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**Research Article**

# Syntheses of [$^{14}$C]BAY 59-7939 and its radiolabeled metabolite M-4

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## Summary

BAY 59-7939 is a novel, oral, direct Factor Xa inhibitor in clinical development for the prevention and treatment of thromboembolic diseases. Radiolabeled BAY 59-7939 was required for drug absorption, distribution, metabolism and excretion (ADME studies). The BAY 59-7939 was labeled with carbon-14 in the carboxamide group in one step in an overall radiochemical yield of 85% starting from 4-{4-[(5*S*)-5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl]phenyl}mor-pholin-3-one and 5-chlorothiophene-2-[$^{14}$C]carboxylic acid. The radiolabeled metabolite M-4 was prepared in 77% yield starting from [1-$^{14}$C]glycine and 5-chlorothiophene-5-carboxylic acid. 

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**Key Words:** Factor Xa inhibitor; carbon-14 synthesis; amide formation

## Introduction

Factor Xa has been identified as a potential target for intervention in the coagulation cascade. It is integral to both the intrinsic and extrinsic pathways of the coagulation cascade, and is the point at which the two pathways converge. Approximately one molecule of Factor Xa results in the generation of up to 1000 thrombin molecules.[1] BAY 59-7939 (see Figure 1) has been shown to selectively and directly inhibit Factor Xa, block thrombin generation and prolong clotting times.[2] Furthermore, results from *in vivo* studies have shown reduced thrombus formation with BAY 59-7939 administered

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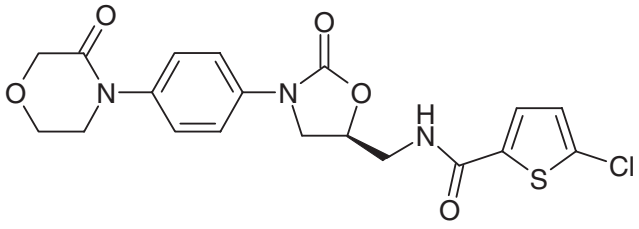


**Figure. 1. BAY 59-7939**

prophylactically in rat and rabbit thrombosis models.[2] In support of a program to develop this new Factor Xa inhibitor synthesis of carbon-14 labeled BAY 59-7939 was required. The radiolabeled metabolite M-4 was synthesized for use as reference standard. This paper describes the syntheses of the two compounds.

## Results and discussion

### *[$^{14}$C]BAY 59–7939*

The approach taken for the synthesis of carbon-14 labeled BAY 59-7939 relies on the previously reported synthesis of BAY 59-7939.[3] The carboxamide formation in the final compound **3** with 5-chloro-2-thiophene[$^{14}$C]carboxylic acid (**1**) and 4-{4-[(5*S*)-5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl]phenyl}-morpholin-3-one (**2**) was realized using *N*'-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC.HCl) and 1-hydroxy-1-benzotriazole (HOBT) in a yield of 85% as shown in Scheme 1. The final compound was purified using a suitable chiral HPLC system to give 7.3 mCi of [$^{14}$C]BAY 59-7939 (**3**) with high chemical and radiochemical purity and an enantiomeric excess of $>99\%$.

### *$^{14}$C-labeled Metabolite M-4 of BAY 59-7939*

For the carboxamide formation in compound **7** the acid chloride (**5**), prepared from 5-chlorothiophenecarboxylic acid (**4**) using thionyl chloride,[4] was reacted with [1-$^{14}$C]glycine (**6**) yielding the metabolite M-4 (**7**) in 77% as shown in Scheme 2. Due to the hydrolytic stability of the acid chloride (**5**) the reaction was carried out in water in the presence of sodium bicarbonate.

## Experimental

### *Materials*

5-Chloro-2-thiophene[$^{14}$C]carboxylic acid was purchased from Amersham Pharmacia Biotech, Cardiff, UK and [1-$^{14}$C]glycine was purchased from



