Chemoenzymatic asymmetric synthesis of fluoxetine, atomoxetine, nisoxetine, and duloxetine

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A B S T R A C T
The asymmetric synthesis of two well-known anti-depressant drugs, fluoxetine and duloxetine has been accomplished in a chemoenzymatic manner. The main highlight of the synthesis is the enantioselective cyanohydrin formation by a plant (R)-HNL (hydroxynitrile lyase). The enantiopure cyanohydrins are then synthetically manipulated into the above two drug molecules and two of their structural analogues, atomoxetine and nisoxetine.

1. Introduction
Selective serotonin re-uptake inhibitors or serotonin-specific reuptake inhibitor (SSRIs) are a class of organic molecules generally used as antidepressants for the treatment of several depression related disorders. They are believed to enhance the extracellular level of serotonin (the well-known neurotransmitter) by inhibiting its reuptake in the presynaptic cell.1 Serotonin–norepinephrine reuptake inhibitors (SNRIs) are another class of organic molecules used mainly in the treatment of major depression. In principle, SNRIs are able to increase the level of two neurotransmitters (serotonin and norepinephrine) in the brain which are known to play an important role in mood related phenomenon.2 Fluoxetine (known as Prozac, Sarafem, Fontrex as brand names) belongs to the SSRI class and is a very well-known antidepressant developed by Eli-Lilly in 1987. Fluoxetine was a blockbuster drug during the early 1990s and its estimated annual sales reached US$350 million within a year, as the drug was off patented in 2001 several generic versions came into the market. Duloxetine (sold under the brand name Cymbalta, Ariclaim, Duzela, Yentreve, and a few others) belongs to the SNRI class and was manufactured and marketed by Eli-Lilly and used mainly in the treatment of major depression and general anxiety disorder (GAD). Atomoxetine is another drug that belongs to the norepinephrine reuptake inhibitor (NRI or NERI) class and acts as a reuptake inhibitor of norepinephrine.3 It blocks the action of norepinephrine transporter receptor and hence leads to enhanced concentration of that neurotransmitter in adrenergic neurotransmission. Atomoxetine was manufactured by Eli-Lilly and sold under the brand name Strattera worldwide and is approved for the treatment of ADHD (attention deficit hyperactivity disorder).4 Nisoxetine, which is structurally similar to fluoxetine and atomoxetine was first synthesized by Eli-Lilly in 1970; it also belongs to the NRI class, and while it was not marketed, it was used widely in biochemical experiments as a selective NRI agent.

All of the aforementioned molecules have a common structural unit, N-methyl-3-aryloxy-3-aryl-proylamine (Fig. 1) hence a common intermediate would be effective for accessing all of these molecules in an efficient way. Numerous synthetic reports exist in the

![Figure 1. Structure of the (R)-enantiomer of fluoxetine, duloxetine, atomoxetine, and nisoxetine.](http://dx.doi.org/10.1016/j.tetasy.2013.06.003)
2. Results and discussion

To the best of our knowledge enantiopure cyanohydrins have not been used as chiral intermediates for the synthesis of the above molecules. Although fluoxetine was sold as a racemic compound, it was later shown that the (R)-enantiomer is the so-called ‘Improved Chemical Entity’ version of the drug. Herein we report the asymmetric synthesis of the (R)-enantiomer of all of these four molecules from enantiopure cyanohydrins. The retrosynthetic analysis is presented below (Scheme 1). One of the most promising and interesting way to synthesize enantiomerically pure cyanohydrins is the HNL catalyzed addition of a cyanide source to the respective carbonyl compounds. HNLs are now widely used as efficient biocatalysts for the asymmetric synthesis of various cyanohydrins. The resulting cyanohydrins are versatile intermediates for a broad variety of chiral synths. The reaction is extremely important, since it allows the synthesis of enantiomerically pure compounds from prochiral substrates in a quantitative yield.

![Scheme 1. Retrosynthetic analysis of fluoxetine, atomoxetine, nisoxetine, and duloxetine by enzymatic hydrocyanation.](image)

We envisioned that (S)-3-(methylamino)-1-phenylpropan-1-ol 5 could act as a common intermediate for the synthesis of the (R)-enantiomer of fluoxetine 1, atomoxetine 3, and nisoxetine 4 by Mitsunobu inversion with the required phenols. In the case of duloxetine 2, the Mitsunobu reaction of 1-naphthol with (S)-3-(methylamino)-1-(thiophen-2-yl)propan-1-ol 6 would lead to the target molecule. The intermediates 5 and 6 were planned to be synthesized from the respective cyanohydrins by straightforward synthetic manipulation. The enantiopure cyanohydrins were accessed from their respective aldehydes by an HNL route developed earlier in our group. Recently we have found a new (R)-HNL from white apricot (shakarpara cultivar, found in the Himalayan region of Nepal and India; Prunus armeniaca). The new enzyme (ParsHNL) exhibits excellent enantioselectivity during the preparation of several cyanohydrins from aliphatic and aromatic carbonyl compounds. By employing this newly found HNL we have synthesized several enantiopure δ,δ-unsaturated cyanohydrins 2-Thiophene-carboxaldehyde was subjected to an asymmetric hydrocyanation reaction by employing similar reaction conditions as stated above. The respective enantiopure cyanohydrin was obtained in 88% yield with excellent enantioselectivity (er = 97%). In general, we followed the modified method developed by Griengl et al. for the HNL catalyzed hydrocyanation reaction (see Section 4 for details).

