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Research Article

Easy preparative scale syntheses of labelled xanthines: Caffeine, theophylline and theobromine

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Abstract: Several easy preparative scale (0.5–1.5 g) syntheses of deuterium labelled caffeine, theophylline and theobromine are described. Some new selective syntheses of theophylline and theobromine have been developed. Labelled xanthines are of great interest in qualitative or quantitative isotope dilution-mass spectrometry, coupled with gas or liquid chromatography, currently performed in anti-doping and forensic laboratories. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: caffeine; theophylline; theobromine; labeled; deuterium

Introduction

The presence of natural xanthines (caffeine, theophylline and theobromine, Figure 1) in biological samples is regulated by International Racing Rules and/or International Sports Authorities. In the horse, caffeine and theophylline are prohibited and for theobromine, a concentration threshold at 2.0 μg/ml in urine has been established.

The use of labelled xanthines as internal standards is recommended for analytical purposes, both in qualitative screening and quantitative analysis in body fluids. As no commercial source was available, it was decided to prepare them. These labelled xanthines should be chemically and isotopically pure (preferably a single isotopomer), highly enriched, with none or trace amounts of unlabelled compound.

As the internal standard may be exposed to strong acid or base during the analytical procedure, labelling should be made on non-exchangeable positions. In the case of methylated xanthines, substitution of one or more N-CH_3 by N-CD_3 is the most appropriate.

The mass spectra of labelled and unlabelled species should exhibit the least possible common fragment ions in order to reduce risk of interference during analyses of biological matrices. The only difference between the mass spectra of N-1 labelled and unlabelled caffeine is at the molecular ion mass.[1] Therefore, caffeine should be labelled at least on N-3 or N-7. Likewise, mass spectra of N-1 and N-3 labelled theophylline[2] indicated that the only difference between N-1 labelled and unlabelled theophylline was the molecular ion mass, labelling of theophylline should be performed at least on N-3. As theobromine exhibits a similar fragmentation pattern, this compound should also be labelled on N-3 or N-7.

Results and discussion

Caffeine

Two different syntheses of labelled caffeine have been published: a total synthesis from [^{15}N]urea[3] and a direct methylation of xanthine (or its methyl derivatives) with $^{13}CH_3I$[4,5] or with CD_3I.[6–9] This direct methylation is obviously the most direct way to caffeine, but a review of the literature highlights several drawbacks: expensive starting materials[4,6–8] use of a large excess of labelled iodomethane,[4,9] low to moderate yields,[6,7,9] reaction leading to a mixture of several methylated xanthines difficult to separate,[5–7] and purification methods incompatible with large-scale synthesis.[5,6]

Because only one equivalent of CH_3I is theoretically required to generate caffeine via N-7 methylation, theophylline **1** was chosen as the starting material. Among the specific N-7 alkylation methods,[4,6,8,10–22] the RX/K_2CO_3/DMF system is the most popular and

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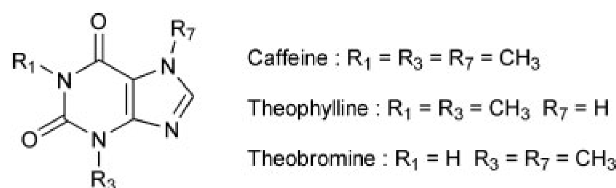

Figure 1 Natural xanthines.

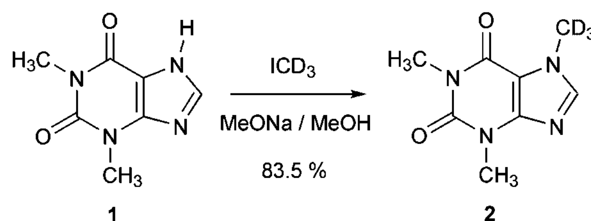

Figure 2 Synthesis of caffeine-*d3*.

seems to be a general one. However, the toxicity of DMF and its high boiling point led us to develop an alternative alkylating process. Caffeine-*d3* **2** was prepared by alkylation of theophylline with CD_3I (1.5 equivalent) in methanol in the presence of sodium methylate (Figure 2).

