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Research Article

Synthesis of tritium and carbon-14 labelled NNC 61-4655: a potent dual-acting PPARα and PPARγ agonist

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Summary

The synthesis of the potent dual-acting PPARα and PPARγ agonist NNC 61-4655 labelled with tritium and carbon-14 is reported. Tritium labelled NNC 61-4655 was obtained in three steps with introduction of tritium through catalytic tritium-halogen exchange of an aryl bromide precursor. This provided [^{3}H]NNC 61-4655 in 39% overall radiochemical yield with a specific activity of 49 Ci/mmol. Carbon-14 labelled NNC 61-4655 was obtained in five steps starting from bromo[1-^{14}C]acetic acid. The synthetic sequence, which included a Horner–Wadsworth–Emmons olefination and a Mitsunobu alkylation, provided [^{14}C]NNC 61-4655 in 33% overall radiochemical yield with a specific activity of 57.4 mCi/mmol. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: carbon-14; tritium; type 2 diabetes; PPARα and PPARγ agonist; HWE olefination.

Introduction

Diabetes mellitus is a metabolic disease affecting a still more significant proportion of people worldwide.[1] Type 2 diabetes is the most prevalent type of diabetes mellitus and is characterized by insulin resistance,

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hyperglycaemia, and often hyperlipidemia. Besides exercise and diet, a number of drugs are available for the treatment of type 2 diabetes. One such class is the peroxisome proliferator–activator receptor agonists, which lowers blood glucose through an insulin sensitizing mechanism ascribed to the activation of the PPARγ receptor subtype.[2] Unfortunately, these drugs also give rise to weight gain in patients,[3] which is problematic, as obesity is a major concern in connection with type 2 diabetes. Activation of the related PPARα receptor subtype leads to lowered plasma triglycerides in humans[4] and has been reported to induce weight loss in rodents.[5] A drug, which improves both insulin sensitivity and effectively decreases hyperlipidemia, would thus be valuable in the management of type 2 diabetes.

Ragaglitazar, a potent dual-acting PPARγ and PPARα agonist, has been investigated in clinical trials for the treatment of type 2 diabetes (Figure 1).[6–8] NNC 61-4655, a structurally related compound, is likewise a potent PPARγ and PPARα agonist with an interesting profile.[9] To further investigate the possible use of NNC 61-4655 as a drug for the treatment of type 2 diabetes, radiolabelled NNC 61-4655 was required.

The biodistribution and metabolic fate of potential drug candidates are routinely investigated in ADME (absorption, distribution, metabolism, and excretion) and WBA (whole-body autoradiography) studies. These studies are commonly performed using tritium and carbon-14 labelled tracers. Labelling with tritium offers the advantage of high specific activity, and generally, the label can be introduced in few synthetic steps making tritium tracers very useful for early exploratory investigations. The high specific activity of tritium also makes tritium

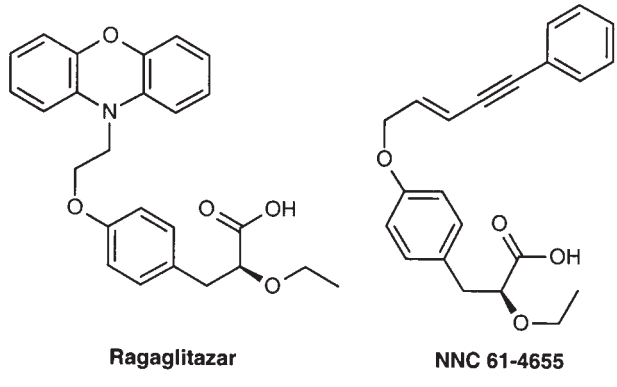


Figure 1. Structure of Ragaglitazar and NNC 61-4655

labelled tracers very useful in screening assays. However, compared to carbon-14, tritium offers considerably less metabolic stability, and for this reason, a carbon-14 labelled tracer is used in most ADME studies. Normally, carbon-14 labelling requires a more lengthy synthetic route in order to incorporate the label in a metabolically stable position.

Here, we report on the labelling of NNC 61-4655 with tritium and carbon-14 for use in *in vitro* and *in vivo* investigations.

