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Synthesis of [$^{13}C_6$]-labelled phenethylamine derivatives for drug quantification in biological samples

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The availability of high-quality ^{13}C-labelled internal standards will improve accurate quantification of narcotics and drugs in biological samples. Thus, the synthesis of 10 [$^{13}C_6$]-labelled phenethylamine derivatives, namely amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxy-*N*-ethylamphetamine, 4-methoxyamphetamine, 4-methoxymethamphetamine, 3,5-dimethoxyphenethylamine 4-bromo-2,5-dimethoxyphenethylamine and 2,5-dimethoxy-4-iodophenethylamine, have been undertaken. [$^{13}C_6$]-Phenol proved to be an excellent starting material for making ^{13}C-labelled narcotic substances in the phenethylamine class, and a developed Stille-type coupling enabled an efficient synthesis of the 3,4-methylenedioxy and 4-methoxy derivatives. The pros and cons of alternative routes and transformations are also discussed. The [$^{13}C_6$]-labelled compounds are intended for use as internal standards in forensic analysis, health sciences and metabolomics studies by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry.

Keywords: [$^{13}C_6$]-labelled standards; amphetamine; methamphetamine; 4-methoxymethamphetamine; 3,4-methylenedioxymethamphetamine; Stille coupling

Introduction

Amphetamine (**1a**) and methamphetamine (**1b**) are some of the most abused drugs in Scandinavia and occur frequently in blood samples from car drivers suspected for driving under influence. The prevalence of amphetamines in a roadside survey made in Norway (2013) of 12000 volunteer candidates shoved 0.3% positive samples.[1] Also, other designer drugs from the class of substituted phenethylamines like 3,4-methylenedioxymethamphetamine (MDMA, **3b**), 3,4-methylenedioxyamphetamine (MDA, **3a**) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA, **3c**), popularized through the rave culture as ecstasy, pose a serious problem worldwide.[2] Probably because of restriction on supply of piperonal and safrole used in the production of the methylenedioxy derivatives **3a**–**c**, an increased illegal production and use of the highly toxic analogues 4-methoxyamphetamine (**2a**) and 4-methoxymethamphetamine (**2b**), currently being sold as fake ecstasy, is seen. As a result, a series of fatal intoxications has been observed in the Scandinavian countries.[3] 4-Bromo-2,5-dimethoxyphenethylamine (**5a**) and 2,5-dimethoxy-4-iodophenethylamine (**6a**) first emerged as legal substitutes of MDMA (**3b**) because of their somewhat similar narcotic effects but are now both listed as illegal drugs in most parts of the world (Figure 1).

Besides the classical methods of synthesis, few 'modern' reports on the preparation of these molecules exist. On the other hand, impurity profiles from seized materials have been used to determine the preferred routes of synthesis in clandestine laboratories.[4–6] The Leuckart reaction seems to be the most common transformation in amphetamine laboratories giving access to the amine function via ketone precursors, while methamphetamine (**1b**) is often synthesized by reduction of ephedrine or by reductive amination of phenyl-2-propanone.[5] (*E*)-1-Methoxy-4-(1-propenyl)benzene (anethole) has been reported as the main precursor of the *para* methoxy derivatives PMA (**2a**) and PMMA (**2b**), which is widely available as the main component (95%) in anise oil.[6] Illegal production of MDMA (**3b**) relies among others on 3,4-methylenedioxyphenyl-2-propanone (**12**) and 5-(2-propenyl)-1,3-benzodioxole (safrole) as starting materials.[4] For many of the synthetic phenethylamines such as 2C-B (**5a**), 2C-I (**6a**) and PMA (**2a**), synthetic procedures have been listed with biological activity in the book *PIHKAL: A Chemical Love Story*,[7] and these methods are also used in clandestine laboratories.

The method of choice for analysis of drugs in biological samples is liquid chromatography-tandem mass spectrometry (LC-MS/MS) due to speed of analysis, sensitivity, selectivity and ease of sample preparation.[8,9] LC-MS/MS instruments frequently show ion suppression or alteration due to matrix compounds, other drugs, preservation agents or other unknown substances

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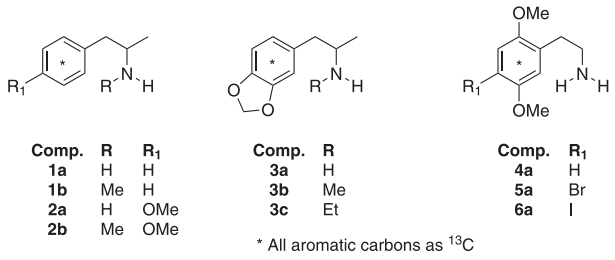


Figure 1. Structure of the synthesized [$^{13}C_6$]-labelled compounds.

contaminating the biological sample.[10] This type of ion suppression/alteration effect generally limits the ruggedness and accuracy of LC-MS/MS methods.[11] Stable isotope internal standards (SIL IS) are added to correct for error in the sample preparation and matrix effects.[12] IS are used to quantify the level of each analyte in the sample. These analyses can be performed using deuterium-labelled IS. However, disadvantages associated with deuterium labelling include the risk of deuterium hydrogen exchange, and that the deuterium-labelled compounds often elute differently from the native, non-labelled compounds in liquid chromatography making them at risk to overlap with matrix compounds.[13] In addition, the deuterium-labelled compounds may have a response factor, which is slightly different from the response factor of the native, non-labelled compounds. Changes in the physicochemical properties of deuterium-labelled compounds as compared with hydrogen analogues can lead to differences in the MS ionization causing ion suppression or alteration of the IS giving false results.[14] This is caused by the isotope effect, lowering the zero point energy of the deuterated IS.[15] ^{13}C-labelled IS have much smaller differences in physicochemical properties[16] and behave almost identical to the unlabelled substance giving no difference in retention times or response factor. Moreover, there is also no risk of exchange. The ^{13}C-labelled IS are particularly suitable for minimizing ion suppression effects in LC-MS/MS analysis; therefore, the quantitative analysis in various biological samples is particularly accurate and reproducible. However, natural matter contains approximately 1.1% ^{13}C; thus, there is a risk of 'overlap' with the natural ^{13}C in the native compound in MS detection. Therefore, the number of labelled atoms must preferably be at least three. Recently, Berg *et al.*[17] demonstrated the success of substituting the deuterated IS for ^{13}C IS in their routine analysis of amphetamine (**1a**) and methamphetamine (**1b**) by SIL IS synthesized in our laboratory. Moreover, the use of ^{13}C as label in analytes for use as IS is widely known and appreciated also in other fields of analytical chemistry.[11,13,18–20]

We herein wish to report our effort to make 10 [$^{13}C_6$]-labelled compounds intended for use as IS in forensic analysis, health sciences and metabolomics studies. To the best of our knowledge, these compounds have not been reported synthesized before with more than three labelled ^{13}C atoms.

Experimental

Chemicals and analysis

Bulk solvents were purchased either from LabScan (Gliwice, Poland) or Merck (Darmstadt, Germany). Deuterated solvents were purchased from CDN Isotopes Inc. All chemicals or reagents used were of highest purity available and purchased from Sigma-Aldrich (Oslo, Norway) or Acros (Geel, Belgium). [$^{13}C_6$]-Benzaldehyde was purchased from Cambridge Isotope Laboratories with isotopic purity >99% (Andover, MA). [$^{13}C_6$]-Phenol was purchased from Campro Scientific (Berlin, Germany) with isotopic purity >99%. All solvents and chemicals were used as is without further purification if otherwise is not stated. Anhydrous solvents were used as is and stored over activated molecular sieves. The silica gel used for flash chromatography was Merck silica gel 60 (230–400 mesh). For chromatography, thin-layer chromatography (TLC) silica gel 60 F_{254} Merck plates/sheets were employed with visualization under ultraviolet light at 254 nm. ^{1}H and ^{13}C NMR spectra were recorded from Bruker Advance DPX instruments (400/100 MHz) (Coventry, United Kingdom). Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane. Because of the high intensity of the ^{13}C-labelled carbons as compared with those unlabelled, some NMR resonances were not detected. In this study, the non-labelled benzylic carbons experiencing extensive coupling, and often also appearing in the solvent region, were difficult to detect. Isotopic purity and accurate mass determination/high resolution mass spectrometry (HRMS) in positive and negative modes on the final product was performed on a 'Synapt G2-S' quadrupole time-of-flight mass spectrometer (Q-TOF) instrument from Waters (En Yvelines Cedex, France), with a resolution of 5 ppm. Samples were ionized by the use of an atmospheric pressure solids analysis probe (ASAP), and no chromatography separation was used previous to the mass analysis. HPLC analysis was performed on an Agilent 1200 with atmospheric pressure chemical ionization (APCI)/electrospray ionization (ESI) multimode ionization and acetonitrile as the mobile phase in combination with water buffered at pH 3 with formic acid (Santa Clara, United States). The column used was XBridge™ C18, 5 μm, 4.6 × 150 mm from Waters or Hilic Plus, 3.5 μm, 4.6 × 100 mm from Agilent. GC-MS analysis was performed on Agilent 6890 with electron impact (EI) ionization, Agilent column HP5MS, 30 m, 0.25 mm internal diameter, 0.25 μm film. GC analyses of the final products (both free amines and hydrochloric acid salts) were performed as their trifluoroacetyl (TFA) derivatives.

