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Efficient Method for the Synthesis of Fingolimod and Impurities

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ABSTRACT

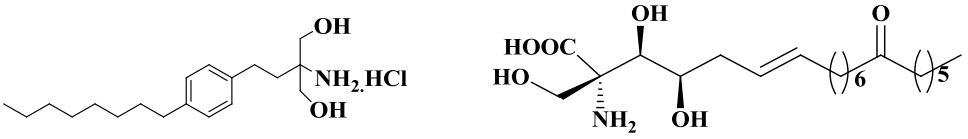
Fingolimod (1b) is an immunomodulating, United States Food and Drug Administration (USFDA) approved Active Pharmaceutical Ingredients (API), which is one of the best drugs available for the treatment of Multiple Sclerosis (MS). Fingolimod was synthesized in seven steps using cheapest commercially inexpensive raw material octanophenone which meets high yield and purity. While developing the process of Fingolimod, some related impurities were observed. These impurities were recognized as Heptyl PNP (2a), Octyl PNP (2b), Nonyl PNP (2c), Nitro heptyl (3a), Nitro octyl (3b), Nitro nonyl (3c), Heptyl (1a) and Nonyl (1c) impurities. The present work explains the synthesis of fingolimod, its impurities and their characterization.

Keywords: Fingolimod, Heptyl, Nonyl, Synthesis, Impurities, AlCl

INTRODUCTION

Fingolimod (1, Gilenya) is a replica drug of myriocin (ISP-1), a natural product which is derived from the fungus *Isaria sinclairii* (Figure 1) [1]. Gilenya, trade name of fingolimod was approved by United States Food and Drug Administration (USFDA) recently for the treatment of Multiple Sclerosis (MS). This drug is basically a structural analogue of sphingosine which is phosphorylated by sphingosine in cells [2-4]. Phospho fingolimod causes the internalization of sphingosine-1-phosphate receptors, which sequesters lymphocytes in lymph nodes, preventing them from moving to central nervous system which results a relapse of multiple sclerosis [5]. A part from that fingolimod also reported as cannabinoid receptor antagonist, a Cytosolic Phospholipases A2 (cPLA2) inhibitor and a ceramide synthase inhibitor.

Fingolimod, whose chemical name is 2-amino-2-[2-(4-octylphenyl)-ethyl-1]-1,3-diol hydrochloride, is generally called as fingolimod hydrochloride, The structural formula is shown in Figure 1.



Fingolimod Hydrochloride (1b) **Myriocin (Thermozymocidin- ISP -1)**

Figure 1: Chemical structure of fingolimod and myriocin (Thermozymocidin-IS-1)

Apart from Existing deceases day to day lot of new deceases are inventing and in this scenario medicine becomes one of the fundamental requirement for human life. Unlike earlier, major percentage of medicines in present years is completely synthetically made which surely contains different type's impurities like chemical, microbial, contaminated particles, mostly chemical impurities. The impurities present in a drug (API) can have a significant bad impact on the quality as well as on safety of the drug products/drug substance. The levels of the impurities in any drug substance are determined as per its biological or toxicological effect on human body. So it's too important from "Regulatory" point of view for the selection of the limitations of "Related Impurities" in any drug while approve. Hence, it is very essential to research carefully about the impurity profile for any drugs (API) and reduce it during the development of the process of a drug product As mentioned in the International Conference on Harmonisation (ICH Q3b) guidelines, any impurities that are forming up to a level of ≥0.10% w/w with respect to the API, should be recognized, and synthesized carrying forward studied thoroughly throughout the progress of the process [6].

Although various synthetic routes for the preparation of fingolimod have been reported [7-17], but we have synthesized fingolimod efficient and cost effective manner (Scheme 1).

The advantage of this route of synthesis is the raw materials for this process are cheap and easily available and removal process after reaction is also simple. In this synthetic route some impurities observed like Heptyl PNP (2a), Octyl PNP (2b), Nonyl PNP (2c), Nitro heptyl (3a), Nitro octyl (3b), Nitro nonyl (3c), Heptyl (1a) and Nonyl (1c) impurities and synthesized and characterized by ^{1}H, ^{13}C-NMR and mass spectroscopy. These impurities and fingolimode shown in Figure 2.