Results and discussion

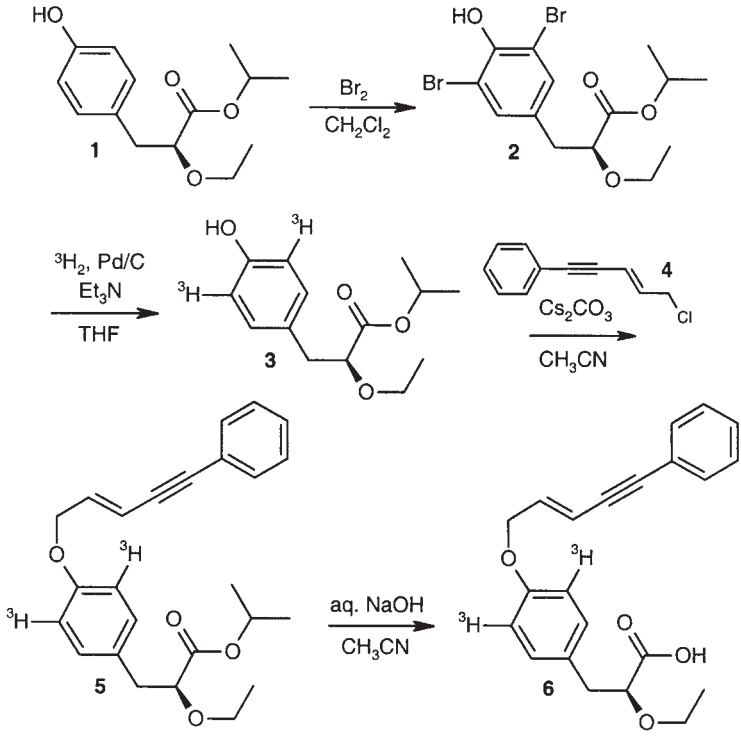
Tritium Labelling

A direct approach to [^{3}H]NNC 61-4655 using catalytic tritium–halogen exchange of an appropriate bromo derivative was not considered feasible, as the target molecule contained two unsaturated moieties prone to reduction. Likewise, NNC 61-4655 was not viewed as suitable substrate for the organoiridium catalysed hydrogen isotope exchange reaction. Therefore, we decided to introduce tritium by tritium–halogen exchange on the dibromo phenolic derivative **2**. Subsequent coupling with the allyllic chloride **4** and hydrolysis would lead to [^{3}H]NNC 61-4655 (**6**) (Scheme 1).

The bromo precursor **2** was prepared by reaction of the phenol **1** with excess bromine in dichloromethane.[†] This gave introduction of two bromines *ortho* to the phenolic moiety and provided **2** as an oil in quantitative yield. Attempted purification of **2** by flash chromatography on silica led to extensive decomposition, and furthermore, the compound proved to be light sensitive. Therefore, it was decided to use the crude material directly in the next step.

Catalytic dehalogenation of **2** using tritium gas and Pd/C in THF proceeded smoothly to give **3** in high yield (82% based on **2**) and good purity. Although **3** could easily be purified at this stage, it is our experience that the crude tritiated compounds after removal of labile tritium can be reacted further without major problems, thereby avoiding an often time-consuming purification step and at the same time minimizing handling and loss of material. Impurities carried forward in the synthetic sequence will most likely be removed in the final purification step. Thus, crude **3** was coupled directly with the allylic

[†]The reaction was also performed using Br_2 in ethanol. This, however, led to extensive transesterfication.