Results and discussion

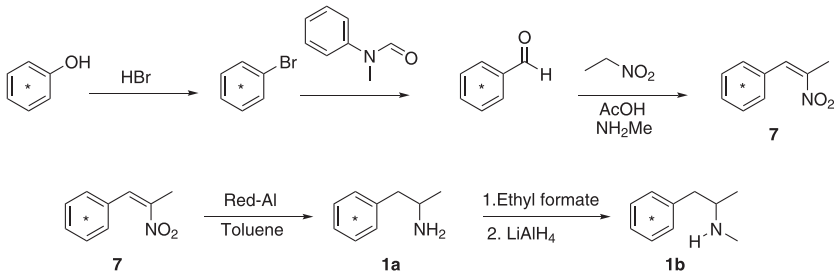
[$^{13}C_6$]-Phenol is readily available from several distributors and relatively cheap compared with other ^{13}C-labelled compounds, and was therefore selected as the general starting material for the synthesis of the phenylethylamine derivatives.

Our first approach to the substituted phenethylamines started by converting phenol to bromobenzene using hydrobromic acid, followed by a modified Bouveault synthesis with *N*-methylformanilide[21] giving [$^{13}C_6$]-benzaldehyde (Scheme 1). However, also [$^{13}C_6$]-benzaldehyde can be bought at a relatively low price so [$_{13}C_6$]-benzaldehyde was used in our later tests to save time. [$_{13}C_6$]Benzaldehyde was then condensed in a classic Henry reaction with nitroethane to give the nitrostyrene **7**. [$^{13}C_6$]-Amphetamine (**1a**) was obtained by a clean Red-Al™ reduction of **7** in toluene. More by-products and lower yields were experienced when using lithium aluminium hydride in this transformation.

Further, **1a** was formylated quantitatively using ethyl formate in a closed pressure reactor, and reduction with lithium aluminium hydride without purification of the formate gave [$^{13}C_6$]-methamphetamine (**1b**) in good yield. Purification of **1b** was performed by crystallization of its hydrochloride salt.

In an alternative strategy, electrolytic reduction of the nitrostyrene **7** with iron in acetic acid gave 1-phenylpropan-2-one.[22] This ketone could successfully be converted to amphetamine (**1a**) by reaction with hydroxylamine to the corresponding ketoxime followed by reduction with sodium metal in ethanol[23] or reductive amination.[24] This route, however, was more time-consuming and also gave lower overall yield.

The 3,4-methylenedioxy derivatives **3a**–**c** were initially targeted by the same type of chemistry as shown in Scheme 1. However, difficulties in preparing the target aldehyde in a straightforward fashion made us look for alternative routes. Different methods

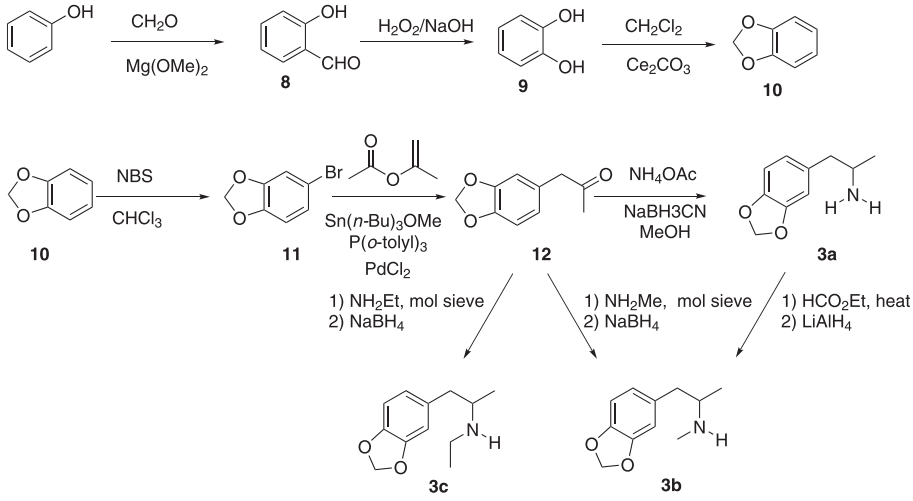


Scheme 1. Synthesis of [$^{13}C_6$]-amphetamine (**1a**) and [$^{13}C_6$]-methamphetamine (**1b**). *All aromatic carbons as ^{13}C.

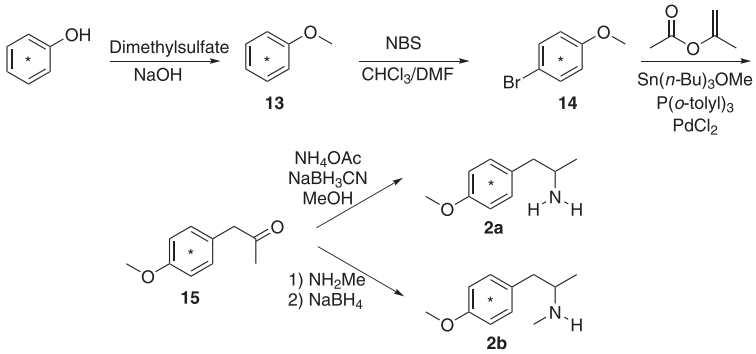
were tested, and in our hands, a new strategy, relying on a Stille coupling,[25] was found to be most useful (Scheme 2).

Our first attempt at synthesizing [$^{13}C_6$]-catechol (**9**) was by the procedure of Ji *et al.*,[26] but this procedure was time-consuming and gave low yield. We then tried the procedure of Thakur *et al.*,[27] but this method gave minimal conversion even after 2 weeks and was also abandoned. [$^{13}C_6$]-Phenol was instead converted to [$^{13}C_6$]-2-hydroxybenzaldehyde (**8**) in 95% yield by an *ortho*-specific formylation with magnesium methoxide and formaldehyde with continuous removal of methanol by a toluene azeotrope.This reaction was superior to several other formylation methods tested.[28,29] Compound **8** could then easily be converted to [$^{13}C_6$]-catechol (**9**) by a Dakin oxidation.[30] The crude product **9** was dark in colour, but could be purified by sublimation, and NMR analysis indicated high purity. [$^{13}C_6$]-1,2-Methylenedioxybenzene (**10**) was then synthesized by a methenylation reaction, and several bases were tested with different dihalomethanes. We found that the use of dichloromethane was preferable over diiodomethane or dibromomethane because of easier product purification. The most efficient transformation was achieved with cesium carbonate as the base leading to 95% yield of good purity product.[31] The use of sodium hydride in hexamethylphosphoramide as reported by Castillo *et al.*[32] also gave good conversion but resulted in a more difficult purification.

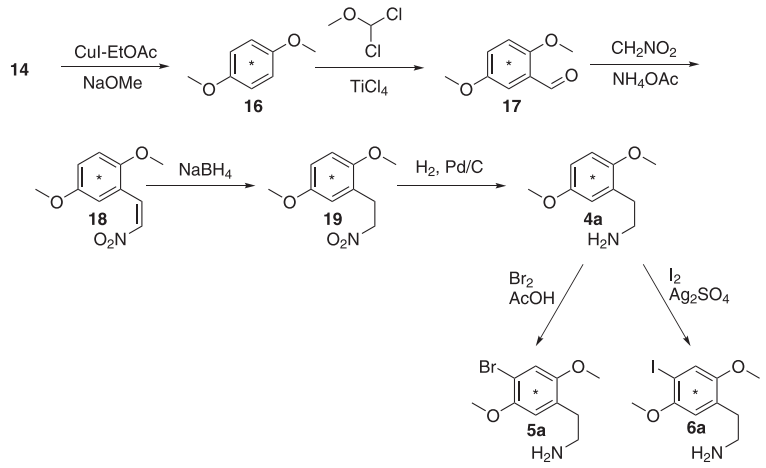
Compound **10** was then converted to the bromide **11** with *N*-bromosuccinimide[33] and taken further to the key intermediate **12** by a Stille-type coupling. This reaction where the tributyltin enolate is generated in situ gave the ketone **12** in one step in over 90% yield. The precursor **12** was then transformed to the respective final products [$^{13}C_6$]-MDMA (**3b**) and [$^{13}C_6$]-MDEA (**3c**) by imine formation in the presence of molecular sieves, and reduction with sodium borohydride in methanol or ethanol,



Scheme 2. Synthesis of the [$^{13}C_6$]-3,4-methylenedioxyamphetamine derivatives **3a**–**c**. *All aromatic carbons as ^{13}C.