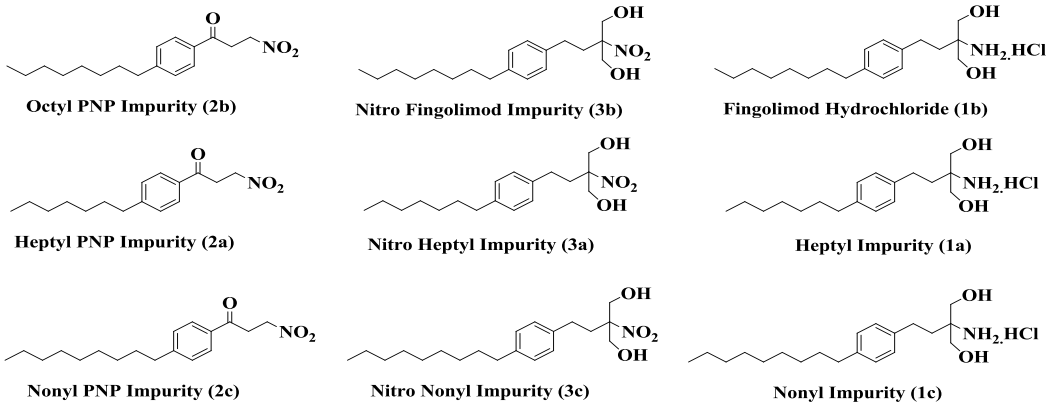
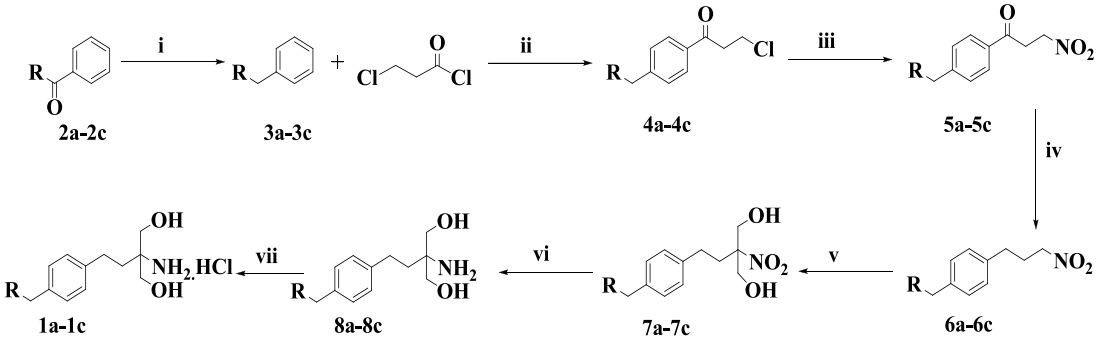


Figure 2: Chemical strictures of fingolimod and impurities

The challenge in the synthesis of fingolimod 1b is the construction of 2-aminopropane-1,3-diol (polar head) group and most of the literature routes have focused on approaches for the construction of the same. An early synthesis of fingolimod 1b by Adachi and coworkers employed alkylation of diethyl 2-acetamidomalonate with 1-(2-bromoethyl)-4-octylbenzene to obtain the Adachi-Fujita intermediate diethyl 2-acetamido-2-(4-octylphenethyl)malonate which was then set out to fingolimod 1b [18]. The other literature synthesis of 1b start from building block such as 4-octylbezaldehyde or 2-(4-octylphenyl)ethanol, which were conjugated to the polar head-group derived from diethyl acetamidomalonate [19], Tris(hydroxymethyl)aminomethane (TRIS) derivatives [20] or from a bis-aldol addition with 2-aryl substituted ethyl nitrate [21]. One of the problems with the late stage introduction of the polar head group is the increased presence of impurities related to the chemistry involved in the introduction of the N-acetamido-1,3-propanediol moiety. Therefore we have decided to start from octanophenone first and then utilize its presence for the synthesis of fingolimod 1b hydrochloride with efficiency and cost-effective manner (Scheme 1).

As a part of our ongoing program on scalable and cost-effective routes for active pharmaceutical ingredients, we were interested in the synthesis of fingolimod 1b since it is a promising oral drug for multiple sclerosis. Some of the literature routes had the disadvantage including complicated steps which gives rise to intermediates as oily substances or various isomeric mixtures. Consequently, isolation and purification of the intermediate products by chromatography techniques rendered the process unviable for large-scale preparation of 1b.

