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# A Urinary Metabolite of $\Delta^1$-Tetrahydrocannabinol. The First Synthesis of 4",5"-Bisnor-$\Delta^1$-Tetrahydrocannabinol-7,3"-dioic Acid, and a Deuterium Labelled Analogue

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**Abstract**—The first synthesis of unlabelled and [$^2H_5$]-labelled 4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid, the major dicarboxylated urinary metabolite of $\Delta^1$-THC in man, is presented (preliminary results of this work have been presented in part at the Melbourne Symposium on Cannabis, Australia, September 1987, Ref. 1). The synthesis of methyl 3-(3,5-dihydroxyphenyl)-[3,3-$^2H_2$]-propanoate (**8**) is described in a nine step sequence from 3,5-dimethoxybenzoic acid in an overall yield of 24%. Compound **8** is condensed with a terpene synthon **9** under acidic conditions, acetylated and hydrolyzed with red HgO and $HgCl_2$ to afford the 1-formyl-4",5",7-trisnor-$\Delta^1$-THC-3"-oic acid derivative (**11**). Compound **11** is oxidized using $NaClO_2$ in 2-methyl-2-butene and hydrolyzed to give (±)-4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid (**12**). The same approach has been used to prepare both the labelled and unlabelled metabolite.

## Introduction

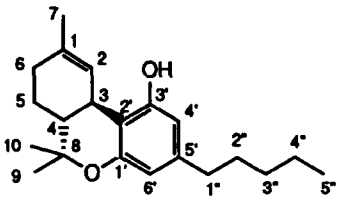
$\Delta^1$-Tetrahydrocannabinol ($\Delta^1$-THC, **1**), the main active constituent of *Cannabis sativa* L. (marijuana), possesses a vast number of pharmacological properties besides the psychotomimetic effect,[2] and is known to undergo an extensive metabolism both in man and in animals.[3,4] The urinary metabolites hitherto identified in man after an oral dose of $\Delta^1$-THC are nearly all acidic and correspond to less than 10% of the given dose.[5] $\Delta^1$-THC-7-oic acid (**2**) is regarded as the major urinary metabolite of $\Delta^1$-THC (**1**), accounting for about 1/3 of the acidic metabolites.[4] 4",5"-Bisnor-$\Delta^1$-THC-7,3"-dioic acid (**3**) is the second most abundant metabolite of $\Delta^1$-THC found in man, accounting for about 8% of the excreted radioactivity.[5] Although elimination via the kidneys is a minor excretory pathway, it is important since the main source for detecting the presence of THC metabolites is urine. Cannabis abuse testing programs have been introduced in many countries due to the increasing concern about the use of marijuana. For example, employees in sensitive positions whose impairment due to marijuana use could create a danger to others are usually screened. Today, the most commonly used technique for screening is based on immunoassays detecting the presence of $\Delta^1$-THC-7-oic acid in urine samples.[6] The speed, low cost and simplicity of immunoassay techniques make them suitable for qualitative and semi-quantitative analysis. Gas chromatography–mass spectrometry (GC–MS) is widely accepted as the method of choice for verification of positive urine samples. The method is both sensitive and more specific. However, there is a risk of interference by endogenous material or other compounds with similar properties and identical mass number as the analyte. The interference of a minor THC metabolite, 6-hydroxy-3",4",5"-trisnor-$\Delta^1$-THC-2"-oic acid, in the analysis of $\Delta^1$-THC-7-oic acid has been reported by Podkowik *et al.*[7] Thus, there is a need for the synthesis of metabolites other than $\Delta^1$-THC-7-oic acid for use as references and internal standards in such determinations. There is also a need to provide enough material to investigate the stability of these polar metabolites upon storage and during work-up prior to analysis, and to study the extent of cross reactivity to other metabolites and cannabinoids in the semi-quantitative immunoassays.

This paper describes the first synthesis of **3**, a principal human dicarboxylic acid metabolite of $\Delta^1$-THC and a deuterium labelled analogue. Only the synthetic procedure for labelled 4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid is described in detail, since the methods used in both cases are identical.

## Results and Discussion

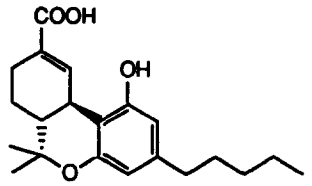
$\Delta^1$-THC-7-oic acid (**2**), the major urinary metabolite in man, is the only acidic metabolite of $\Delta^1$-THC synthesized so far. Compound **2** has been prepared both in unlabelled and labelled form, as well as in racemic and optically active form, using various methods.[8–13] There is a lack of methodology for the preparation of $\Delta^1$-THC metabolites, particularly the 7-oxygenated THC derivatives. The undesired isomerization of the double bond in the $\Delta^1$-position to $\Delta^6$ presents a significant synthetic problem. Procedures applicable to

the formation of the more thermodynamically stable $\Delta^6$-metabolites are most often not compatible with $\Delta^1$-THC metabolites which makes the preparation of the latter more difficult.



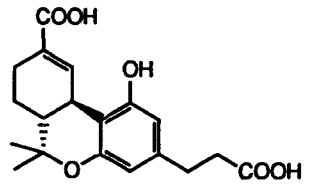
$\Delta^1$-THC

(1)



$\Delta^1$-THC-7-oic acid

(2)



4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid

(3)

**Figure 1.** Structures of $\Delta^1$-THC and two major human urinary metabolites.

4",5"-Bisnor-$\Delta^1$-THC-7,3"-dioic acid was first isolated and identified by Nordqvist *et al.* as the major non-conjugated acidic metabolite of $\Delta^1$-THC in rabbit urine.[14] The synthesis of [$^2H_5$]-4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid has been achieved by utilizing the general procedure previously described for the synthesis of [$^2H_{10}$]-$\Delta^1$-THC-7-oic acid.[12] This paper further extends the versatility of the procedure first developed by Uliss *et al.*[15] for the synthesis of some $\Delta^1$-THC metabolites, mainly dihydroxylated.[16,17] The introduction of the 1,3-dithiane masking group stabilizes the double bond in $\Delta^1$-position inhibiting the isomerization to the more stable $\Delta^6$-derivative.

