Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 28 September 2011, Revised 27 October 2011, Accepted 9 December 2011 Published online 23 January 2012 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1959

Proton exchange reactions in isotope chemistry (I)

Zhigang Jian,* Tapan Ray, Amy Wu, and Lawrence Jones

Direct H–D exchange reactions were applied to the preparations of stable isotope-labeled TKI258 and two TKI258 metabolites. Each compound was made in one single H–D exchange reaction with excellent isotope incorporation. The number of deuterium incorporation and deuterium distribution in the molecules was similar in all three compounds. Stable isotope-labeled TKI258 was also prepared from d_8-methylpiperazine in a multistep synthesis.

Keywords: deuterium; exchange; stable isotope

Introduction

TKI258 is an inhibitor of types III to V RTKs (Receptor Tyrosine Kinase) that mediate both endothelial and tumor cell proliferation and survival and are under development as an antitumor agent. Stable isotope-labeled TKI258 and its two metabolites, TKI258 N-oxide and desmethyl TKI258, are needed for bio-analytical studies. Stable isotope-labeled TKI258 was initially prepared from deuterium N-methylpiperazine in a multistep synthesis (Figure 1).

Whereas deuterium TKI258 N-oxide can be prepared from deuterium TKI258, the stable isotope-labeled desmethyl TKI258 has to be made from deuterium-labeled N-protected piperazine through a multiple-step reaction.

Proton exchange reactions have found wide application in isotope chemistry for both tritium-labeled and deuterium-labeled compounds.[1,2] Very often, a target compound can be tritiated or deuterated in one single step with only a few milligrams of material. We were interested to see if we can label all three compounds by H–D exchange reactions.

After evaluating all the available options we have, we decided to use deuterium D_3PO_4–BF_3 complex as the reagent for our purpose because of its strong capability to catalyze relatively complete proton exchange reactions to compounds with different structures, as long as they are not acid–base sensitive,[2–9] just like the three compounds we have.

Results and discussion

Each of the three compounds was taken up in deuterium D_3PO_4–BF_3 complex and was subject to microwave at 120 C for 60 min (Figure 2). The reaction mixture was then cooled down to room temperature, diluted with water, and neutralized with sodium hydroxide solution. Any labile deuterium atoms should have been removed during this work-up process. The product was extracted into ethyl acetate, and solvent removal left solid that was either purified by prep-HPLC or used as is. The final product was analyzed by LC–MS and NMR.

Earlier studies used traditional heating for these types of reactions,[2–9] and it usually takes many hours and even days for the reaction to complete. We decided to use microwave for the heating and found that the reactions were completed in just 60 min.

LC–MS analysis showed that all six aromatic protons participated in the deuterium exchange. The most abundant molecular ions are M + 4 with trace of M + 0 (<0.2%), indicating a very high incorporation of four deuterium atoms in each case (Figure 3). The isotope clusters in the molecule ion area for all three compounds are relatively narrow, unlike most other H–D exchange methods that usually generate products with wide distribution of deuterium-labeled molecule ions resulting from incomplete H–D exchange. NMR data indicated that deuterium exchange occurred mostly in *o* and *p* positions of amino groups, in line with the reaction mechanism proposed.[3]

Bio-analytical studies require stable isotope-labeled compounds to contain an enrichment of at least three mass units relative to the unlabeled analyte. In addition, the stable isotope-labeled internal standard should contain <0.5% of the unlabeled isotopomer. Hence, one of the principal challenges of utilizing H–D reactions is the generation of isotope clusters that result from incomplete H–D exchange and the presence of significant amounts of unlabeled parent in the sample. However, in our case, when utilizing a single exchange cycle, although we did observe an isotope cluster of variable deuterium incorporation, the principal species observed in the mass spectrum was D4. In addition, MS analysis for all three compounds prepared indicated that <0.2% of the unlabeled parent was present in the samples, thus making these tracers suitable internal standards.