The cost of raw materials like a reducing agent lithium aluminum hydride is very high as well as having great potential safety hazard in the production of lithium aluminum hydride. Some intermediate 2-(4-octyl phenyl)-ethyl chloride in some route is inconstant and not easy to store, which leads to difficulty to meet the growing market demand. The key of synthetic steps is that, there commonly exists with low yield and difficult treatment of two splicing fragment reaction. The present invention is to provide a new and well synthetic route of fingolimod hydrochloride. The route does having very good advantages like scalable reaction conditions and lower cost of reagents and not involving the reagents like as titanium tetrachloride, lithium aluminum hydride, lithium hydroxide and so on. To achieve the above-mentioned advantages and conditions, the following schemes provides a new synthetic route of fingolimod hydrochloride.



Reagents, conditions: (i) $NaBH_4$, $AlCl_3$, THF, 0-5°C-60-65°C, 16 h; (ii) $AlCl_3$, -5 to -10°C-25-30°C, 2 h; (iii) $NaNO_2$, DMF, 0-5°C-25-30°C, 5 h; (iv) $NaBH_4$, $AlCl_3$, THF, 0-5°C-60-65°C, 16 h; (v) $(HCHO)_n$, K_2CO_3, Toluene, Reflux, 5 h; (vi) 10% Pd/C, MeOH, RT, 16 h; (vii) NH_4Cl, MeOH, 25-30°C, 4 h

Scheme 1: Synthesis of fingolimod (1b) and impurities

Herein, we report a seven step synthesis of fingolimod 1b (Scheme 1) starting from the easily available starting material octanophenone 2b. Unlike hitherto known routes, which involved the insertion of the polar head group at the first, we decided to start the synthesis from the octanophenone 2b. The first step involving the reduction of 2b to get 3b then Friedel-Crafts acylation followed by nucleophilic substitution with sodium nitrite to afford 5b, which will undergo reduction and Henrry reaction to form 7b and hydrogenated to get the fingolimod 1b.

MATERIALS AND METHODS

All the reagents and solvents were used in this process are of commercial grade and used without any additional purification. Reactions were monitored by Thin Layer Chromatography (TLC) based on silica gel plates (60 F254), Spots seen with iodine spray or ultraviolet light (254 nm). Column chromatography was performed on silica gel (60-120 mesh) using a proper eluent. ^{1}H and ^{13}C-NMR spectra were determined in Deuterated Chloroform ($CDCl_3$) and Dimethyl Sulfoxide (DMSO) solutions (Varian MR 400 MHz) spectrometers. Proton chemical shifts (δ) are relative to Tetramethylsilane (TMS, δ=0.0) as internal standard and expressed in ppm. Spin multiplicities are denoted as δ=0.0) as internal standard and expressed in ppm.

Spin multiplicities are denoted as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as br (broad), coupling constants (J) are given in Hertz (Hz). Infrared spectra were recorded on a Jasco FTIR-4200 spectrometer. Melting points were measured by using a Buchi Melting Point B-540 apparatus. Mass spectra were recorded and analysed using an Agilent 6430 triple quadruple mass spectrometer.

Experimental procedures

General procedure for 3-nitro-1-(4-heptylphenyl)propan-1-one (5a-c)

To a solution of heptanophenone (25 g, 131.3 mmol) and aluminum chloride (52.52 g, 393.9 mmol) in tetrahydrofuran (125 ml) is added sodium borohydride (14.88 g, 393.9 mmol) with in 1 h at a temperature 0-5°C. The reaction mixture stirred for 2 h at 0-5°C. The reaction mixture was stirred for about 16 h at 60°C to 65°C. After completion of reaction, concentration of reaction mass and quenched in mixture of water (125 ml) and hydrochloric acid (25 ml) is added to the reaction mixture at a temperature 10-20°C. Extracted with toluene and organic layer was separated. Solvents removed under reduced pressure to afford the compound 3a. This crude compound 3a in dichloromethane 125 ml cooled to -5°C to -10°C. A solution of 3-chloropropanyl chloride (18.33 g, 144.4 mmol) was added dropwise, followed by addition of aluminum chloride (22.75 g, 170.6 mmol) in portion to control the reaction temperature. The reaction mixture allowed to 25-30°C and stirred for 2 h. The reaction mixture was poured into pre cooled water (250 ml) and extracted with dichloromethane (125 ml). Solvent was evaporated under reduced pressure to get the compound 4a. The obtained crude was dissolved in 125 ml of N,N-dimethylformamide and added sodium nitrite (13.58 g, 196.9 mmol) at a temperature 0-5°C. The reaction mixture was allowed to 25-30°C and stirred for 5 h. The reaction mixture was poured into 250 ml ice-water extracted with ethyl acetate 125 × 2 ml. The organic layer was combined, washed with brine and evaporated the solvent under reduced pressure to afford a yellow liquid, which was purified by flash chromatography (EA: Hex=2: 8) to afford title compound 5a. R_f=0.3 (30% EtOAc/Hexane).