Scheme 3. Synthetic route to derivatives [$^{13}C_6$]-PMA (**2a**) and [$^{13}C_6$]-PMMA (**2b**). *All aromatic carbons as ^{13}C.



Scheme 4. Synthetic route to the [$^{13}C_6$]-labelled derivatives **4a**, **5a** and **6a**. *All aromatic carbons as ^{13}C.

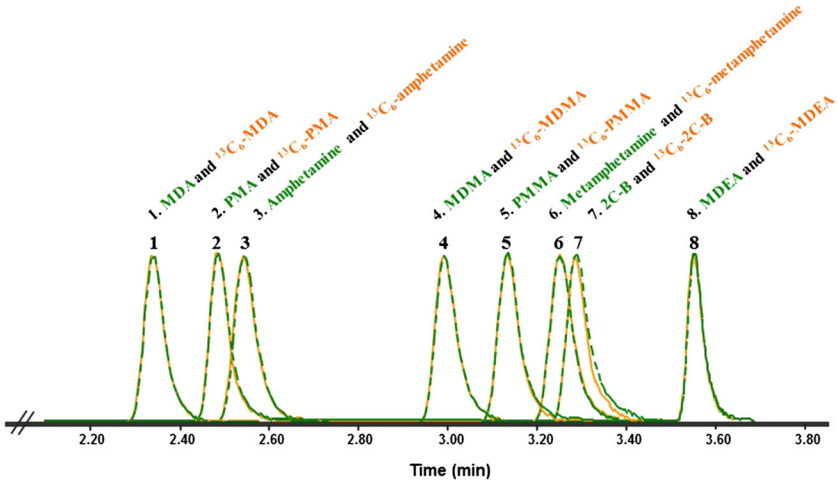


Figure 2. Ultra performance liquid chromatography (UPLC) chromatogram showing overlap of the [$^{13}C_6$]-labelled (yellow) and unlabelled compounds (green). This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr

which facilitates the reaction in one pot. MDA (**3a**) was synthesized by a reductive amination using sodium cyanoborohydride and ammonium acetate as amine source, giving lower yield than the method discussed earlier. [$^{13}C_6$]-MDMA (**3b**) was also made from **3a** by formylation and reduction in 84% yield.

The amphetamine derivatives **2a**–**b**, **4a**, **5a** and **6a** were all made via 4-bromo-[$^{13}C_6$]-anisole (**14**) obtained by methylation of [$^{13}C_6$]-phenol with methylsulfate and *N*-bromosuccinimide bromination (Scheme 3). To prepare the 4-methoxy derivatives **2a**–**b**, **14** was converted to the key intermediate **15** by a Stille reaction in quantitative yield. Compound **15** was then transformed to the respective amines [$^{13}C_6$]-PMA (**2a**) and [$^{13}C_6$]-PMMA (**2b**) as described in the case of **3a**–**b**.

Synthesis of 2,5-dimethoxy-[$^{13}C_6$]-phenethylamine (**4a**), 4-bromo-2,5-dimethoxy-[$^{13}C_6$]-phenethylamine (**5a**) and 2,5-dimethoxy-4-iodo-[$^{13}C_6$]-phenethylamine (**6a**) is shown in Scheme 4. First, 4-bromo-[$^{13}C_6$]-anisole (**14**) was transformed to 1,4-dimethoxy-[$^{13}C_6$]-benzene (**16**) in a copper iodide/ethyl acetate-catalysed nucleophilic aromatic substitution.[34] Then, a Rieche formylation with titanium tetrachloride and dichloromethyl methyl ether by the procedure of Mancini *et al.*[35] gave the aldehyde **17**. Compound **17** was also synthesized by an *ortho* formylation of 4-hydroxyanisole and methylation with dimethylsulfate. Although both steps were high yielding, this pathway was judged more labour-intensive than the Rieche formylation.

The aldehyde **17** was further condensed with nitromethane in a Henry reaction, and the nitrostyrene **18** was reduced in two steps to the target **4a**. Direct hydrogenation of **18** to the primary amine **4a** with palladium on barium sulfate, palladium on carbon and platinum (IV) oxide was also tested. In the best cases using platinum (IV) oxide, the yield was around 45%, and several non-identified by-products were seen. Compound **4a** was then selectively brominated and iodinated[36] in position 4 yielding [$^{13}C_6$]-2C-B (**5a**) and [$^{13}C_6$]-2C-I (**6a**), respectively.

For all the final products, yields were sacrificed because of the need of achieving a final purity >99%. Crystallization from various solvents including diethyl ether, acetonitrile and alcohols and a mixture of these was efficient. The identity of all products and intermediates were confirmed by co-elution with unlabelled materials, high-resolution MS and NMR spectroscopy. A representative UPLC chromatogram is seen in Figure 2.

Conclusion

Synthetic pathways have been designed for [$^{13}C_6$]-amphetamine, [$^{13}C_6$]-methamphetamine, [$^{13}C_6$]-MDA, [$^{13}C_6$]-

MDMA, [$^{13}C_6$]-PMA, [$^{13}C_6$]-PMMA, [$^{13}C_6$]-2C-B, [$^{13}C_6$]-2C-I and [$^{13}C_6$]-2C-H. To the best of our knowledge, these $^{13}C_6$-labelled compounds have been prepared for the first time. Several methods were tested at each step to find synthetic routes that were efficient, fast, high yielding and allowing for a straightforward purification. The identified processes rely on [$^{13}C_6$]-phenol as starting material and use some common intermediates and similar process steps, thus allowing for this series of compounds to be prepared in a fast and economic way. Synthesized [$^{13}C_6$]-amphetamine and [$^{13}C_6$]-methamphetamine have been found superior over deuterated analogues for use as SIL IS in the field of forensic analysis and toxicology.

Synthesis of DL-[$^{13}C_6$]-amphetamine (1a) and DL-[$^{13}C_6$]-methamphetamine (1b)

*Synthesis of 2-nitro-1-propene-1-yl-[$^{13}C_6$]-benzene (**7**)*

[$^{13}C_6$]-Benzaldehyde (1.00 g, 8.92 mmol) was added to nitroethane (5 mL) in a 20-mL round-bottom flask with a magnetic stirrer. Anhydrous ammonium acetate (0.16 g, 2.1 mmol) was added, and the solution was warmed to 80 °C before methylamine (33% in ethanol, 0.1 mL) was added in one portion. After 2 h, full conversion was obtained as observed by TLC (silica gel eluting with toluene) and by GC-MS. The solvent from the reaction mixture was evaporated on a rotary evaporator, and the resulting crude product was recrystallized from hot 2-propanol (3 mL). After slowly cooling to 4 °C overnight, the crystals were isolated on a Büchner funnel and washed with a small amount of cold 2-propanol. The light yellow crystals obtained were dried thoroughly to remove volatiles, yielding 1.10 g (6.51 mmol, 73%) based on [$^{13}C_6$]-benzaldehyde. MS-EI (*m/z*): 169.1 (18), 152.1 (12), 137.0 (6), 121.1 (100), 111.0 (30), 95.1 (16); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.47 (s, 3H), 7.15–7.34 (m, 3H), 7.55–7.75 (m, 2H), 8.10 (br.t, J = 3.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0, 128.0–130.0 (m, 4C), 131.7–133.2 (m), 147.8.

*Synthesis of DL-[$^{13}C_6$]-amphetamine sulfate (**1a**)*

2-Nitro-1-propene-1-yl-[$^{13}C_6$]-benzene (**7**) (1.10 g, 6.50 mmol) was dissolved in dry toluene (20 mL). The resulting solution was added drop wise to a mixture of Vitride (Red-Al™, sodium *bis*(methoxyethoxy)aluminium hydride) solution (70% in toluene, 5 mL) and dry toluene (22.5 mL) at 70 °C. After all the nitrostyrene solution had been added, the resulting reaction mixture was stirred for overnight at room temperature for the completion of the reaction. Sodium hydroxide (5% aqueous solution) was then added carefully, and the toluene layer was separated. The water phase was washed with toluene (2 × 25 mL), and the combined organic phases were washed with saturated sodium bicarbonate and brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated to yield 930 mg of the free base as light yellow oil. This oil was distilled on a Kugelröhr apparatus. The distilled free base was dissolved in anhydrous diethyl ether (20 mL), followed by a slow addition of concentrated sulfuric acid in diethyl ether (10%) until the pH was 4.5. The precipitated salt was isolated by filtration and washed with cold diethyl ether (2 × 10 mL). After drying under reduced pressure, 751 mg (3.14 mmol, 48%) of the product was obtained as white powdery sulfate salt; mp. 310–311 °C; purity 99% (TFA derivative, GC-MS); MS-EI (*m/z*): 140.0 (100), 124.1 (90), 97.1 (55), 69.0 (28); ^{1}H NMR (400 MHz, D_2O) δ: 1.22 (d, J = 6.6 Hz, 3H), 2.82–2.90 (m, 2H), 3.55 (qd, J = 6.9, 2.3 Hz, 1H), 6.87–7.75 (m, 5H); ^{13}C NMR (100 MHz, D_2O) δ: 17.5, 39.0–40.1 (m), 49.1, 126.5–130.5 (m, 5C), 134.7–137.3 (m); HRMS (ES$^+$): calcd for $^{13}C_6C_3H_{14}N$ [M]$^+$: 142.1322; found 142.1328.