Experimental

Materials and methods

^{1}H NMR spectra were recorded on a 500 MHz Bruker NMR spectrometer. Chemical shifts are reported in ppm relative to

Novartis Pharmaceuticals Corporation, NIBR-DMPK-DMBA-IL, East Hanover, NJ 07936, USA.

**Correspondence to: Zhigang Jian, DMPK/DMBA-IL, NIBR, Novartis Pharmaceuticals Corporation, B435/R3149, One Health Plaza, East Hanover, NJ 07936, USA. E-mail: zhigang.jian@novartis.com*

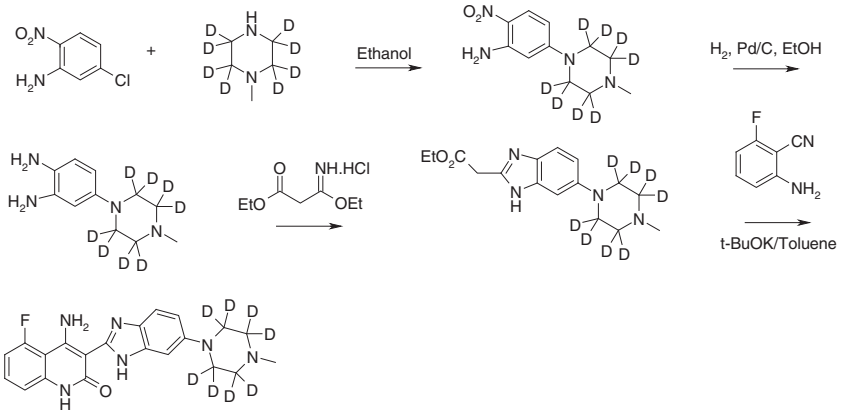


Figure 1. Original synthesis of stable isotope-labeled TKI258.

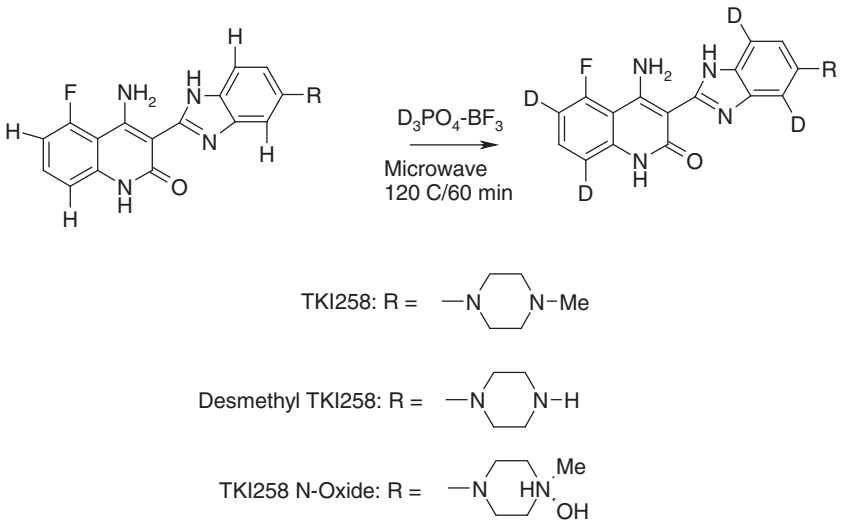


Figure 2. H–D exchange reactions.

TMS (Tetramethylsilane). The LC–MS analysis was performed on an LCQ Advantage mass spectrometer coupled with a Waters Alliance HPLC instrument. Analytical HPLC conditions: column, Phenomenex Polar RP-C18, 4 μm, 4.6 × 150 mm; mobile phase A, 10 mM ammonium acetate with 0.5% acetic acid; mobile phase B, acetonitrile; gradient, 18 min from 80% A–20% B to 20%A–80% B; flow rate, 1 ml/min; UV, 297 nm. Prep-HPLC conditions: column, XBridge C-18, 5 μm, 30 × 150 mm; mobile phase A, 20 mM ammonium acetate with 0.1% acetic acid; mobile phase B, acetonitrile; gradient, 20 min from 90%A–10%B to 10%A–90%B; UV, 254 nm; flow rate, 40 ml/min. Microwave heating was carried out on a CEM Discover with fixed power output of 150 W. The chemicals and solvents were reagent grade obtained from Sigma Aldrich without further purification.