3,5-Dimethoxybenzoic acid (**4**) was esterified and reduced to the corresponding alcohol **5** using LiAl[$^2H_4$], introducing two deuterium atoms into the side-chain. Compound **5** was treated with $SOCl_2$ and the resulting chloride was used for the alkylation of sodiodiethyl malonate[18] thus furnishing **6**. Alkaline hydrolysis of **6** to the free diacid, followed by decarboxylation at 145 °C to the monoacid and subsequent esterification provided the methyl 3-(3,5-dimethoxyphenyl)-[3,3-$^2H_2$]propanoate (**7**). Demethylation with $BBr_3$ in $CH_2Cl_2$ at –63 °C using a modified procedure from that described by Pitt *et al.*[19] resulted in the formation of 3-(3,5-dihydroxyphenyl)-[3,3-$^2H_2$]propanoic acid which was esterified before condensation with (±)-[$^2H_3$]-**9**. The total yield of **8** from 3,5-dimethoxy benzoic acid was 24%. The chemical shifts for unlabelled compounds **5–8** are almost identical with that of the labelled compounds.[20]

The syntheses of the unlabelled and deuterium labelled terpene synthon **9** have previously been described in detail.[12,15] Deuterium was introduced in the geminal methyl groups of the terpene synthon by using [$^2H_3$]-MeMgI. The deuterium incorporation was 95.1% (m/z 295) according to mass spectrometry.[12]

The acid-catalyzed condensation of [$^2H_2$]-**8** with [$^2H_3$]-**9** afforded the thioketal **10a** in 10% yield.[12] The reaction resulted in numerous compounds according to TLC. However, one major by-product in the reaction was **10b** in which the condensation took place *ortho* rather than *para* to the side-chain on the aromatic ring. The reaction mixture was chromatographed on an open bed column and subsequently on a Lobar column using a low pressure pump eluting with a gradient of ether–petroleum ether and 5% MeCN–$CCl_4$, respectively. The latter gives inverted retention volumes for **10a** and **10b** which results in an excellent separation. In the $^1H$ spectrum of **10a** H-3 is observed as a double doublet at $\delta_H$ 3.27 ppm. The coupling constants ($J_{3,4}$ = 11 Hz, $J_{2,3}$ = 2 Hz) are consistent with the presence of diaxially positioned hydrogens. Consequently, the A and B rings are fused in a *trans* fashion. This is further shown in the signal for H-4 which is observed as a triplet of doublets at $\delta_H$ 1.70 ppm ($J_{4,3}$ = 11 Hz). Several attempts were made to optimize the yield in this reaction and to find conditions that would be reproducible. Despite the use of a wide range of Lewis acids (*p*-TSA, $BF_3$–$Et_2O$, $MeSO_3H$), together with variation of reaction time, solvents and temperature, no optimum conditions were found in which the yield was significantly improved. For example, the reaction of compounds **8** and **9** with $BF_3$–$Et_2O$ as catalyst gave as the major product a dehydrated benzofuran derivative where the phenolic oxygen has attacked the double bond and formed a furan ring.[21] The structure of this product has previously been tentatively suggested by Siegel *et al.*[10] The phenolic group of the condensation product **10a**, was protected by acetylation and the 1,3-dithiane masking group removed by reaction with red HgO and $HgCl_2$ in aq. MeCN to give **11**. The method described by Siegel *et al.*[22] using a *t*-butyldimethylsilyl derivative of **10a** in the hydrolysis reaction was unsuccessful in our hands. Our attempts to hydrolyze the 1,3-dithiane group to the corresponding aldehyde only resulted in decomposition of the *t*-butyldimethyl silyl derivative of **10a**.

The aldehyde **11** was oxidized using $NaClO_2$ in the presence of excess of 2-methyl-2-butene as a hypochlorous acid scavenger,[22] followed by deprotection of the phenol under alkaline conditions resulting in (±)-[$^2H_5$]-4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid (**12**).

The $^1$H NMR for deuterium labelled **12** was consistent with that of unlabelled **12** except for the absence of resonance for C-1" protons and the collapse of the C-2" methylene triplet to a singlet. The formation of two diastereomeric forms due to the incorporation of deuterium in the geminal methyl groups is represented in the $^1$H NMR spectrum by two singlets appearing at $\delta_H$ 1.10 and 1.41 ppm. The $^1$H NMR data for unlabelled and labelled methyl ester of **12** were in agreement with those for the isolated unlabelled metabolite first reported by Nordqvist *et al.*[14] The assignments given by Nordqvist *et al.*[14,23] for the peaks in the aromatic region of the $^1$H NMR representing the atoms in the 4'- and 6'-positions were not well defined. In order to establish the correct shift values, INEPT long range technique[24] in combination with FLOCK experiments[25] were used in addition to the regularly used NMR techniques. Irradiation of the phenolic group in the 3'-position in the INEPT long range experiment gave a response at 107.4 ppm, confirming that this peak comes from C-4'. The peak at 109.7 ppm therefore originates from C-6'. Throughout, $^{13}$C NMR spectra of the labelled derivatives were consistent with those of the unlabelled compounds with the notable difference that the resonance for the C-1" deuterated carbon was almost absent due to the much less effective relaxation of carbon by deuterium than by protons. Depending on the resolution, two peaks very close to each other were occasionally seen in the $^{13}$C NMR spectrum representing the two diastereomeric forms. Mass spectral analysis of the dimethyl ester of **12** (see Fig. 2) gives a molecular ion at m/z 379 and a base peak at m/z 364 [$M^+$-*gem*-$CH_3$]. Other diagnostic fragments are m/z 361 and 320 corresponding to [$M^+$-*gem*-C[$^2H_3$]] and [$M^+$-$COOCH_3$], respectively. The mass spectrum indicates a high deuterium incorporation. The content of unlabelled compound is less than 3% in the deuterium labelled final product showing that the label is stable throughout the reaction sequence. Unlabelled **12** derivatized as the dimethyl ester and TMS ether gives a molecular ion at m/z 446. The fragmentation is in agreement with that reported by Nordqvist *et al.*[14,23] The fragmentation is similar to that of the labelled compound with the addition of a peak at m/z 374 from the loss of the TMS group.

This paper describes the first synthesis of unlabelled and [$^2H_5$]-labelled 4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid, a major dicarboxylated urinary metabolite of $\Delta^1$-THC in man. While the achievements of these syntheses are substantial, there are some drawbacks associated with them. First, the overall yield is low (1–2%), in part due to the great number of steps involved. Second, the method used provides a racemic final product. For our primary purposes this is not a problem since chiral compounds are not a requirement when used as chemical references and as internal standards in quantitative measurements. For quantitative determinations of $\Delta^1$-THC metabolites using GC–MS analysis (SIM technique), analogues labelled with stable isotopes are most suitable as internal standards. The deuterated internal standard should have an incorporation of at least three deuterium atoms to avoid interference of the M-1 and M-2 ions of the standard with the $M^+$ ion of the unlabelled analyte.[26] A report by Joern shows that when an internal standard containing six deuterium atoms was used in the analysis of $\Delta^1$-THC-7-oic acid a considerably increased linearity was achieved.[27] The side-chain and the geminal methyl positions (C-9/C-10) of the cannabinoids are stable to fragmentation and were therefore chosen as the sites of incorporation of two and three deuterium atoms, respectively.