*Synthesis of DL-[$^{13}C_6$]-methamphetamine hydrochloride (**1b**)*

Amphetamine (**1a**) free base (100 mg, 0.71 mmol) was dissolved in ethyl formate (10 mL). The solution was heated in an ACE pressure reactor (Vineland, New Jersey, United States) for 2 h at 100 °C. The reaction mixture was cooled to ambient temperature, and the solvent was evaporated under reduced pressure. The resulting carbamate derivative was dissolved in dried diethyl ether (5 mL). The solution was added drop wise to a suspension of lithium aluminium hydride (40.4 mg, 1.07 mmol) in dry diethyl ether (10 mL). The reaction mixture was refluxed for 5 h. After cooling to 0 °C, water was carefully added. The lithium salt was filtered over Celite™ in a Büchner funnel. The granules obtained were washed thoroughly with excess diethyl ether. The combined ether phases were washed with 5% sodium hydroxide and brine before being dried with magnesium sulfate. The solvent was evaporated to yield 96 mg of **1b** as free base. The free base was re-dissolved in diethyl ether (10 mL), and the solution was cooled down to 10 °C before hydrochloric acid gas dissolved in 2-propanol (1.85 mL) was added in small portions until the pH reached 4. The resulting hydrochloride salt was filtered off, washed with diethyl ether and dried to yield to 110 mg (0.57 mmol, 81%) of the product; mp. 133.5–134.1 °C; purity > 98.8% (TFA derivative, GC-MS); MS-EI (*m/z*): 154.0 (100), 124.1 (26), 110.0 (28), 97.1 (16), 69.0 (11); ^{1}H NMR (400 MHz, D_2O) δ: 1.19 (d, J = 6.6 Hz, 3H), 2.62 (s, 3H), 2.74–3.08 (m, 2H), 3.41–3.51 (m, 1H), 6.81–7.74 (m, 5H); ^{13}C NMR (100 MHz, D_2O) δ: 14.8, 30.0, 38.5–39.0 (m), 56.4, 126.4–30.2 (m, 5C), 134.5–136.7 (m); HRMS (ES$^+$): calcd for $^{13}C_6C_4H_{16}N$ [M]$^+$: 156.1479; found 156.1491.

Synthesis of DL-[$^{13}C_6$]-4-methoxyamphetamine derivatives 2a–b

*Synthesis of [$^{13}C_6$]-anisole (**13**)*

[$^{13}C_6$]-Phenol (3.00 g, 30.0 mmol) was dissolved in water (30 mL). Sodium hydroxide (1.21 g, 30.3 mmol) was dissolved in water (20 mL) and added to the phenol solution under an argon atmosphere. The reaction was stirred for 30 min and was then cooled to 0 °C. Dimethyl sulfate (3.82 g, 30.3 mmol) was added slowly through a cannula and septum. The reaction mixture was gradually warmed to 50 °C and stirred at this temperature for 1 h. Then, the reaction mixture was extracted with dichloromethane (3 × 20 mL), and the combined organic phases were washed with 5% sodium hydroxide solution (20 mL) and brine (20 mL), and dried over magnesium sulfate. The solvent was evaporated to yield 3.38 g (29.7 mmol, 99%) of [$^{13}C_6$]-anisole (**13**) as a clear liquid. MS-EI (*m/z*): 114.1 (100), 99.1 (14), 84.1 (57), 70.1 (45); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.77 (d, J = 4.2 Hz, 3H), 6.61–6.79 (m, 1H), 7.00–7.19 (m, 1H), 6.7 (dm, 156 Hz, 1H), 7.47 (quin, 8.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.1 (dt, J = 6.6, 2.2 Hz), 113.1–114.9 (m, 2C), 120.6 (tdt, J = 56.1, 9.1, 2.8 Hz), 129.5 (tdd, J = 56.4, 9.4, 2.2 Hz, 2C), 159.6 (td, J = 66.7, 9.1 Hz).

*Synthesis of 4-bromo-[$^{13}C_6$]-anisole (**14**)*

[$^{13}C_6$]-Anisole (**3**) (3.03 g, 26.6 mmol) was dissolved in chloroform (15 mL) and *N,N*-dimethylformamide (2 mL). Then, *N*-bromosuccinimide (4.73 g, 26.6 mmol) was added in small portions at 70 °C with intensive magnetic stirring. Upon reaction completion, more chloroform (50 mL) was added, and the

organic phase was washed with water (50 mL). The solvent was evaporated, and the crude product was purified by bulb-to-bulb distillation (70 °C, 1.7 mbar^{-1}) yielding 4.21 g (21.8 mmol, 82%) of clear oil. MS-EI (*m/z*): 194.0 (100), 192.0 (100), 179.0 (45), 177.0 (45), 150.0 (36), 148.0 (36); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.77 (d, *J* = 4.2 Hz, 3H), 6.56 (dm, *J* = 169.0 Hz, 2H), 7.15 (dm, *J* = 169.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.4 (td, *J* = 6.6, 2.2 Hz), 112.8 (tdt, *J* = 64.7, 9.5, 1.8 Hz), 115.7 (tm, *J* = 64.7, 6.6 Hz, 2C), 132.2 (tm, *J* = 62.7, 2.2 Hz, 2C), 158.7 (td, *J* = 68.1, 9.5 Hz).

*Synthesis of [$^{13}C_6$]-4-methoxyphenyl-2-propanone (**15**)*

4-Bromo-[$^{13}C_6$]-anisole (**14**) (1.00 g, 5.18 mmol) was dissolved in toluene (20 mL) in a 100-mL reaction flask with a stirring bar and septum. Tri-(*o*-tolyl)phosphine (94 mg, 0.31 mmol), tributyltinmethoxide (2.48 g, 7.72 mmol) and *iso*-propenyl acetate (0.79 g, 27.2 mmol) were added, and the flask was flushed with argon by the aid of ultrasound degassing and vacuum. Palladium(II) chloride (27 mg, 0.15 mmol) was added rapidly under a blanket of argon, and the mixture was heated at 90 °C for 4 h. The solvent was then evaporated under vacuum, and the crude product was purified by dry flash chromatography on a plug of silica gel (60 mL) using heptane/ethyl acetate (85/15). This gave 873 mg (5.13 mmol, 99%) of [$^{13}C_6$]-4-methoxyphenyl-2-propanone as an oil; purity > 99% (GC-MS). MS-EI (*m/z*): 170.1 (16), 127.1 (100), 96.1 (5), 83.1 (10); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.13 (s, 3H), 3.62 (q, *J* = 5.4 Hz, 2H), 3.78 (d, *J* = 4.2 Hz, 3H), 6.62–6.72 (m, 1H), 6.90 (q, *J* = 8.7 Hz, 1H), 7.01–7.11 (m, 1H), 7.25–7.36 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.1, 50.1 (ddt, *J* = 44.7, 7.5, 3.4 Hz), 55.2 (td, *J* = 4.0, 2.2 Hz), 113.4–115.0 (m, 2C), 126.2 (tdt, *J* = 58.1, 8.7, 2.2 Hz), 129.7–131.1 (m, 2C), 158.7 (td, *J* = 67.3, 8.7 Hz), 206.9.

*Synthesis of DL-[$^{13}C_6$]-4-methoxyamphetamine hydrochloride (DL-[$^{13}C_6$]-PMA-HCl, **2a**)*

4-Methoxy-[$^{13}C_6$]-phenyl-2-propanone (**15**) (300 mg, 1.76 mmol) was dissolved in methanol (10 mL), and ammonium acetate was added (1.36 g, 17.6 mmol) under an argon atmosphere. To this solution was added sodium cyanoborohydride (113 mg, 1.8 mmol) in small portions under stirring, and the reaction was further stirred overnight at 22 °C. Then, the solvent was evaporated, and hydrochloric acid (3 M, 30 mL) was added. The water phase was then washed with diethyl ether (30 mL) and basified by addition of a concentrated sodium hydroxide solution until pH was 10. The product free base was extracted from the aqueous phase with diethyl ether (3 × 30 mL). The combined organic phases were dried over magnesium sulfate, and the solvent was evaporated yielding DL-[$^{13}C_6$]-4-methoxyamphetamine free base. The compound was dissolved in anhydrous diethyl ether (10 mL), and hydrochloric acid gas was slowly bubbled through the solution until pH was 4. The solvent was evaporated and the product recrystallized twice from acetonitrile yielding 233 mg (1.12 mmol, 64%) of [$^{13}C_6$]-PMA hydrochloride as white crystals; mp. 200.6–201.2 °C; purity 99% (GC-MS); MS-EI of TFA derivative (*m/z*): 267.1 (8), 154.1 (35), 127.1 (100); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.09 (d, *J* = 6.6 Hz, 3H), 2.54–2.65 (m, 2H), 2.85–2.96 (m, 1H), 3.74 (d, *J* = 4.3 Hz, 3H), 6.66–6.74 (m, 1H), 6.91–7.02 (m, 1H), 7.06–7.14 (m, 1H), 7.29–7.39 (m, 1H), 7.93 (br.s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 17.7, 48.1, 55.9, 113.9 (ddd, *J* = 65.9, 55.5, 8.3 Hz, 2C), 127.6–131.1 (m, 3C), 158.1 (td, *J* = 66.6, 8.1 Hz); the benzylic carbon was not detected in ^{13}C NMR of the [$^{13}C_6$]-labelled compound; HRMS (ES$^+$): calcd for $^{13}C_6C_4H_{16}NO$ [M]$^+$: 172.1428; found 172.1441.

