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# 10th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds—Wiley Young Scientist Awards

Session 10, Tuesday, June 16, 2009

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**Abstract:** Addresses by the three of the four Wiley Young Scientist Award winners are highlighted.

**Keywords:** Anthocyanins; polyphenols; Radioiodo-de-stannylation; Iodine-125; Steroid fatty acid esters; Ionic liquid; Microwave

## STUDIES TOWARDS THE SYNTHESIS OF $^{13}$C-LABELLED ANTHOCYANINS

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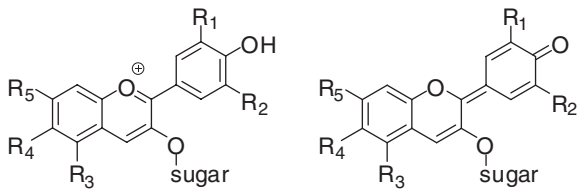
**Summary:** The anthocyanins are a class of polyphenols found in nature, which are widely distributed throughout the plant kingdom and are thought to possess antioxidant properties. Methodology previously developed in our group for the regioselective placement of $^{13}$C-atoms into aromatic rings is being applied to the synthesis of $^{13}$C-labelled anthocyanins–namely cyanidin-3-glucoside and delphinidin-3-glucoside.

**Keywords:** anthocyanins; cyanidin; delphinidin; polyphenols

**Introduction:** Over 500 different anthocyanins have been isolated from plants, and are found mainly in flowers, fruits and vegetables. They are responsible for the colours observed in these species, which can range from red to blue and purple. This change in colour is due to the difference in structure observed in response to pH changes, as shown in **Scheme 1**.[1] The anthocyanins are based on the core structure of the flavylium cation, which itself is based on the benzopyrylium cation. They possess a positive charge, most commonly with $Cl^-$ as the counterion. Chemically, the anthocyanin group consists of the sugar-free anthocyanidins and the anthocyanin glycosides which are water-soluble.




Under acidic conditions (red) Under basic conditions (blue)

**Scheme 1.** Changes in anthocyanin structure due to change in pH.

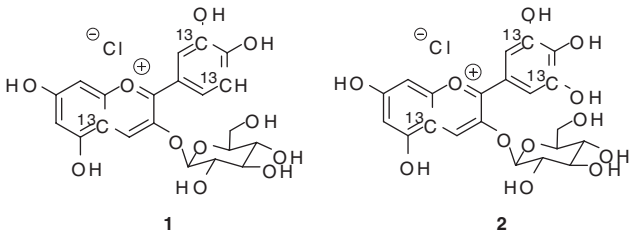
The anthocyanins are powerful antioxidants, protecting the plant from radicals formed either by UV light or during metabolic processes. Interest in anthocyanin pigments has intensified over recent years due to the possible health benefits to humans. One crucial factor is that the antioxidant property is retained after consumption, and so fruits and vegetables containing anthocyanins

are a good source of dietary antioxidants. Consumption of anthocyanin-containing foods may therefore help to prevent illnesses such as coronary heart disease, cancer and strokes. This backs up a popular theory that the drinking of red wine can result in much lower rates of coronary heart disease in countries such as France and Italy, where the consumption of red wine is higher than in North American and North European countries–many of which have significantly higher rates of coronary heart disease.[2]

Other health benefits have been suggested, however the effects of anthocyanins on human health are still poorly understood. Studies into the biological effects of natural products such as these require accurate quantification of the compounds in plasma samples, with LC-MS and GC-MS being the common analytical techniques used. The accuracy and reproducibility of these techniques is greatly improved by the use of stable isotope labelled standards for calibration, as the standard will have similar chemical and physical properties to the analyte.[3] New synthetic routes to isotopically labelled anthocyanins are therefore required.