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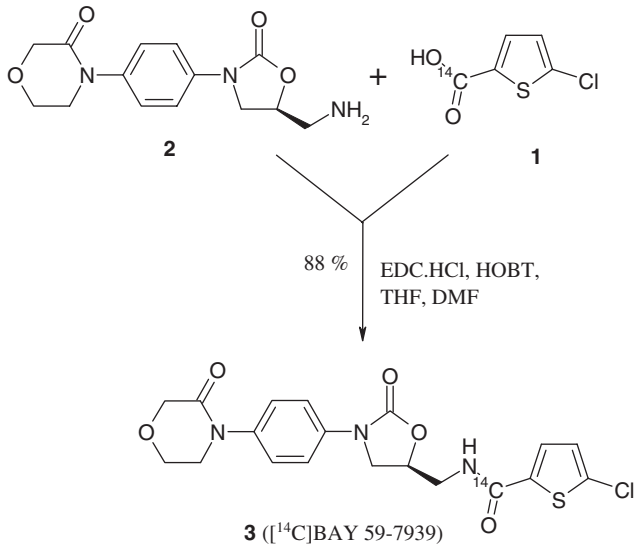
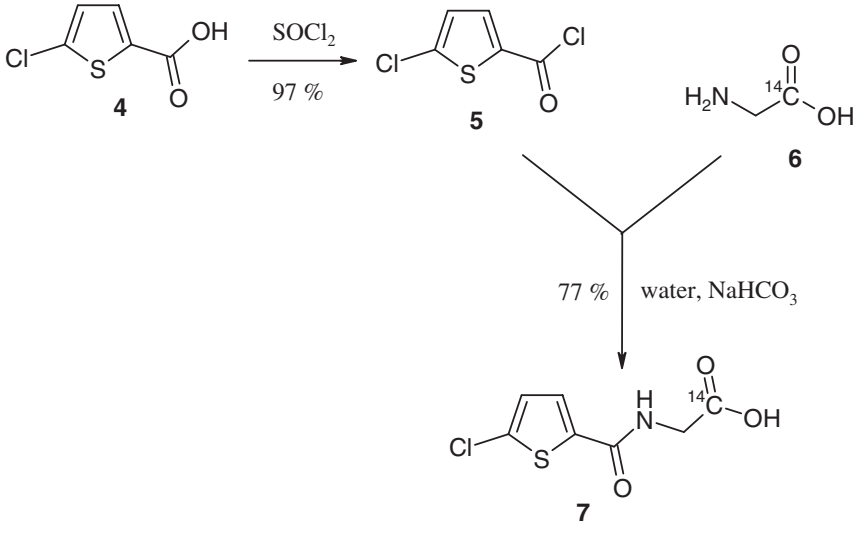



**Scheme 1. Synthesis of [$^{14}$C]BAY 59-7939**




**Scheme 2. Synthesis of the metabolite M-4 of BAY 59-7939**

American Radiolabeled Compounds, Inc., St. Louis, USA. Compounds **2** and **4** were obtained from the Chemical Development or Chemical Research Departments, Bayer HealthCare AG. All remaining reagents and solvents were purchased from commercial suppliers and were used as received.



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*Liquid scintillation counting*

Quantification of radioactivity was performed using a PerkinElmer TRI-CARB® 2500 TR liquid scintillation analyzer, with Ultima Gold™ cocktail used throughout.

*High-performance liquid chromatography*

All final compounds were analyzed by HPLC using a HP 1050 system Series II (Hewlett-Packard, Waldbronn, Germany) with a Ramona® Cell 2270 (Raytest, Straubenhard, Germany) for radioactivity detection. The LC-MS analytics were obtained at a PE Sciex/API III with MacIntosh Quadra® 900 (PerkinElmer Sciex Instruments, Thornhill, Canada). The following HPLC system was used for the purity check:

(1) Cosmosil® C18-5 AR, 250 × 4.6 mm (Waters GmbH, Eschborn Germany), column temperature: 45°C, flow rate: 1 ml/min, gradient: A: 0.2% phosphoric acid, B: acetonitrile, 0 min: 30% B, 0–20 min: 40% B, 20–35 min 90% B, 35–40 min 90% B, detection: UV 250 nm.
(2) Chiracel OD-H® AD, 250 × 4.6 mm (J.T. Baker B.V., Deventer, Netherlands), column temperature: 50 °C, flow rate: 1 ml/min, eluent: *n*-heptane/ethanol 70 + 30 (v + v), detection UV 250 nm.

*NMR spectra*

The NMR spectra were recorded on a Bruker DRX 400 spectrometer (Bruker, Rheinstetten, Germany).