The disappearance of the 3H signal at 3.93 ppm indicates complete labelling of the N-7 methyl, in good agreement with published data.[6] The mass spectrum of caffeine-*d3* exhibits a 3 amu shift for all characteristic fragments, in good agreement with the fragmentation pattern of labelled caffeine.[1]

Theophylline

Three synthetic methods for labelled theophylline have been published. The first method proceeds via hydrogen/tritium exchange in presence of palladium leading to low isotope enrichment (12%).[23] The second method proceeds via a N-7 pivaloyloxymethyl protection of 1-or 3-methylxanthine followed by alkylation of the unsubstituted nitrogen atom with CD_3I and subsequent removal of the protecting group.[2] The third method consists of the direct methylation of xanthine using $^{13}CH_3I/CH_3I$, leading to a mixture of mono-, di- and trimethylxanthines, which were separated.[5]

Unfortunately, these methods are not suitable for large-scale synthesis for the following reasons: low yield,[2] reactions leading to a mixture of substituted xanthines and purification process incompatible with large-scale preparation (preparative TLC[2] and semi-preparative liquid chromatography).[5]

Several selective syntheses of theophylline starting from 6-amino-uracil derivatives have also been published.[20,24–27] Likewise, numerous syntheses of 1,3-disubstituted xanthine have been described.[10–13, 28–30]

The first procedure we tested is an improvement of the historic Traube xanthine synthesis starting from 6-aminouracil[24] (Figure 3). Indeed, this compound is inexpensive and, if needed, can easily be prepared.[24,31,32] The 5-nitroso derivative **5** was prepared from 6-aminouracil **3** according to a classical procedure[14,24,30,33] and reduced to the corresponding air-sensitive 1,3-dimethyl-5,6-diamino-uracil-*d6* **6** via catalytic hydrogenation[10,14,30] instead of the original Zn/H_3O^+ reduction. Imidazole ring closure was performed in one step[33,34] instead of the original two step procedure (formylation followed by alkaline ring closure).[35] This one step procedure using triethylorthoformate (or formamide, dimethylethylacetate, diethoxymethylacetate) is now widely used for both xanthines and purines.[21,36,37] Unfortunately, the combined yields of the two last steps were low (20%), leading to an extensive loss of labelled material.

In order to overcome low yield, we have developed a second procedure starting from guanosine (Figure 4). Guanosine **8** was easily converted into 7-benzylxanthine **10**.[28] The classical CD_3I/K_2CO_3/DMF procedure was found to be the most efficient for the conversion of **10** into 1,3-bis(trideuteromethyl)-7-benzylxanthine **11**. The alkylation of **10,** using $(CD_3)_2SO_4$ in a K_2CO_3/THF (or acetone) mixture, gave **11** with a lower yield. All attempts to alkylate **10,** using CD_3I/MeOH/MeONa (or K_2CO_3), led to a mixture of mono- and dimethylated compounds (complete N-3 alkylation, partial N-1 alkylation). Alkylation using $(CD_3)_2SO_4$ in tetra-*n*-butylammonium fluoride/THF[22] rapidly

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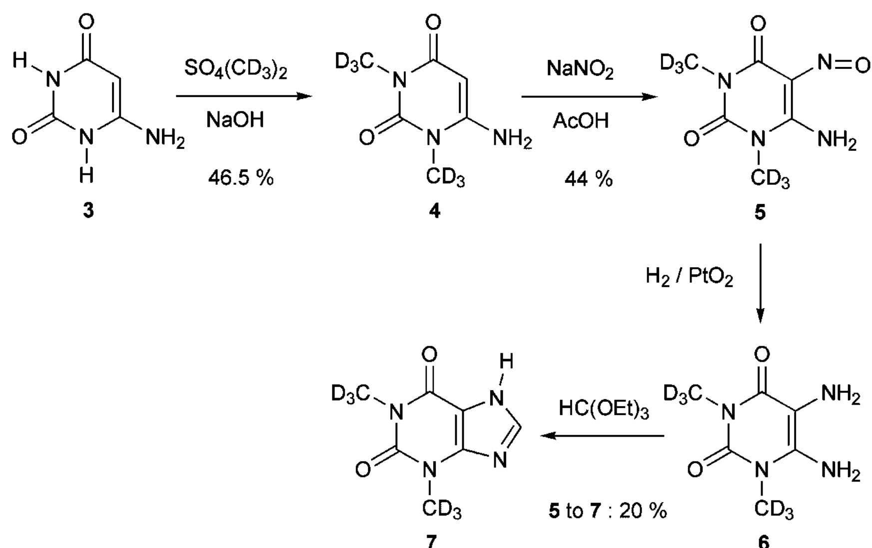

Figure 3 Synthesis of theophyline-*d6*, method A.