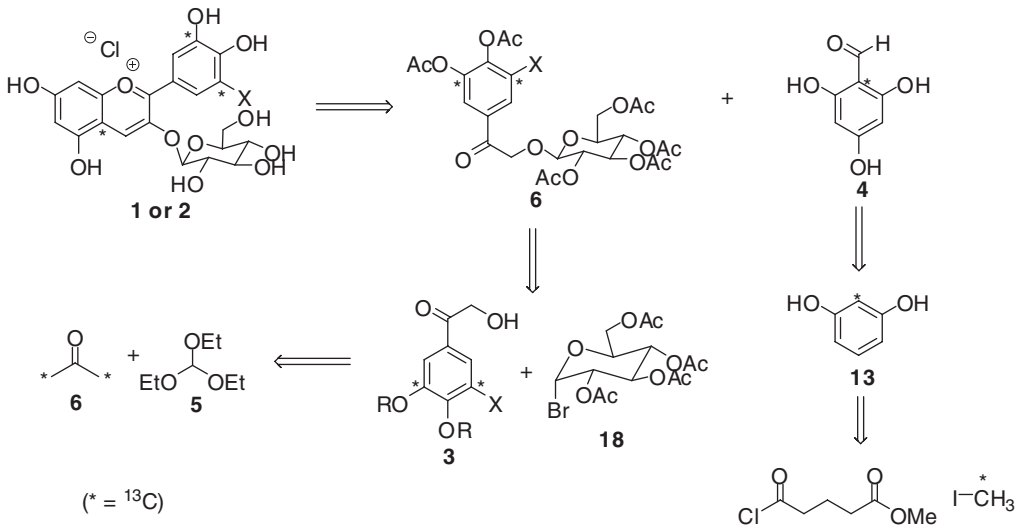
For the isotope chemist, these new synthetic routes must also be applicable to the incorporation of isotopically-labelled atoms through commercially available compounds. Isotopically-labelled small molecules are more widely available and cheaper than uniformly-labelled aromatic rings, and their use can allow the regioselective incorporation of labelled atoms into the structure, as previously demonstrated in work by our group.[3]

An efficient route towards the synthesis of two anthocyanins, namely cyanidin-3-glucoside **1** (found in strawberries) and delphinidin-3-glucoside **2** (found in grapes), (Scheme 2) is being examined, with the unlabelled synthesis being carried out initially in order to optimise the conditions. The synthetic route builds on the strategy developed in the synthesis of [1,3,5–$^{13}C_3$]gallic acid,[3] with regioselective incorporation of $^{13}$C-atoms in the B-ring using either [1,3–$^{13}C_2$]acetone or triethyl [$^{13}$C]orthoformate, as desired. The third $^{13}$C-atom will be introduced using [$^{13}$C]methyl iodide during the synthesis of the A-ring.



**Scheme 2.** Final target compounds.

**Results and Discussion:** Our aim was to apply the synthetic route and labelling strategy used in the synthesis of [1,3,5–$^{13}C_3$]gallic acid to the synthesis of our two target anthocyanins,[3] the retrosynthetic analysis of which is shown in Scheme 3. Using this approach the $^{13}$C-labels are situated in the A and B rings, rather than the central heterocyclic, C-ring. This means that such derivatives would also have utility in studies for the investigation of the metabolism and pharmacokinetics of anthocyanins. The A and B rings usually remain intact during metabolism, whereas the C-ring is broken down, so that $^{13}$C-labels in the C-ring are often lost.



**Scheme 3.** Retrosynthetic analysis of compounds 1 and 2.

Scheme 4 shows the preparation of the initial component **3** starting with synthesis of the 4H-pyran-4-one **4** from triethyl orthoformate **5** and acetone **6** via the intermediate enone **7**. The proposed $^{13}$C-labelled synthesis will make use of [1,3–$^{13}C_2$]acetone to introduce the two $^{13}$C-atoms into the ring.[3] Using our previously optimised procedure, the required phenol 4-hydroxyacetophenone **8** was produced from the reaction of the pyranone with the pronucleophile acetylacetone under basic conditions, followed by acidic hydrolysis to yield the aromatic product in 95% yield.[3] Bromination was achieved by one of two methods, which gave either mono- or di-brominated **9** as required, with acetic acid/sodium acetate/bromine giving di-bromination in quantitative yield and dichloromethane/bromine giving mono-bromination in quantitative yield.[3] Subsequent substitution of bromine with hydroxyl groups was carried out in aqueous sodium hydroxide in the presence of copper sulfate to give **10**.[3] Protection of the hydroxyl groups as the