*Synthesis of DL-[$^{13}C_6$]-4-methoxymethamphetamine hydrochloride (DL-[$^{13}C_6$]-PMMA-HCl, **2b**)*

4-Methoxy-[$^{13}C_6$]-phenyl-2-propanone (**15**) (300 mg, 1.76 mmol) was dissolved in methanol (10 mL), and molecular sieves 4 Å (200 mg) were added together with methylamine (322 mg, 3.53 mmol) in ethanol (33 wt.%). The reaction was stirred for 2 h, followed by addition of sodium borohydride (84 mg, 2.22 mmol) in small portions. Then, the reaction mixture was stirred overnight, and the quenched by adding 3 M hydrochloric acid (50 mL) followed by additional stirring for 1 h. The water phase was washed with diethyl ether (30 mL) and basified with 50% sodium hydroxide solution (pH 11). The product was extracted from the water phase with diethyl ether (3 × 30 mL). The organic phase was washed with brine (30 mL) and dried over magnesium sulfate. The solvent was evaporated yielding DL-[$^{13}C_6$]-4-methoxymethamphetamine free base, which was dissolved in anhydrous diethyl ether (10 mL), and hydrochloric acid gas was slowly bubbled through the solution until pH was 4. The solvent was evaporated, and the solid obtained was recrystallized twice from 2-propanol/diethyl ether yielding 292 mg (1.32 mmol, 75%) of [$^{13}C_6$]-PMMA hydrochloride as white crystals; mp. 176.6–176.7 °C; purity > 99% (GC-MS). MS-EI of TFA derivative (*m/z*): 281.1 (3), 154.1 (100), 127.1 (65), 110.1 (15); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.08 (d, *J* = 6.6 Hz, 3H), 2.57 (s, 3H), 2.54–2.62 (m, 2H), 3.01–3.11 (m, 1H), 3.74 (d, *J* = 4.0 Hz, 3H), 6.66–6.76 (m, 1H), 6.91–7.04 (m, 1H), 7.06–7.15 (m, 1H), 7.30–7.42 (m, 1H), 8.69 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 17.7, 40.8, 58.3, 59.5, 114.0 (ddd, *J* = 65.9, 55.5, 8.3 Hz, 2C), 127.4–131.2 (m, 3C), 158.1 (td, *J* = 66.6, 8.1 Hz); the CH_3-N signal could not be found; HRMS (ES$^+$): calcd for $^{13}C_6C_5H_{18}NO$ [M]$^+$: 186.1584; found 186.1598.

Synthesis of DL-[$^{13}C_6$]-3,4-methylenedioxyamphetamine derivatives 3a–c

*Synthesis of [$^{13}C_6$]-o-salicylaldehyde (**8**)*

Magnesium turnings (627 mg, 25.8 mmol) were added to a three-necked round-bottom flask together with methanol (7.8 mL) and a small iodine crystal. When all the metal had dissolved, the solution was cooled to 40 °C, and [$^{13}C_6$]-phenol (5.00 g, 49.9 mmol) was added slowly as a solution in toluene (40 mL) under argon atmosphere. The reaction mixture was warmed to 75 °C, and most of the methanol was distilled out. When the temperature reached 95 °C, paraformaldehyde (4.60 g, 155 mmol) was added in small portions, and the temperature was controlled at 95 °C. Vacuum was applied for short intervals to assure that the methanol was distilled out continuously, and toluene was added to maintain solvent volume. After 30 min at 100 °C, full conversion was confirmed by TLC analysis (heptane/acetone, 6/4). The mixture was cooled to 20 °C, 10% sulfuric acid solution (90 mL) was added and the product was extracted with toluene (3 × 30 mL). The combined organic phases was washed with water and dried over magnesium sulfate. After solvent evaporation, the residue was dry flashed with silica gel (heptane/acetone, 4/1) yielding 5.25 g (40.97 mmol 95%) of [$^{13}C_6$]-*o*-salicylaldehyde; purity: 98% (GC-MS); MS-EI (*m/z*): 128.1 (100), 127.1 (99), 110.1 (17), 99.1 (20), 82.1 (15), 70.1 (24); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.76–6.88 (m, 1H), 7.14–7.41 (m, 2H), 7.68–7.81 (m, 1H), 9.86–9.95 (dm, *J* = 19.9 Hz, 1H), 11.01–11.07

(m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 117.5 (dddt, *J* = 66.4, 58.3, 6.6, 2.9 Hz), 119.8 (tm, *J* = 57.1 Hz), 120.6 (tm, *J* = 58.9 Hz), 133.7 (tdd, *J* = 58.8, 6.5, 1.5 Hz), 136.9 (dddd, *J* = 58.3, 54.5, 6.5, 1.5 Hz), 161.6 (ddd, *J* = 66.4, 60.8, 8.2 Hz), 196.5 (dtd, *J* = 54.5, 5.4, 2.2 Hz).

*Synthesis of 1,2-dihydroxy-[$^{13}C_6$]-benzene (**9**)*

To a stirred solution of [$^{13}C_6$]-*o*-salicylaldehyde (**8**) (4.87 g, 38.0 mmol) in water (50 mL) under an argon atmosphere was rapidly added sodium hydroxide (1.52 g, 38.0 mmol) in water (50 mL). A solution of hydrogen peroxide (4.63 g, 47.6 mmol, 35%) in water was then added slowly from a dropping funnel, and the temperature was controlled with a water bath so that the temperature did not exceed 40 °C. The reaction was then stirred at 30 °C for 30 min before quenching with 15% sulfuric acid solution to pH 3–4. The water phase was then saturated with magnesium sulfate, and the product was extracted out with diethyl ether (5 × 50 mL). The organic phase was dried with magnesium sulfate and stirred overnight with active carbon (0.5 g). The black suspension was filtered, the solvent evaporated and the residue was crystallized from toluene (30 mL). The crystals were then sublimated (105 °C, 1.4 mbar) to a final yield of 2.77 g (23.9 mmol, 63%) as a white crystalline solid; mp. 104.7–105.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.26–6.57 (m, 2H), 6.68–6.98 (m, 2H), 8.76 (br.s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 114.4–116.8 (m, 2C), 118.3–120.1 (m, 2C), 145.2 (dtt, *J* = 27.3, 23.0, 3.6 Hz, 2C).

*Synthesis of 3,4-methylenedioxy-[$^{13}C_6$]-benzene (**10**)*

To a stirred solution of 1,2-dihydroxy-[$^{13}C_6$]-benzene (**9**) (2.76 g, 23.8 mmol) in dimethylsulfoxide (30 mL), cesium carbonate (15.50 g, 47.6 mmol) was added under an argon atmosphere. The reaction was then stirred at 80 °C for 2 h. The reaction was quenched by ice water (120 mL), and then, the product was steam distilled from the water suspension by two 100-mL water portions. The water/product distillate was extracted with diethyl ether (3 × 50 mL). The combined ether extracts were dried over magnesium sulfate, and the diethyl ether was evaporated. This yielded 3.04 g (23.8 mmol, 99%) based on 1,2-dihydroxy-[$^{13}C_6$]-benzene. MS-EI (*m/z*): 128 (62), 127 (100), 68.1 (20); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.91 (t, *J* = 2.0 Hz, 2H), 6.49–6.81 (m, 2H), 6.82–7.11 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 100.6 (t, *J* = 3.5 Hz), 107.5–109.8 (m, 2C), 120.8–122.5 (m, 2C), 146.5–148.3 (m, 2C).

