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Research Article

Synthesis of [^{11}C]levofloxacin

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Summary

Levofloxacin, the pure S enantiomer of the fluoroquinolone antibiotic ofloxacin, was labeled via methylation of the corresponding, *des*-methyl, secondary amine with N.C.A. [^{11}C]methyl iodide. The methylation reaction was regioselective, giving predominantly the preferred methyl amine at high temperature in DMF, while otherwise giving predominantly the methyl ester of a free carboxylic acid also present in the molecule. Levofloxacin was obtained in 80% chemical yield after a 45 min synthesis. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: levofloxacin; antibiotic; methyl iodide; regioselective; carbon-11

Introduction

Levofloxacin 2 is the purified levorotatory, or S, enantiomer of ofloxacin, (–)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, a fluoroquinolone antibiotic compound (Figure 1). The antibiotic has broad spectrum activity against gram-positive and gram-negative, aerobic and anaerobic bacteria. It is used to treat chronic bronchitis, skin infections, gonorrhea, chlamydia, urinary and prostate infections, maxillary sinusitis, and pneumonia. The racemate, ofloxacin, has been

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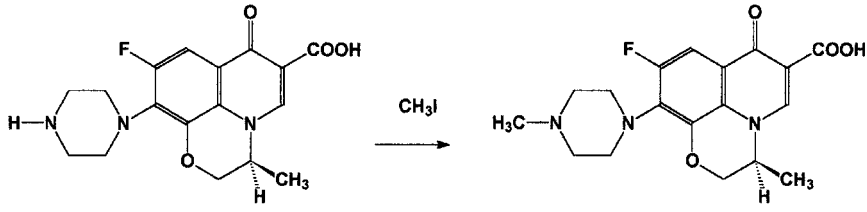


1 *des*-methyl levofloxacin *2* levofloxacin

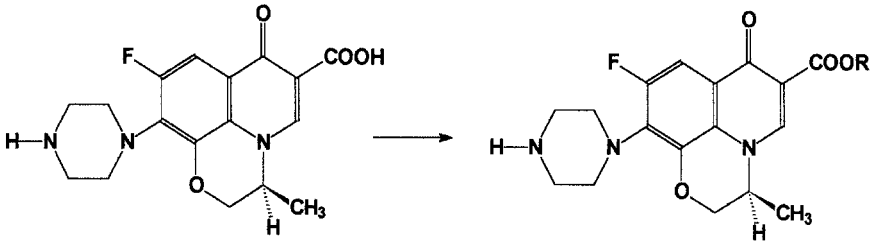
Figure 1.

used for similar clinical indications. In addition to oral or injected administration, the drug may be administered by inhalation to treat infection in the airways. Inhaled administration has the advantage of placing a high concentration of the drug onto the airway surfaces with a relatively low systemic burden.

The low systemic burden is desirable to limit the potential to induce resistance to the antibiotic in bacteria that may be present in non-target areas of the body. When developing formulations and methods for drug inhalation, PET imaging to measure the deposition and retention of drug at target sites[1–4] can be useful. It allows one to evaluate the necessary dosage for successful treatment and the corresponding systemic burden before beginning clinical trials. Of course, imaging studies of drug distribution require labeled drug. The goal of this work was to produce levofloxacin labeled with carbon-11 in sufficient quantity to be used as a tracer for PET studies[5–7] of the biodistribution and regional kinetics of proposed levofloxacin formulations. The levofloxacin molecule contains a convenient methyl amine function on the piperazine ring (Figure 1), so labeling by methylation of the 4-*des*-methyl piperazine precursor **1** with methyl iodide was a logical potential route. The carboxylic acid at the 6-position of the quinolone was a potentially interfering group, as it could compete for reaction with methyl iodide to produce the labeled methyl ester **3** (Figure 2).

Results and discussion

Levofloxacin, the drug of interest, is the resolved S enantiomer of the racemic drug, ofloxacin. We therefore used resolved S enantiomers. However, it is clear that though this might have an effect on the drug's regional pharmacokinetics, it has no significance for the radiolabeling.