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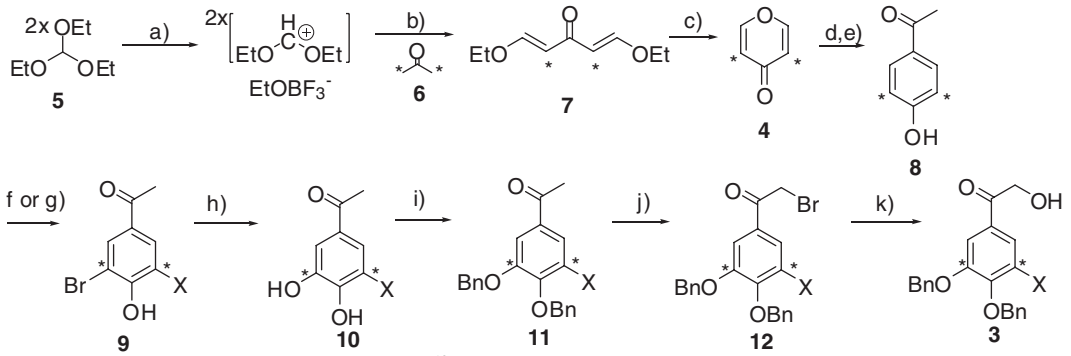
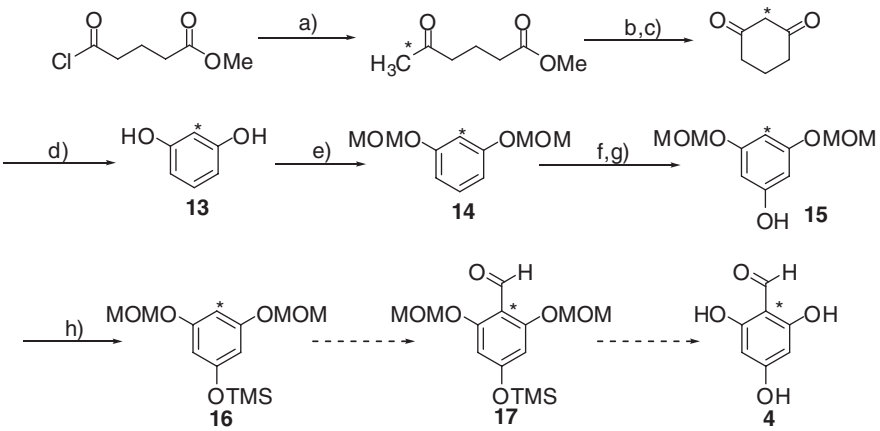
benzyl ethers **11**, produced the first requirement for purification during the synthesis of component **3**.[4] Bromination on the methyl group using tetrabutylammonium tribromide and subsequent substitution of the bromine with a hydroxyl group followed by purification gave component **3** in either the tri- or di-benzyloxy form as desired (29% tri-benzyloxy, 37% di-benzyloxy overall).[5] This is now being repeated with the use of $^{13}$C-labelled starting materials to give the labelling pattern as indicated in **Scheme 4**.

The synthesis of component **4** (**Scheme 5**) was then carried out, which began with the preparation of $^{13}$C-labelled resorcinol **13** as previously developed in our group.[6] The use of [$^{13}$C]methyl iodide in the first stage of this synthesis gives the $^{13}$C-label at the 2 position of the resorcinol. MOM protection[7] of the two hydroxyl groups was then achieved without any problems to give **14** in 67% yield.