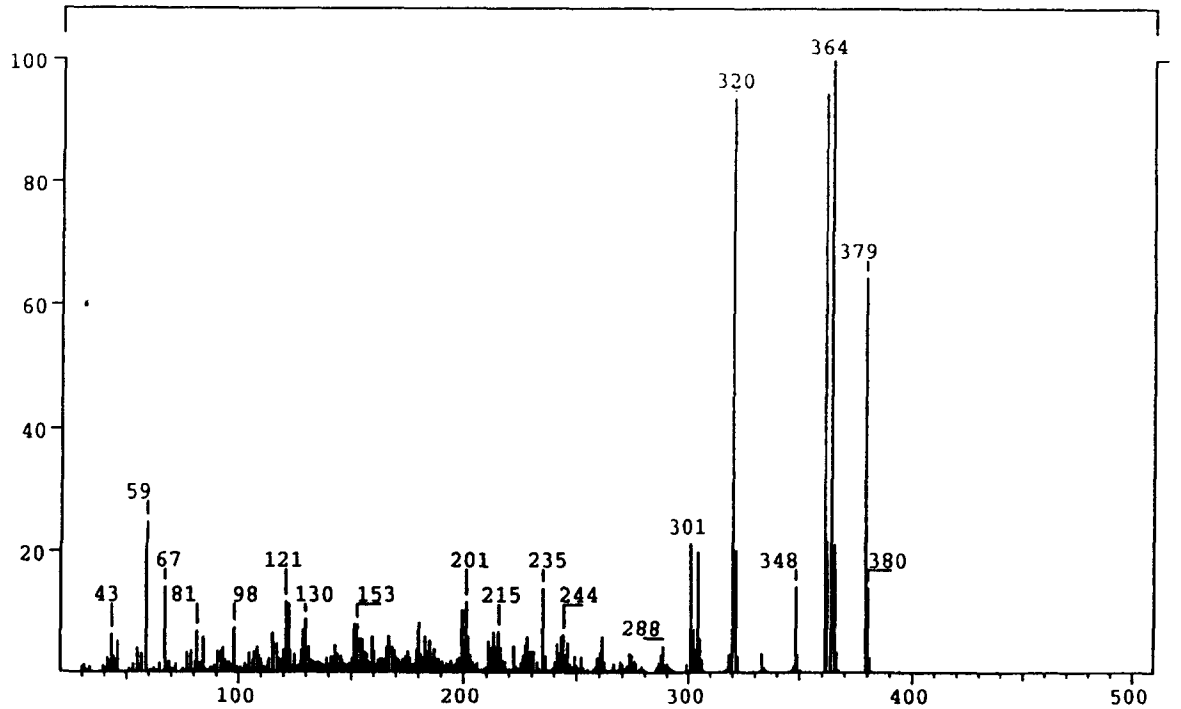



**Figure 2.** Mass spectrum of the methyl ester of [$^2H_5$]-labelled 4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid (**12**).

The present synthesis is significant in that it provides an additional standard useful in the testing procedures for cannabis abuse. The urinary metabolites manifest a considerably longer time course and therefore provide a good indicator of previous smoking compared to analysis of THC in plasma. Evaluating the concentration of $\Delta^1$-THC-7-oic acid together with other acidic metabolites such as 4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid, should provide a better accuracy. The main advantage of the synthetic route described is that the molecules can readily be labelled with isotopes. Minor modifications in the synthesis also permit the synthesis of other 7-substituted metabolites.

## Experimental

### *Material and methods*

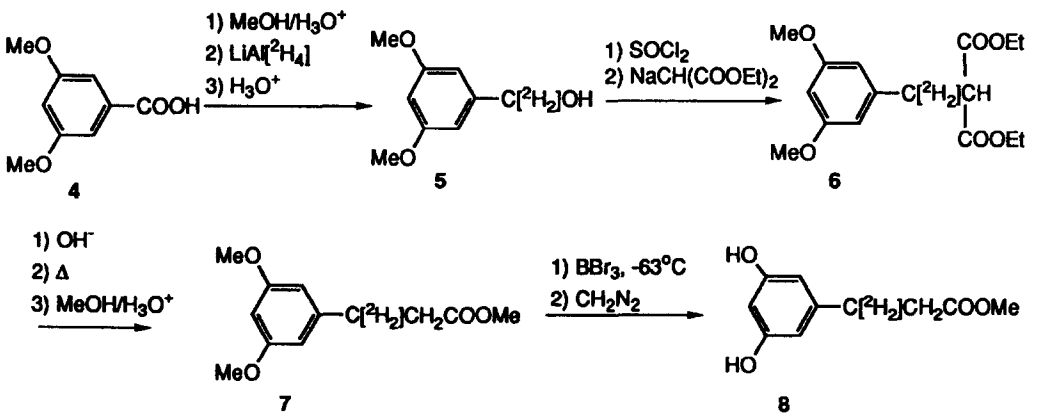
3,5-Dimethoxybenzoic acid was purchased from Aldrich-Chemie, Germany. LiAl [$^2H_4$] (99 atom % deuterium) was obtained from Ciba–Geigy, Switzerland. Melting points were determined on an Electrothermal Digital melting point apparatus and are uncorrected. IR spectra were recorded on a Polaris FT-IR spectrometer (Mattson Instruments, U.K.). $^1$H NMR and $^{13}$C NMR spectra were recorded on a Jeol JNM-EX 270 MHz instrument, a Varian Gemini 300 spectrometer or a Varian Unity 300. Spectra were recorded with TMS as the internal standard in [$^2$H]-chloroform, unless otherwise stated. Two-dimensional NMR spectroscopy was performed with standard C–H heteronuclear correlation. Attached Proton Test (APT)[28] was used to determine the carbon multiplicity. The isotopic distribution was estimated by mass spectrometric analyses using a Finnigan MAT TSQ 70 (San José, CA, U.S.A.) equipped with a Finnigan MAT thermospray source. Ammonium acetate was used as the ionizing agent. Mass spectra were also recorded using GC–MS equipped with an HP Ultra 2 column (5% phenylmethylsilicone; 25 m × 0.32 mm × 0.52 μm) connected to a Finnigan MAT SSQ 700 (San José, CA, USA). The molecular mass was determined by electron impact (EI) mass spectrometry using a Finnigan MAT 95Q (Bremen, Germany) double focusing mass spectrometer. Perfluorkerosine was used as reference. Silica gel 60 (70–230 mesh, Merck) was used for open bed liquid chromatography. Low pressure liquid chromatography was performed using a pre-packed normal phase (Si 60, 40–63 mm) Lobar column (Merck). All synthetic and analytical operations were initially performed with unlabelled compounds, and the structures of unlabelled intermediates and products were confirmed spectroscopically. The monoterpenoid numbering system is used throughout this paper. For numbering of the atoms in molecules **10–12** see Scheme 2.