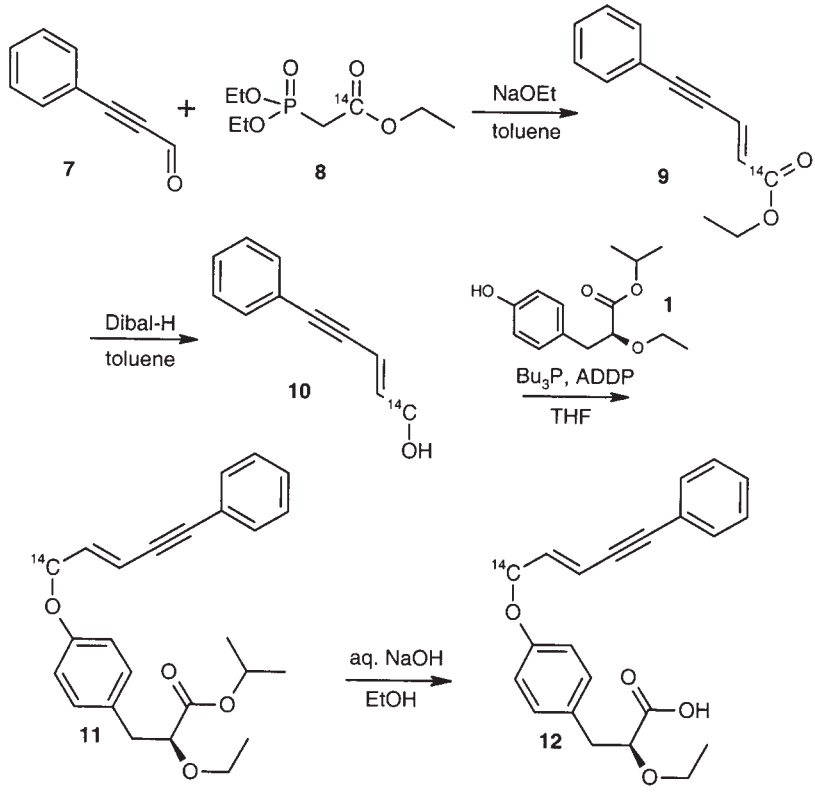


Scheme 1. Synthesis of [^{3}H]NNC 61-4655

chloride **4** in the presence of cesium carbonate[8] to provide crude **5** in quantitative yield. Hydrolysis of the reaction mixture followed by purification on HPLC provided [^{3}H]NNC 61-4655 (**6**) (47%) in 39% overall yield with a radiochemical purity > 98%. The specific radioactivity was determined to 49 Ci/mmol by mass spectroscopy. A method for the analysis of the enantiomeric purity was not developed, as previous experiments have shown that the chiral center in the phenyl propionic acid moiety does not racemise under the reaction conditions employed.[8,10]

Carbon-14 Labelling

For the labelling of NNC 61-4655 with carbon-14, it was decided to introduce the label near the alkene moiety, as this was viewed to be a metabolically stable position compatible with the planned ADME studies. The labelling could be accomplished by subjecting phenyl propargylaldehyde to a Horner–Wadsworth–Emmons olefination (HWE), which eventually would place the label in the allylic position.



Scheme 2. Synthesis of [^{14}C]NNC 61-4655

Although triethyl phosphonoacetate, the desired HWE reagent, is commercially available in its carbon-14 labelled form, it is an expensive starting material, and therefore, we looked for a cheaper alternative. Bromo[1-^{14}C]acetic acid is a cheap commercially available starting material, which can be converted into triethyl [1-^{14}C]phosphonoacetate in one step.[11] Thus, triethyl [1-^{14}C]phosphonoacetate (**8**) was prepared from bromo[1-^{14}C]acetic acid by esterfication with HCl in ethanol followed by reaction with triethyl phosphite. Subsequently, the product was employed in a stereoselective[9] HWE reaction with phenyl propargylaldehyde (**7**) and gave the ester **9** in 75% yield as the only observed isomer (Scheme 2).‡

Reduction of the ester functionality in **9** was accomplished with diisobutylaluminium hydride (Dibal-H), which gave the allylic alcohol

‡We did encounter some problems in reproducing this reaction. In our opinion, most likely this is due to the Arbuzov reaction between ethyl bromo[1-^{14}C]acetate and triethyl phosphite being very sluggish. Currently, this is being investigated in our laboratories.

10 in good yield (68%). Conversion to the allylic chloride would then set the stage for coupling with the phenol **1**. However, this would introduce an additional step compared to a direct Mitsunobu coupling between **10** and **1**, and we decided to pursue the latter strategy. Thus, reaction of **10** and **1** with tributyl phosphine and 1,1′-(azodicarbonyl)dipiperidine (ADDP) proceeded to give the ester **11** in good yield (77%) after flash chromatography. The use of the Mitsunobu reaction was also investigated for the preparation of the tritium labelled compound **5**. However, the small scale employed when working with tritium makes anhydrous chemistry difficult, and in our hands, the Mitsunobu coupling between **3** and the corresponding alcohol failed to react most likely due to the difficulty of keeping the reaction sufficiently dry.