Preparation of D_3PO_4–BF_3 complex

To 48 g of phosphorus pentoxide cooled in an ice bath and under nitrogen was added 20 ml of deuterium oxide dropwise over 1 h. The reaction mixture was stirred overnight to form a homogeneous viscous liquid, and then BF_3 gas was introduced until no more absorption could be observed.

H–D exchange reaction with D_3PO_4–BF_3 complex

Substrate (0.25 mmol) was taken up in D_3PO_4–BF_3 (5 ml), and the reaction mixture was subject to microwave at 120 C for 60 min. The reaction mixture was cooled to room temperature and added dropwise to 10 ml of 50% sodium hydroxide cooled in an ice bath. The precipitate formed was collected by filtration and washed with ethyl acetate (3 × 10 ml). The filtrate was extracted with ethyl acetate (3 × 10 ml). Ethyl acetate washings and extracts were combined, washed with water (3 × 5 ml), and dried over anhydrous sodium sulfate. Solvent removal left a solid that was further dried under high vacuum for 2 h.

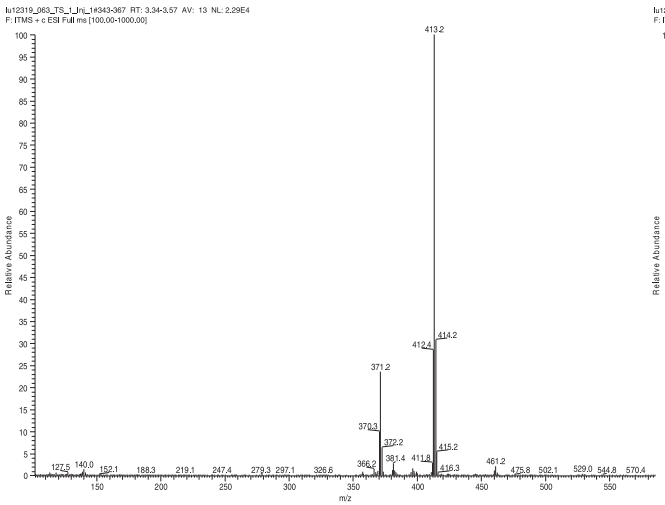
[M + 4] TKI258

No further purification was needed after direct deuterium exchange reaction. Yield: 65%. ^{1}H NMR (DMSO-d_6) δ 2.2 (s, 3H), 2.43 (m, 4H), 3.06 (m, 4H), 7.39 (s), 7.49 (m), 7.66 (m); MS (ESI+): *m/z* 394 (M + 0, <02%), 396 (M + 2, 27%), 397 (M + 4, 100%), 398 (M + 5, 33%), 399 (M + 6, 9%).

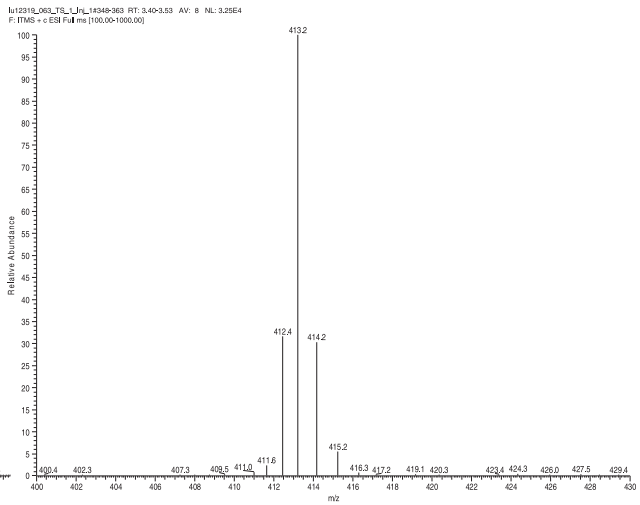
[M + 4] TKI258 N-oxide

The crude product after direct deuterium exchange reaction was purified by prep-HPLC. Yield: 10%. ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H),