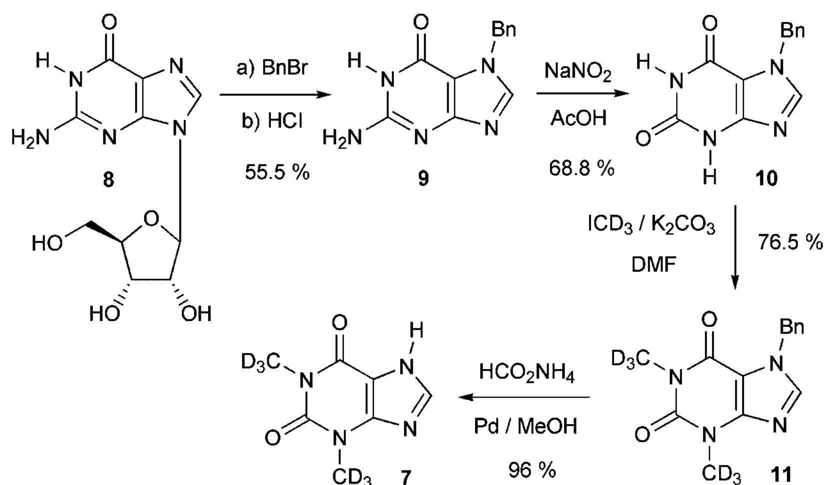

Figure 4 Synthesis of theophyline-*d6*, method B.

gave complete dialkylation, however the ammonium salt was difficult to separate from the alkylated xanthine.

Among the published N-3 or N-7 benzyl cleavage procedures ($AlCl_3$,[18] Na/NH_3,[38] H_2SO_4/TFA/anisole,[13,14] H_2/Pd),[18,28,29,38] catalytic low-pressure hydrogenation was tested first and gave theophylline-*d6* **7** after chromatographic purification. Debenzylation of 1,3-dimethyl-7-benzylxanthine-*d6* **11** by catalytic transfer hydrogenation[39] gave, after recrystallization, pure theophylline-*d6* **7**. This first application of catalytic hydrogen transfer to xanthine debenzylation was found to be superior to the catalytic low-pressure hydrogenation. This procedure gives a higher yield, avoids use of hazardous hydrogen gas and avoids chromatographic purification.

The disappearance of 3H signals at 3.18 and 2.94 ppm indicates complete labelling of N-1 and N-3 methyl groups. The mass spectrum of the theophylline-*d6* exhibits a 6 amu shift of the molecular ion and a 3 amu shift of the main fragments in good agreement with published data.[1]

Theobromine

The sole reported synthesis of labelled theobromine consists in the direct alkylation of xanthine with CD_3I leading to a mixture of mono-, di-and trimethylated

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xanthines, which are separated using semi-preparative liquid chromatography.[5] As this purification is not convenient for large-scale preparation, we decided to develop a new method suitable for preparative amounts of theobromine-*d3*. The major drawback of the classical synthetic method for theobromine (methylation of xanthine or 3-methylxanthine)[20,21,25] is that it affords theobromine contaminated with small amounts of 3-methylxanthine and caffeine (separation of 3-methylxanthine and theobromine is difficult).

Several selective syntheses of theobromine have been published: starting from adenine,[38] from caffeine via N-1 aminodemethylation[40] and from xanthine via methylation of its triethylsilyl derivative.[41] Likewise, selective syntheses of 3,7-xanthine derivatives have been reported: from xanthine via its trimethylsilyl derivative[42] and from 1-substituted-6-aminouracil.[34]

N-1 aminodemethylation of caffeine was the first procedure tested because the preparation of caffeine-*d3* (N-7 labelled) is easy. The conversion of caffeine-*d3* into 1-amino-3,7-dimethylxanthine-*d3* and then into theobromine-*d3* was very disappointing in our hands; the yield of aminodemethylation was subject to large variations, leading to a quite low yield of theobromine-*d3* (<15%).

The synthesis of theobromine-*d6* was then attempted via alkylation of the tris(trimethylsilyl)xanthine with iodomethane at the N-3 and N-7 positions, while the N-1 position remains unchanged.[41] Xanthine was readily silylated, by heating under reflux in hexamethyldisilazane, to give tris(trimethylsilyl)xanthine as a white solid. After dissolution in toluene and addition of CD_3I, the mixture was heated at 75°C. GC/MS monitoring established that 7-methylation was completed in less than 1 h. The second methylation at N-3 was completed after several days of heating and required the use of up to 50 equivalent of CD_3I. This result led us to test the same procedure starting from 3-methylxanthine instead of xanthine.

