

Research Article

Synthesis of [³H] vardenafil, Levitra[®], using a new labeling technique

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Summary

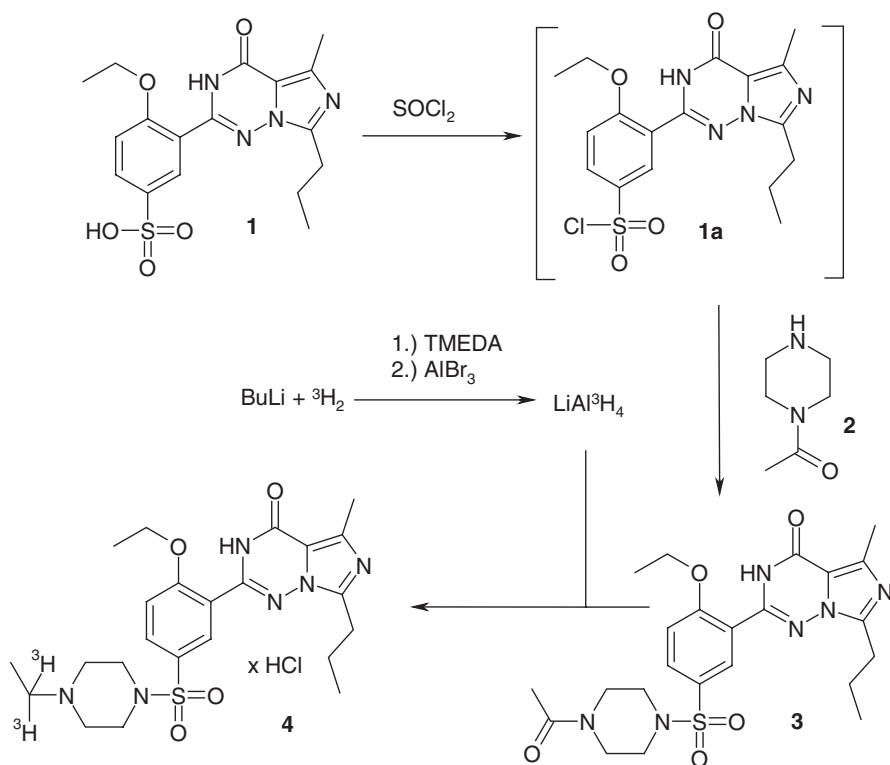
Tritium was introduced into the new orally active, selective phosphodiesterase type V (PDE V) inhibitor vardenafil (Levitra[®]), by reduction of a suitable amide precursor with freshly prepared lithium aluminum tritide. A specific activity of 52.7 Ci/mmol (1.95 TBq/mmol) was achieved. In order to overcome the usual technical difficulties during the preparation of complex tritides a new and easy labeling technique which has considerable potential for various tritiation procedures, was developed. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: Levitra[®]; PDE V inhibitor; complex tritide; tritium labeling technique

Introduction

Receptor binding studies of vardenafil,¹ Levitra[®], a phosphodiesterase type V inhibitor against erectile dysfunction, required the synthesis of the tritium labeled compound **4**. The complete synthesis is depicted in Scheme 1. For that very reason a special amide precursor **3** was synthesized in order to reduce the amide to the tertiary amine with lithium aluminum tritide, which was freshly prepared starting from butyl lithium.² Hydrogenation of butyl lithium with tritium in the presence tetramethylethylenediamine (TMEDA) yielded lithium tritide,

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Scheme 1. Synthesis of $[^3\text{H}]$ varденаfil

which was subsequently reacted with aluminum tribromide, yielding lithium aluminum tritide.

During this process a gas mixture of tritiated butane and excess tritium gas and solvents (mainly an ether/cyclohexane mixture) had to be removed. For disposal of this radioactive mixture, the reaction vessel is usually frozen in liquid nitrogen and the remaining tritium gas absorbed on a tritium uranium getter and the solvent vacuum transferred to a cooled trap.³ This procedure has the disadvantage that the remaining tritium gas contains traces of $[^3\text{H}]$ butane and solvents which inhibit reabsorption of the tritium gas on the getter. Furthermore, the disposal of highly radioactive solvents is difficult. Furthermore, additional tritium was formed due to the decomposition of the excess lithium aluminum tritide. This tritium gas contained hydrogen and also traces of solvents. A safe disposal of this radioactive waste is also difficult.