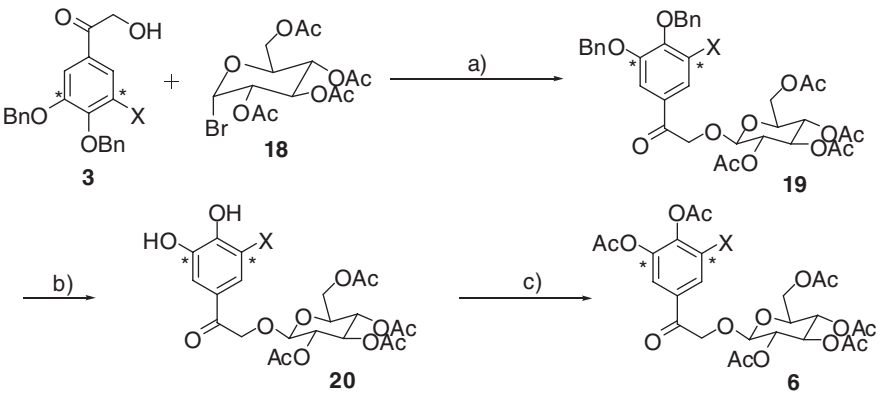


**Scheme 4.** Preparation of component **3**- *indicates proposed point of $^{13}$C introduction and labelled positions. Reagents and Conditions: (a) $BF_3 \cdot OEt_2$, DCM; (b) $^{i}Pr_2NEt$, acetone (or [1,3-$^{13}C_2$]acetone), quant; (c) Aq. HCl, EtOH, efflux, 24 h, quant; (d) Acetylacetone, KO$^{t}$Bu, $^{t}$BuOH, reflux, 20 h; (e) 1 M HCl, reflux, 1 h (95%); (f) AcOH, NaOAc, $Br_2$ 1 h (99%, X = Br); (g) DCM, $Br_2$; (h) $CuSO_4 \cdot 5H_2O$, Aq. NaOH, reflux, 18 h (88% X = OH), 82% X = H); (i) BnBr, 18-crown-6, $K_2CO_3$, acetone, reflux, 6 h (69% X = OBn, 73% X = H); (j) Tetrabutylammonium tribromide, DCM, MeOH, 3 h (99% X = OBn or H); (k) Sodium formate, EtOH, reflux, 18 h (52% X = OBn, 66% X = H).



**Scheme 5.** Studies towards the preparation of component **4** - *indicates proposed point of $^{13}$C introduction and labelled positions. Reagents and Conditions: (a) $CH_3I$ (or $^{13}CH_3I$), Li, CuI, $Et_2O$ (55%); (b) THF, KO$^{t}$Bu, reflux, 6 h; (c) Aq. HCl (74%); (d) 10% Pd/C, xylene, reflux, 3 h (20%); (e) MOMCl, DMF, $Et_2O$, NaH (67%); (f) $B_2$ $Pin_2$, d$^{t}$bpy, $[Ir(OMe)(COD)]_2$, *iso*-hexane, reflux, 18 h; (g) Aq. oxone, acetone (61%); (h) TMSCl, pyridine, DMAP (54%).

The key step was the introduction of a third hydroxyl group *meta* to both MOM ethers (**15**), using a procedure originally reported by Maleczka,[8] and developed further in our group for this purpose. TMS protection to yield **16** was also straightforward. It is now proposed that regioselective formylation of the TMS-protected compound (**17**) followed by global deprotection will yield component **4**, where the $^{13}$C-label will be present adjacent to the formyl group as indicated by the asterisk in **Scheme 5**.



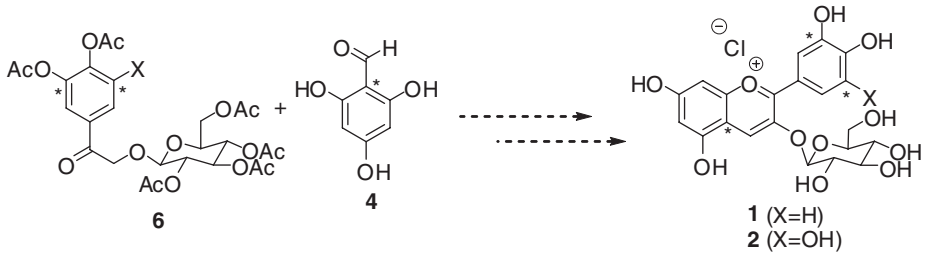
**Scheme 6.** Coupling of components **3** and **5** to give component **18**- *indicates proposed point of introduction of $^{13}$C label and labelled positions. Reagents and Conditions: (a) $Hg(CN)_2$, 4 Å mol. sieves, $Na_2SO_4$, toluene, reflux, 2 h (34% X = OBn, 47% X = H); (b) $H_2$(g), 10% Pd/C, THF, 18 h (95% X = OH, 47%, X = H); (c) Acetic anhydride, pyridine, 18 h (55% X = OAc, 10% X = H).