Following the same procedure as illustrated for 5a, the other compounds 5b and 5c were prepared from the corresponding compounds otanophenone and nonanophenone respectively. The physical, spectral and analytical data for these compounds are mentioned as follows.

3-Nitro-1-(4-heptylphenyl)propan-1-one (5a)

Color less oil, Yield: 29.16 g (80.0%); ^{1}H-NMR (400 MHz, $CDCl_3$), δ=7.91-7.88 (d, 2H), 7.31-7.29 (d, 2H), 4.84-4.81 (t, 2H), 3.66-3.63 (t, 2H), 2.69-2.65 (t, 2H), 1.65-1.61 (m, 2H), 1.34-1.25 (m, 7.8H), 0.89-0.86 (t, 3H); ^{13}C-NMR (DMSO-d6), δ=194.0,147.5,131.8,127.0, 126.5,77.3,77.0,76.6,67.9,33.8,32.9,29.7,29.1, 27.1,27.0, 20.6,12.3; Mass (m/z): 278 (M+1).

3-Nitro-1-(4-octylphenyl)propan-1-one (5b)

Light yellow color oil, Yield: 26.91 g (75.5%); ^{1}H-NMR (400 MHz, $CDCl_3$), δ=7.90-7.86 (d, 2H), 7.21-7.19 (d, 2H), 4.80-4.79 (t, 2H), 3.62-3.61 (t, 2H), 2.66-2.62 (t, 2H), 1.62-1.59 (m, 2H), 1.31-1.22 (m, 7.8H), 0.89-0.86 (t, 3H); ^{13}C-NMR (DMSO-d6), δ=194.2, 147.4, 131.6, 127.1, 126.4, 77.2, 77.1, 76.5, 67.7, 33.6, 32.7, 29.5, 29.2, 27.3, 27.2, 20.4, 12.2; Mass (m/z): 292 (M+1).

3-Nitro-1-(4-nonanylphenyl)propan-1-one (5c)

Yellow color oil, Yield: 27.27 g (78.0%); ^{1}H-NMR (400 MHz, $CDCl_3$), δ=7.91-7.88 (d, 2H), 7.31-7.29 (d, 2H), 4.84-4.81 (t, 2H), 3.66-3.63 (t, 2H), 2.69-2.65 (t, 2H), 1.65-1.61 (m, 2H), 1.31-1.26 (m, 12H), 0.89-0.86 (t, 3H); ^{13}C-NMR (DMSO-d6), δ=139.8, 139.1, 128.4, 128.2, 89.5, 63.3, 36.1, 31.9, 31.3, 29.7, 29.4, 29.2, 28.83, 28.81, 22.5, 14.1; Mass (m/z): 306 (M+1).

General procedure for 2-nitro-2-(4-heptylphenylethyl)propane-1,3-diol (7a-c)

To a solution of compound-5a (16 g, 57.68 mmol) and aluminum chloride (23.07 g, 173.04 mmol) in tetrahydrofuran (160 ml) is added sodium borohydride (6.54 g, 173.04 mmol) with in 1 h at a temperature at 0°C to 5°C. The reaction mixture stirred for 2 h at 0°C to 5°C. The reaction mixture was stirred for about 16 h at 60°C to 65°C. After completion of reaction, concentration of reaction mass and quenched in mixture of water (80 ml) and hydrochloric acid (16 ml) is added to the reaction mixture at a temperature 10-20°C. Extracted with toluene and organic layer was separated. Solvents removed under reduced pressure to afford the compound 6a. The crude compound-6a and 80 ml of toluene were charged in a flask. Then, was added paraformaldehyde (6.0 g, 201.8 mmol) and potassium carbonate (15.94 g, 115.3 mmol). The reaction mixture was allowed to stir at 100-110°C for 6 h, filtered the solid. Filtrate solvent was evaporated under reduced pressure to afford the crude compound-7a, which was purified by flash chromatography (EA: Hex=4: 6) to afford a white solid. R_f=0.3 (30% EtOAc/Hexane).