### *3,5-Dimethoxy[α,α-$^2H_2$]benzyl alcohol (5, Scheme 1)*

Methyl 3,5-dimethoxybenzoic acid (9.00 g, 45.9 mmol), prepared by standard procedures, was dissolved in anhydrous THF (180 mL) under $N_2$ and cooled to 0 °C. LiAl[$^2H_4$] (1.4 g, 33.3 mmol; alternatively $LiAlH_4$ for preparation of the unlabelled compound) was added and stirred at room temperature for 30 min and then at reflux for 2 h. The reaction mixture was cooled to 0 °C and $H_2O$ (10 mL) was added dropwise. The precipitate was filtered off, washed and the aqueous phase extracted with toluene (3 × 150 mL). The combined organic layers were washed with $H_2O$, dried over $Na_2SO_4$ and evaporated under reduced pressure to give **5** as a pale yellow oil (95% yield) which crystallized upon standing; mp 48.5–49.1 °C. IR (KBr) 3368, 1598 cm$^{-1}$; $^1$H NMR δ 6.52 (*d*, *J* = 2 Hz, 2H, H-2 and H-6), 6.38 (*dd*, *J* = 2 Hz, 1H, H-4), 3.79 (*s*, 6H, -$OCH_3$), 1.90 (*br s*, OH); $^{13}$C NMR δ 161.0 (C-5; C-3), 143.3 (C-1), 104.6 (C-2; C-6), 99.7 (C-4), 55.3 (-O<u>C</u>H$_3$); MS m/z 171 ([M$^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (169–171): 170, 0.1%; 171, 99.9%. HRMS m/z [M$^+$] calculated for $C_9O_3H_{10}[^2H_2]$ 170.0912, found 170.09. $^1$H NMR and IR data for the unlabelled compound were in agreement with those found in the literature.[29]

### *Diethyl 2-(3,5-dimethoxy[α,α-$^2H_2$]benzyl)malonate (6)*

Pyridine (1.2 mL) was added to a solution of **5** (8.14 g, 47.9 mmol) in anhydrous ether (100 mL) while stirring. $SOCl_2$ (10.9 mL) in anhydrous ether (50 mL) was added dropwise during 1 h and then refluxed for 30 min. The reaction mixture was cooled with ice and the aqueous




**Scheme 1.** Preparation of methyl 3-(3,5-dihydroxyphenyl)-[3,3-$^2H_2$]propanoate (**8**).

phase was washed with ether (2 × 50 mL). The combined ether extracts were washed with aq. sat. $NaHCO_3$ to neutral pH, and with brine, dried over $Na_2SO_4$ and then evaporated to give crystalline 3,5-dimethoxy[α,α-$^2H_2$]benzyl chloride in 90% yield (8.13 g, 43.1 mmol); mp 42.7–43.1 °C. $^1H$ NMR δ 6.52 (*d*, *J* = 2 Hz, 2H, H-2 and H-6), 6.40 (*dd*, *J* = 2 Hz, 1H, H-4), 3.77 (*s*, 6H, -$OCH_3$); $^{13}C$ NMR δ 160.9 (C-3; C-5), 139.5 (C-1), 106.5 (C-2; C-6), 100.4 (C-4), 55.4 (-O<u>C</u>$H_3$); MS m/z 153 ([$M^+$] – Cl). The isotopic distribution according to mass spectrometry was, m/z (186–188): 186, 0.4%; 188, 99.6%. $^1H$ NMR and IR data for the unlabelled compound were in agreement with previously published data.[30]

NaH (0.41 g) was added to diethylmalonate (4.65 g) in anhydrous THF (20 mL) while cooling. The 3,5-dimethoxy[α,α-$^2H_2$]benzyl chloride (3.0 g, 15.9 mmol) dissolved in anhydrous THF (10 mL) was added slowly and the mixture was refluxed for 4.5 h and then evaporated. The structure was confirmed from $^1H$ and $^{13}C$ NMR data after purification; $^1H$ NMR δ 6.36 (*d*, *J* = 2 Hz, 2H, H-2 and H-6), 6.32 (*dd*, *J* = 2 Hz, 1H, H-4), 4.17 (*dq*, *J* = 7 Hz, 4H, -C<u>H</u>$_2$$CH_3$), 3.76 (*s*, 6H, -$OCH_3$), 3.62 (*s*, 1H, -C[$^2H_2$]C<u>H</u>-), 1.23 (*t*, *J* = 7 Hz, 6H, -$CH_2$C<u>H</u>$_3$); $^{13}C$ NMR δ 168.8 (-<u>C</u>OOH), 160.8 (C-3; C-5), 140.2 (C-1), 106.7 (C-2; C-6), 98.7 (C-4), 61.5 (-C[$^2H_2$]<u>C</u>H-), 55.3 (-O<u>C</u>$H_3$), 53.6 (-<u>C</u>$H_2CH_3$), 14.0 (-$CH_2$<u>C</u>$H_3$); MS m/z 313 ([$M^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (311–313): 312, 0.2%; 313, 99.8%. $^1H$ NMR data for the unlabelled compound were in agreement with those found in the literature.[31]

*Methyl 3-(3,5-dimethoxyphenyl)-[3,3-$^2H_2$]propanoate (7)*

The crude diester **6** (3.00 g, 9.6 mmol) was hydrolyzed with KOH (7.0 g) in a 2:1 (v/v) mixture of EtOH/$H_2O$ (30 mL). The reaction mixture was stirred vigorously for 1 h with reflux. The solution was concentrated, diluted with $H_2O$ (50 mL), acidified with 2 N HCl, extracted with ether (3 × 50 mL) and dried over $Na_2SO_4$ before evaporating to dryness. IR (KBr) 2900, 1703, 1597 $cm^{-1}$; $^1H$ NMR ([$^2H_5$]-pyridine) δ 11.06 (*br s*, -COOH), 6.90 (*d*, *J* = 2 Hz, 2H, H-2 and H-6), 6.57 (*dd*, *J* = 2 Hz, 1H, H-4), 4.37 (*s*, 1H, -C[$^2H_2$]C<u>H</u>-), 3.65 (*s*, 6H, -$OCH_3$); $^{13}C$ NMR ([$^2H_5$]-pyridine) δ 172.6 (-<u>C</u>OOH), 161.5 (C-3; C-5), 142.5 (C-1), 107.6 (C-2; C-6), 99.2 (C-4), 55.3 (-C[$^2H_2$]<u>C</u>H-), 55.1 (-O<u>C</u>$H_3$); MS m/z 212 ([$M^+$] – $CO_2$). The isotopic distribution according to mass spectrometry was, m/z (211–213): 213, 100%.