*Synthesis of 3,4-methylenedioxy-[$^{13}C_6$]-1-bromobenzene (**11**)*

3,4-Methylenedioxy-[$^{13}C_6$]-benzene (**10**) (3.04 g, 23.8 mmol) was dissolved in chloroform (15 mL). *N*-Bromosuccinimide (4.23 g, 23.8 mmol) was added in small portions with intensive magnetic stirring. The reaction mixture was refluxed for 2 h. The obtained solids were removed by filtration and washed with two small portions of cold chloroform. The combined organic fractions were evaporated, and diethyl ether (150 mL) was added before washing with water (100 mL). The organic phase was purified by dry flash chromatography on a plug of silica gel in a Büchner funnel using diethyl ether as the mobile phase. The yield of 3,4-methylenedioxy-[$^{13}C_6$]-1-bromobenzene was 4.38 g (21.2 mmol, 89%) based on methylenedioxy-[$^{13}C_6$]-benzene. MS-EI (*m/z*): 206.0 (100), 147.1 (5), 68.1 (35); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.96 (t, *J* = 2.0 Hz, 2H), 6.42–7.20 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 101.6 (t, *J* = 3.1 Hz), 108.7–110.3 (m), 111.3–113.9 (m, 2C), 123.45–125.1 (m), 146.1–149.5 (m, 2C).

*Synthesis of 3,4-methylenedioxy-[$^{13}C_6$]-phenyl-2-propanone (**12**)*

3,4-Methylenedioxy-[$^{13}C_6$]-1-bromobenzene (**11**) (3.66 g, 17.7 mmol) was dissolved in toluene (30 mL) in a 100-mL reaction flask with a stirring bar and septum. Tri-(*o*-tolyl)phosphine (322 mg, 1.06 mmol), tributyltinmethoxide (8.51 g, 26.5 mmol) and *iso*-propenyl acetate (2.72 g, 27.17 mmol) were added, and argon was flushed in by the aid of ultrasound degassing and vacuum. Palladium(II) chloride (94 mg, 0.53 mmol) was added rapidly under a blanket of argon. The reaction mixture was then heated at 90 °C for 4 h, followed by evaporation of solvent. The crude product was purified by dry flash chromatography on a plug of silica gel in a Büchner funnel using heptane/ethyl acetate (85/15) as the mobile phase. The yield of 3,4-methylenedioxy-[$^{13}C_6$]-phenyl-2-propanone (**12**) was 2.95 g (16.0 mmol, 91%) as a colourless oil. MS-EI (*m/z*): 184.1 (35), 141.1 (100), 111.1 (5), 82.1 (11); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.14 (s, 3H), 3.59 (q, *J* = 5.9 Hz, 2H), 5.93 (t, *J* = 2.0 Hz, 2H), 6.39–7.03 (m, HC); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.1, 50.2–50.9 (m), 101.3 (t, *J* = 3.7 Hz), 107.6–110.5 (m, 2C), 122.5 (dddd, *J* = 61.8, 57.8, 6.6, 1.5 Hz), 127.8 (dddt, *J* = 61.8, 59.1, 7.3, 2.2 Hz), 146.6 (ddtd, *J* = 66.0, 65.5, 7.3, 1.5 Hz), 147.9 (ddtd, *J* = 69.7, 65.5, 8.1, 2.2 Hz), 206.4.

*Synthesis of DL-[$^{13}C_6$]-3,4-methylenedioxyamphetamine hydrochloride (DL-[$^{13}C_6$]-MDA-HCl, **3a**)*

3,4-Methylenedioxy-[$^{13}C_6$]-phenyl-2-propanone (**12**) (313.4 mg, 1.70 mmol) was dissolved in methanol (10 mL), and ammonium acetate was added (1.31 g, 17.0 mmol). An argon atmosphere was introduced at this stage. To this solution was added sodium cyanoborohydride in small portions under stirring (113 mg, 1.80 mmol). The reaction was then stirred overnight at room temperature. The solvent was evaporated, and hydrochloric acid (3 M, 30 mL) was added. The water phase was then washed with diethyl ether (30 mL) and basified by addition of a concentrated sodium hydroxide solution until pH was 10. The product free base was extracted from the aqueous phase with diethyl ether (3 × 30 mL). The combined organic phases were dried over magnesium sulfate, and the solvent was evaporated yielding DL-[$^{13}C_6$]-MDA free base (394 mg). This was dissolved in anhydrous diethyl ether (10 mL), and hydrochloric acid gas was slowly bubbled trough the solution until pH was 4. The solvent was evaporated, and the obtained solid was recrystallized twice from acetonitrile yielding 122.0 mg (0.55 mmol, 32%) [$^{13}C_6$]-MDA hydrochloride as glistening white crystals; mp. 181.1–181.6 °C; purity > 99% (MS-EI, TFA derivative); MS-EI (*m/z*): 281.1 (25), 168.1 (46), 141.1 (100), 111.0 (5), 82.1 (10); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.11 (d, *J* = 6.3 Hz, 3H), 2.55–2.66 (m, 1H), 2.81–2.93 (m, 1H), 3.59 (tq, *J* = 7.2, 6.3 Hz, 1H), 5.99 (t, *J* = 2.0 Hz, 2H), 6.40–7.21 (m, 3H), 7.93 (br.s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 17.7, 50.2–50.9 (m), 100.9 (t, *J* = 3.6 Hz), 107.6–110.5 (m, 2C), 122.8 (ddd, *J* = 60.0, 57.1, 7.3 Hz), 130.7 (dddt, *J* = 60.0, 58.3, 7.3, 2.2 Hz), 145.7–148.7 (m, 2C); the benzylic carbon was not detected in ^{13}C NMR of the [$^{13}C_6$]-labelled compound/within solvent region; HRMS (ES^+): calcd for $^{13}C_6C_4H_{14}NO_2$ $[M]^+$: 186.1220; found 186.1234.

*Synthesis of DL-[$^{13}C_6$]-3,4-methylenedioxy-N-methylamphetamine hydrochloride (DL-[$^{13}C_6$]-MDMA-HCl, **3b**) from **3a***

DL-[$^{13}C_6$]-MDA (**3a**) as a free base (100 mg, 0.54 mmol) was dissolved in ethyl formate (10 mL) and heated in an ACE pressure reactor for 2 h at 100 °C. The reaction mixture was cooled to 22 °C, and the solvent was evaporated under reduced pressure. The

carbamate derivative obtained was dissolved in dry diethyl ether (5 mL) and added drop wise to a suspension of lithium aluminium hydride (31 mg, 0.81 mmol) in diethyl ether (10 mL). The reaction mixture was refluxed for 5 h and cooled to 0 °C, and then, water was added carefully. The lithium salt formed was filtered through Celite™ on a Büchner funnel, and the granules were washed thoroughly using an excess of diethyl ether. The ether phases were combined and washed with 5% sodium hydroxide and brine before drying over magnesium sulfate. The solvent was evaporated to yield **3b** as a free base (96 mg). The product was re-dissolved in diethyl ether and cooled down to 10 °C before hydrochloric acid dissolved in 2-propanol (1.85 M) was added in small portions until the pH was 4. The salt formed was filtered off, washed with diethyl ether and dried to yield DL-[$^{13}C_6$]-MDMA hydrochloride (110 mg, 0.47 mmol, 86%) based on DL-[$^{13}C_6$]-MDA; mp. 249.5–250.1 °C; purity > 99% (GC-MS, TFA derivative). MS-EI of TFA derivative (*m/z*): 295.1 (16), 168.1 (86), 154.0 (100), 141.1 (59), 110.0 (30), 82.1 (10); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.09 (d, *J* = 6.6 Hz, 3H), 2.55 (br.s, 4H), 2.97–3.13 (m, 1H), 3.26–3.30 (m, 1H), 6.01 (s, 2H), 6.40–7.20 (m, 3H), 8.84 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 17.8, 29.6, 50.2–50.9 (m), 100.9 (t, *J* = 3.6 Hz), 107.7–110.6 (m, 2C), 122.4 (dddd, *J* = 61.8, 57.8, 6.6, 1.5 Hz), 130.2 (dddd, *J* = 61.8, 59.1, 7.3, 2.2 Hz), 146.0 (tt, *J* = 65.4, 7.1 Hz), 146.7 (tt, *J* = 65.1, 7.1); the benzylic carbon was not detected in ^{13}C NMR of the [$^{13}C_6$]-labelled compound/within solvent region; HRMS (ES$^+$): calcd for $^{13}C_6C_5H_{14}NO_2$ [M]$^+$: 200.1377; found 200.1391.