**Scheme 6** shows the coupling of component **3** with sugar component **18**–obtained from α-*D*-glucose and hydrogen bromide in acetic acid. The optimal conditions for this coupling were found to require the use of mercuric cyanide in toluene under rigorously anhydrous conditions[9] to give **19** in 34% yield (X = OBn) and 47% yield (X = H). Deprotection of the benzyl groups using hydrogen in the presence of palladium on carbon (**20**) followed by subsequent acetyl protection gave component **6**. Once again, either the tri- or di-acetoxy compounds could be obtained depending on the route followed in **Scheme 1**.

The final stage in the synthesis will involve the coupling of components **4** and **6**, and subsequent cyclisation to construct the anthocyanin ring system. Final deprotection of all the hydroxyl groups, on the benzene rings and the sugar, gives the target anthocyanins **1** and **2** as shown in **Scheme 7** below.[9] Currently this has yet to be achieved unlabelled, but the synthesis will follow good literature precedent.[9] When completed in $^{13}$C-labelled form, the target compounds will possess three $^{13}$C-labels–introduced by the use of [1,3–$^{13}C_2$]acetone and [$^{13}$C]methyl iodide–one in the A-ring and two in the B-ring.




**Scheme 7.** Proposed coupling of components **6** and **4** to give target compounds **1** and **2**. (∗ indicates proposed position of labelling).

**Conclusion:** Studies towards the synthesis of $^{13}$C-labelled anthocyanins cyanidin-3-glucoside **1** and delphinidin-3-glucoside **2** are underway, with the synthesis of the unlabelled target compounds nearing completion. Optimisation of the route has led to high yields in the majority of steps, with very little purification being required in the synthesis of component **3**. Both of these factors are beneficial to syntheses employing $^{13}$C-labelled materials as it ensures the minimum amount of losses of material. The application of our previous methodology[3] to this synthesis will allow the regioselective placement of $^{13}$C-atoms into the anthocyanin core, thus producing multiply-labelled anthocyanins for use in biological testing involving GC-MS and LC-MS analysis.

**Acknowledgements:** Financial support from the BBSRC and GlaxoSmithKline is gratefully acknowledged.

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## RADIOIODODESTANNYLLATION OF NOVEL GHB NEURORECEPTOR LIGANDS

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**Abstract:** A series of 4-substituted gamma-hydroxybutyric acid (GHB) analogues have previously been synthesized and characterized for binding to specific high-affinity GHB sites in rat brain cortical membranes.[1,2] Within this series some halogenated analogues displayed unprecedented high affinity and selectivity for GHB sites, and would potentially be valuable as pharmacological tools if radiolabelled. Here we present the iodine-125 labelling of one of these analogues by radioiodo-destannylation.

**Keywords:** Radioiododestannyllation; GHB; iodine-125; chloramines-T

**Introduction:** Gamma-hydroxybutyric acid (GHB) is a neuroreceptor ligand with weak affinity for gamma-aminobutyric acid subtype B ($GABA_B$) receptors and high affinity for specific GHB sites which to date still have unclear pharmacological and physiological importance. GHB is a metabolite of GABA and is found in micromolar concentrations in the mammalian brain.[3] The compound is listed as an illicit drug because of its drug-abuse potential (so-called: Fantasy) and examples of uses as a date

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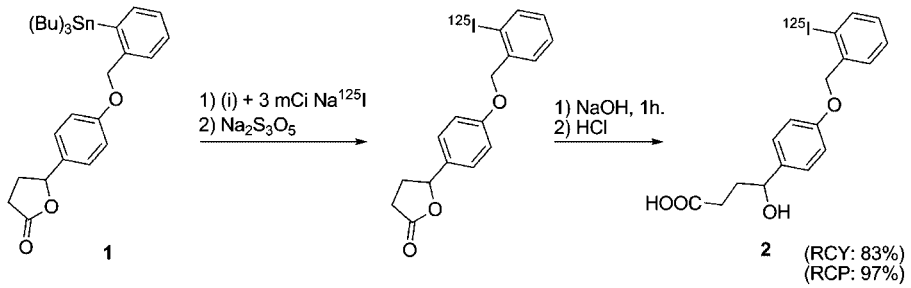
rape drug.[4] GHB is also a registered drug for the treatment of excessive daytime sleepiness and cataplexy associated with the disorder narcolepsy,[5,6] formulated as the sodium salt (Xyrem®).[7] Because of the public concern relating to the misuse of GHB along with the pharmaceutical use and its natural occurrence in the mammalian brain, investigation of its exact mechanism of action is needed.