3-Methylxanthine **15** was readily prepared in three steps via the stable hydrochloride salt of 1-methyl-5-6-diaminouracil **14**,[43] silylated by heating under reflux in hexamethyldisilazane and treated with CD_3I (13 equivalents) to give theobromine-*d3* **17** (Figure 5).

Direct methylation of 3-methylxanthine was tested using a CD_3I/K_2CO_3/DMF mixture (with 1.5 equivalent

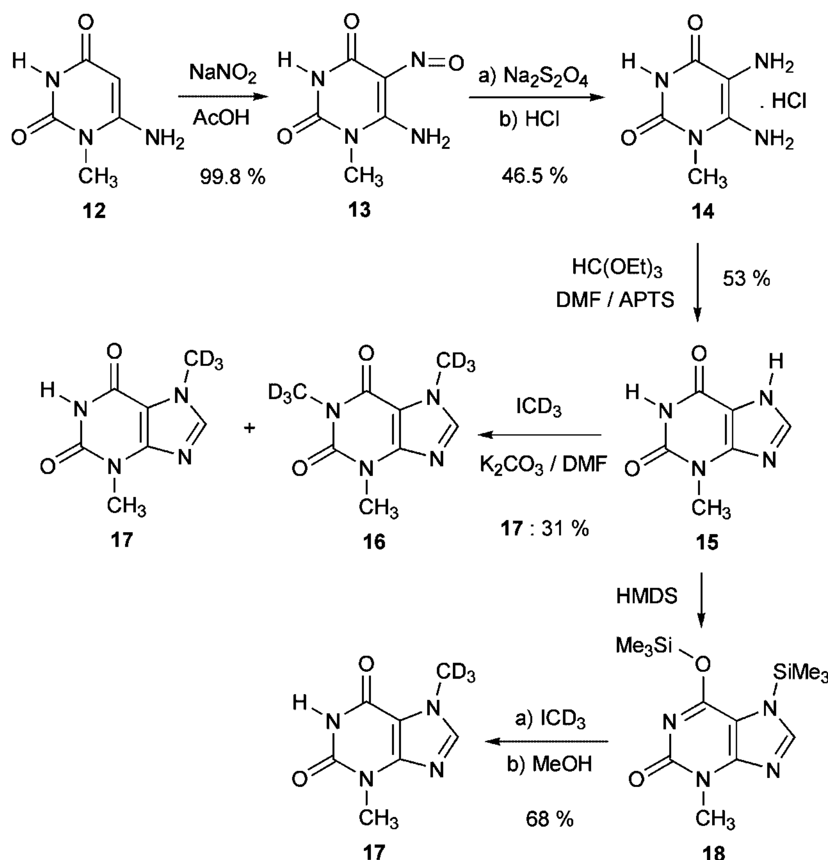

Figure 5 Synthesis of theobromine-*d3*, methods A and B.

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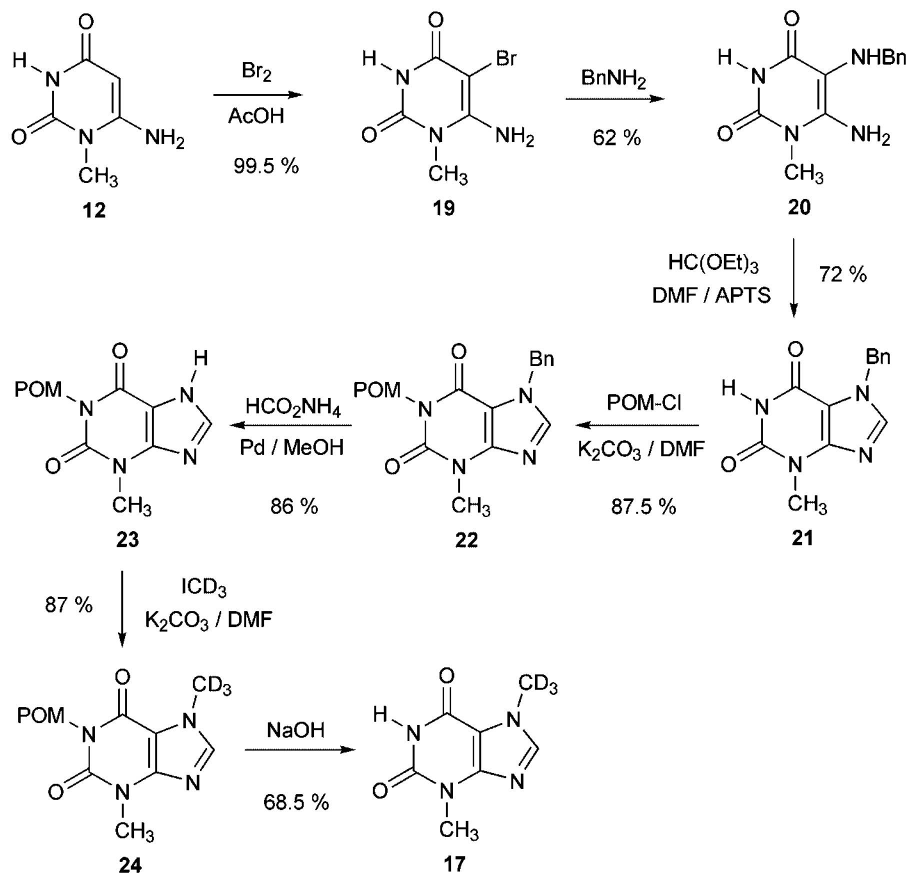