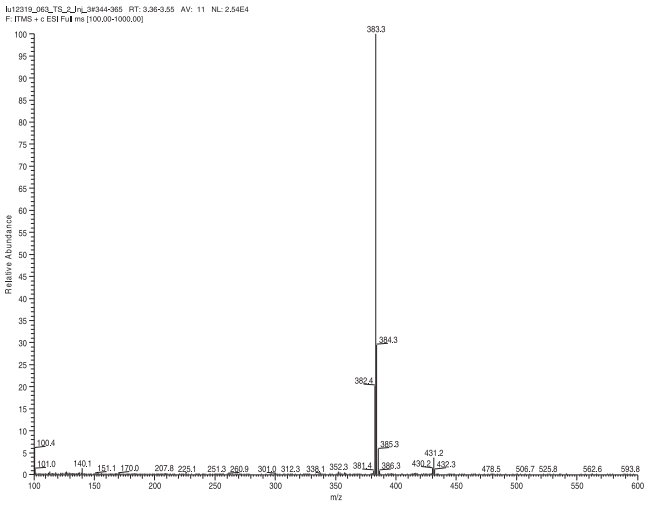
M+4 TKI-258 N-Oxide (Mz+100-600)



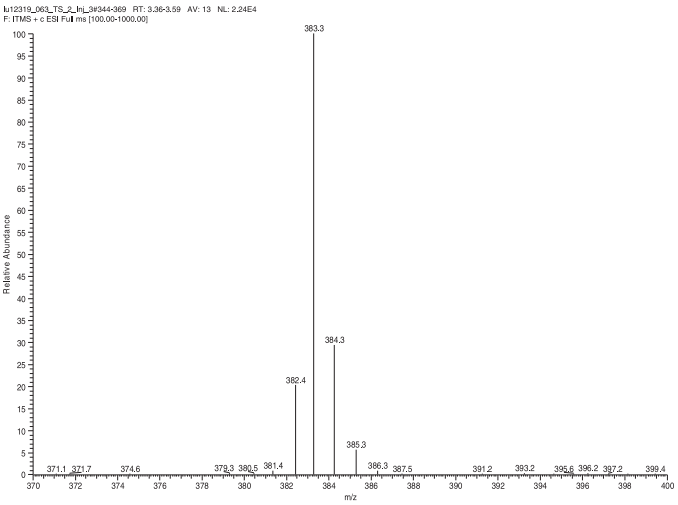
M+4 TKI-258 N-Oxide (Mz+ 400-430)



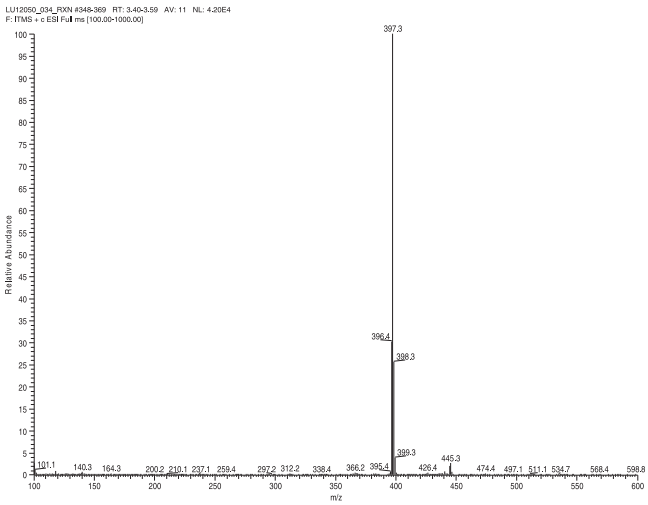
M+4 TKI258 Des-Methyl (Mz+100-600)



