Organic Process Research & Development

Technical Note

pubs.acs.org/OPRD

Simple Modification To Obtain High Quality Fludarabine

Siddheshwar W. Kshirsagar, Mangesh S. Deshpande, Swapnil P. Sonawane,* Golak C. Maikap, and Mukund K. Gurjar

API R & D Centre, Emcure Pharmaceuticals Ltd, I.TBT Park, Phase-II, M.IDC Hinjewadi, Pune-411057, India

ABSTRACT: A simple and improved debenzylation process is described to obtain fludarabine in greater than 99.8% purity and 90−95% yield.

INTRODUCTION

Fludarabine is the fluorinated nucleotide analog of the antiviral agent vidarabine[1] used in the treatment of chronic lymphocytic leukemia. Fludarabine is a purine analog and can be given both orally and intravenously.[2,3] Being phosphorylated, fludarabine is ionized at physiologic pH and is effectually trapped in blood. This provides some level of specificity for blood cells, both cancerous and healthy.[4] This metabolite appears to act by inhibiting DNA polymerase α, ribonucleotide reductase, and DNA primase, thus inhibiting DNA synthesis.

RESULTS AND DISCUSSION

During the process development of fludarabine phosphate, significant impurities were appearing in the drug substance, and it was extremely difficult to remove these impurities without significant loss in yield. Upon investigation it was observed that the quality of fludarabine phosphate is dependent on its precursor, fludarabine. A commonly adapted commercial synthetic route[3] is displayed in Scheme 1, in which several researchers have attempted the debenzylation of **2** using various cryogenic reaction conditions in less than 90% purity. The potential impurity observed was Ara-A, which is not a part of any pharmacopoeial specifications.

Montgomery et al.[3] disclose a debenzylation reaction by employing palladium or palladised charcoal or sodium/liquid ammonia, and a 34% yield was obtained. The use of catalytic hydrogenation using hydrogen gas along with palladium/carbon always results in defluorination and gives a mixture of fludarabine and defluorinated fludarabine (Ara-A). Further, the use of sodium/ammonia or catalytic hydrogenation using a pressure reactor (autoclave) for a debenzylation reaction is quite tedious and produces an unsatisfactory product with respect to quality and yield.

Debenzylation with boron trichloride in dichloromethane was also disclosed by the same authors.[5] Even though boron trichloride produces a minimum of defluorinated product, however, the method is limited to the laboratory scale, as the reaction is performed at −80 °C and boron trichloride liberates hydrochloric acid and boric acid in the presence of moisture or alcoholic solvent and therefore requires stringent anhydrous conditions for implementation on an industrial scale.

To overcome these issues, Blumbergs et al.[6] disclosed a process for preparation of 2,6-(2,3,5,-tri-*O*-benzyl-β-D-arabinofuranosyl) purine, which is further treated with fluoroboric acid and sodium nitrite in the presence of tetrahydrofuran as solvent. Further, debenzylation with palladium chloride/charcoal produces fludarabine in 81% yield; however, the use of hydrogen gas necessitates the utilization of a pressure reactor (autoclave), which makes the reaction tedious and unfriendly for commercial application. In addition to the above drawbacks, the use of methoxyethanol as solvent is not recommended because of its toxic effect on bone marrow and testicles; high exposure causes granulocytopenia macrocytic anemia, oligospermia, and others.[7]

We observed that all the prior art methods lack consistency in getting the desired quality of debenzylated product, i.e. fludarabine, on a large scale. Moreover, being an antineoplanstin, these products are very expensive, and the yields reported by various researchers may not be acceptable for a commercial process. Thus, there was a strong need to develop a method which produces the desired quality and quantity of fludarabine and its phosphate salt. Therefore, to overcome these issues, the present authors have modified the debenzylation method by replacing the catalytic hydrogenation−debenzylation with transfer hydrogenation,[8] utilizing ammonium formate as an in situ hydrogen donor. This process is reproducible, easily implemented industrially, cost-effective, and environmental friendly. With this simple modification reported in this Note, the degradation impurities were reduced to an undetectable level in greater than 90% yield (Table 1).

