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**Research Article**

# Synthesis of a $^{11}$C-labelled taxane derivative by [1-$^{11}$C]acetylation

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## Summary

The $^{11}$C-labelling of the taxane derivative BAY 59-8862 (**1**), a potent anticancer drug, was carried out as a module-assisted automated multi-step synthesis procedure. The radiotracer **[$^{11}$C]1** was synthesized by reacting [1-$^{11}$C]acetyl chloride (**6**) with the lithium salt of the secondary hydroxy group of precursor **3** followed by deprotection. After HPLC purification of the final product **[$^{11}$C]1**, its solid-phase extraction, formulation and sterile filtration, the decay-corrected radiochemical yield of **[$^{11}$C]1** was in the range between 12 and 23% (related to [$^{11}$C]$CO_2$; $n = 10$). The total synthesis time was about 54 min after EOB. The radiochemical purity of **[$^{11}$C]1** was greater than 96% and the chemical purity exceeded 80%. The specific radioactivity was $16.8 \pm 4.7$ GBq/μmol ($n = 10$) at EOS starting from 80 GBq of [$^{11}$C]$CO_2$. 

**Key Words:** taxane; anticancer drug; [1-$^{11}$C]acetyl chloride; [$^{11}$C]BAY 59-8862; positron emission tomography

## Introduction

Taxanes are an important class of antitumor agents. These compounds bind to the microtubuli and inhibit their depolymerization into tubulin. Subsequently, taxanes arrest cell-cycle progression in mitosis by interacting directly with tubulin and interfering with microtubule spindle function.[1]

The non-invasive investigation of such antitumor agents, labelled with a positron emitting radionuclide, by means of positron emission tomography (PET) has proved as valuable tool to assess general pharmacokinetics of the

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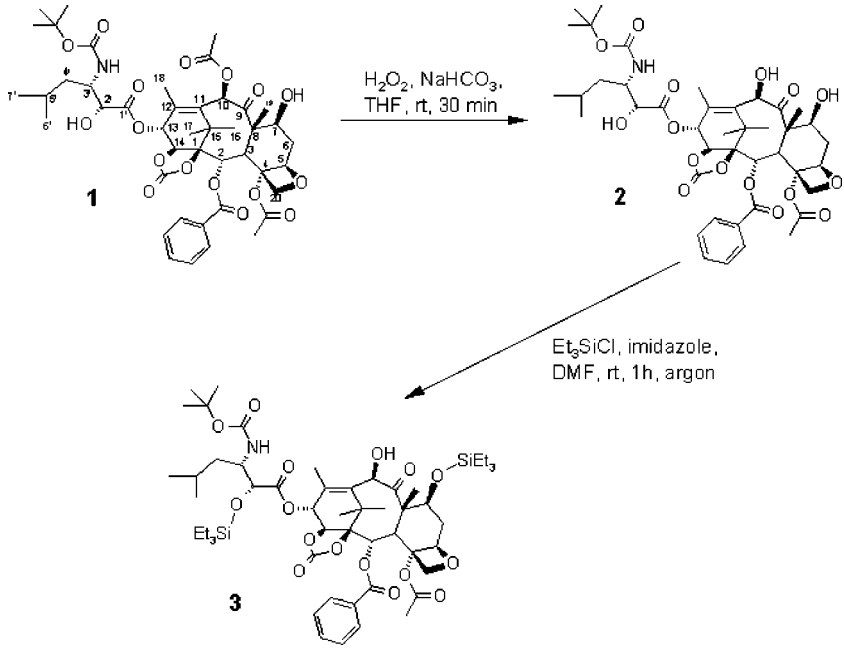



**Scheme 1. Synthesis of the precursor 3**

drug *in vivo*. Moreover, PET also allows monitoring the body's response after treatment with such drugs.