Figure 6 Synthesis of theobromine-*d3*, method C.

of CD_3I, in order to avoid any incomplete methylation of 3-methylxanthine), leading to a mixture of theobromine-*d3* **17** and caffeine-*d6* **16** which was removed by washing with chloroform.

Since the four previous preparations of labelled theobromine were only partially successful we decided to develop a new synthetic method (Figure 6). The key intermediate **22** (N-1 protected theobromine precursor) was prepared by action of chloromethyl pivalate on 3-methyl-7-benzylxanthine **21**. The pivaloyloxymethyl protecting group was used because all attempts to cleave 1-benzyltheobromine via catalytic hydrogenation failed. After debenzylation and trideuteromethylation, **24** was deprotected by alkaline hydrolysis, leading to theobromine-*d3* **17**, which was easily purified by sublimation under vacuum.

The disappearance of the 3H signal at 3.54 ppm indicates the complete labelling of the N-7 methyl. The mass spectrum of the theobromine-*d3* exhibits a 3 amu shift of all the characteristic fragments in good agreement with the fragmentation pattern of methylated xanthine.[1]

Experimental

All reagents were obtained from Sigma-Aldrich (St. Quentin Fallaviers, France) except guanosine hydrate (Lancaster, Strasbourg, France) and were used without further purification. NMR spectra were recorded on a Bruker ARX250 instrument. GC/MS analysis were performed using Agilent GC5890/MSD5972, equipped with a 25 m DB-5MS column (J & W Scientific).

Caffeine-*d3* 2

CD_3I (1 ml, 16 mmol) was added in one portion to a solution of theophylline (**1**, 1.91 g, 10.7 mmol) and sodium methylate (730 mg, 12.8 mmol) in methanol (100 ml) at room temperature. After 4 days stirring in a sealed flask in the dark, water (200 ml) was added and the mixture was extracted with chloroform (2×100 ml). The organic fractions were combined, dried with brine (100 ml) and anhydrous sodium sulfate. Evaporation of chloroform gave **2** as a white solid. Caffeine-*d3* was

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dissolved in chloroform (30 ml), filtered and crystallized by slow addition of diethyl ether. The caffeine-*d3* **2** was collected by filtration, washed with diethyl ether and dried under vacuum (1.31 g). The mother liquor was evaporated, the remaining solid was dissolved in chloroform (10 ml) and crystallized in the same way to give 0.19 g of **2** (white crystals, combined: 1.50 g, 71%).

^{1}H NMR ($CDCl_3$) ppm: 7.60 (s, 1H), 3.52 (s, 3H), 3.34 (s, 3H). MS (EI), m/z (%): 197 (100), 168 (6), 139 (5), 112 (60), 85 (26), 70 (29), 67 (24), 58 (42), 45 (13).

Caffeine-*d3* prepared from 250 mg of theophylline (1.38 mmol), 97 mg of sodium methylate (1.66 mmol), and CD_3I (447 μl, 6.9 mmol) in methanol (15 ml) was obtained after 24 h stirring at room temperature (white crystals, 228 mg, 83.5%).

Theophylline-*d6* 7, method A

6-Amino-1,3-dimethyluracil-*d6* 4. $(CD_3)_2SO_4$ (10 g, 75.7 mmol) was added in one portion to a suspension of 6-aminouracil **3** (4.27 g, 33.6 mmol) in a mixture of 32% sodium hydroxyde solution (6.3 ml) and water (26 ml), cooled in an ice bath. After 4.5 h stirring, the solid was collected by filtration, washed with water (30 ml) and dried (white solid, 2.53 g, 46.7%).