Developing a suitable radioligand with binding affinity to GHB sites is an important tool for the further understanding of the GHB action and mechanism and we therefore set out to develop a radioligand with high affinity for GHB receptors. Several cold analogues were tested and the iodine-127 version of 2 (**Scheme 1**) showed superior binding to GHB receptor rich regions of the rat brain. The iodine-125 compound was prepared according to the following.

Radioiododestannyllation has proved to be a widely applicable method for labelling organic molecules with radioiodine.[8] Electrophilic iodine can be created (as $I^{\delta+}$-$Cl^{\delta-}$) *in situ* by the reaction of NaI with the oxidant Chloramine-T.[9] In this example we have used a palladium coupling reaction for the synthesis of the organostannate using hexa-*n*-butylditin and tetrakistriphenylphosphine-palladium.

**Results and Discussion:** Iodine-125 labelled compound 2 was readily prepared and is currently undergoing pharmacological evaluation. The tributyl-stannate precursor in its lactone form 1 was reacted with electrophilic iodine-125 (generated from carrier free $Na^{125}I$ in the presence of chloramine-T). Efficient labelling was achieved within 30 min, and subsequent deprotection was achieved by lactone hydrolysis (**Scheme 1**). RP-HPLC purification and subsequent reformulation using SEP PAK C18 cartridges provided the labelled compound 2 in excellent radiochemical yield (RCY 83%) and high radiochemical purity (RCP 97%).




**Scheme 1.** (i) Chloramine-T in a mixture of 94% EtOAc, 4% DMF, 1% AcOH and 1% water. Room temperature, 30 min.

**Experimental: 4-hydroxy-4-[4-(2-[125-iodo]benzyloxy)phenyl]butanoic acid. (2)** Chloramine-T trihydrate (40.8 mg, 145 μmole) was suspended in a mixture of EtOAc (4.75 mL), DMF (0.25 mL), acetic acid (50 μL) and a small amount of water was added (50 μL) to dissolve the compound. This reagent solution (1000 μL) was added to the stannyl precursor **1** (1.19 mg, 2.1 μmole) in a small HPLC vial, the vial was capped and shaken to dissolve the compounds. I-125 NaI (30 μl, 3 mCi) was added to the vial which was quickly reclosed. The vial was shaken and the contents left to react for 30 min.

Sodium metabisulfite (9.70 mg) in water (350 μL) was added to quench excess Chloramine-T and the reaction mixture was washed with purified water (3 × 300 μL). The organic layer was evaporated to dryness under an argon flow.

Subsequently, the reaction mixture was dissolved in acetonitrile (500 μL) and NaOH (20 μL, 4M) the latter to hydrolyse the lactone. The sample was shaken and left over night at 5°C, neutralized with HCl (80 μL, 1 M) and immediately subjected to HPLC purification.

The desired product **2** was collected at 24.7 min, and analysed to have a radiochemical purity of 97% and a total activity of 2.48 mCi (83%). HPLC conditions: Buffer A (4.9% MeCN, 95% Water, 0.1% TFA), Buffer B (99.9% MeCN, 0.1% TFA). Gradient: 0 min to 15 min (30% B); 15 min to 35 min (gradient to 100% B); 35 min to 45 min (100% B); 45 min to 46 min (gradient to 30% B).

The product was diluted five times with ultra purified water and loaded onto a pre-activated C-18 Sep-PAK (160 mg). The loaded cartridge was washed with ultra purified water (5 mL), eluted with EtOH (5 mL) and subsequently diluted to a concentration of approximately 5 MBq/ml with a dilute NaOH solution (0.001 M).