*Synthesis of DL-[$^{13}C_6$]-3,4-methylenedioxy-N-methylamphetamine hydrochloride (DL-[$^{13}C_6$]-MDMA-HCl, **3b**) from **12***

3,4-Methylenedioxy-[$^{13}C_6$]-phenyl-2-propanone (**12**) (500 mg, 2.72 mmol) was dissolved in methanol (10 mL), and molecular sieves 4 Å (1.00 g) were added together with methylamine (511 mg, 5.43 mmol) in ethanol (33 wt.%). The reaction was stirred for 2 h. Sodium borohydride (130 mg, 3.44 mmol) was added in small portions, and the reaction mixture was stirred overnight. Water was added (60 mL) followed by careful addition of hydrochloric acid (3 M, 50 mL), and the reaction was stirred for 1 h. The water phase was washed with diethyl ether (30 mL) and basified with 50% sodium hydroxide solution (pH 11). The product was extracted from the water phase with diethyl ether (3 × 30 mL), and organic phase was washed with brine (30 mL) and dried over magnesium sulfate. The solvent was evaporated yielding DL-[$^{13}C_6$]-3,4-methylenedioxy-*N*-methylamphetamine free base, which was purified by short path vacuum distillation to (533 mg, 2.67 mmol). The product was re-dissolved in diethyl ether and cooled down to 10 °C before hydrochloric acid dissolved in 2-propanol (1.85 M) was added in small portions until the pH was 4. The salt formed was filtered off, washed with acetone (5 mL) and diethyl ether (10 mL), recrystallized from isopropanol/ether and dried to yield DL-[$^{13}C_6$]-MDMA hydrochloride as white needles (540 mg, 2.29 mmol); purity 99% (GC-MS, TFA derivative); the product had the same chromatographic and spectroscopic data as that described earlier.

*Synthesis of DL-[$^{13}C_6$]-3,4-methylenedioxy-N-ethylamphetamine hydrochloride (DL-[$^{13}C_6$]-MDEA-HCl, **3c**)*

3,4-Methylenedioxy-[$^{13}C_6$]-phenyl-2-propanone (**12**) (338 mg, 1.84 mmol) was dissolved in methanol (10 mL), and molecular sieves 4 Å (1.00 g) were added together with ethylamine (752 mg, 3.67 mmol) in ethanol (22 wt.%). The reaction was stirred for 2 h. Sodium borohydride (87 mg, 2.30 mmol) was added in small portions, and the reaction mixture was stirred overnight. Hydrochloric acid (3 M, 50 mL) was added, and the reaction was stirred for 1 h. The water phase was washed with diethyl ether (30 mL) and basified with 50% sodium hydroxide solution (pH 11). The product was extracted from the water phase with diethyl ether (3 × 30 mL), and organic phase was washed with brine (30 mL) and dried over magnesium sulfate. The solvent was evaporated yielding DL-[$^{13}C_6$]-MDEA free base. This was dissolved in anhydrous diethyl ether (10 mL), and hydrochloric acid gas was slowly bubbled through the solution until pH was 4. The solvent was evaporated, and the solid obtained was recrystallized twice from 2-propanol/diethyl ether (15 mL/6 mL) yielding 260 mg (1.04 mmol, 58%) [$^{13}C_6$]-MDEA hydrochloride as white crystals; mp. 200.2 °C (dec); purity 99% (GC-MS); MS-EI of TFA derivative (*m/z*): 309.1 (9), 168.1 (100), 140.0 (24), 82.1 (6); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.09 (d, *J* = 6.6 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H), 2.09 (s, 2H), 2.92–3.13 (m, 3H), 6.01 (t, *J* = 2.0 Hz, 2H), 6.41–7.18 (m, 3H), 8.61 (br.s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.7, 17.7, 32.7, 50.7, 100.9 (t, *J* = 3.6 Hz), 107.9–110.7 (m, 2C), 122.8 (dddd, *J* = 61.2, 57.1, 7.3, 1.5 Hz), 130.7 (dddt, *J* = 61.2, 59.1, 7.3, 2.2 Hz), 145.6–148.7 (m, 2C); the benzylic carbon was not detected in ^{13}C NMR of the [$^{13}C_6$]-labelled compound/within solvent region; HRMS (ES$^+$): calcd for $^{13}C_6C_6H_{18}NO_2$ [M]$^+$: 214.1533; found 214.1548.

Synthesis of 2,5-dimethoxy-[$^{13}C_6$]-phenethylamine derivatives 4a, 5a and 6a

*Synthesis of 1,4-dimethoxy-[$^{13}C_6$]-benzene (**16**)*

4-Bromo-[$^{13}C_6$]-anisole (1.30 g, 6.73 mmol) (**14**) was dissolved in methanol (10 mL) and *N,N*-dimethylformamide (10 mL). To this solution, freshly made sodium methoxide (10 mL, 5 M) was added together with ethyl acetate (2 mL). Copper iodide (1.4 g, 7.4 mmol) was added and the reaction stirred at 80 °C in a sealed tube under an argon atmosphere for 48 h. Sulfuric acid (aq 10%, 100 mL) was added, and the product was extracted with diethyl ether (3 × 30 mL). The organic phase was washed with water (2 × 20 mL) and dried over magnesium sulfate. The solvent was evaporated yielding 837 mg (5.81 mol, 86%) of 2,4-dimethoxy-[$^{13}C_6$]-benzene as white platelets after drying; purity: 99% (GC-MS); MS-EI (*m/z*): 144.1 (80), 129.1 (100), 114.1 (3), 100.1 (30), 85.1 (5), 69.1 (8); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.77 (d, *J* = 4.3 Hz, 6H), 6.62 (dm, *J* = 160.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.8–55.9 (m, 2C), 113.7–115.5 (m, 4C), 152.5–155.0 (m, 2C).

*Synthesis of 2,4-dimethoxy-[$^{13}C_6$]-benzaldehyde (**17**)*

2,4-Dimethoxy-[$^{13}C_6$]-benzene (**16**) (837 mg, 5.81 mmol) was dissolved in dichloromethane (50 mL) in a septum-sealed flask, and dichloromethyl methyl ether (667 mg, 5.8 mmol) was added. This solution was cooled to −5 °C, and argon was flushed in. Titanium tetrachloride (1.82 g, 9.60 mmol) was added slowly with good stirring through a cannula. After 1 h at −5 °C, the reaction was allowed to stir for 16 h at 4 °C. After this time, the reaction mixture was quenched rapidly over crushed ice and allowed to stir for 1 h. The product was extracted with dichloromethane (3 × 30 mL), and the combined organic phases were washed with brine (20 mL) and dried over magnesium sulfate. The solvent was evaporated and the crude product purified by silica-gel flash chromatography (toluene/ethyl acetate, 92/8), followed by crystallization from pentane. This gave 912 mg (5.30 mmol,

91%) of 2,4-dimethoxy-[$^{13}C_6$]-benzaldehyde as white needles; purity: 99% (GC-MS); MS-EI (*m/z*): 172.1 (100), 157.0 (32), 143.0 (10), 126.1 (18), 112.1 (15), 100.1 (20); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.82 (d, *J* = 4.2 Hz, 3H) 3.91 (d, *J* = 4.0 Hz, 3H), 6.94 (dm, *J* = 152.8 Hz, 1H), 7.15 (dm, *J* = 159.3 Hz, 1 H), 7.34 (dm, *J* = 154.1 Hz, 1H), 10.46 (ddd, *J* = 22.5, 3.0, 1.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.8 (td, *J* = 4.0, 2.2 Hz), 56.2 (td, *J* = 4.8, 2.7 Hz), 110.4 (dddt, *J* = 69.5, 62.3, 4.5, 1.5 Hz), 113.3 (ddt, *J* = 66.5, 60.40, 4.4 Hz), 123.5 (ddt, *J* = 68.0, 60.5, 4.7 Hz), 124.9 (ddtd, *J* = 69.7, 62.3, 4.5, 1.5 Hz), 153.6 (dddd, *J* = 69.5, 68.0, 7.0, 1.5 Hz), 156.7 (dddd, *J* = 69.7, 66.3, 7.0, 1.5 Hz), 189.1–189.9 (m).

*Synthesis of 2-nitro-1-propene-1-yl-2,4-dimethoxy-[$^{13}C_6$]-benzene (**18**)*

2,4-Dimethoxy-[$^{13}C_6$]-benzaldehyde (**17**) (130 mg, 0.76 mmol) was dissolved in nitromethane (10 mL), and ammonium acetate (33.5 mg, 0.44 mmol) was added. The reaction was warmed at 70 °C for 2 h. After this, the reaction was poured over a plug of silica gel (50 mL) wetted with heptanes in a Büchner funnel, and the product was eluted with (20 mL) fractions of toluene. The fractions containing the product were concentrated yielding 166 mg (0.77 mmol, ≥100%) as yellow crystals. Some trace amount of toluene was detected by ^{1}H NMR. MS-EI (*m/z*): 215.1 (100), 168.0 (45), 153 (36), 139.0 (45), 124.0 (15), 110.0 (15), 95.1 (10), 81.0 (17); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.80 (d, *J* = 4.3 Hz, 3H), 3.90 (d, *J* = 4.2 Hz, 3H), 6.69–7.28 (m, 3H), 7.85 (dd, *J* = 13.6 Hz, 1H), 8.12 (dt, *J* = 13.6, 5.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 55.8 (td, *J* = 4.0, 2.2 Hz), 56.0 (td, *J* = 4.8, 2.7 Hz), 112.4 (ddt, *J* = 68.3, 60.0, 4.7 Hz), 116.2 (ddtd, *J* = 68.8, 62.9, 4.7, 2.1 Hz), 119.1 (ddt, *J* = 67.5, 60.0, 4.6 Hz), 119.5 (ddtd, *J* = 69.8, 62.9, 5.1, 1.6 Hz), 153.5 (dddd, *J* = 68.8, 67.5, 7.1, 1.6), 154.0 (dddd, *J* = 69.8, 68.3, 7.1, 2.1 Hz). The sp^2 carbons residing at 135 and 138 ppm in the unlabelled compound could not be observed in this analysis.