The crude malonic acid derivative was decarboxylated by heating at 145 °C for about 0.5 h. The resulting brown oil was dissolved in 2 M NaOH (30 mL), washed with ether (2 × 25 mL), acidified with 2 N HCl to pH 3 and extracted with ether (3 × 30 mL). The organic layer was dried over $Na_2SO_4$ and evaporated *in vacuo* to give white crystals in 88% yield from **6**; mp 55.5–56.3 °C; $^1H$ NMR δ 11.10 (*br s*, 1H, -COOH), 6.36 (*d*, *J* = 2 Hz, 2H, H-2 and H-6), 6.32 (*d*, *J* = 2 Hz, 1H, H-4), 3.75 (*s*, 6H, -$OCH_3$), 2.65 (*s*, 2H, -C[$^2H_2$]C<u>H</u>-); $^{13}C$ NMR δ 179.4 (-<u>C</u>OOH), 160.9 (C-3; C-5), 142.5 (C-1), 106.3 (C-2; C-6), 98.3 (C-4), 55.2 (-O<u>C</u>$H_3$), 35.3 (-C[$^2H_2$]<u>C</u>H-); MS m/z 213 ([$M^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (211–213): 213, 100%. $^1H$ NMR data for the unlabelled compound were in agreement with previously published data.[32]

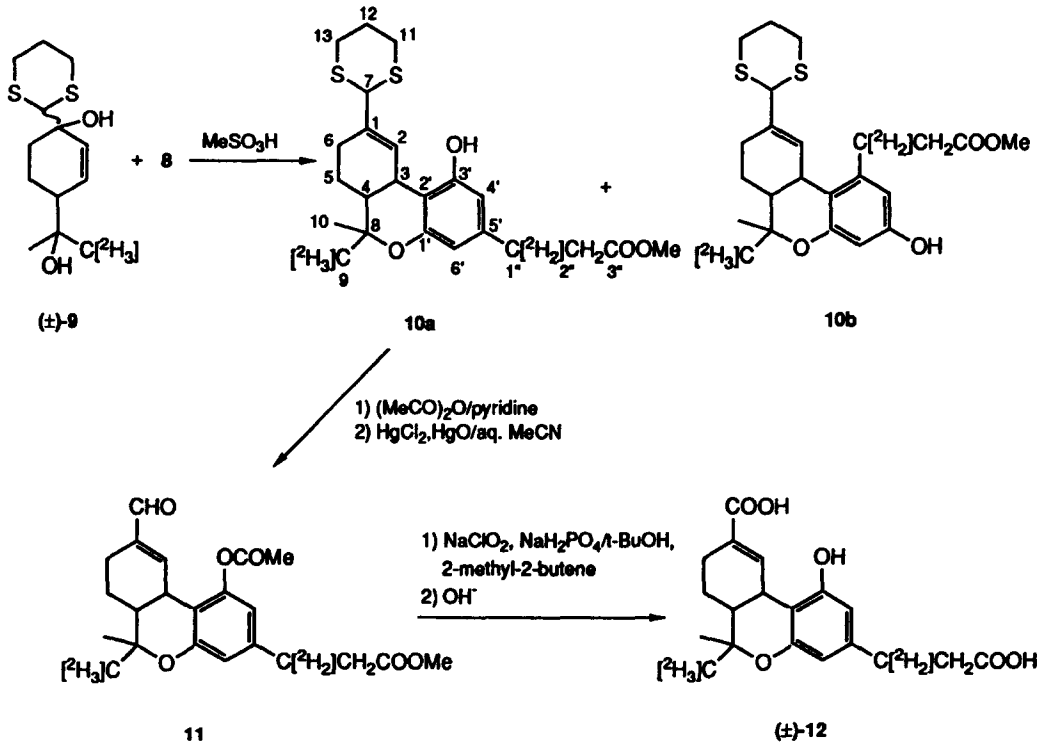
3-(3,5-Dimethoxyphenyl)-[3,3-$^2H_2$]propanoic acid (2.0 g, 9.44 mmol) was dissolved in MeOH, added to a solution of conc. $H_2SO_4$ (7.5 mL) in MeOH (40 mL) and refluxed for 4 h. The reaction mixture was concentrated, poured onto ice water (50 mL) and extracted with ether (3 × 150 mL). The combined organic layer was washed with aq. sat. $NaHCO_3$ to neutral pH, and with brine, dried over $Na_2SO_4$ and evaporated *in vacuo*. The crude mixture was chromatographed on silica with 1:4 EtOAc:toluene as eluent to give **7** in 91% yield. $^1H$ NMR δ 6.36 (*dd*, *J* = 2 Hz, 2H, H-2 and H-6), 6.32 (*d*, *J* = 2 Hz, 1H, H-4), 3.76 (*s*, 6H, -$OCH_3$), 3.67 (*s*, 3H, -COOC<u>H</u>$_3$), 2.61 (*s*, 2H, -C[$^2H_2$]C<u>H</u>$_2$-); $^{13}C$ NMR δ 173.5 (-<u>C</u>OOH), 160.9 (C-3; C-5), 142.8 (C-1), 106.3 (C-2; C-6), 55.3 (-O<u>C</u>$H_3$), 51.7 (-COO<u>C</u>$H_3$), 35.4 (-C[$^2H_2$]<u>C</u>H-); MS m/z 227 ([$M^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (225–227): 227, 100%.

*Methyl 3-(3,5-dihydroxyphenyl)-[3,3-$^2H_2$]propanoate (8)*

Compound **7** (4.52 g, 20.0 mmol) was dissolved in $CH_2Cl_2$ (100 mL) and cooled to –63 °C with stirring under $N_2$. $BBr_3$ (60 mL, 60.0 mmol, 1 M in $CH_2Cl_2$) was added dropwise and the temperature was kept at –63 °C for 1 h, allowed to rise to 0 °C and kept at 0 °C for 1 h. The reaction mixture was stirred overnight at room temperature and quenched by adding 10% aq. $NaHSO_3$ (400 mL) while cooling with ice water. The aqueous phase was extracted with EtOAc (3 × 300 mL), dried over $Na_2SO_4$ and evaporated *in vacuo*. The mixture was dissolved in ether:MeOH (10:1) and esterified at room temperature with 1 eq. $CH_2N_2$. After evaporation the crude mixture was chromatographed on silica with 1:9 EtOAc:toluene as eluent giving **8** as an oil in 55% yield. $^1H$ NMR ([$^2H_4$]-MeOH) δ 6.21 (*d*, *J* = 2 Hz, 2H, H-2 and H-6), 6.17 (*dd*, *J* = 2 Hz, 1H, H-4), 3.70 (*s*, 3H, -COOC<u>H</u>$_3$), 2.62 (*s*, 2H, -C[$^2H_2$]C<u>H</u>$_2$-); $^{13}C$ NMR ([$^2H_4$]-MeOH) δ 176.0 (-<u>C</u>OOH), 160.2 (C-3; C-5), 144.7 (C-1), 108.2 (C-2; C-6), 102.0 (C-4), 52.4 (-COO<u>C</u>H3), 36.6 (-C[$^2H_2$]<u>C</u>H-); MS m/z 199 ([$M^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (197–199): 199, 100%. $^1H$ NMR data for the unlabelled compound were in agreement with previously published data.[33]

*(±)-[$^2H_5$]-Methyl-[4",5",7-trisnor-1-(1,3-dithianyl)]-$\Delta^1$-tetrahydrocannabinol-3"-oate (**10a, 10b**, Scheme 2)*

All glassware was carefully dried before use. Compound **8** (1.78 g, 8.98 mmol) was dissolved in dry $CH_2Cl_2$ and dry benzene (150 mL, 1:1 v/v) with heating to 45 °C. Crystalline **9** (2.49 g, 8.98 mmol)[12] and 0.1 eq. $MeSO_3H$