To decide which solvent system is suitable for debenzylation so that catalyst can be easily separated from product, the solubility of fludarabine in different solvents was carefully studied, and we observed that it is highly soluble in a 70:30 methanol/water mixture. The choice of utilizing other hydrogen donors such as hydrazinium monoformates[9,10] was ruled out due to the safety concerns of hydrazine. After studying various parameters such as dilution, ratio of methanol/water, catalyst, and resin, the ratio of methanol/water and dilution were found to be crucial parameters for debenzylation. During reflux, mild sublimation of ammonium formate was noticed at the edges of the condenser, which was easily removed by water during reflux. The resin was used for removing residual amines, as the last step involves reaction of triethyl phosphate and phosphorus oxychloride; removal of the amines also helped in improving the purity. Thus, Table 1

Received: February 29, 2012
Published: April 2, 2012

dx.doi.org/10.1021/op3000509 | *Org. Process Res. Dev.* 2012, 16, 840−842

Scheme 1. Synthesis of Fludarabine Phosphate

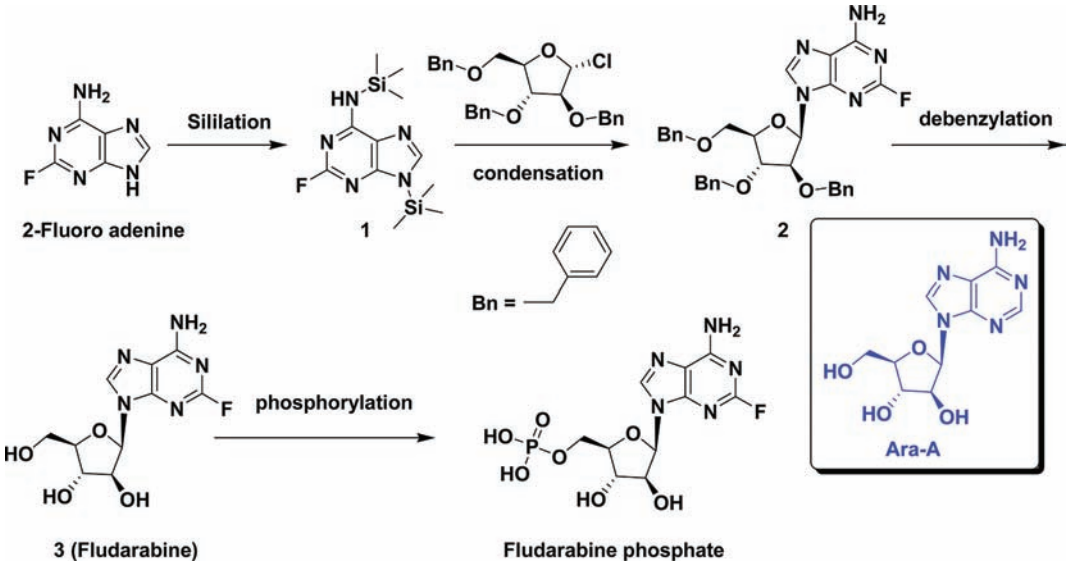
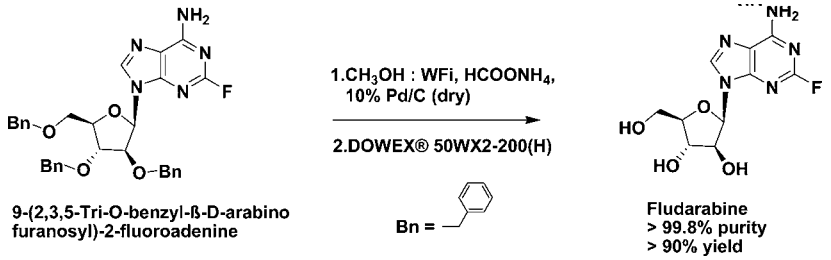


Table 1. Optimization of Reaction Conditions[a]



entry	methanol/ water ratio	dilution	time (h)	resin (w/w)	isolated yield (%)	purity (%)
1	50:50	20	2		no reaction	*b*
2	10:90	20	2		no reaction	*b*
3	90:10	10	2	1	60	99.95
4	80:20	10	2	1	85	99.94
5	**70:30**	**20**	**2**	**1**	**94**	**99.95**
6	30:70	20	2	1	no reaction	*b*
7	70:30	20	2	0.7	95	98.22

[a]Reaction on 10 mmol, 10% Pd/C 20% w/w, ammonium formate 1:1 w/w w.r.t. **2**. [b]Starting material recovered due to solubility issues.

reiterates that entry 5 of was the best experiment so far in this series.

To avoid waste production, all effluents along with 10% Pd/C and resins are recycled and reused. In conclusion, an environmentally safe and cost efficient modification was developed to obtain high purity fludarabine and thereby fludarabine phosphate.

EXPERIMENTAL SECTION

HPLC Method. Gradient program; column, Sunfire C-18 (4.6 mm × 250 mm) 5 μ or equivalent; flow rate, 1.0 mL/min; wavelength, 210 nm; injection volume, 10 μL; run time, 45 min; mobile phase-A, potassium dihydrogen phosphate (1.36 g) in Millipore water (1000 mL); mobile phase-B, acetonitrile and methanol (50:50); diluent, homogenous mixture of methanol and Millipore water (9:1).