Hydrolysis of **11** with aqueous sodium hydroxide furnished [^{14}C]NNC 61-4655 (**12**) (85%) in 33% overall yield with a radiochemical purity $>98\%$ after purification by HPLC. The specific radioactivity was determined to 57.4 mCi/mmol by mass spectroscopy.

Conclusion

In summary, we have developed synthetic routes for the labelling of the dual acting PPARα and PPARγ agonist NNC 61-4655 with tritium and carbon-14. NNC 61-4655 was labelled with tritium in the phenyl propionic acid moiety in 39% overall yield with tritium introduced by a tritium–halogen exchange reaction performed on the aryl bromide precursor **2**. Likewise, NNC 61-4655 was labelled with carbon-14 in the allylic position in 33% overall yield starting from bromo[1-^{14}C]acetic acid. Following HPLC purification, the products were obtained in high radiochemical purity ($>98\%$ in both cases) and with high specific radioactivity (49 Ci/mmol and 57.4 mCi/mmol for tritium and carbon-14, respectively).

Experimental

General

Bromo[1-^{14}C]acetic acid (specific activity: 58 mCi/mmol) was supplied by Amersham Biosciences, UK. Reactions using tritium gas were performed on a custom build tritium handling unit from RC Tritec AG,

Switzerland, who also supplied the tritium gas. Tritium gas was stored in the form of U^3H_3 and was prepared *in situ* by heating the uranium bed. Phenyl propargylaldehyde, isopropyl (*S*)-3-(4-hydroxyphenyl)-2-ethoxypropionate, and (*E*)-(5-chloro-pent-3-en-1-ynyl)-benzene were supplied by Chemical Development, Novo Nordisk A/S. All reagents and solvents were of analytical grade and used without further purification.

HPLC was performed using a Merck Hitachi Intelligent Pump L6200A equipped with a Merck Hitachi Column Thermostat T6300 (operated at 40°C) with a Rheodyne injector and Merck Hitachi UV Detector L4000A (detection at 273 nm). Detection of carbon-14 and tritium was performed on a Canbarra Packard Flow Detector A-500. The following system was used for analytical HPLC: RP C18 column (4.6 × 250 mm, 5 μm, OdDMeSi 120 Å, Novo Nordisk) using a flow of 1.0 ml/min with a gradient running from 60:40 to 40:60 A/B over 40 min followed by 0:100 A/B for 10 min (A: 10% acetonitrile in 0.1% aq. TFA, B: 90% acetonitrile in 0.1% aq. TFA). Purifications were carried out using the same system employing 4.6 × 250 mm and 10 × 250 mm columns for tritium (1.0 ml/min) and carbon-14 (5.0 ml/min), respectively. Radioactivity measurements were performed on a Packard Tri-Carb 1000 liquid scintillation analyzer using Ultima Flo™ M (Packard Bioscience) as liquid scintillation cocktail. Specific activities were determined on a Sciex API 300 mass spectrometer equipped with an ionspray interface. ^{1}H NMR spectra were recorded on a Bruker DRX 300 spectrometer. Flash chromatography was performed with silica gel 60 Å (Merck, 230 – 400 mesh) and radio-TLC was performed on a Bioscan Imaging Scanner System 200A using Merck F_{254} precoated silica plates.

*Isopropyl (S)-2-ethoxy-3-(3,5-dibromo-4-hydroxyphenyl)-propionate (**2**)*

A solution of Br_2 in dichloromethane (0.20 g/ml, 1.1 ml, 1.4 mmol) was added to a solution of isopropyl (*S*)-2-ethoxy-3-(4-hydroxyphenyl)-propionate (**1**) (67.4 mg, 0.27 mmol) in dichloromethane (10 ml) under protection from light. The reaction mixture was stirred for 1.5 h at rt and then added further Br_2 in dichloromethane (0.20 g/ml, 1.1 ml, 1.4 mmol) followed by stirring for 1 h. Concentration *in vacuo* provided **2** directly as light sensitive orange oil (0.15 g, 100%), which was pure as seen by ^{1}H NMR. Further purification by flash chromatography on silica gel lead to extensive decomposition of **2**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (3 H, t), 1.21 (3 H, d), 1.25 (3 H, d), 2.89 (2 H, d), 3.36

(1 H, m), 3.63 (1 H, m), 3.91 (1 H, dd), 5.06 (1 H, m), 5.80 (1 H, br s), 7.36 (2 H, s).