**Scheme 2.** Preparation of (±)-[$^2H_5$]-4",5"-bisnor-$\Delta^1$-THC-7,3"-dioic acid (**12**).

were added with stirring under $N_2$. The reaction was quenched after 6 h with $H_2O$ (25 mL) and 5% aq. $NaHCO_3$ (10 mL) resulting in a brown coloured solution. The aqueous layer was extracted with ether (3 × 50 mL) and the combined organic layers were washed with brine, dried over $Na_2SO_4$ and evaporated *in vacuo*. The crude mixture was chromatographed on silica with a gradient of ether–petroleum ether (0–40%). Fractions containing the isomeric **10a** and **10b** were pooled, evaporated to dryness, dissolved in a small volume of the eluent and filtered through a Bond Elut extraction cartridge (Si, 3 mL, Analytichem International). The eluate was chromatographed on a Lobar column (size B) with 5% MeCN–$CCl_4$ (2 mL $min^{-1}$) using a low pressure pump. The recovery of pure **10a** and **10b** by this procedure were 10% of each compound. After evaporation *in vacuo* **10a** appeared as an oil and **10b**[34] as white foamy crystals. $^1H$ NMR **10a** δ 6.95 (*d*, *J* = 1.6 Hz, 1H, H-2), 6.24 (*d*, *J* = 1.6 Hz, 1H, H-6'), 6.16 (*d*, *J* = 1.6, 1H, H-4'), 4.59 (*s*, 1H, H-7), 3.68 (*s*, 3H, -COOC$\underline{H}_3$), 3.27 (*dd*, *J* = 11, 2 Hz, 1H, H-3), 2.80–3.00 (*m*, 4H, H-11 and H-13), 2.57 (*s*, 2H, H-2"), 2.37–2.47 (*m*, 2H, H-6), 1.78–2.18 (*m*, H-5 and H-12), 1.70 (*td*, *J* = 11, 2 Hz, 1H, H-4), 1.40 (*s*, 2H, H-10), 1.35–1.50 (*m*, H-5), 1.07 (*s*, 1H, H-9);[35] $^{13}C$ NMR **10a** δ 173.7 (C-3"), 155.0 (C-1'), 154.7 (C-3'), 140.2 (C-5'), 135.1 (C-1), 129.5 (C-2), 109.6 (C-6'), 108.8 (C-2'), 107.4 (C-4'), 53.4 (C-7), 51.7 (-COO$\underline{C}H_3$), 44.9 (C-4), 35.1 (C-2"), 33.8 (C-3), 31.6 (C-11 and C-13), 27.7 (C-6), 27.4 (C-10), 25.5 (C-12), 24.6 (C-5), 19.2 (C-9);[35] MS m/z 440 ([$M^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (435–440): 438, 4.3%; 439, 5.9%; 440, 89.8%.

*(±)-[$^2H_5$]-Methyl-(3'-acetoxy-1-formyl-4",5",7-trisnor)-$\Delta^1$-tetrahydrocannabinol-3"-oate (11)*

Compound **10a** (0.186 g, 0.42 mmol) was treated with acetic acid anhydride (4.05 mL) in pyridine (12.2 mL) at room temperature over night, poured onto ice water (50 mL) and extracted with ether (3 × 50 mL). The combined ethereal layers were washed with 2 N HCl to neutral pH, with aq. sat. $NaHCO_3$ (10 mL), and with brine and then dried over $Na_2SO_4$ and by azeotropic distillation with benzene, giving an oil in quantitative yield (0.20 g, 0.42 mmol). $^1H$ NMR acetylated **10a** δ 6.58 (*d*, *J* = 1.5 Hz, 1H, H-2), 6.56 (*d*, *J* = 1.8 Hz, 1H, H-6'), 6.45 (*d*, *J* = 1.8 Hz, 1H, H-4'), 4.45 (*s*, 1H, H-7), 3.67 (*s*, 3H, -COOC$\underline{H}_3$), 3.12 (*br d*, 1H, H-3), 2.78–2.98 (*m*, 4H, H-11 and H-13), 2.58 (*s*, 2H, H-2"), 2.35–2.46 (*m*, H-6), 2.36 (*s*, -OCOC$\underline{H}_3$), 1.72–2.22 (*m*, H-5 and H-12), 1.69 (*td*, *J* = 11 Hz, 1H, H-4), 1.43 (*s*, 2H, H-10), 1.07 (*s*, 1H, H-9);[36] $^{13}C$ NMR acetylated **10a** δ 173.2 (C-3"), 168.9 (-O$\underline{C}$OCH$_3$), 154.7 (C-1'), 149.5 (C-3'), 140.4 (C-5'), 136.7 (C-1), 128.4 (C-2), 115.2 (C-6'), 114.7 (C-2'), 114.0 (C-4'), 52.4 (C-7), 51.7 (-COO$\underline{C}H_3$), 44.6 (C-4), 35.0 (C-2"), 34.2 (C-3), 31.7; 31.8 (C-11 and C-13), 29.7 (C-6), 28.1 (C-10), 25.6 (C-12), 24.5 (C-5), 21.4 (-OCO$\underline{C}H_3$), 19.2 (C-9);[36] MS m/z 499 ([$M^+$] + $NH_4$). The isotopic distribution according to mass spectrometry was, m/z (494–499): 497, 4.7%; 498, 2.4%; 499, 92.8%.

Acetylated **10a** (0.20 g, 0.42 mmol) in 80% aq. MeCN (15 mL) was added with stirring at room temperature to $HgCl_2$ (0.25 g, 0.93 mmol) in 80% aq. MeCN (3 mL). Red HgO (0.10 g, 0.47 mmol) was added and the

orange suspension was heated under reflux for 1 h under $N_2$. After cooling, the suspension was centrifuged for 5 min at 1000 rpm. The combined organic layers were washed with 5 M aq. ammonium acetate, with $H_2O$, and with brine and dried over $Na_2SO_4$ to give **11** (0.135 g, 0.34 mmol) in 81% yield. $^1$H NMR **11** δ 9.45 (*s*, 1H, H-7), 7.36 (*d*, *J* = 2 Hz, 1H, H-2), 6.60 (*d*, *J* = 1.8 Hz, 1H, H-6'), 6.50 (*d*, *J* = 1.8 Hz, 1H, H-4'), 3.67 (*s*, 3H, -COOC$\underline{H}_3$), 3.34 (*br d*, *J* = 12 Hz, 1H, H-3), 2.58 (*s*, 2H, H-2"), 2.30 (*s*, 3H, -OCOC$\underline{H}_3$), 1.73 (*t*, *J* = 12 Hz, 1H, H-4), 1.43 (*s*, 2H, H-10), 1.12 (*s*, 1H, H-9);[37] $^{13}$C NMR **11** δ 193.8 (C-7), 173.5 (C-3"), 168.6 (-O$\underline{C}$OCH$_3$), 154.9 (C-1'), 151.7 (C-2), 149.3 (C-3'), 141.6 (C-5')*, 141.2 (C-1)*, 115.5 (C-6')**, 114.1 (C-4')**, 113.0 (C-2'), 51.7 (-COO$\underline{C}$H$_3$), 44.8 (C-4), 35.6 (C-3), 34.9 (C-2"), 27.2 (C-10), 23.5 (C-6), 22.3 (C-5), 21.2 (-OCO$\underline{C}$H$_3$), 19.0 (C-9) (* and ** indicate interchangeable shifts);[37] MS m/z 409 ([M$^+$] + NH$_4$). The isotopic distribution according to mass spectrometry was, m/z (404–409): 404, 0.1%; 405, 0.9%; 406, 0.5%; 407, 1.5%; 408, 1.8%; 409, 95.3%.