M+4 TKI-258 Des-Methyl (Mz+ 370-400)



M+4 TKI258 (Mz+100-600)



M+4 TKI-258 (Mz+ 380-410)

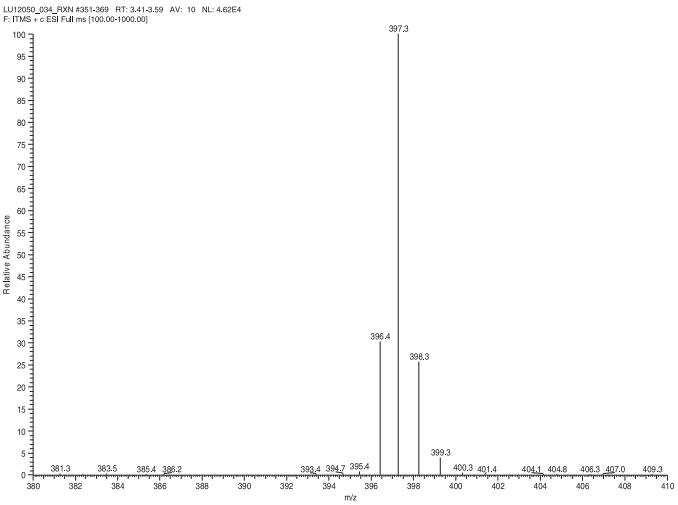


Figure 3. Mass spectra for the deuterium-exchanged compounds.

3.72 (m, 4H), 4.06 (m, 4H), 7.58 (m), 7.61 (s); MS (ESI+): *m/z* 409 (M + 0, 0.2%), 411 (M + 2, 30%), 413 (M + 4, 100%), 414 (M + 5, 43%), 415 (M + 6, 5%).

[M + 4] desmethyl TKI258

No further purification was needed after direct deuterium exchange reaction. Yield: 54%. ^{1}H NMR ($CDCl_3$) δ 2.44 (m, 2H), 2.83 (m, 2H), 2.97 (m, 4H), 7.29 (s), 7.49 (m), 7.66 (m); MS (ESI+): *m/z* 379 (M + 0, 0.2%), 382 (M + 3, 9%), 383 (M + 4, 100%), 384 (M + 5, 16%), 385 (M + 6, 4%).

Acknowledgements

The authors would like to thank Novartis DMPK management for their support and other Novartis Isotope Lab colleagues for their technical assistants.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] J. Atzrodt, V. Deerdau, T. Fey, J. Zimmermann, *Angew. Chem. Int. Ed.* **2007**, *46*, 7744–7765.
[2] R. Voges, J. R. Heys, T. Moenius, Preparation of Compounds Labeled with Tritium and Carbon-14, John Wiley and Sons, Ltd, Great Britain, **2009**, pp. 47–98.
[3] P. M. Yavorsky, *J. Am. Chem. Soc.* **1962**, *84*, 1071–1072.
[4] K. Wahala, S. Rasku, *Tetrahefron Lett.* **1977**, *38*, 7287–7290.
[5] S. Rasku, K. Wahala, J. Koskimies, T. Hase, *Tetrahedron* **1999**, *55*, 3445–3454.
[6] S. Rasku, K Wahala, *Tetrahedron* **2000**, *56*, 913–919.
[7] S. Rasku, K. Wahala, *J. Labelled Compd. Radiopharm.* **2004**, *47*, 25–30.
[8] P. S. Kiuru, K. Wahala, *Steroids* **2006**, *71*, 54–60.
[9] M. Pohjoispaa, E. Leppala, K. Wahala, *J. Labelled Compd. Radiopharm.* **2007**, *50*, 521–522.