The taxane derivative paclitaxel (Taxol®) is an effective anticancer drug against solid tumors, which was labelled with carbon-11 by reacting [α-$^{11}$C]benzoyl chloride with the corresponding primary amine precursor.[2] Recently, another $^{11}$C-labelling procedure involving a free amino group of a taxane derivative has been reported by van Tilburg *et al.*[3] Docetaxel (Taxotere®) as an accepted chemotherapeutic agent for the treatment of breast cancer and non-small cell lung cancer was labelled with $^{11}$C in the carbamate moiety using [$^{11}$C]*tert*-butanol via [$^{11}$C]*tert*-butyl-1,2,2,2-tetrachloroethyl carbonate.[3]

This report describes the $^{11}$C-labelling of the taxane derivative BAY 59-8862 (**1**) using [1-$^{11}$C]acetyl chloride. Compound **1**, 5*β*, 20-epoxy-1,2α,4,7*β*,13α, 14*β*-heptahydroxytax-11-en-9-one-1,14-carbonate-4,10-diacetate-2-benzoate 13-[(2*R*,3*S*)-3-(*N*-*tert*-butoxycarbonyl)-amino-2-hydroxy-5-methylhexanoate] (see Scheme 1) was shown to be a new potent anticancer drug[4] which is intended for the therapy of brain metastases of breast cancer.

## Results and discussion

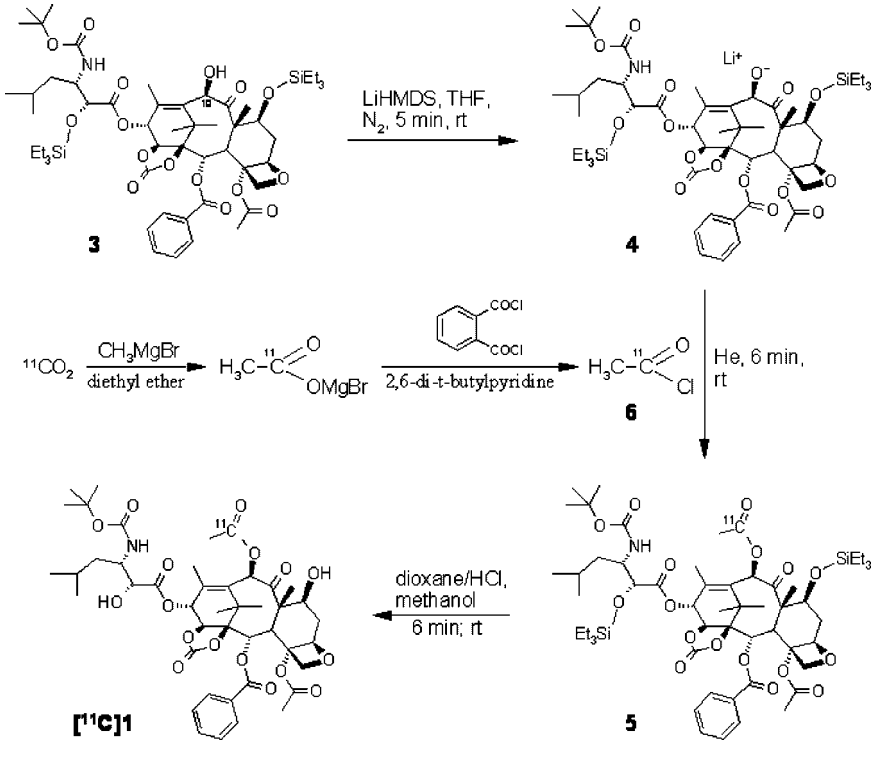
The acetyl moiety of compound **1** at position 10 (Scheme 1) was selected to introduce carbon-11 by reaction of a suitable 10-deacetyl precursor compound

with [1-$^{11}$C]acetyl chloride. With respect to drug metabolism, experiments with human hepatocytes revealed sufficient metabolic stability of the selected acetyl group.

For the synthesis of the required precursor **3** taxane **1** (BAY 59-8862) was selectively deacetylated in position 10 by treatment with sodium hydrogencarbonate and hydrogen peroxide to give compound **2** in 46% yield (Scheme 1). To avoid the known epimerization of the hydroxy group in position 7 and to stabilize the ester group in position 13 the 2′ and 7 hydroxy groups of **2** were selectively protected as triethylsilyl ethers.[5] This protection procedure was carried out using triethylsilyl chloride and imidazole in DMF to afford compound **3** in 59% yield. Precursor **3** contains the sterically hindered hydroxy group in position 10.

[$^{11}$C]BAY 59-8862 (**[$^{11}$C]1**) was synthesized in a multi-step procedure according to Scheme 2 using an automated synthesis module. The schematic diagram of this module is demonstrated in Figure 1. The capability of this module includes the radiosynthesis of [1-$^{11}$C]acetyl chloride (**6**) and its conversion with the lithium salt **4** of precursor **3** into intermediate **5**, acidic hydrolysis of **5** to crude **[$^{11}$C]1**, HPLC purification and solid-phase extraction of the final product **[$^{11}$C]1** as well as its formulation.