5-Nitroso-6-amino-1,3-dimethyluracil-*d6* 5. Sodium nitrite (430 mg, 6.2 mmol) in water (2 ml) was slowly added to a stirred solution of 6-amino-1,3-dimethyluracil-*d6* **4** (500 mg, 3.1 mmol) in acetic acid (5 ml) and water (5 ml), at 80°C over a period of 1h. After stirring for additional 30 min, the reaction mixture was cooled in ice and stored overnight at 4°C. The purple precipitate of **5** was collected by filtration, washed with cold water (10 ml) and dried over phosphorus pentoxide (purple solid, 260 mg, 44%).

Theophylline-*d6* 7. A stream of hydrogen was passed through a stirred mixture of 255 mg (1.34 mmol) of 5-nitroso-6-amino-1,3-dimethyluracil-*d6* **5** and platinum (IV) oxide (20 mg) in methanol (15 ml) using a fritted pipe. After 3.5 h, the catalyst was removed by filtration through a celite pad which was rinsed with dichloromethane (100 ml). The filtrate was evaporated to give 5,6-diamino-1,3-dimethyluracil-*d6* **6** as a dark oil, which was dissolved in triethylorthoformate (15 ml) and heated under reflux for 4 h. Triethylorthoformate was removed by distillation and the oily solid was purified by chromatography on a silica gel column (20 × 3 cm, dichloromethane/methanol 2–10%) to give theophylline-*d6* **7** (white solid, 51 mg, 20%).

Theophylline-*d6* 7, method B

7-Benzylguanine Hydrochloride 9. Guanosine hydrate **8** (10 g, 33.2 mmol) was dissolved in dimethylsulfoxide (50 ml) at 70°C. After cooling at room temperature, benzylbromide (10.0 ml, 84.1 mmol) was added in one portion and the solution was stirred at room temperature in a sealed flask for 4 h. Concentrated hydrochloric acid (37%, 25 ml) was added to the stirred solution in one portion without cooling. After stirring for an additional 1 h, the reaction mixture was poured into well-stirred cold methanol (−10°C, 300 ml). After another 1 h stirring, the white crystalline precipitate was collected by filtration, washed with cold methanol (2 × 100 ml) and dried under vacuum (white crystalline solid, 5.01 g, 55.5%).

7-Benzylxanthine 10. 7-Benzylguanine hydrochloride **9** (5 g, 18 mmol) was dissolved in a mixture of acetic acid (120 ml) and water (20 ml) at 100°C. After cooling to 50°C, sodium nitrite (4.97 g, 72 mmol) in water (20 ml) was added dropwise and stirring was continued for 4 h at room temperature. The precipitate, formed upon cooling, was collected by filtration, dried, washed with diethyl ether (2 × 100 ml) and dried under vacuum (white solid, 3.0 g, 68.8%).

1,3-Dimethyl-7-benzylxanthine-*d6* 11. 7-Benzylxanthine **10** (400 mg, 1.65 mmol), potassium carbonate (920 mg, 6.7 mmol) and CD_3I (400 μl, 6.43 mmol) in dry DMF (5 ml) were stirred at 70°C for 3 h in a sealed flask in the dark. After cooling at room temperature, water (25 ml) was added with stirring and the white precipitate was collected by filtration, washed with water (2 × 25 ml) and dried under vacuum at 80°C (white solid, 349 mg, 76.5%).

Theophylline-*d6* 7. Solid ammonium formate (1.5 g, 23.8 mmol) was added in one portion to a mixture of 1,3-dimethyl-7-benzylxanthine-*d6* **11** (660 mg, 2.39 mmol) and palladium on carbon (10%, 750 mg) in dry methanol (50 ml) heated under reflux. After 3 h, the palladium was removed by filtration through a celite pad which was thoroughly washed with methanol (100 ml). The solvent was removed by evaporation and a tan solid was obtained. This solid was dissolved in hot chloroform (10 ml) and filtered. After evaporation of chloroform, the white solid was dissolved in methanol (50 ml) and slowly cooled to −20°C to yield (after 4 days) white crystals (348 mg). The solid was filtered, washed with cold methanol (−20°C, 20 ml) and dried under vacuum. The mother liquor was evapored, the collected solid was dissolved in boiling methanol (8 ml) and

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crystallized similarly to give 79 mg of **7** (white crystals, combined: 427 mg, 95.9%).

^{1}H NMR (D_2O / DCl) ppm: 8.44 (s, 1H). MS (EI), *m/z* (%): 186 (100), 156 (7), 126 (6), 98 (68), 71 (45), 53 (32), 44 (20).