1 *3,4,5* R= $^{11}CH_3$, CH_3, $C(CH_3)_3$

Figure 2.

The single optical center is not involved in the chemistry and the compound does not racemize under the conditions used. Our results would therefore be equally applicable to the R enantiomer and to the racemate.

Initial attempts to react *des*-methyl levofloxacin **1** with labeled methyl iodide in THF, CH_2Cl_2, $CHCl_3$, CH_3CN, acetone, and DMSO using sodium hydroxide, carbonate or bicarbonate, trialkylamines and tetraalkylammonium hydroxides at temperatures from room temperature to 100°C did not methylate as desired. Rather, methylation was predominantly at the 6-carboxylic acid position to produce the labeled methyl ester of *des*-methyl levofloxacin **3** (Figure 2) in 2–30% yield. This constituted a regioselective and useful synthesis of **3**, possibly for other purposes. Levofloxacin **2** was a minor product of these reactions, with 0–2% yield. As a protection measure, the unlabeled methyl ester of *des*-methyl levofloxacin **4** was prepared, though with unusual difficulty. Attempts to produce **4** by direct acid- or base-catalyzed esterification and DCC-mediated esterification[8] failed to give any desired product. Ultimately, methyl esterification via the acid chloride intermediate[9] produced *in situ* by thionyl chloride gave **4** in good yield. Labeling of **4** with methyl iodide then afforded the desired levofloxacin methyl ester **6** (not shown). However, deprotection under convenient conditions (acid and basic, hydrous and anhydrous ethanolic) was unsatisfactory, and attempts to produce the more labile *t*-butyl ester **5** via isobutene or direct esterification[10–12] failed to form detectable product. Finally, a more extensive search for reaction conditions revealed a temperature-dependent regiospecificity of the reaction of **1** and methyl iodide in DMF, and a useful procedure consisting of reaction of **1** in DMF at 150°C. The radiochemical

(uncorr.) yield of **3** was 25% EOS, chemical yield was 80% with respect to methyl iodide, and the synthesis time of 45 min included HPLC purification. No radiochemical or chemical impurities were detectable in the HPLC-purified products, and the maximum product specific activity at EOS was 110 TBq/mmol (3 Ci/μmol), depending on target condition and irradiation parameters. It should be noted that the specific activity of the product is irrelevant to its major potential use as a tracer for therapeutic formulations of levofloxacin or ofloxacin products in which micrograms to milligrams of drug may be administered. Similarly, the final processing and formulation will be determined by particular drug formulations of interest. However, **3** is easily redissolved in aqueous or ethanolic solution for formulation. If necessary it can be sterile filtered, though sterile filtration is not necessary for an inhaled formulation.

Experimental

Reagents were obtained from Aldrich and Fisher and were used without additional purification unless otherwise noted. CH_3CN was freshly distilled from calcium hydride.

Analytical HPLC was performed using a Hewlett-Packard HP 1050 system with diode array UV detector and 2.5×7 cm NaI radiation detector, with an Alltech C-18 reverse phase (250×4.6 mm) column eluted at 2 ml/min with 10 mM NH_4OAc in 40% acetonitrile; rt: *des*-methyl levofloxacin, 6.2 min; levofloxacin, 9.2 min; *des*-methyl levofloxacin methyl ester, 4.4 min; levofloxacin methyl ester, 6.4 min. Preparative HPLC was performed using a (250×4.6 mm) silica column eluted with 40% MeOH in CH_2Cl_2 rt: levofloxacin 4.6 min, *des*-methyl levofloxacin 10.5 min. TLC was performed on Merck F-254 silica gel 60 plates eluted with chloroform–methanol–acetic acid–distilled water (15 : 5 : 2 : 1, v/v) Rf: *des*-methyl levofloxacin, 0.3; levofloxacin, 0.4. Mass spectra and 300 MHz NMR spectra were obtained at the CWRU Major Analytical Instrument Facility (MAIF), infrared spectra were obtained on a Perkin-Elmer Spectrum 1000 instrument.