**Scheme 2. Synthesis of [$^{11}$C]1**

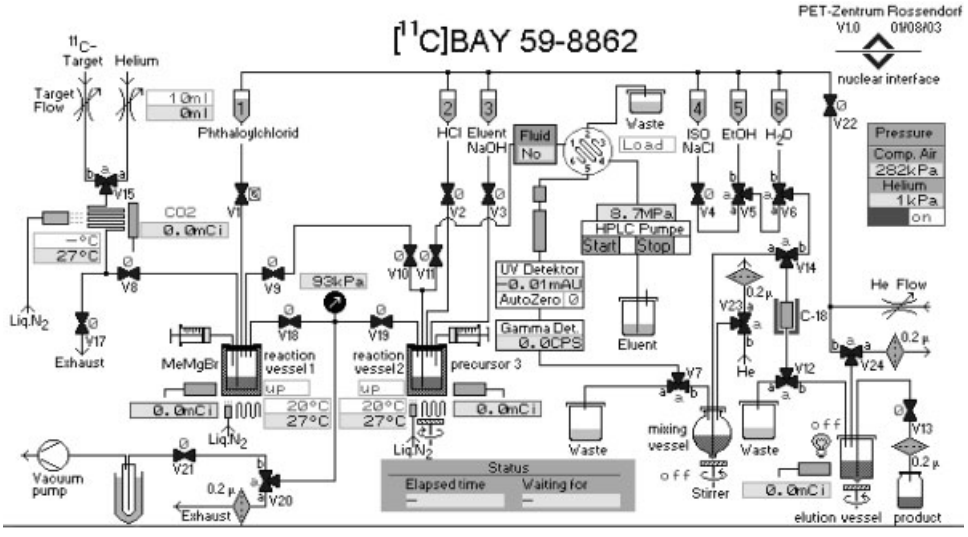



**Figure 1. Schematic diagram of the module for synthesizing [$^{11}$C]1**

The first step of this procedure was the radiosynthesis of [1-$^{11}$C]acetyl chloride (**6**) by conversion of [$^{11}$C]$CO_2$ with MeMgBr in diethylether followed by quenching the resulting [$^{11}$C]acetate solution with phthaloyl dichloride and 2,6-di-*t*-butylpyridine[6] according to Scheme 2. The synthesis of **6** is described in literature using different Grignard reagents before addition of phthaloyl dichloride: MeMgBr in diethylether,[6] MeMgBr in THF,[7] MeMgCl or MeLi in diethylether[8] and MeMgCl in THF.[9] We have tested the several Grignard/solvent combinations and found out, that only the use of MeMgBr in diethylether[6] gave a radiochemical pure **6**. The other combinations caused a non-identified radioactive by-product of up to 50%. The reason for undesirable labelled products can be a side reaction of carbonated methylmagnesium halide with excess methylmagnesium halide as discussed by Pike *et al.*[10].

The release of **6** out of the reaction mixture was carried out by means of a fractionated distillation.[11] The first fraction up to 85°C (reactor temperature) contained the diethylether with interfering phthaloyl dichloride and was evaporated in the exhaust. The main radioactivity of **6** was released at 85–100°C, although the boiling point of acetyl chloride is 51°C. This issue was probably caused by boiling point elevation due to the presence of phthaloyl dichloride and 2,6-di-*t*-butylpyridine.

It was shown that traces of phthaloyl dichloride substantially prevent the [$^{11}$C]acetylation reaction. Therefore phthaloyl dichloride contaminations should be avoided, and distillation of pure **6** was achived by carefully flushing a helium gas stream over the surface of the quenched Grignard reaction mixture. Mixing by direct bubbling the He stream through

the quenched Grignard reaction mixture was only performed within very short time intervals. This bubbling was necessary for satisfactory radiochemical yields of **6**.

