of intermediate **10** resulted from the Et₃Sid reduction of ketone **9** to give a D₆ labeled product. Extent of deuterium incorporation on the ring was ~86%.

¹H-NMR of compound **10** in CDCl₃



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

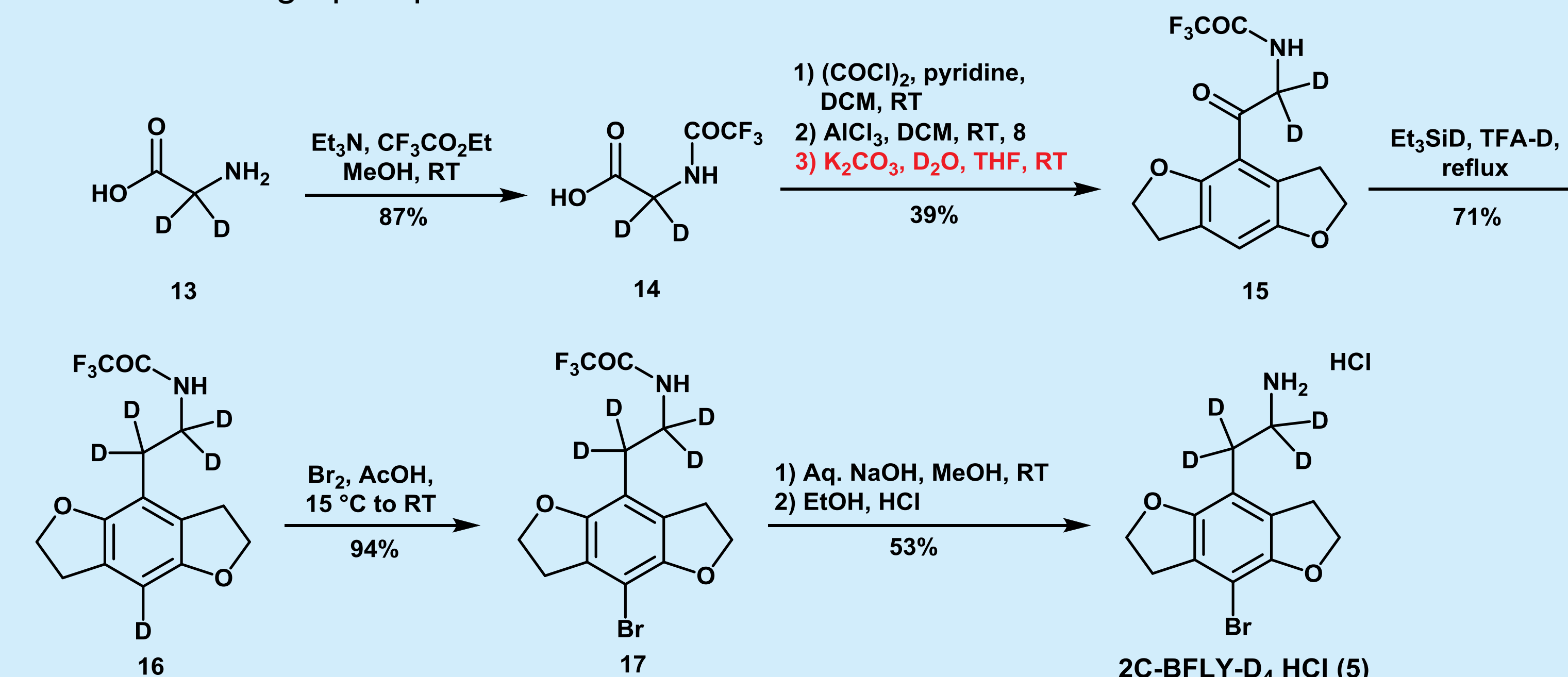
Isotopic Distribution of (±)-Bromo-DragonFLY-D₅

D ₅	98.048%
D ₄	1.902%
D ₃	0.006%
D ₂	0.043%
D ₁	0.000%
D ₀	0.000%

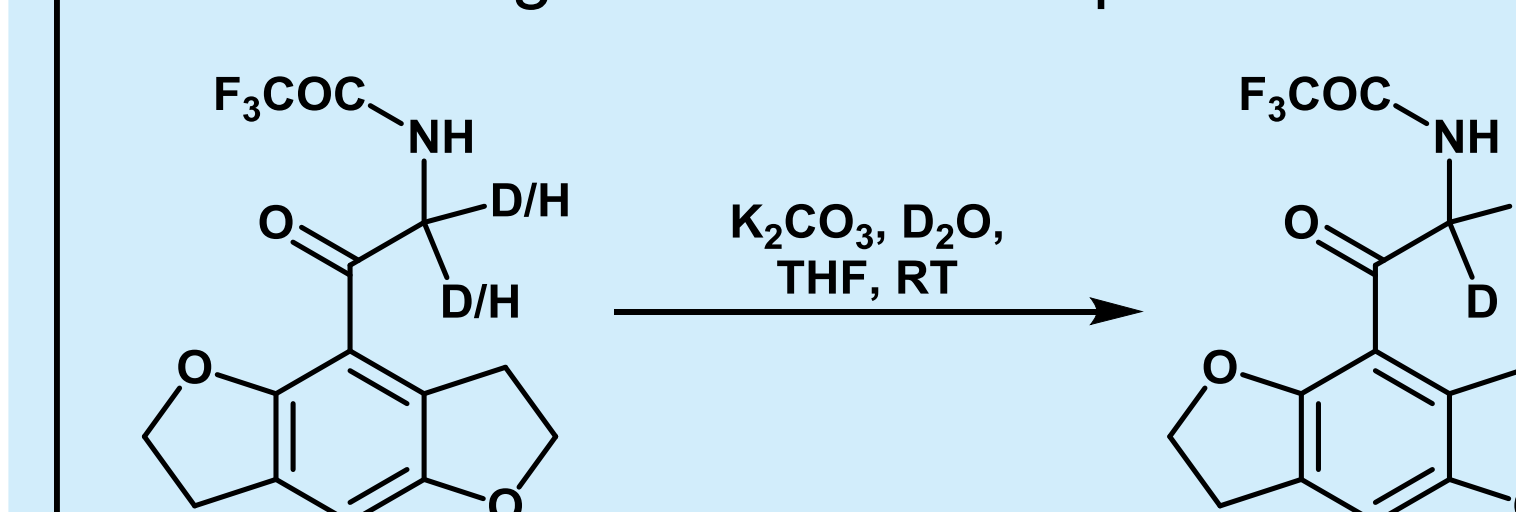
Final isotopic purity was determined to be ~98% D₅ with D₀/D₅ = 0%. Chromatographic purity by GC/FID and HPLC was > 99%

Synthesis of 2C-B-FLY-D₄ HCl

2C-B-FLY-D₄ HCl (**5**) was prepared in an analogous fashion to (±)-Bromo-DragonFLY-D₅ HCl (**4**) by utilizing glycine-2,2-D₂ (**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 D₂O 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 ₂	60.635%	94.223%
D ₁	31.513%	5.308%
D ₀	7.851%	0.291%

Isotopic Distribution of 2C-B-FLY-D₄

D ₄	95.854%
D ₃	3.829%
D ₂	0.338%
D ₁	0.009%
D ₀	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% D₄ with D₀/D₄ = 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-D₅ HCl (**4**) and 2C-B-FLY-D₄ 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.