Theobromine-*d3* 17, method A

6-Amino-5-nitroso-1-methyluracil 13. A solution of sodium nitrite (7.12 g, 103.2 mmol) in water (40 ml) was added dropwise to a stirred suspension of 6-amino-1-methyluracil **12** (10.01 g, 70.9 mmol) in water (50 ml) and acetic acid (20 ml) at room temperature. The suspension was then heated at 50°C for 1 h, stirred at room temperature for 3 h and the purple solid was collected by filtration. This solid was washed with ice-water (20 ml), cold ethanol (100 ml) and dried under vacuum at 90°C (purple solid, 11.68 g, 99.8%).

5,6-Diamino-1-methyluracil Hydrochloride 14. Solid sodium hydrosulfite (technical grade 85%, 20.01 g, 100 mmol) was slowly added to a suspension of 6-amino-5-nitroso-1-methyluracil **13** (11.7 g, 68.8 mmol) in water (150 ml). After complete addition, the mixture was heated at 70°C for 1 h, then cooled to room temperature and filtered. The collected yellow solid was washed with water (2 × 100 ml), dried and added in small portions to well stirred hot concentrated hydrochloric acid (37%, 50 ml, 90°C). After stirring for an additional 15 min, the suspension was cooled to room temperature and filtered. The solid was washed with acetone (100 ml) and dried under vacuum (white crystalline solid, 6.15 g, 46.5%).

3-Methylxanthine 15. 5,6-Diamino-1-methyluracil hydrochloride **14** (6.15 g, 31.9 mmol) was heated at 70°C in a mixture of dry DMF (40 ml), triethylorthoformate (13 ml, 71.8 mmol) and *p*-toluenesulfonic acid monohydrate (50 mg) for 3.5 h (under nitrogen). After cooling in an ice bath, water (100 ml) was slowly added with vigorous stirring. The precipitate was collected by filtration, washed with cold water (2 × 100 ml), acetone (100 ml) and dried under vacuum at 80°C (white solid, 2.81 g, 53%).

Theobromine-*d3* 17. 3-Methylxanthine **15** (1.72 g, 10.3 mmol), potassium carbonate (3.0 g, 21.7 mmol) and CD_3I (1 ml, 16 mmol) in dry DMF (20 ml) were heated at 80°C for 2 h in a sealed flask in the dark. After 12 h at 4°C, water (50 ml) was added and the solution was neutralized with acetic acid. The precipitate was collected by filtration, washed with water (2 × 50 ml), acetone (2 × 50 ml), chloroform (2 × 50 ml), diethyl ether (2 × 50 ml) and dried under vacuum at 90°C (white solid, 590 mg, 31%).

Theobromine-*d3* 17, method B

Theobromine-*d3* 17. 3-Methylxanthine **15** (440 mg, 2.66 mmol) was heated under reflux in hexamethyldisilazane (15 ml) under dry nitrogen until 3-methylxanthine was completely dissolved (24–48 h). Hexamethyldisilazane was removed by evaporation and the white crystals were suspended in dry toluene (20 ml). CD_3I (2.15 ml, 34.5 mmol) was added and the reaction mixture was stirred at 75°C in a sealed flask in the dark for 3 days. After cooling at room temperature, acetone (50 ml) was added with vigorous stirring, followed by methanol (5 ml, dropwise). The precipitate was collected by filtration, washed with acetone (50 ml), dried under vacuum and crystallized from a minimum volume of boiling water (white solid, 330 mg, 68%).

Theobromine-d3 17, method C

6-Amino-5-bromo-1-methyluracil 19. Sodium acetate trihydrate (1.50 g, 11.0 mmol) was added to a suspension of 6-amino-1-methyluracil **12** (1.25 g, 8.8 mmol) in acetic acid (40 ml) at 125°C. After cooling at 80°C, bromine (0.50 ml, 9.7 mmol) in acetic acid (5 ml) was added dropwise to the suspension and heating was stopped. The suspension was stirred for 48 h at room temperature, the precipitate of **19** was collected by filtration, washed with acetic acid (2 × 25 ml) and dried in vacuum at 90°C (white solid, 1.94 g, 99.5%).

6-Amino-5-benzylamino-1-methyluracil 20. 6-Amino-5-bromo-1-methyluracil **19** (11.0 g, 50 mmol) was suspended in benzylamine (100 ml) at 100°C for 2 h. After cooling at room temperature (precipitation occured), ice-water (200 ml) was added with vigorous stirring and the precipitate was collected by filtration. The solid **20** was washed with cold water (3 × 100 ml) and dried in vacuum at 90°C (white solid, 7.63 g, 62%).