After transfer of the purified gaseous **6** into a cooled THF solution of precursor **3** and lithium hexamethyldisilazide (LiHMDS) in a second reaction vessel, the [$^{11}$C]acetylation reaction to form intermediate **5** was carried out at room temperature. After evaporation of the solvent *in vacuo*, the triethylsilyl protecting groups of **5** were removed by means of hydrochloric acid in MeOH/dioxane at room temperature. After neutralization of the reaction mixture, crude **[$^{11}$C]1** was purified by semi-preparative RP HPLC and solid-phase extraction[12] using an RP–18-cartridge. The resulting ethanolic solution of **[$^{11}$C]1** was transferred through a sterile filter followed by a isotonic saline solution to obtain the final formulation of **[$^{11}$C]1** in pharmaceutical quality. The stepwise transfer of these solutions prevented the loss of **[$^{11}$C]1** by absorption on the membrane of the sterile filter.

The decay-corrected radiochemical yield of **[$^{11}$C]1** ranged from 12 to 23% (related to [$^{11}$C]$CO_2$; $n = 10$) in its final formulation. The total synthesis time of the process was 54 min. The radiochemical purity of **[$^{11}$C]1** was higher than 96%. The chemical purity ranged between 80 and 90% based on UV absorption at 235 nm. The specific radioactivity of the final product was $16.8 \pm 4.7$ GBq/μmol ($n = 10$) at EOS starting from 80 GBq of [$^{11}$C]$CO_2$.

## Experimental

### *Materials and methods*

The $^1$H-NMR spectrum of the precursor **3** was recorded on a Bruker DRX NMR spectrometer at 400 MHz. Chemical shifts are reported in ppm relative to TMS as internal standard.

The LC-MS analyses for the compounds **2** and **3** were performed using a PE/API III with MacIntosh Quadra® 900 from Perkin-Elmer Sciex Instruments.

To determine the radiochemical and chemical purity of the reaction product, and the specific radioactivity of **[$^{11}$C]1**, an Agilent 1100 HPLC system with an UV detector (235 nm) coupled in series with a radioactivity detector GABI (raytest, Straubenhardt, Germany) was used. The HPLC analyses were performed on a Purospher RP-18 column (5 μm, 125 mm × 3 mm, Merck) eluted isocratically with acetonitrile/water (60/40) containing 0.1 M ammonium formate at a flow rate of 0.5 ml/min.

The specific radioactivity of **[$^{11}$C]1** was calculated by means of a calibration curve using different concentrations of the nonradioactive **1** in relation to their UV absorbance response and the final radioactivity of **[$^{11}$C]1**.

The used chemicals were reagent grade obtained from commercial suppliers. BAY 59-8862 **(1)** and the reference compound of **2** (10-deacetyl BAY 59-8862) was delivered by Indena (I-20090 Milan, Italy).

The THF (Fluka, waterfree) for the radiochemical conversions was freshly distilled over Na/benzophenone in a nitrogen atmosphere. The 0.15 M solution of methylmagnesium bromide in diethyl ether was made from a 3 M ethereal MeMgBr solution (Aldrich) by dilution with dry diethyl ether (Fluka, 99.8%, puriss., over molecular sieve) under nitrogen. The grade and source of the other reagents for the synthesis of the final product **[$^{11}$C]1** were as follows: phthaloyl dichloride, for synthesis, Merck; 2,6-di-*t*-butylpyridine, $\geqslant$97%, Aldrich; lithium hexamethyldisilazide, 1 M in THF, Aldrich; hydrochloric acid, 4 M in dioxane, Fluka; methanol, $\geqslant$99.5%, extra pure, Merck; NaOH, 1 M, Ph Eur, Merck; acetonitrile, p.a., Merck; water, for injection use, Serumwerk Bernburg AG, Germany; ammonium formate, 99%, Acros Organics; ethanol, 96%, extra pure, Merck; isotonic saline solution, for intravenous infusion, DeltaSelect GmbH, Pfullingen, Germany.

The sterile filtration were done by means of a sterile filter 'Millex®-GP' (Millipore, 0.22 μm, Ø = 25 mm).

The used module, outlined in Figure 1, was a commercially available module for $^{11}$C methylation from Nuclear Interface (Münster, Germany). It was modified in terms of program and hardware.

Before starting the two-pot synthesis, a manual cleaning procedure and two cleaning programs for the module were carried out:

The two contaminated reaction vessels were removed and manually purified by means of acetone, ethanol (96%) and water. The purified reaction vessels were filled with acetone (extra pure, Merck) and installed at the module. All contaminated tubing and valves were rinsed with acetone from storage vessel 1 via valve 1–reaction vessel 1–valve 18–valve 20a–exhaust and via valve 1–reaction vessel 1–valve 9–valve 10–reaction vessel 2–valve 19–valve 20a–exhaust.