Radiochemical analysis (Figure 1) was performed on the same HPLC system as the preparation with another HPLC method. A Carroll Ramsey S105 equipped with a silicon diode was used as radio detector and UV-detection confirmed a chemically pure product.

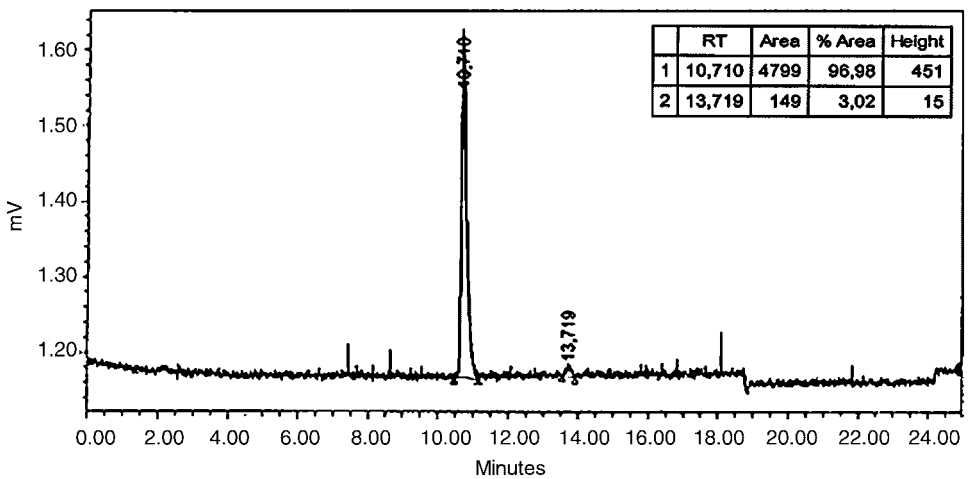



**Figure 1.** HPLC Radiogram for RCP of **2**.



**Material and Methods:** Chemicals were used as arrived from the supplier. Carrier free sodium[I–125]iodine was purchased from Perkin Elmer with a radioactive concentration of 10 mCi/100 μL.

**Acknowledgements:** The work on design and synthesis of GHB ligands has been supported by The Danish Medical Research Council, Beckett-fonden and 'Carl og Ellen Hertz Legat for Dansk Natur- og Lægevidenskab' and is an ongoing collaboration with Drs. Hans Bräuner-Osborne, Rasmus P. Clausen and Bente Frølund (Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen) who are gratefully acknowledged. Radiolabelling experiments were performed at the Hevesy Laboratory, Radiation Research Division, Risø-DTU, The Technical University of Denmark, who are gratefully thanked for the supply of radioisotopes and instruments necessary for the labelling.

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## SYNTHESIS OF [$^3$H] STEROID FATTY ACID ESTERS IN IONIC LIQUIDS UNDER MICROWAVE IRRADIATION

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**Abstract:** Fatty acid esters of steroid hormones are naturally occurring compounds but their physiological role is not fully understood. These esters have potential therapeutic uses. To study their properties a reference compound was required. Radiolabelled analogues of these compounds can serve as a standard. In this work we report the synthesis of long chain fatty acid ester derivatives of tritiated steroids by microwave irradiation in an ionic liquid.

**Keywords:** fatty acid ester; oleate; linoleate; steroid; estradiol; dehydroepiandrosterone; testosterone; tritium label; microwave; ionic liquid

**Introduction:** Fatty acid esters (FAE) of steroid hormones (SH) occur naturally. Since the hydroxyl group is protected as an ester they are metabolically stable, act as a hormone reservoir and can release active hormone to the target tissues as required by the action of esterase.[1,2] SH-FAE are found in different tissues, but their biological significance is not known completely. *In vitro* studies suggest that in blood estradiol ($E_2$)-FAE increases the anti-oxidant potential of lipoprotein.[3] Ovarian follicular fluid contains a mixture of different $E_2$-FAE, comprising predominantly of unsaturated FAE. Since estradiol has action on ovaries, it is expected that $E_2$-FAE may also have an important role in the female reproductive system.[4,5] Concentration of dehydroepiandrosterone fatty acid esters (DHEA-FAE) in the adrenal gland is three times higher than that of free DHEA.[1] In blood DHEA-FAE are produced in high density lipoprotein (HDL) by lecithin-cholesterol acyltransferase (LCAT) and then transferred to low density lipoprotein (LDL) and very low density lipoprotein (VLDL).[6,7] *In vitro* studies show that DHEA-FAE is formed in the ZR-75-1 breast cancer cells and they may act as a prehormone for other steroids.[8,9] These findings are significant for estrogen responsive breast cancer. Testosterone esters occur in the fat and testes of male rat.[10] In rat testosterone is esterified in the brain also and esterification may play an important role in the development of rat brain.[11]