*(±)-[$^2H_5$]-4",5"-Bisnor-Δ$^1$-tetrahydrocannabinol-7,3"-dioic acid (12)*

80% $NaClO_2$ (349 mg) and $NaH_2PO_4$ (332 mg) were dissolved in $H_2O$ (9 mL) and added in portions to a solution of **11** (0.13 g, 0.33 mmol) in *t*-BuOH (16.6 mL) and 2-methyl-2-butene (16.6 mL). The two phase mixture was stirred at room temperature until the reaction was complete. The reaction mixture was concentrated and dissolved in $CH_2Cl_2$:$H_2O$ (80 mL, 1:1 v/v). The aqueous phase was extracted with $CH_2Cl_2$ (2 × 25 mL). The combined organic layers were washed with 0.1 M HCl, and with $H_2O$, dried over $Na_2SO_4$ and evaporated *in vacuo* to give an orange oil.[23] $^1$H NMR acetylated **12** δ 7.72 (*d*, *J* = 2 Hz, 1H, H-2), 6.59 (*d*, *J* = 1.7 Hz, 1H, H-6'), 6.49 (*d*, *J* = 1.7 Hz, 1H, H-4'), 5.30 (*s*, -OH), 3.68 (*s*, 3H, -COOC$\underline{H}_3$), 3.25 (*br d*, *J* = 10 Hz, 1H, H-3), 2.59 (*s*, 2H, H-2"), 2.31 (*s*, 3H, -OCOC$\underline{H}_3$), 1.96–2.07 (*m*, H-5), 1.72 (*t*, *J* = 11 Hz, 1H, H-4), 1.44 (*s*, 2H, H-10), 1.35–1.50 (*m*, H-5), 1.11 (*s*, 1H, H-9); $^{13}$C NMR acetylated **12** δ 173.2 (C-3"), 172.0 (C-7), 168.8 (-O$\underline{C}$OCH$_3$), 154.7 (C-1'), 149.2 (C-3'), 142.8 (C-2), 140.9 (C-5'), 130.0 (C-1), 115.4 (C-6'), 114.1 (C-4'), 113.3 (C-2'), 77.2 (C-8), 51.7 (-COO$\underline{C}$H$_3$), 43.9 (C-4), 34.9 (C-3), 34.6 (C-2"), 27.2 (C-10), 24.8 (C-6), 24.0 (C-5), 21.1 (-OCO$\underline{C}$H$_3$), 19.1 (C-9); MS m/z 425 ([M$^+$] + NH$_4$). The isotopic distribution according to mass spectrometry was, m/z (420–425): 421, 0.2%; 422, 0.2%; 423, 0.9%; 424, 1.8%; 425, 96.8%.

The crude acetylated **12** (50 mg, 0.12 mmol) was dissolved in a solution of KOH (226 mg) in EtOH (3.9 mL) and $H_2O$ (0.23 mL) and stirred over night at room temperature. The reaction mixture was concentrated, diluted with $H_2O$ and extracted with ether (3 × 25 mL). The aqueous phase was acidified with 2 N HCl and extracted with ether (3 × 25 mL) and then with EtOAc (3 × 25 mL). The organic layers were combined, dried over $Na_2SO_4$ and evaporated to dryness to give **12** (28 mg, 0.08 mmol) in 65% yield. $^1$H NMR **12** ([$^2H_6$]-acetone) δ 8.64 (*br s*, -OH), 8.11 (*d*, *J* = 2 Hz, 1H, H-2), 6.34 (*d*, *J* = 1.7 Hz, 1H, H-4'), 6.21 (*d*, *J* = 1.7 Hz, 1H, H-6'), 3.37 (*m*, 1H, H-3), 2.53 (*s*, H-2"), 1.67 (*td*, *J* = 11, 1.8 Hz, 1H, H-4), 1.41 (*s*, 2H, H-10), 1.10 (*s*, 1H, H-9);[38] $^{13}$C NMR **12** ([$^2H_6$]-acetone) δ 174.1 (C-3"), 168.9 (C-7), 156.8 (C-1'), 155.9 (C-3'), 143.4 (C-2), 141.8 (C-5'), 129.9 (C-1), 109.7 (C-6'), 108.4 (C-2'), 108.1 (C-4'), 77.4 (C-8), 45.5 (C-4), 35.5 (C-2"), 35.3 (C-3), 27.8 (C-10), 26.3 (C-6), 25.0 (C-5), 19.3 (C-9);[38] MS m/z 352 ([M$^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (347–352): 347, 0.2%; 348, 1.6%; 349, 0.2%; 350, 2.6%; 351, 2.6%; 352, 92.7%.

In order to facilitate the purification, **12** was dissolved in MeOH:ether (1:10) and esterified with 1 eq. $CH_2N_2$. After evaporation to dryness the sample was dissolved in 5% MeCN–$CCl_4$ and filtered through a Bond Elut extraction cartridge (Si, 3 mL, Analytichem International) and purified using low pressure liquid chromatography with 5% MeCN–$CCl_4$ (2 mL min$^{-1}$) as eluent. $^1$H NMR dimethyl ester of **12** δ 8.01 (*d*, *J* = 2 Hz, 1H, H-2), 6.25 (*d*, *J* = 2 Hz, 1H, H-6'), 6.22 (*d*, *J* = 2 Hz, 1H, H-4'), 6.04 (*br s*, 1H, -OH), 3.75 (*s*, 3H,-COOC$\underline{H}_3$), 3.68 (*s*, 3H, H-4"), 3.36 (*m*, 1H, H-3), 2.57 (*s*, 2H, H-2"), 2.35–2.65 (*m*, H-6), 1.97–2.06 (*m*, H-5), 1.71 (*td*, *J* = 12 Hz, H-4), 1.43 (*s*, 2H, H-10), 1.35–1.50 (*m*, H-5), 1.10 (*s*, 1H, H-9); $^{13}$C NMR dimethyl ester of **12** δ 173.7 (C-3"), 168.5 (C-7), 155.2 (C-1'), 154.8 (C-3'), 142.9 (C-2), 140.7 (C-5'), 129.1 (C-1), 109.7 (C-6'), 107.7 (C-2'), 107.4 (C-4'), 77.2 (C-8), 51.8 (-COO$\underline{C}$H$_3$), 44.3 (C-4), 35.1 (C-2"), 34.6 (C-3), 27.5 (C-10), 25.5 (C-6), 24.3 (C-5), 19.1 (C-9); MS m/z 380 ([M$^+$] + H). The isotopic distribution according to mass spectrometry was, m/z (375–380): 376, 1.2%; 377, 0.1%; 378, 0.4%; 379, 0.5%; 380, 97.8%.