To overcome these problems we developed a special trap made from stainless steel (Figure 1) which is filled with a mixture of platinum oxide and charcoal. The trap is evacuated before the start of the labeling

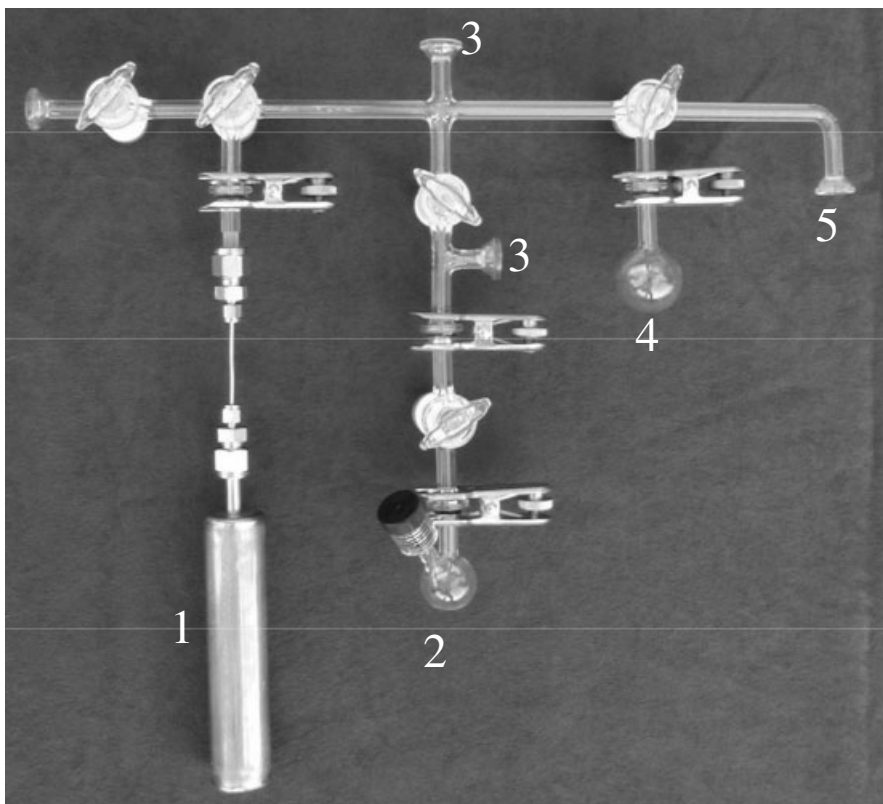


Figure 1. Tritium labeling apparatus: 1, steel trap; 2, two-neck reaction vessel; 3, connections to manometers, 4 reservoir, 5 connection to tritium storage

process and is cooled with liquid nitrogen during the synthesis. The capacity is big enough to absorb all volatile components including tritium gas, solvents and even the air to evacuate the labeling apparatus. Pumps for the vacuum were not required during the whole synthesis. After the labeling all radioactive volatiles, including the solvents from the exchange of the labile tritium, were trapped in the steel container. The trap is connected via 1/16 in steel capillary and Swagelok[®] connectors to the labeling apparatus. At the end of the labeling the trap was disconnected and closed tightly by cutting the capillary with diagonal pliers. The tightness of the trap after cutting was tested by many experiments conducted before. But for safety reasons, the head of the trap was additionally sealed with epoxide resin. We tried to use a 1/8 in capillary because the diameter would have been better for the transfer into the trap but this capillary could not be closed tightly during the cutting process.

Here we describe in considerable detail the development of this new technology which is likely to have wide application for various tritiation procedures. The example chosen is that of the tritiation of vardenafil.

