JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2006; **49**: 683–686.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1085

Note

Synthesis and HPLC-purification of [^{77}Br]TMC125-R165335 (etravirine), a new anti-HIV drug of the DAPY-NNRTI class

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Summary

[^{77}Br]TMC125-R165335 (etravirine) was synthesized for imaging studies by SPECT. Labelling was performed with bromine-77 by electrophilic substitution of the desbromo-precursor 4-{6-amino-2-[(4-cyanophenyl)amino]pyrimidin-4-yloxy}-3,5-dimethylbenzenecarbonitrile using carrier-free $^{77}Br^-$ and chloramine-T (CAT) as oxidizing agent. The reaction proceeded in 10 min at room temperature in aqueous DMSO as solvent. Purification was performed by HPLC, giving a chemically and radiochemically pure [^{77}Br]TMC125-R165335 (etravirine) in aqueous ethanol. A final radiolabelling yield of 50% is obtained.

Received 27 March 2006; Accepted 24 April 2006

Key Words: [^{77}Br]TMC125-R165335 (etravirine); anti-HIV DAPY-NNRTI; bromine labelling; HPLC purification

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Introduction

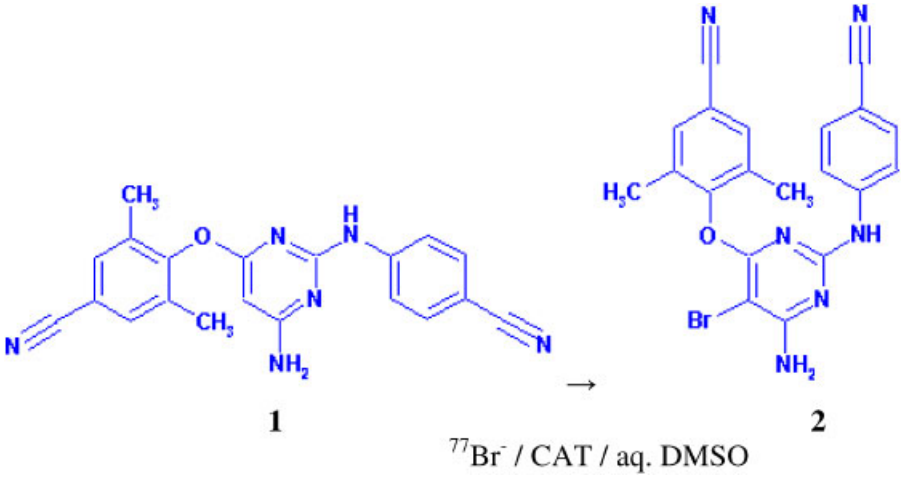
TMC125-R165335 (etravirine) is a novel drug substance, intended for the treatment of HIV-infected patients. It belongs to a new generation of non-nucleoside reverse transcriptase inhibitors (NNRTIs), the diarylpyrimidine (DAPY) derivatives, showing increased efficacy with improved virological potency against current NNRTI-resistant HIV-1 mutants.[1–9]

To further elucidate the mechanism of action and to support on-going ADME pharmaco-kinetic studies, the need arose to synthesize the corresponding [^{77}Br] compound, which can be used in SPECT imaging. In this paper, we describe the synthesis and HPLC purification of [^{77}Br]TMC125-R165335 (etravirine) **2**.

Results and discussion

The precursor desbromo-TMC125 was obtained from Tibotec, Mechelen, Belgium. Production of the ^{77}Br-isotope was through the ^{75}As(α,2n)^{77}Br reaction, using As_2O_3 as target material.[10] The carrier-free $^{77}Br^-$ was eluted from an anion-exchange column by 1 M $NaHSO_4$.[11]

The desbromo-TMC125 precursor **1** was dissolved in DMSO, to which the carrier-free aqueous $^{77}Br^-$ solution is added, followed by the addition of aqueous chloramine-T (CAT) solution and acetic acid (Scheme 1). After mixing, the reaction was allowed to proceed for 5–10 min at ambient room temperature. After the synthesis, semi-preparative HPLC purification was performed by reversed-phase chromatography, using an aqueous acetate buffer–ethanol mixture as isocratic mobile phase. This HPLC system allowed the production of a purified radiopharmaceutical drug in a non-toxic solvent. The retention times were, respectively, approximately 9 min for the



Scheme 1. Synthesis of [^{77}Br]TMC125-R165335 (etravirine)

DOI: 10.1002/jlcr

desbromo-precursor and approximately 14 min for TMC125-R165335 (etravirine). Typical chromatograms (UV at 254 nm followed by NaI-radioactivity detection) are given in Figure 1.

Experimental

Preparation of [$^{77}Br^{-}$]solution

As_2O_3 (400 mg) was irradiated with α-particles (30 MeV, 8 μA; yield of 0.2 mCi/μA.h)[10]. Purification was obtained by anion-exchange chromatography using Dowex AG 1×8 resin (200–400 Mesh; Bio-Rad Laboratories): the carrier-free $^{77}Br^{-}$ is eluted in 1 ml of 1 M $NaHSO_4$ [11].

Preparation of [^{77}Br]TMC125-R165335 (etravirine)

The desbromo-precursor 4-{6-amino-2-[(4-cyanophenyl)amino]pyrimidin-4-yloxy}-3,5-dimethylbenzenecarbonitrile **1** (1 μmol) was dissolved in 200 μl DMSO. Carrier-free 77-bromide solution (20–30 μl) was added and mixed, followed by the addition of chloramines-T solution (2 μmol dissolved in 100 μl water) and acetic acid (5 μl). After mixing, the reaction was allowed to proceed for 5–10 min at ambient room temperature.

HPLC purification

After the synthesis, the resultant mixture was subjected to HPLC purification applying following chromatographic conditions:
Column: Alltech Alltima C-18, 5 μm, 250×4.6 mm
Mobile phase: 50 + 50% v/v ethanol + acetate buffer (10 mM ammonium acetate + 0.1% acetic acid)
Flow rate: 1.2 ml/min
Detection: UV254 nm + NaI radioactivity detector

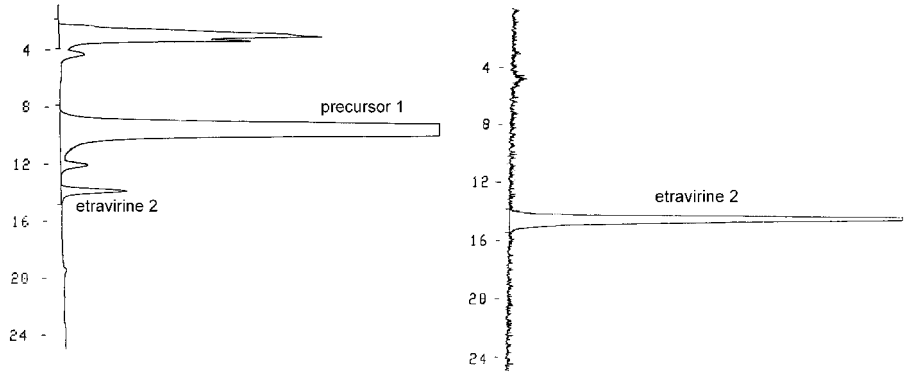


Figure 1. Typical HPLC chromatogram, with $UV_{254\,nm}$ (left) and NaI-radioactivity (right) detection

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Acknowledgements

Structures given in Scheme 1 are derived from the anti-HIV chemical compound structures dbase (NIAID, NIH), applying Marvin and JChem functionalities (ChemAxon). Compound **1** is AIDS#108490, while compound **2** is AIDS#105156.

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DOI: 10.1002/jlcr