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20. Chemical shift for the benzylic carbon in the unlabelled intermediates **5**–**8**: 65.2 (**5**); 46.3 (3,5-dimethoxy[α,α-$^2H_2$]benzyl chloride); 34.9 (**6**); 36.2 (2-(3,5-dimethoxy[α,α-$^2H_2$]benzyl)malonic acid); 31.2 (3-(3,5-dimethoxy-phenyl)-[3,3-$^2H_2$]propionic acid); 31.2 (**7**); 31.3 (**8**).

21. Spectral data for unlabelled major condensation by-product using $BF_3$–$Et_2O$ as catalyst: $^1$H NMR δ 6.28 (*d*, *J* = 2 Hz, 1H, H-4'), 6.14 (*d*, *J* = 2 Hz, 1H, H-6'), 5.76 (*br s*, 1H, OH), 4.81 (*m*, 1H, H-2), 4.70 (*d*, *J* = 2 Hz, 1H, C=C$\underline{H}_2$), 4.54 (*d*, *J* = 2 Hz, 1H, C=C$\underline{H}_2$), 4.35 (*d*, *J* = 10.5 Hz, 1H, H-7), 3.67 (*s*, 3H, -COOC$\underline{H}_3$), 2.75–3.00 (*m*, 5H, H-3, H-11 and H-13), 2.54 (*t*, *J* = 8 Hz, 2H, H-2"), 1.85–2.22 (*m*, H-1, H-5 and H-12), 1.76 (*s*, C-C$\underline{H}_3$), 1.33–1.73 (*m*, H-6); $^{13}$C NMR δ 174.2 (C-3"), 160.3 (C-1'), 156.4 (C-3'), 147.1 (C-5'), 138.2 (C-2'), 125.0 (C-8), 113.4 (C=$\underline{C}H_2$), 107.1 (C-6'), 97.1 (C-4'), 83.6 (C-2), 51.7 (-COO$\underline{C}H_3$), 49.7 (C-4), 49.2 (C-7), 43.1 (C-3), 42.8 (C-1), 35.1 (C-2"), 30.0 (C-6), 29.6 (C-11 and C-13), 28.4 (C-1"), 26.0 (C-12), 24.4 (C-5), 18.9 (C-$\underline{C}H_3$); MS 452 ([M$^+$] + $NH_4$).

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34. Spectral data for **10b**: $^1$H NMR δ 6.29 (*d*, *J* = 2.5 Hz, 1H, H-4'), 6.21 (*d*, *J* = 1.6 Hz, 1H, H-2), 6.19 (*d*, *J* = 2.6 Hz, 1H, H-6'), 6.12 (*br s*, 1H, -OH), 4.54 (*s*, 1H, H-7), 3.65 (*s*, 3H, -COOC$\underline{H}_3$), 3.23 (*dd*, *J* = 10.9, 2 Hz, 1H, H-3), 2.78–2.98 (*m*, 4H, H-11 and H-13), 2.62, 2.68 (AB, *dd*, *J* = 16.2 Hz, H-2"), 2.40–2.44 (*m*, 2H, H-6), 2.04–2.14 (*m*, 1H, H-12), 1.90–2.00 (*m*, 1H, H-5), 1.74–1.90 (*m*, 1H, H-12), 1.70 (*td*, *J* = 10.9, 2 Hz, H-4), 1.38 (*s*, 2H, H-10), 1.04 (*s*, 1H, H-9); $^{13}$C NMR δ 174.3 (C-3"), 155.8 (C-1'), 155.4 (C-5'), 141.0 (C-3'), 136.5 (C-1), 132.4 (C-2), 115.7 (C-2'), 109.1 (C-4')*, 102.0 (C-6')*, 77.1 (C-8), 52.1 (C-7), 52.0 (-COO$\underline{C}H_3$), 46.5 (C-4), 35.2 (C-2"), 34.8 (C-3), 31.6 (C-11 and C-13), 27.5 (C-6), 27.3 (C-10), 25.6 (C-12), 24.7 (C-5), 18.6 (C-9) (*interchangeable shifts). The isotopic distribution according to mass spectrometry was, m/z (435–440): 439, 4.0%; 440, 96.0%. HRMS m/z calcd for $C_{23}O_4S_2H_{25}[^2H_5]$ 439.1900; found 439.196.

35. Significant peaks in $^1$H NMR for unlabelled **10a** δ 6.96 (*d*, *J* = 1.5 Hz, 1H, H-2), 2.78 (*t*, *J* = 8 Hz, 2H, H-1"), 2.57 (*t*, *J* = 8 Hz, 2H, H-2"), 1.40 (*s*, 3H, H-10), 1.07 (*s*, 3H, H-9); $^{13}$C NMR unlabelled **10a** δ 30.4 (C-1"), 27.5 (C-10), 19.3 (C-9).

36. Significant peaks in $^1$H NMR for unlabelled acetylated **10a** δ 6.58 (*d*, *J* = 1.8 Hz, 1H, H-2), 2.80–2.98 (*m*, 6H, H-11, H-13 and H-1"), 2.55–2.60 (*t*, *J* = 8 Hz, 2H, H-2"), 1.40 (*s*, 3H, H-10), 1.07 (*s*, 3H, H-9); $^{13}$C NMR unlabelled acetylated **10a** δ 30.3 (C-1"), 27.3 (C-10), 19.2 (C-9).

37. Significant peaks in $^1$H NMR for unlabelled **11** δ 9.46 (*s*, 1H, H-7), 7.37 (*s*, 1H, H-2), 2.87 (*t*, *J* = 8 Hz, 2H, 1"-H), 2.61 (*t*, *J* = 8 Hz, 2H, 2"-H), 1.43 (*s*, 3H, H-10), 1.13 (*s*, 3H, H-9); $^{13}$C NMR unlabelled **11** δ 30.3 (C-1"), 27.3 (C-10), 19.1 (C-9).

38. Significant peaks in $^1$H NMR for unlabelled **12** ([$^2H_4$]-MeOH) δ 8.01 (*d*, H-2), 2.73 (*t*, H-1"), 2.55 (*m*, H-2"), 1.39 (*s*, H-10), 1.06 (*s*, H-9); $^{13}$C NMR unlabelled **12** ([$^2H_4$]-MeOH) δ 31.7 (C-1"), 27.9 (C-10), 19.3 (C-9).

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