*Synthesis of 5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-1,3-oxa-zolidin-5-yl}methyl)-2-thiophene-[$^{14}$C]carboxamide (**3**)*

A solution of 5-chloro-2-thiophene[$^{14}$C]carboxylic acid (**1**) (544 mg, 3.3 mmol, total radioactivity: 200 mCi, specific activity: 59 mCi/mmol, radiochemical purity: 99.1%), 1-hydroxy-1-benzotriazole (498 mg, 3.7 mmol) and *N*'-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide hydrochloride (706 mg, 3.7 mmol), dissolved in 10 ml tetrahydrofuran and 6.7 ml dimethyl formamide was stirred at room temperature for 1 h. A solution of 4-{4-[(5*S*)-5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl]-phenyl}morpholin-3-one (**2**) (975 mg, 3.3 mmol) in 8.6 ml dimethyl formamide and 4.5 ml tetrahydrofuran, was slowly added. The reaction mixture was stirred for 20 h at room temperature and subsequently for 1 h at 45°C. After addition of water (60 ml) the reaction mixture was stirred for 1 h. The precipitate was filtered off and washed several times with about 100 ml of water overall. The white crystals were dried under high vacuum yielding 1260 mg of compound **3** (88%) with a total radioactivity



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of 170.2 mCi. The UV purity was 98.3%, the radiochemical purity was 98.2% and the enantiomeric excess was $>87.1\%$.

LC-MS : $m/z = 436\,[M + H]^+(3.1\%)$ and 438 $[^{14}C\text{-}M + H]^+(96.9\%)$

A portion of compound **3** (1080 mg), dissolved in 29 ml tetrahydrofuran and 25 ml dimethyl formamide, was diluted with non-radioactive BAY 59–7939 (900 mg). After addition of 108 ml water the precipitate was filtered off, washed with 200 ml water and dried under high vacuum yielding 1898 mg of compound **3** showing a specific activity of 32.4 mCi/mmol. From this batch 10 mg aliquots were purified using the following chiral HPLC conditions: column: filled with poly(*N*-methacryloyl-*L*-leucine-*tert*-butylamide)silica composite,[5] 10 μm, 250 × 20 mm (Bayer AG, Wuppertal, Germany),[5] eluent: ethyl acetate; flow rate: 13 ml/min; detection: UV 270 nm; retention time: 13.7 min. Using this method approximately 130 mg of crude product was purified to give compound **3** (98 mg, 7.3 mCi) with chemical purity, radiochemical purity and enantiomeric excess of $>99\%$.

$^1$H-NMR (400 MHz, $D_6$-DMSO) $\delta$ 8.95 (t, 1H), 7.69 (d, 1H), 7.56 (d, 2H), 7.40 (d, 2H), 7.19 (d, 1H), 4.83 (m, 1H) 4.23–4.14 (m, 3H), 3.95 (t, 2H), 3.84 (m, 1H), 3.70 (t, 2H), 3.59 (t, 2H) ppm.

*Synthesis of N-[(5-chloro-2-thienyl)carbonyl][1-$^{14}$C]glycine (**7**)*

A mixture of 5-chlorothiophene-2-carboxylic acid (**4**) (500 mg, 3.1 mmol) and 2.5 ml thionyl chloride was heated to 60 °C for 2 h under stirring. The reaction mixture was evaporated to dryness under vacuum. The residue was dissolved in 5 ml toluene and evaporated to dryness. This process was repeated yielding 541 mg of compound **5** (97%). The GC analysis showed a chemical purity of $>99\%$.

Non-radioactive glycine (48.5 mg, 0.65 mmol) and sodium bicarbonate (279 mg, 3.32 mmol) and subsequently compound **5** (147 mg, 0.81 mmol) and 1 ml water were added to a solution of [1-$^{14}$C]glycine (**6**) (1.36 mg, 18.1 μmol, total radioactivity: 1 mCi, specific activity: 55 mCi/mmol, radiochemical purity $>99\%$) in 1 ml 0.01% hydrochloric acid. The reaction mixture was stirred for 23 h at room temperature. The pH value was adjusted to pH = 1 with half concentrated hydrochloric acid. The precipitate was filtered off, washed four times with each 0.5 ml water and dried under high vacuum giving 113 mg of compound **7** with a total radioactivity 0.77 mCi and a specific activity of 1.5 mCi/mmol corresponding to 77% yield.

GC − MS : $m/z = 219\,[^{12}C - M]^+$

$^1$H-NMR (400 MHz, $D_6$-DMSO) $\delta$ 12.69 (s, 1H), 8.96 (t, 1H), 7.66 (d, 1H), 7.20 (d, 1H), 3.89 (d, 2H) ppm.



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