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

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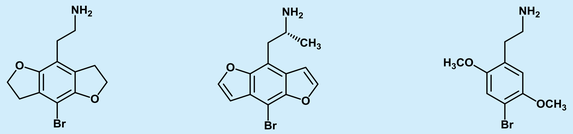
Background

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.



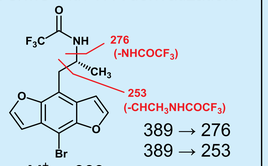
2C-B-FLY (1) (R)-(-)-Bromo-DragonFLY (2) 2C-B (3)

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**.

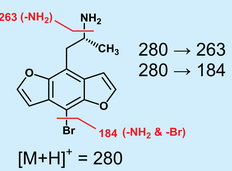
Bromo-DragonFLY:

GC/MS with TFA derivatization:



389 → 276
389 → 253
$M^{+\cdot}$ = 389

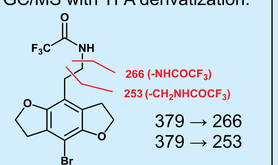
LC/MS/MS:



280 → 263
280 → 184
$[M+H]^+$ = 280

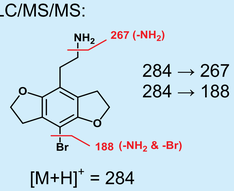
2-C-B-FLY:

GC/MS with TFA derivatization:

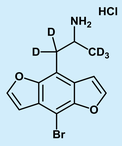


379 → 266
379 → 253
$M^{+\cdot}$ = 379

LC/MS/MS:



284 → 267
284 → 188
$[M+H]^+$ = 284



(±)-Bromo-DragonFLY-D_5 HCl (4)

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

2C-BFLY-D_4 HCl (5)

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)

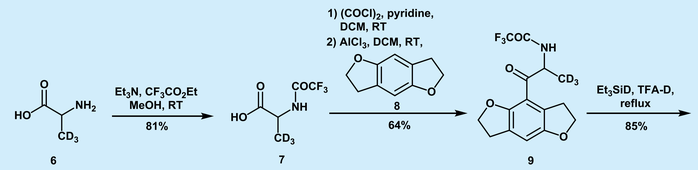
Temp	t=1 week	t=1 year
Freezer	99.87%	99.86%
Refrigerator	99.87%	99.84%
Room temp	99.79%	99.88%
40 °C	99.87%	99.91%

Solution stability data for 2-C-B-FLY HCl
1.0 mg/mL in ACN with 10% H_2O (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%

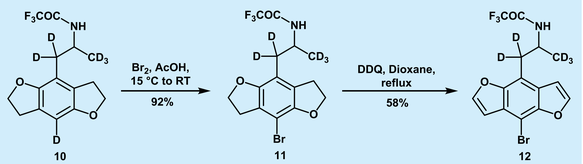
Synthesis of (±)-Bromo-DragonFLY-D_5 HCl

The synthesis of (±)-Bromo-DragonFLY-D_5 HCl (**4**) was based on the literature method to prepare (R)-(-)-Bromo-DragonFLY (**1**).[2] DL-alanine-3,3,3-D_3 (**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 ^{1}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.



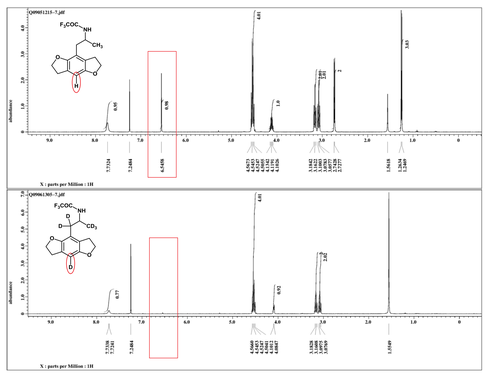
Isotopic Distribution of Compound 10

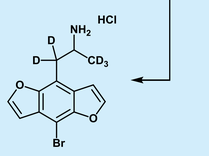
D_6	85.937%
D_5	13.661%
D_4	0.329%
D_3	0.073%
D_2	0.000%
D_1	0.000%
D_0	0.000%



Extra deuterium incorporation on the phenyl ring of intermediate **10** resulted from the Et_3SiD reduction of ketone **9** to give a D_6 labeled product. Extent of deuterium incorporation on the ring was ~86%.

^{1}H-NMR of compound **10** in $CDCl_3$





(±)-Bromo-DragonFLY-D_5 HCl (4)

Isotopic Distribution of (±)-Bromo-DragonFLY-D_5

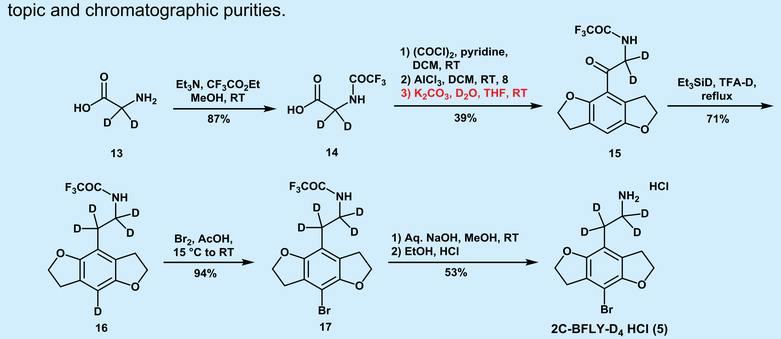
D_5	98.048%
D_4	1.902%
D_3	0.006%
D_2	0.043%
D_1	0.000%
D_0	0.000%

Final isotopic purity was determined to be ~98% D_5 with D_0/D_5 = 0%. Chromatographic purity by GC/FID and HPLC was > 99%

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

Synthesis of 2C-B-FLY-D_4 HCl

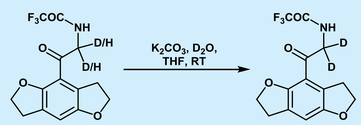
2C-B-FLY-D_4 HCl (**5**) was prepared in an analogous fashion to (±)-Bromo-DragonFLY-D_5 HCl (**4**) by utilizing glycine-2,2-D_2 (**13**) as the starting labeled amino acid. While no H/D exchange was observed during the TFA protection in step one, the Friedel-Crafts reaction in step two resulted in 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_2O to improve the isotopic distribution. Ultimately, the desired compound **5** was prepared in five linear steps and with acceptable isotopic and chromatographic purities.



Isotopic Distribution of 2C-B-FLY-D_4

D_4	95.854%
D_3	3.829%
D_2	0.338%
D_1	0.009%
D_0	0.000%

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%

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_4 with D_0/D_4 = 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_5 HCl (**4**) and 2C-B-FLY-D_4 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

1. *J. Med. Chem.* **1196**, *39*, 2953-2961.
2. *J. Med. Chem.* **2001**, *44*, 1003-1010.
3. *J. Med. Chem.* **1998**, *41*, 5148-5149.
4. *Forensic Toxicol.* **2010**, *28*, 9-18.