[^{11}C]levofloxacin 2

Des-methyl levofloxacin **1** (1 mg, 2.9 μmol, obtained commercially) was dissolved in 200 μl DMF, or alternatively in DMA, freshly distilled from

$MgSO_4$ and 1 µl 1 N NaOH (aq.) was added. The solution was cooled in a bath of dry ice/acetonitrile, typically at −45°C. [^{11}C]Methyl iodide was prepared by the $LiAlH_4$/HI method,[13,14] as reported previously, distilled and collected in the chilled solution. The reaction vessel was then sealed and placed in a hot block at 150°C for 10 min. The solvent was evaporated under He gas flow, 2×200 µl EtOH added and evaporated, and the residue taken up in 40% MeOH in CH_2Cl_2 for HPLC injection. Labeled product (80% from MeI) co-eluted with levofloxacin on HPLC and TLC. Final processing of the tracer will depend upon the drug formulation to be traced. The purified product collected from HPLC was evaporated and could then be easily taken up in water or ethanol for further analysis or formulation, and was not removed from solution by passage through sterilizing filters.

Des-methyl levofloxacin [^{11}C]methyl ester 3

Reaction was performed as for **2**, but using DMF at 100°C, with $NaHCO_3$ (1 µmol) as base. Up to 2% of **2** was present before HPLC. After purification, **3** was obtained in 30% radiochemical yield EOS (uncorr).

Des-methyl levofloxacin methyl ester 4

Compound **4** was obtained via the acid chloride[9] produced *in situ* by thionyl chloride. Compound **1** (50 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (2 ml) and DMF (20 µl), with 10 mg Na_2CO_3 and 36 mg methanol (1.1 mmol). Thionyl chloride (33 mg, 0.28 mmol) in CH_2Cl_2 was added dropwise at 0°C, the mixture was stirred overnight at room temperature, then refluxed 2 h, extracted with water, dried over $MgSO_4$ and crystallized from ethanol/ethyl acetate to yield **4**, 16%. IR: 1718. Mass spec: M^+ 375. This material, in addition to its use as a precursor for labeling, was used as an analytical standard to identify **3**.

Conclusion

Levofloxacin was successfully labeled in sufficient yield and purity for use in PET studies by regiospecific methylation at high temperature in DMF. By simple alteration of the reaction conditions, labeled *des-*

methyl levofloxacin methyl ester could also be obtained sufficiently to be useful as a radiotracer.

References

1. Bergstrom M, *et al*. *Clin Pharmacokinet* 1999; **36**(Suppl 1): 33–39.
2. Cass LM, *et al*. *Clin Pharmacokinet* 1999; **36**(Suppl 1): 21–31.
3. Pike VW, *et al*. In PET for Drug Development and Evaluation, Comar D (ed). Kluwer Academic Publishers: Dordrecht; 1995; 341–352.
4. Sihver W, *et al*. *J Neurochem* 1998; **71**: 1750–1760.
5. Berridge MS, *et al*. *J Nucl Med* 2000; **41**: 1603–1611.
6. Berridge MS, *et al*. *J Nucl Med* 1998; **39**: 1972–1977.
7. Berridge MS, *et al*. *Curr Pharm Design* 2000; **6**: 1631–1651.
8. Hassner A, Alexanian V. *Tetrahedron Lett* 1978; **46**: 4475–4478.
9. Brenner M, Huber W. *Helvetíca Chímíca Acta* 1953; **36**: 1109.
10. Ertan M, *et al*. *Arzneimittelforschung* 1992; **42**(1): 70–72.
11. Roeske RW. *J Org Chem* 1963; **28**: 1251–1257.
12. Wright SW, Hageman DL. *Tetrahedron Lett* 1997; **38**: 7345–7348.
13. Berridge MS, *et al*. *Appl Radiat Isot* 1995; **46**: 173–175.
14. Langstrom B, *et al*. *J Nucl Med* 1987; **28**: 1037–1040.