Following the same procedure as illustrated for 7a, the other compounds 7b and 7c were prepared from the corresponding compounds 5b and 5c respectively. The physical, spectral, and analytical data for these compounds are mentioned as follows.

2-Nitro-2-(4-heptylphenylethyl)propane-1,3-diol (7a)

Half white color solid, Yield: 15.85 g (85.0%); ^{1}H-NMR (400 MHz, DMSO-d6), δ=7.09 (m, 4H), 5.34-5.31 (t, 2H), 3.85- 3.76 (m, 4H), 2.53-2.46 (m, 4H), 2.10-2.06 (m, 2H), 1.54-1.50 (t, 2H), 1.26-1.23 (m, 10H), 0.86-0.82 (t, 3H); ^{13}C-NMR (DMSO-d6), δ=139.5, 138.3, 128.4, 128.2, 95.1, 60.6, 34.6, 31.5, 31.4, 31.2, 28.6, 28.4, 28.2, 22.1, 14.0; Mass (m/z): 324 (M+1).

2-Nitro-2-(4-octylphenylethyl)propane-1,3-diol (7b)

Half white color solid, Yield: 15.19 g (82.0%); ^{1}H-NMR (400 MHz, DMSO-d6), δ=7.09 (m, 4H), 5.34-5.31 (t, 2H), 3.85- 3.76 (m, 4H), 2.53-2.46 (m, 4H), 2.10-2.06 (m, 2H), 1.54-1.50 (t, 2H), 1.26-1.23 (m, 10H), 0.86-0.82 (t, 3H); ^{13}C-NMR (DMSO-d6), δ=139.9, 138.2, 128.2, 128.0, 95.0, 60.8, 34.75, 31.8, 31.2, 31.0, 28.8, 28.6, 28.5, 22.0, 13.93; Mass (m/z): 338 (M+1).

2-Nitro-2-(4-nonanylphenylethyl)propane-1,3-diol (7c)

Half white color solid, Yield: 14.53 g (79.0%); ^{1}H-NMR (400 MHz, DMSO-d6), δ=7.09 (m, 4H), 5.34-5.31 (t, 2H), 3.85-3.76 (m, 4H), 2.53-2.46 (m, 4H), 2.10-2.06 (m, 2H), 1.54-1.50 (t, 2H), 1.26-1.23 (m, 10H), 0.86-0.82 (t, 3H); ^{13}C-NMR (DMSO-d6), δ=139.4,138.0, 128.4, 128.2, 95.2, 60.6, 34.4, 31.6, 31.4, 31.2, 28.6, 28.4, 28.2, 22.2, 14.1; Mass (m/z): 352 (M+1).

General procedure for 2-amino-2-(4-heptylphenylethyl)propane-1,3-diol HCl (1a-c)

To a 500 ml autoclave reactor, was added compound-7a (8 g, 24.73 mmol) in methanol (80 ml) and 800 mg of 10% Pd/C at temperature 25-30°C. The reaction mixture was allowed stirred less than 1.0 kg/cm^3 hydrogen pressure. Stir the reaction mixture for 16 h at 25-30°C. The reaction was filtered through celite bed. The solvent was evaporated under reduced pressure to afford the crude compound-8a, which was purified by flash chromatography (MeOH: DCM=0.2: 9.8) to afford a white solid. (6.9 g, yield: 95.0%). The compound 8a (6.5 g, 22.15 mmol) was dissolved in methanol (65 ml) and added ammonium chloride (1.18 g, 22.15 mmol) at a temperature 25-30°C. The reaction mixture stirred for 3 h at 25-30°C. Filter the solid to afford the titled compound (1a, 6.5 g, yield: 90.0%). R_f=0.1(10% MeOH/DCM).

Following the same procedure as illustrated for 1a, the other compounds 1b and 1c were prepared from the corresponding compounds 7b and 7c respectively. The physical, spectral and analytical data for these compounds are mentioned as follows.