*Synthesis of 2-nitro-1-propane-1-yl-2,4-dimethoxy-[$^{13}C_6$]-benzene (**19**)*

Sodium borohydride (92 mg, 2.44 mmol) was dissolved in a mixture of ethyl acetate (10 mL) and ethanol (2 mL) and cooled to 0 °C. Then, 2-nitro-1-propene-1-yl-2,4-dimethoxy-[$^{13}C_6$]-benzene (**18**) (175 mg, 0.81 mmol) was added slowly as a solution in ethyl acetate (5 mL). The reaction was stirred at 22 °C for 1 h, and a water solution of sulfuric acid (30 mL, 10%) was added. The product was isolated by extraction with diethyl ether (2 × 20 mL), and the combined organic phases were washed with brine (20 mL) and dried over magnesium sulfate. The solvent was evaporated to give 160 mg (0.74 mmol, 91%) of yellow oil, which was pure enough to be used in the next step; purity: 97% (GC-MS); MS-EI (*m/z*): 217.1 (83), 170.1 (100), 156.1 (72), 141.1 (36), 127.1 (21), 111.1 (11); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.23–3.34 (m, 2H), 3.76 (d, *J* = 4.3 Hz, 3H), 3.81 (d, *J* = 4.3 Hz, 3H), 4.61 (td, *J* = 7.3, 3.5 Hz, 2H), 6.49–6.69 (m, 2H), 6.88–7.07 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.5, 55.7 (dd, *J* = 5.1, 2.2 Hz), 56.0 (td, *J* = 4.8, 2.7 Hz), 74.6, 111.1 (dddt, *J* = 66.2, 59.5, 4.7, 4.7, 1.9 Hz) 112.9 (ddtd, *J* = 67.2, 59.5, 4.5, 4.5, 2.5 Hz) 116.9 (ddtd, *J* = 68.2, 62.4, 4.5, 4.5, 1.9 Hz) 124.8 (ddt, *J* = 68.8, 62.4, 4.7, 4.7 Hz) 151.6 (dddd, *J* = 68.8, 67.2, 7.5, 1.9 Hz) 153.5 (dddd, *J* = 68.2, 66.2, 7.5, 2.5 Hz). This reaction was repeated to provide sufficient material for further synthesis.

*Synthesis of 2,5-dimethoxy-[$^{13}C_6$]-phenethylamine hydrochloride (**4a**)*

2-Nitro-1-propane-1-yl-2,4-dimethoxy-[$^{13}C_6$]-benzene (**19**) (240 mg, 1.11 mmol) was dissolved in methanol (20 mL), and palladium on carbon (10%, 72 mg) was added. Hydrogen gas was added to the mixture for 4 h. The reaction mixture was filtered using a plug of Celite™, and hydrochloric acid gas dissolved in methanol was added until the pH was 3, measured on a moist pH paper. The reaction mixture was evaporated, and the residue was recrystallized with 2-propanol (1 mL) and diethyl ether (3 mL). The crystals formed were then isolated by filtration and recrystallized from 2-propanol (2 mL) and ethanol (5 mL) yielding 218 mg (1.16 mmol, 88%) of 2,5-dimethoxy-[$^{13}C_6$]-phenethylamine hydrochloride salt as white needles; mp. 136.5–137.3 °C; purity > 99% (GC-MS); MS-EI of TFA derivative (*m/z*): 283.1 (100), 170.0 (92), 157.1 (45), 127.1 (49), 97.1 (11), 83.1 (7); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.77–2.88 (m, 2H), 2.88–3.02 (m, 2H), 3.70 (d, *J* = 4.3 Hz, 3H), 3.75 (d, *J* = 4.3 Hz, 3H), 6.78 (dm, *J* = 157.0 Hz, 2H), 6.90 (dm, *J* = 157.0 Hz, 1H), 7.97 (br.s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 39.0, 55.3, 55.8, 111.3–113.5 (m), 116.8 (tm, *J* = 64.2 Hz), 119.1 (tm, *J* = 64.9 Hz), 126.5 (tm, *J* = 65.5 Hz), 150.8–154.4 (m, 2C); the benzylic carbon could not be identified; HRMS (ES$^+$): calcd for $^{13}C_6C_4H_{16}NO_2$ [M]$^+$: 188.1377; found 188.1390.

*Synthesis of 4-bromo-2,5-dimethoxy-[$^{13}C_6$]-phenethylamine hydrochloride (**5a**)*

2,5-Dimethoxy-[$^{13}C_6$]-phenethylamine hydrochloride salt (**4a**) (103 mg, 0.46 mmol) was dissolved in acetic acid (10 mL). Bromine (73.5 mg, 0.46 mmol) was added as a solution in acetic acid (1 mL) over 30 min, and the reaction was stirred for 16 h at 22 °C. The reaction mixture was then poured onto a 15% sodium hydroxide solution (40 mL), and the product was extracted with dichloromethane (2 × 30 mL). The combined organic phases were washed with brine (20 mL) and dried over magnesium sulfate. The solvent was evaporated and the crude product dissolved in dry diethyl ether. The pH was adjusted with hydrochloric acid gas in 2-propanol to 4 where the product precipitated as a white solid. The solid was isolated by filtration and then recrystallized from acetonitrile containing some methanol. Filtration and drying gave 80.9 mg (0.27 mmol, 57%) of 4-bromo-2,5-dimethoxy-[$^{13}C_6$]-phenethylamine as the hydrochloride salt; mp. 224–225 °C (dec); purity > 99% (HPLC); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.79–2.87 (m, 2H), 2.93–3.02 (m, 2H), 3.77 (d, *J* = 4.0 Hz, 3H), 3.80 (d, *J* = 4,0 Hz, 3H), 6.06–7.94 (m, 2H), 7.96 (br.s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.2, 56.0, 108.8 (tm, *J* = 68.4 Hz), 115.4 (qm, *J* = 67.3 Hz, 2C), 125.4 (tm, *J* = 62.2 Hz), 149.3 (tm, *J* = 73.2 Hz), 151.5 (tm, *J* = 66.6 Hz); the two methylene carbons were not identified; NHRMS (ES$^+$): calcd for $^{13}C_6C_4H_{15}NO_2Br$ [M]$^+$: 266.0482; found 266.0493.

*Synthesis of 4-iodo-2,5-dimethoxy-[$^{13}C_6$]-phenethylamine hydrochloride (**6a**)*

2,5-Dimethoxy-[$^{13}C_6$]-phenethylamine hydrochloride (**4a**) (99.3 mg, 0.45 mmol) was dissolved in ethanol (10 mL) under argon atmosphere. To this solution was added under constant stirring silver sulfate (140.3 mg, 0.45 mmol) and iodine (114 mg, 0.45 mmol). The conversion halted after stirring for 24 h; thus, additional silver sulfate (140 mg, 0.45 mmol) and iodine (114 mg, 0.45 mmol) were added, followed by 24-h extended reaction time. The reaction mixture was then poured on a 5%

sodium hydroxide solution (40 mL), and the product was extracted with dichloromethane (2 × 30 mL). The combined organic phases were washed with brine (20 mL) and dried over magnesium sulfate. The solvent was evaporated, and the crude product dissolved in dry diethyl ether (10 mL). The pH was adjusted with hydrochloric acid gas in 2-propanol to 4 where the product precipitated as a white solid. This solid was re-dissolved in hot 2-propanol (10 mL) and acetonitrile (4 mL) and crystallized at 20 °C to remove unconverted starting material. The yield of 4-iodo-2,5-dimethoxy-[$^{13}C_6$]-phenethylamine hydrochloride was 75 mg (0.21 mmol, 48%) as fluffy white needles; mp. 244.0 °C (dec); purity 99% (HPLC); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.79–2.87 (m, 2H), 2.93–3.02 (m, 2H), 3.77 (d, J = 4,0 Hz, 3H), 3.80 (d, J = 4,0 Hz, 3H), 6.63–7.58 (m, 2H), 7.86 (br.s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 39.1, 55.3, 55.6, 113.9 (tm, J = 64.2 Hz), 119.1 (tm, J = 62.2 Hz), 121.3 (tm, J = 68.1 Hz), 126.3 (tm, J = 66.6 Hz), 151. 9 (tm, J = 62.2 Hz, 2C); the benzylic carbon could not be identified; HRMS (ES$^+$): calcd for $^{13}C_6C_4H_{15}NO_2I$ [M]$^+$: 314.0343; found 314.0357.

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Conflict of Interest

The authors did not report any conflict of interest.

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