3-Methyl-7-benzylxanthine 21. 6-Amino-5-benzylamino-1-methyluracil **20** (7.5 g, 30 mmol), triethylorthoformate (14 ml, 77.3 mmol) and *p*-toluenesulfonic acid monohydrate (150 mg) were heated at 70°C for 48 h in dry DMF (60 ml) under nitrogen. After cooling (ice bath), ice-water (200 ml) was added and the suspension was stirred for 15 min. The precipitate of **21** was collected by filtration, washed with ice-water (3 × 100 ml), acetone (3 × 100 ml), and dried in vacuum at 50°C (white solid, 5.60 g, 72 %).

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1-Pivaloyloxymethyl-7-benzyl-3-methylxanthine 22. A mixture of 3-methyl-7-benzylxanthine **21** (5.5 g, 21.4 mmol), potassium carbonate (3.60 g, 26 mmol) and chloromethyl pivalate (3.4 ml, 23.6 mmol) in dry DMF (60 ml) was heated at 80°C for 2 h under nitrogen. After evaporation of the solvent, the yellow gum was dissolved in chloroform (100 ml) and filtered through a silica gel pad, yielding a yellow oil. This oil was dissolved in diethyl ether (100 ml), hexane (50 ml) was added with vigorous stirring (precipitation occurred) and diethyl ether was slowly evaporated at room temperature. The precipitate of **22** was collected by filtration and dried (white solid, 6.97 g, 87.5%).

1-Pivaloyloxymethyl-3-methylxanthine 23. Solid ammonium formate (2.5 g, 39.7 mmol) was added in one portion to a mixture of 1-pivaloyloxymethyl-7-benzyl-3-methylxanthine **22** (2.5 g, 6.75 mmol) and palladium on carbon (10%, 2 g) in dry methanol (100 ml) at 60°C under nitrogen. After 1 h heating under reflux, the catalyst was removed by filtration, washed with hot methanol (2 × 100 ml) and the combined methanolic solutions were evapored to give **23** (white solid, 1.63 g, 86%).

1-Pivaloyloxymethyl-3-7-dimethylxanthine-*d3* 24. A mixture of 1-pivaloyloxymethyl-3-methylxanthine **23** (1.63 g, 5.81 mmol), potassium carbonate (1,7 g, 12.3 mmol) and CD_3I (720 µl, 11.5 mmol) in dry DMF (15 ml) was stirred for 2 h at 70°C and then for 2 days at room temperature in a sealed flask in the dark. The solvent was evaporated, the remaining yellow oil was dissolved in chloroform (100 ml) and filtered. The solid was washed with chloroform (50 ml). After evaporation down to 30 ml, the combined chloroform solutions were filtered through a silica gel pad and evaporated, yielding a clear yellow oil. This oil was dissolved in diethyl ether (50 ml), hexane (50 ml) was added and a crystalline precipitate was formed. The volume was reduced to 50 ml, and hexane (50 ml) was added. The solution was evaporated down to 50 ml at room temperature and stored at 4°C for 12 h. White crystals of **24** were collected by filtration, washed with cold hexane and dried under vacuum at 90°C (white crystals, 1.51 g, 87%).

Theobromine-*d3* 17. 1-Pivaloyloxymethyl-3-7-dimethylxanthine-*d3* **24** (1.4 g, 4.71 mmol) in 2.5 M sodium hydroxide (50 ml) was heated under reflux for 2 h. After cooling in an ice bath, the pH was adjusted to 5 with concentrated hydrochloric acid (precipitation occurred). This suspension was stored at 4°C for 12 h and the precipitate of **17** was collected by filtration, washed with cold water (2 × 25 ml) and dried in vacuum at 120°C (white solid, 590 mg, 68.5%). The purification of theobromine-*d3* was achieved by sublimation under vacuum.

^1H NMR (D_2O / NaOD) ppm: 7.35 (s, 1H), 3.16 (s, 3H). MS (EI), *m/z* (%): 183 (100), 140 (6), 139 (6), 112 (29), 85 (28), 70 (39), 67 (25), 58 (45), 45 (11).

Conclusion

Simple and efficient preparations of labelled caffeine, theophylline and theobromine have been performed, including new syntheses of theophylline and theobromine.

The main advantages of these procedures are: excellent chemical and isotopic purities, highly reproducible yields (from labelled starting materials), facile processing and purification, low cost and possibility of labelling with other isotopes of hydrogen or carbon.

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