Results and discussion

In order to introduce tritium into vardenafil a suitable starting compound had to be synthesized. The sulfonic acid **1** was reacted with thionyl chloride yielding the sulfochloride **1a** as intermediate which was directly converted with *N*-acetylpiperazine **2** to give the starting material **3** for the radiosynthesis. The acetyl moiety was reduced with freshly prepared lithium aluminum tritide [1] to the ethyl functionality.

The total radioactivity was determined by LS counting to be 15.5 mCi (573 MBq). The distribution of tritium was determined by LC-MS (Table 1).

According to this distribution the specific activity was calculated as 52.7 Ci/mmol (1.95 TBq/mmol). The MS fragmentation pattern corresponds to that for the non-labeled reference compound. The chemical and radiochemical purities were both > 99.0%. The low yield was most likely caused by traces of moisture in one of the solvents because in pre-experiments with deuterium significantly better yields were achieved. However, this yield was sufficient for the further studies. The most important result of this experiment was the safe handling of tritium and its volatile compounds during this multistep synthesis. The whole volatile radioactivity during the labeling was completely captured in a tightly closed stainless steel trap which is accepted by official deposits for radioactive waste in Germany.

Experimental

General

All reagents were purchased from commercial suppliers and were used without further purification. The cylindrical trap made from stainless

Table 1.

m/z	489	491	493
Ion	[M + H] ⁺	[³ HM + H] ⁺	[³ H ₂ M + H] ⁺
Percentage	1.8%	15.2%	83.0%

steel with a wall thickness of 1.6 mm, and prepared at Bayer AG was 12 cm long and had an inner diameter of 2.4 cm which gives a total volume of about 54 ml. This trap was filled with a mixture of charcoal (5 g) (Merck AG, Darmstadt, Germany) and platinum oxide (250 mg) (Sigma-Aldrich, Taufkirchen, Germany). A metal frit U10C-413, 10 µm (ass-CHEM GmbH, Bad Homburg, Germany) in the Swagelok[®] connector ensured that no charcoal or catalyst powder could escape from the trap during the evacuation. The trap is connected via its 1/4 in tube (3 cm long), the Swagelok[®] connector and the 1/16 in steel capillary with an inner diameter of 1 mm to the labeling apparatus. For cutting the capillary an ordinary diagonal pliers was used. The epoxide was UHU plus[®] (UHU Vertrieb GmbH, Buehl, Germany) consisting of two components which were mixed short before. The epoxide is solid after 5 min.

Analytical methods

A Supelcosil ABZ Plus column, 5 µm, 250 × 4.6 mm, (Sigma-Aldrich, GB Supelco, Deisenhofen, Germany), flow rate: 1.0 ml/min, and UV detection (Hewlett Packard 1050 diode array spectrophotometer) at 254 nm and an on-line radioactivity flow detector Ramona[®] (Raytest, Straubenhardt, Germany) were employed with the following gradient:

Eluent A: 0.2% trifluoroacetic acid; eluent B: acetonitrile; 0 min 10% B; 0–30 min 20% B; 30–40 min 40% B; 40–45 min 90% B; 45–50 min 10% B; retention time for vardenafil: 36.3 min.

LC–MS was performed using a PE/Sciex/API III with MacIntosh Quadra[®] 900 (Perkin-Elmer Sciex Instruments; Thornhill, Ont., Canada); Infusion: acetonitrile/0.2% trifluoroacetic acid 90+10 (v+v); LC–MS conditions: the interface was maintained at 4.8 kV and the nebulizing nitrogen pressure at 265 kPa. The atmospheric pressure ion source was used in the positive ion mode. The scan rate was 2.5 s per scan with data collected in 0.2 amu steps from 480 to 500.

Radiochemical counting was performed on a Liquid Scintillation Analyzer TRI-CARB[®] 2500 TR using Ultima Gold[™] cocktail.