Relative Retention Times. Compound **3**, 1.0; compound **2**, about 3.5; toluene, about 3.0.

Preparation of Fludarabine (3). In a clean vessel, a solution of water for injection (22.5 L) and methanol (70 L) was prepared. To a slurry of Pd/C (1 kg) in water for injection (2.5 L) was added a suspension of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (**2**) (5 kg, 8.99 mol) in the previously prepared aqueous methanol (75 L). The reaction vessel was swept with nitrogen. A solution of ammonium formate (5 kg) in water for injection (5 L) was added over a period of 25 to 45 min at 25 to 35 °C; the addition pot was then rinsed with previously prepared aqueous methanol (17.5 L). The reaction mixture was stirred for 2 h at 70 to 75 °C temperature and monitored by HPLC. After completion, the reaction mass was cooled to 60−65 °C and the catalyst was removed by filtration.[11] The filtrate was concentrated to a volume of 30−35 L under reduced pressure.[12] The solid was filtered and the wet cake was suspended in water for injection (100 L) and cation exchange resin (2.5 kg, DOWEX 50WX2-200(H)).[13] The mixture was heated to 90−95 °C. The resulting solution was filtered[14] and cooled to 25 to 30 °C. Pure fludarabine was isolated by filtration and drying at 50 to 55 °C under reduced pressure to give a yield of 2.45 kg (95% yield, purity, ≥99.8%). mp 265−267 °C (dec);[5] ^{1}H NMR (400 MHz,

dx.doi.org/10.1021/op3000509 | *Org. Process Res. Dev.* 2012, 16, 840−842

DMSO d^6) δ 8.16 (s, 1H), 7.76 (br s, 2H), 6.11−6.09 (d, 1 H, J = 5 Hz), 5.62−5.60 (d, 1 H, J = 5.24 Hz), 5.51−5.49 (d, 1 H, J = 4.56 Hz), 5.05 (t, 1 H, J = 5.2 Hz), 4.16−4.09 (m, 2 H), 3.78−3.75 (m, 1 H), 3.69−3.24 (m, 2H); MS (CI): Calcd for $C_{10}H_{12}FN_5O_4$ m/z: 286.24 (M + H). Found: m/z: 286.2.

AUTHOR INFORMATION

Corresponding Author

*Fax: +91-20-39821445. E-mail: Swapnil.Sonawane@emcure.co.in.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Samit S. Mehta, Emcure Pharmaceuticals Ltd, for generous support and constant encouragement. Finally, it is a pleasure to acknowledge the reviewers and editor for valuable suggestions.

REFERENCES

(1) (a) Lee, W. W.; et al. *J. Am. Chem. Soc.* **1960**, *82*, 2648. (b) Reist, E. J.; et al. *J. Org. Chem.* **1962**, *27*, 3274.
(2) (a) Ulf, T.; Thomas, S.; Klaus, N. U. S. Patent 7,547,776, 1999. (b) Electronic orange book http://www.accessdata.fda.gov/scripts/cder/ob/docs/patexclnew.cfm?Appl_No=022273&Product_No=001&table1=OB_Rx.
(3) (a) Montgomery, J.; Hewson, K. *J. Med. Chem.* **1969**, *12*, 498−504. (b) Montgomery, A. U. S. Patent 4,188,378, 1980.
(4) Fludarabine. http://en.wikipedia.org/wiki/Fludarabine.
(5) (a) Montgomery, A.; Clayton, S. D.; Shortnacv, T. A. *J. Heterocycl. Chem.* **1979**, *16*, 157−160. (b) Montgomery, A. U. S. Patent 4,210,745, 1980.
(6) Blumbergs, P.; Khan, M. S.; Kalamas, R. L. U. S. Patent 5,110,919, 1992; U. S. Patent 5,296,589, 1992.
(7) Occupational exposure guidelines http://www.cdc.gov/niosh/pdfs/91-103b.pdf.
(8) Anwer, K. M.; Sherman, B. D.; Roney, G. J.; Spatola, F. A. *J. Org. Chem.* **1989**, *54*, 1284−1289.
(9) Gowda, S.; Gowda, C. D. *Tetrahedron* **2002**, *11*, 2211−2213.
(10) Gowda, C. D. *Tetrahedron Lett.* **2002**, *43*, 311−313.
(11) Catalyst was recycled and reused after activation. Activation was done by Monarch. http://www.monarchcatalyst.com.
(12) Distillate was recycled and reused after adjusting the ratio of water for injection and methanol.
(13) Purchased from Sigma-Aldrich.
(14) Filtrate was distilled and reused after adjusting the ratio of water for injection and methanol.