*Isopropyl (S)-2-ethoxy-3-([3,5-$^3H_2$]-4-hydroxyphenyl)-propionate (**3**)*

Pd/C (10%, 6.5 mg) and Et_3N (10 µl, 72 µmol) were added to a solution of isopropyl (*S*)-2-ethoxy-3-(3,5-dibromo-4-hydroxyphenyl)-propionate (**2**) (7.9 mg, 19.3 µmol) in THF (0.4 ml). The reaction mixture was degassed by three freeze/thaw cycles and stirred overnight with tritium gas (8.1 Ci). Excess tritium gas was reabsorbed on to an uranium waste bed followed by filtration of the reaction mixture and lyophilisation with ethanol (3 × 1 ml) in order to remove labile tritium. Finally, the residue was dissolved in acetonitrile (5 ml) to give **3** (772 mCi, 49 Ci/mmol, 82% based on **2**) with a radiochemical purity of 87%.

*Isopropyl (E)(S)-2-ethoxy-3-[[3,5-$^3H_2$]-4-(5-phenyl-pent-2-en-4-ynyloxy)-phenyl]-propionate (**5**)*

Cs_2CO_3 (32.2 mg, 98.8 µmol) and isopropyl (*S*)-2-ethoxy-3-([3,5-$^3H_2$]-4-hydroxyphenyl)-propionate (**3**) (77.2 mCi, 1.58 µmol) in acetonitrile (0.5 ml) were added to a solution of (*E*)-(5-chloro-pent-3-en-1-ynyl)-benzene (**4**) (8.1 mg, 45.9 µmol) in acetonitrile (1 ml). The reaction mixture was stirred for 2 h at 60°C. This provided **5** (77.2 mCi, 100%) with a radiochemical purity of 75%. The reaction mixture was used directly in the next step.

*(E)(S)-2-Ethoxy-3-[[3,5-$^3H_2$]-4-(5-phenyl-pent-2-en-4-ynyloxy)-phenyl]-propionic acid (**6**)*

Aqueous NaOH (1N, 0.5 ml) was added to a solution of isopropyl (*E*)(*S*)-2-ethoxy-3-[[3,5-$^3H_2$]-4-(5-phenyl-pent-2-en-4-ynyloxy)-phenyl]-propionate (**5**) (77.2 mCi, 1.58 mmol) in acetonitrile (1.5 ml), and the reaction mixture was stirred for 3 h at 60°C. The reaction was quenched by addition of aqueous HCl (2N, 1.0 ml). The reaction mixture was purified by HPLC, which provided **6** (36.6 mCi, 47%) in 39% overall yield with a radiochemical purity > 98%. The specific radioactivity was determined to be 49 Ci/mmol (MS).

*Triethyl [1-^{14}C]phosphonoacetate (**8**)*[11]

A solution of HCl in ethanol (0.5 ml-made by passing HCl (g) through ethanol) was added to a solution of bromo[1-^{14}C]acetic acid (26.6 mCi, specific activity: 58 mCi/mmol) in ethanol (1 ml) and the mixture was stirred for 7.5 h. Approximately half of this solution was transferred to a

5 ml pressure vial, added $P(OEt)_3$ (2 ml), and the lid was tightly closed. The mixture was stirred at 140°C for 5.5 h and stored at −20°C overnight. The mixture was transferred to a pear-shaped flask using ethanol (2 ml) and concentrated (1 mm Hg, bath temperature 55°C) to a yellow oil (approx. 0.2 ml), which was dissolved in toluene (1 ml) and used directly in the next step.