2-[2-Ethoxy-5-(4-acetylpiperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one, (3)

A mixture of sulfonic acid **1** (2.0 g, 5.1 mmol) dried under vacuum at 60°C for 1 h, thionyl chloride (4 ml) and dimethylformamide (20 µl) was

warmed up to a temperature of 60°C and stirred for 1.5 h at that temperature. After addition of *p*-xylene (10 ml) the mixture was stirred at 85°C for 15 min and subsequently evaporated to dryness. To remove traces of thionyl chloride *p*-xylene (10 ml) was added and repeatedly evaporated to dryness. To the sulfochloride **1a** dissolved in *p*-xylene (1.5 ml), *N*-acetyl-piperazine **2** (1.5 g, 11.7 mmol) in *p*-xylene (3.2 ml) was added over the course of 30 min. This reaction mixture was stirred at 40°C for 2 h and over a weekend at room temperature. After addition of water (12 ml) the pH was adjusted to 1.0 with concentrated hydrochloric acid (1.5 ml). The aqueous phase was separated and acetone (4 ml) was added. The pH was adjusted to 7.5 with sodium hydroxide solution and the mixture cooled in an ice bath for 1.5 h. The precipitate was filtered off, washed with a mixture of acetone: water 3:1 and dried in a desiccator. Yield: 1.33 g **3** corresponding to 53% of the theory, the chemical purity was >99% as determined by HPLC.

LC-MS: [M + H]⁺ m/z = 503.

*2-[2-Ethoxy-5-(4-[1-³H₂]ethyl-piperazine-1-sulfonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one hydrochlorid, [³H]var-denafil, (**4**)*

The labeling apparatus (Figure 1) was evacuated by cooling the pre-evacuated steel trap in liquid N₂. A vacuum of 10⁻¹ mbar was achieved within 10–15 min. Subsequently tritium gas from the storage was transferred to the frozen mixture of butyl lithium/hexane solution (125 µl, 1.6 mol) in hexane (0.9 ml) and tetramethylethylenediamine (TMEDA) (400 µl, 250 µmol) in a 5 ml two-neck vessel attached to the apparatus. The mixture was warmed up to room temperature and stirred for 60 min at a tritium partial pressure of about 38 kPa (380 mbar). Then the non-reacted tritium and the solvent was adsorbed in the trap filled with platinum oxide and charcoal at the temperature of liquid nitrogen. To the solid residue in the evacuated two-neck vessel aluminum bromide (14.2 mg, 50 µmol) dissolved in ether (500 µl) was added by means of a syringe and via the septum of the second neck of the vessel. After stirring for 5 min compound **3** (5.3 mg, 10 µmol), dissolved in tetrahydrofuran (1125 µl) was added in the same way. The reaction mixture was stirred for 15 min. To decompose the surplus lithium aluminum tritide 200 µls of methanol were added. The released tritium and the solvent were adsorbed in the same trap used for the preparation of the lithium tritide. To remove the labile tritium the dry

residue was dissolved/suspended in ethanol (1 ml) and the solvent was adsorbed in the trap as well. This process was repeated twice. At the end all the solvents and the tritium gas used were in the trap. The trap was disconnected by diagonal pliers and sealed with epoxide resin.

The dry residue was dissolved with 1.0 ml of a mixture of acetonitrile/0.2% trifluoroacetic acid 20:80 (v + v) and purified in 18 equal portions under the following conditions: column: SupelcosilTM, SPLC-ABZ, 250 × 10 mm, (Sigma-Aldrich, GB Supelco, Deisenhofen, Germany); eluent: A = acetonitrile, B = 0.2% trifluoroacetic acid, gradient: 0 min 18% A, 0–40 min 20% A, 40–41 min 50% A, 41–45 min 50% A, 45–46 min 15% A, 46–50 min 15% A; flow rate: 5 ml/min; UV detection: 254 nm; retention time for [³H]vardenafil: 22.5–23.5 min. The eluates containing [³H]vardenafil were fractionated on the basis of the UV signal. The eluates were evaporated under vacuum and hydrochloric acid (10 µl 0.5 mol) was added to the residue in order to prepare the hydrochloride. The solution was lyophilized and the remaining solid was dissolved in 10 ml ethanol to form the stock solution.

Yield: 144 µg (0.29 µmol) **3** corresponding to 2.9% of theory, the chemical purity was >99% as determined by HPLC; specific activity: 52.7 Ci/mmol (1.95 TBq/mmol); total radioactivity: 15.5 mCi (573 MBq).

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