The first cleaning program rinsed the synthesis part of the module with acetone (storage vessels 1 and 2) and water (storage vessel 3). The second cleaning program rinsed the synthesis part of the module with acetone (storage vessels 1–3) and the solid-phase extraction part of the module with ethanol (storage vessels 4–6). The module was dried by means of a helium gas stream and vacuum.

### *Chemistry*

*Synthesis of compound* **2**. A solution of compound **1** (500 mg, 573 μmol) in tetrahydrofurane (48 ml), hydrogenperoxide (30%, 24 ml) and sodium hydrogencarbonate (48 mg, 56.9 μmol) were stirred at room temperature for 30 min. Ethyl acetate (50 ml) and a sodium thiosulfate solution (5%, 50 ml) were added

and the phases were separated. The organic phase was washed twice with a sodium thiosulfate solution (5%, 50 ml), following with a sodium chloride solution (50 ml) and subsequently with water (20 ml). After drying with sodium sulfate the solution was evaporated to dryness and the crude product was purified by HPLC in 14 portions under the following conditions: column: Nucleosil® C18, 120 × 16 mm, (Knauer GmbH, Berlin, Germany), eluent: acetonitrile/water 60/40, flow rate: 6 ml/min, UV detection: 228 nm, retention time for **2**: about 8 min. The separated fractions of **2** were combined and evaporated to dryness. Yield: 220 mg (265 μmol, 46%); LC-MS: *m/z* $[M+H]^+ = 831$, calculated for $C_{42}H_{55}O_{16}N$: M = 830.

The HPLC retention time of **2** was identical to the reference compound IDN 5236 (10-deacetyl BAY 59-8862) from Indena.

*Synthesis of precursor* **3**. A mixture of compound **2** (155 mg, 186 μmol), imidazole (508 mg, 75 μmol) and triethylsilyl chloride (1.25 ml, 75 μmol) in dimethyl formamide (14 ml) was stirred under an argon atmosphere at room temperature for 1 h. After addition of water (15 ml) the mixture was extracted three times with ethyl acetate (each 5 ml). The organic phases were combined, washed with sodium chloride solution (5 ml) and water (5 ml), dried over sodium sulfate and evaporated to dryness. The raw material was purified by HPLC in 12 portions using the following conditions: column: LiChrosorb® RP18, 250 × 25 mm, (Merck, Darmstadt, Germany), eluent: acetonitrile/water 95/5, flow rate: 15 ml/min, UV detection: 230 nm, retention time for **3**: about 26 min.The separated fractions of **3** were combined and evaporated to dryness. Yield: 116 mg (110 μmol, 59%); LC-MS: *m/z* $[M+H]^+ = 1059$, calculated for $C_{48}H_{71}O_{16}NSi_2$: M = 1058; $^1$H-NMR (400 MHz, $CDCl_3$)δ8.04 (d, 2H, *J* = 8.4 Hz, benzoyl$_{ortho}$), 7.63 (t, 1H, *J* = 7.4 Hz, benzoyl$_{para}$), 7.50 (q, 2H, *J* = 8.4 Hz, benzoyl$_{meta}$), 6.40 (d, 1H, *J* = 7.2 Hz, H-13), 6.11 (d, 1H, *J* = 7.4 Hz, H-2), 5.10 (s, 1H, H-10), 4.95 (dd, 1H, *J* = 9.7 Hz, 2.4 Hz, H-5), 4.86 (d, 1H, *J* = 7.2 Hz, H-14), 4.61 (d, 1H, *J* = 10.0 Hz, NH-3′), 4.41 (d, 1H, *J* = 3.9 Hz, H-2′), 4.40 (dd, 1H, *J* = 10.9, 6.7 Hz, H-7), 4.33 (d, 1H, *J* = 8.7 Hz, H-20a), 4.26 (d, 1H, *J* = 8.7 Hz, H-20b), 4.17 (tt, 1H, *J* = 10.0, 4.2 Hz, H-3′), 3.80 (d, 1H, *J* = 7.4 Hz, H-3), 2.54 (s, 3H, $COCH_3$), 2.49 (m, 2H, H-6), 1.96 (s, 3H, H-18), 1.80 (s, 3H, H-19), 1,70 (m, 1H, H-5′), 1.54 (m, 2H, H-4′), 1.43 (s, 9H, $OC(CH_3)_3$), 1.35 (s, 3H, H-16), 1.25 (s, 3H, H-17), 1.01 (d, 6H, *J* = 6.7 Hz, H-6′, 7′), 0.98 (m, 18H, $SiCH_2\mathbf{C}\mathbf{H}_3$), 0.74–0.64 (m, 12H, $Si\mathbf{C}\mathbf{H}_2CH_3$).