*Ethyl [1-^{14}C]-5-phenyl-pent-2-en-4-ynoate (**9**)*

A solution of triethyl [1-^{14}C]phosphonoacetate (**8**) (13.3 mCi, 0.23 mmol) in toluene (1 ml) was added to a slurry of sodium ethoxide (24.9 mg, 0.42 mmol) in dry toluene (2 ml) under nitrogen resulting in a clear solution, which was stirred for 40 min at rt. Phenyl propargylaldehyde (**7**) (40.3 mg, 0.31 mmol) in toluene (1 ml) was added, and the resulting thick slurry was stirred for 1.5 h. The reaction mixture was quenched with 10% aqueous H_2SO_4 (5 ml) followed by addition of toluene (25 ml). The phases were separated and the aqueous phase further extracted with toluene (25 ml). The combined organic phases were washed with sat. $NaHCO_3$ (5 ml), dried ($MgSO_4$) and concentrated to an oil, which was dissolved in ethanol (20 ml). This provided **9** (9.91 mCi, 75%) with a radiochemical purity of 88%.

*(E)-[1-^{14}C]-5-Phenyl-pent-2-en-4-yn-1-ol (**10**)*

Diisobutylaluminium hydride (1.2 M in toluene, 0.5 ml, 0.60 mmol) was added to a solution of ethyl [1-^{14}C]-5-phenyl-pent-2-en-4-ynoate (**9**) (4.95 mCi, 0.085 mmol) in dry toluene (5 ml) under nitrogen and stirred for 1 h at rt. The reaction mixture was quenched with 10% aqueous H_2SO_4 (5 ml) and stirred for 10 min followed by addition of dichloromethane (25 ml). The phases were separated and the aqueous phase further extracted with dichloromethane (25 ml). The combined organic phases were washed with brine (5 ml), dried ($MgSO_4$) and concentrated to an oil, which was dissolved in ethanol (10 ml). This provided **10** (3.39 mCi, 68%) with a radiochemical purity of >98%.

*Isopropyl (E)(S)-2-ethoxy-3-[4-([1-^{14}C]-5-phenyl-pent-2-en-4-ynyloxy)-phenyl]-propionate (**11**)*

A solution of isopropyl (*S*)-2-ethoxy-3-(4-hydroxyphenyl)-propionate (**1**) (26.4 mg, 0.10 mmol) in dry THF (1.0 ml) was added to a solution of (*E*)-[1-^{14}C]-5-phenyl-pent-2-en-4-yn-1-ol (**10**) (2.77 mCi, 0.048 mmol) in dry THF (3 ml) under nitrogen. The mixture was cooled to 0 °C, and tributyl phosphine (70 µl, 0.28 mmol) was added followed by a solution

of 1,1′-(azodicarbonyl)dipiperidine (55.8 mg, 0.22 mmol) in dry THF (1.0 ml). The mixture was stirred for 1 h, during which a slow precipitation was observed. The mixture was concentrated to dryness and dissolved in dichloromethane (60 ml). The organic phase was washed with water (2 × 15 ml) followed by re-extration of the aqueous phases with dichloromethane (15 ml). The combined organic phases were washed with brine (20 ml), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (ethyl acetate/heptane 2:8) gave a clear oil, which was dissolved in ethanol (5 ml). This provided **11** (2.14 mCi, 77%) with a radiochemical purity of >98%.

*(E)(S)-2-Ethoxy-3-[4-([1-^{14}C]-5-phenyl-pent-2-en-4-ynyloxy)-phenyl]-propionic acid (***12***)*

Aqueous NaOH (1 N, 2 ml) was added to a solution of isopropyl (*E*)(*S*)-2-ethoxy-3-[4-([1-^{14}C]-5-phenyl-pent-2-en-4-ynyloxy)-phenyl]-propionate (**11**) (2.14 mCi, 0.037 mmol) in ethanol (5 ml), and the mixture was stirred at 70°C for 1.5 h. The mixture was cooled to rt, and aqueous HCl (1 N, 2.5 ml) was added (pH < 2).

The crude reaction mixture was purified by HPLC. The combined fractions containing product were concentrated to dryness[§] to give a white solid, which was dissolved in ethanol (5 ml). This provided **12** (1.82 mCi, 85%) in 33% overall yield with a radiochemical purity of >98%. The specific activity was determined to 57.4 mCi/mmol (MS).

Acknowledgements

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[§]Co-concentration with acetonitrile in order to remove the last traces of TFA is recommended, as the free acid readily forms the ethyl ester upon standing in ethanol, when strong acid is present.

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