2-Amino-2-(4-heptylphenylethyl)propane-1,3-diol HCl (1a)

White color solid, Yield: 6.55 g (80.0%); ^{1}H-NMR(400 MHz, DMSO-d6), δ=7.12-7.10 (m, 4H), 3.52 (s, 4H), 2.57-2.50 (m, 2H), 1.80-1.75 (m, 2H), 1.54-1.51 (m, 2H), 1.26-1.19 (m, 8H), 0.86-0.83 (t, 3H); ^{13}C-NMR($CDCl_3$), δ=139.6,138.6, 128.3, 128.1, 60.8, 60.1, 34.4, 33.3, 31.3, 31.0, 28.7, 28.5, 27.6, 22.1, 14.0; Mass (m/z): 330 (M+1).

2-Amino-2-(4-octylphenylethyl)propane-1,3-diol HCl (1b)

White color solid, Yield: 6.76 g (83.0%); ^{1}H-NMR(400 MHz, DMSO-d6), δ=7.96 (br s, 3H), 7.11-7.06 (m, 4H), 5.42-5.39 (t, 2H), 3.56-3.48 (m, 4H), 2.59-2.55 (m, 2H), 2.52-2.48 (m, 2H), 1.80-1.75 (m, 2H), 1.53-1.50 (m, 2H), 1.25-1.22 (m, 10H), 0.85-0.82 (t, 3H); ^{13}C-NMR($CDCl_3$), δ=139.7, 138.9, 128.2, 128.0, 60.9, 60.3, 34.7, 33.2, 31.2, 31.0, 28.8, 28.6, 27.9, 22.0, 13.94; Mass (m/z): 344 (M+1).

2-Amino-2-(4-nonanylphenylethyl)propane-1,3-diol HCl (1c)

White color solid, Yield: 6.92 g (85.0%); ^{1}H-NMR(400 MHz, DMSO-d6), δ=7.12-7.08 (m, 4H), 3.59 (s, 4H), 2.59-2.52 (m, 2H), 1.81-1.76 (m, 2H), 1.54-1.52 (m, 2H), 1.26-1.23 (m, 12H), 0.86-0.83 (t, 3H); ^{13}C-NMR ($CDCl_3$), δ=139.5, 138.8, 128.4, 128.2, 60.7, 60.2, 34.6, 33.1, 31.2, 31.0, 28.6, 28.7, 27.7, 22.1, 14.1; Mass (m/z): 358 (M+1).

RESULTS AND DISCUSSION

Reduction of the alkanophenone 2 was carried out using sodium borohydride and aluminum chloride in tetrahydrofuran to obtain phenyl alkane 3 in very good yield and with high purity. During our initial screening, we employed triethylsilane for reduction. However, sodium borohydride was milder and easier to handle and a scalable process. Subsequently, the phenyl alkane 3 was taken up for Friedel-Crafts acylation with 3-chlorpropanoyl chloride to obtain the desired *para*-acylated product 4 exclusively in good yield. This process was further optimized on a multigram scale and the purification of the product was carried out without resorting to column chromatography. Similar results were observed when $SnCl_4$ or $TiCl_4$ were employed for the reaction, but the yields were slightly lower compared to the reaction mediated by $AlCl_3$.

Nucleophilic substitution of compound 4 with consistent yields and reproducibility was achieved using sodium nitrite in N,N-dimethyl formamide as the solvent. Under these conditions, we were able to prepare compound 5 conveniently in a 75.5% yield, without the formation of styrene or impurities, because the reason may be the chlorine is a good leaving group and the elimination of hydrochloric acid to form an enone rather the nucleophilic substitution was preferred for compound 4. Reduction of the compound 5 was carried out using sodium borohydride and aluminum chloride in tetrahydrofuran to obtain compound 6 in very good yield and with high purity. The gem-hydroxyl methyl moiety was installed to compound 6 on the alpha carbon to form compound 7 by Henrry reaction with high yield. To complete the synthesis of Fingolimod (1b), the nitro group in compound 7 was reduced to amine with Pd/C, followed by a salt formation with ammonium chloride.

In summary, a concise route for the synthesis of Fingolimod was reported from commercially available octanophenone in 7 steps with an overall yield of 58.0%.

CONCLUSION

In conclusion, high-yield, reproducible, a safe and robust route for the synthesis of fingolimod 1b starting from octanophenone is reported [22]. The current work demonstrates that final installation of the polar head group can extensively reduce the impurities associated with the polar head group in the initial steps. Prevention of isomeric mixture, expensive catalysts, hazardous reagents and difficult purification methods renders this route a cost-effective process for fingolimod 1b, which can be translated into an industrially feasible process.

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