*Radiochemistry*

The storage vessels 1–6, the mixing vessel and the two reaction vessels of the module were supplied with the needed reagent solutions or solvents according to Figure 1.

[$^{11}$C]$CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on the PET cyclotron 'CYCLONE 18/9' (IBA, Belgium). The nitrogen target was irradiated with a beam current of 25 μA for 40 min giving, on average, 80 GBq of [$^{11}$C]carbon dioxide at the end of the bombardment.

After trapping at −165°C the [$^{11}$C]carbon dioxide was released from the cooling trap at –20°C using a helium gas flow of 8 ml/min into reaction vessel 1 containing a cooled solution (–20°C) of methylmagnesium bromide in diethyl ether (500 μl; 0.15 M). When the radioactivity in the vessel reached a plateau, phthaloyl dichloride (100 μl; 692 μmol) and 2,6-di-*t*-butylpyridine (100 μl; 445 μmol) were added. By means of a helium gas stream (50 ml/min) the diethyl ether was evaporated up to 85°C in the exhaust (via V18 and V20a). After reaching 85°C, the helium gas stream was switched from the exhaust to reaction vessel 2 (via V9 and V10). Then in the range of 85–100°C gaseous **6** was transferred from reaction vessel 1 into reaction vessel 2 containing a cooled (−50°C) THF solution (250 μl) of **3** (1.8 mg; 1.7 μmol) and lithium hexamethyldisilazide (4 μl; 1 M in THF). The helium gas stream (50 ml/min) in reaction vessel 1 was driven over the quenched Grignard reaction mixture for the most time. It was driven through the quenched Grignard reaction mixture only in nine intervals of each with 4 s over a time of 6 min. The [$^{11}$C]acetylation took place at room temperature for 6 min. After evaporation of the THF *in vacuo* at room temperature a solution of hydrochloric acid in dioxane (60 μl; 4 M) and MeOH (400 μl) were added for cleavage of the triethylsilyl protecting groups at room temperature. After 6 min, the reaction mixture was neutralized with NaOH (200 μl; 1 M), diluted with HPLC eluent (1.5 ml) and injected to a semipreparative HPLC column (Kromasil-100, RP-18, 7 μm, 300 × 8 mm, Knauer, including a precolumn 30 × 8 mm). The final product **[$^{11}$C]1** was eluted with acetonitrile/water (65/35) containing 0.1 M ammonium formate (pH 7) at a flow rate of 6 ml/min with a retention time of about 8 min. The detection was carried out by means of a UV detector (235 nm) and a radioactivity detector.

The separated fraction of **[$^{11}$C]1** was diluted with water (20 ml) and collected by solid-phase extraction at an RP–18 cartridge. After washing the cartridge with water (10 ml), **[$^{11}$C]1** was eluted with ethanol (1.4 ml). Then the ethanolic solution of **[$^{11}$C]1** and a isotonic saline solution (12.6 ml) were transferred successively through a sterile filter 'Millex®-GP' to obtain the formulated final solution of **[$^{11}$C]1**. In this way a clear, colorless, sterile, pyrogen-free, isotonic NaCl solution of **[$^{11}$C]1** within a volume of 14 ml and containing 10% ethanol was obtained.

## Conclusion

[$^{11}$C]BAY 59-8862 (**[$^{11}$C]1**) was synthesized in a four-step procedure using a commercially available module for $^{11}$C methylation. The base of the

$^{11}$C-labelling was the synthesis of [1-$^{11}$C]acetyl chloride (**6**) in high radiochemical and chemical purity. The [1-$^{11}$C]acetylation using **6** was carried out with the lithium salt of the suitable taxane precursor **3** at the sterically hindered, secondary hydroxy group in position 10. After a deprotection step, crude **[$^{11}$C]1** was purified by semi-preparative RP HPLC and solid-phase extraction. The yield, the radiochemical and chemical purity and the specific radioactivity of the formulated **[$^{11}$C]1** was suitable for human PET studies.

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