All these studies indicate that SH-FAE may have some unique actions. A better understanding of their physiological role may be therapeutically helpful. Studies were actively carried out to reveal their biological effects requiring a standard compound. Radiolabelled analogue of these compounds can serve as a standard. We have synthesized FAE of [$^3$H] labeled SH for use as standard in biological studies.[12]

**Results and discussion:** [$^3$H] labelled estradiol 17b-oleate (**1**), DHEA oleate (**2**), DHEA linoleate (**3**) and testosterone linoleate (**4**) were synthesized in mg scale from the [$^3$H] labelled steroid in an ionic liquid solvent under microwave irradiation (MW) in high yield. All the starting materials were converted to the corresponding esters in a very short time.



  

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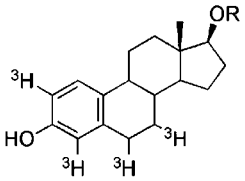
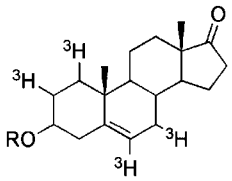
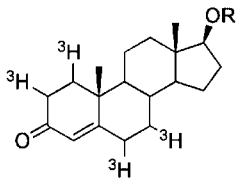



**1** R= $CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$-*cis*




**2** R= $CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$-*cis*
**3** R= $CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7CO$-*cis,cis*




**4** R= $CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7CO$-*cis,cis*

**Figure 1**. Tritum-labeled fatty acid eters of Stroud synthesized in Mw/IL.

Selective esterification at C-17 of estradiol was done in one pot. After preparation of the diester, the product was not isolated. KOH was added to the same vial and the mixture irradiated again to give selectively the C-3 monoester. Since esterification and selective saponification were both done in the same reaction vessel no significant loss of the costly[$^3$H] labelled materials occurs. This methodology is very effective for synthesis with isotopically labeled materials, where cost of the materials compels one to run the reactions on a small scale.

**Experimental:** We synthesized tritium-labelled fatty acid esters of steroids from [1,2,6,7-$^3$H]-DHEA (94.5 Ci/mmol), [2,4,6,7-$^3$H]-estradiol (70 Ci/mmol) obtained from PerkinElmer, Boston and [1,2,6,7-$^3$H]-testosterone (74 Ci/mmol) obtained from Amersham.

*General procedure for synthesis of fatty acid esters of steroids*

A mixture of $7.1 \times 10^{-6}$ mmol of steroid, catalytic amount 4-(dimethylamino)pyridine, 1-butyl-3-methylimidazolium chloride[B-mim]Cl and excess of fatty acid chloride in dry pyridine was MW irradiated at 40°C with 20 W power for 15 min. After the reaction, the mixture was acidified with 1N HCl. The aqueous phase was extracted three times with EtOAc. The combined organic phase was washed successively with a $NaHCO_3$ solution and brine and dried over $Na_2SO_4$. The crude product was purified on a short silica gel column eluting with a mixture of 8:1 or 3:1 n-hexane/EtOAc depending on the procduct.

For the synthesis of estradiol-17b-oleate, after irradiation at 40°C, an excess of KOH and toluene was added to the vial and irradiated at 80°C for a further 5 min. The product was then isolated by the procedure described above.

The resulting[$^3$H] steroid esters gave a single radioactive spot coincident with authentic cold steroid esters on thin layer chromatography.

**Conclusion:** Tritium labeled steroid fatty acid esters were synthesized with the assistance of IL and MW. The method is rapid and high yielding.

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