With the enantiopure cyanohydrins in hand, we then proceeded further for the total synthesis of the target molecules. (R)-Mandelonitrile was protected as its TBS (tert-butyldimethyl silyl) ether by treatment with imidazole and TBS–Cl at 25 °C to afford the protected cyanohydrin 7 in 88% yield. Compound 7 was not purified further, and so the crude mixture of 7 was subjected to DIBAL–H treatment at −78 °C for 2 h, which yielded the corresponding aldehyde 8 in almost quantitative yield. The aldehyde after usual work-up (no chromatographic separation is needed) was subjected to one carbon Wittig olefination with Ph3P=CH2 to afford olefin 9 in...
82% yield. Compound 9 upon hydroboration with BH₃ SMe₂ afforded the corresponding alcohol 10 in 86% yield (no chromatographic separation was needed). Alcohol 10 was then treated with methanesulfonyl chloride (Ms-Cl) and Et₃N to furnish the respective mesylate 11 in 90% yield (no chromatographic separation was needed). Mesylate 11 upon treatment with an aq. solution of 40% MeNH₂ in refluxing THF afforded the corresponding amine 12 in 78% yield. Desilylation (TBS deprotection) of compound 12 was achieved by treatment with TBAF/THF to afford aminoalcohol 5 in 88% yield (no chromatographic separation was needed). The Mitsunobu reaction of 5 with 1-naphthol afforded the target molecule (R)-duloxetine (overall yield = 21% from compound 13).

3. Conclusion

In conclusion, we have reported an efficient and general synthetic strategy for the enantiopure synthesis of a few important antidepressant drug molecules. The main highlight of our synthesis is the enzymatic formation of enantiopure cyanohydrins from their respective aldehydes by a plant HNL (ParsHNL). The enantiopure cyanohydrins were then synthetically manipulated to the target molecules. It is also noteworthy that out of the nine synthetic steps employed, we have used chromatographic purification methods in only three steps. As a result, the synthesis is cost effective and green also, as chromatographic separation in some steps is avoided.

4. Experimental

4.1. General

Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. Benzaldehyde and thiophene-2-carbaldehyde were freshly distilled or

Scheme 2. Reagents and conditions: (a) HCN in iPr₂O, ParsHNL, citrate buffer (pH 4.0), 92% (90% for 13); (b) imidazole, TBS–Cl, rt, 88% (92% for 14); (c) DIBAL-H, −78 °C; (d) LHMDS, Ph₃P Me⁺, 0 °C, 82% (75% for 16); (e) BH₃ SMe₂, 0 °C, H₂O₂, NaOH, 86% (84% for 17); (f) MeSO₂Cl, Et₃N, DMAP, 90% (88% for 18); (g) MeNH₂ (40% aq), THF, 78% (72% for 19); (h) TBAF, THF, 88% (90% for 6); (i) Ph₃P, DIAD, THF, Ar-OH.
washed with an aq NaHCO₃ solution in order to minimize the amount of free acid, which is supposed to inhibit HNL activity. The enzymatic hydrocyanation reactions were performed in well ventilated fume hoods with proper glassware, hardware (gloves), and using a mask if necessary. Any excess HCN remaining after the reaction was destroyed cautiously by treatment with an excess of bleach solution and disposed of in a proper way. Reactions were monitored by TLC, carried out on 0.25 mm silica gel plates (Merck) with UV light, ethanolic anisaldehyde, and phosphomolybdic acid/heat as developing agents. Silica gel 100–200 mesh was used for column chromatography. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. NMR spectra were recorded on 200 MHz spectrometer at 25 °C in CDCl₃ using TMS as the internal standard. Chemical shifts are referenced to CDCl₃. 1H NMR (200 MHz, CDCl₃ and using TMS as the internal standard. Chemical shifts are referenced to CDCl₃.

4.2. (R)-2-Hydroxy-2-phenylacetoinitrile

To a solution of benzaldehyde (1.06 g, 1.0 mol) in DIPE (60 ml), a solution of PürshNL (300 IU/10 mmol of aldehyde, approximately 10 ml of crude enzyme solution) was added and the resulting mixture was stirred vigorously until an emulsion was formed. The pH of the enzyme solution was previously adjusted to 4.0 with 10% citric acid solution. Freshly prepared HCN in DIPE (2 equiv) was added to it, and the reaction temperature was kept at 10 °C. After completion of the reaction, it was extracted thoroughly with ether several times and the organic layer was dried over MgSO₄. Evaporation of the solvent yielded the crude cyanohydrin in 92% yield. The (R)-mandelonitrile obtained provides comparable spectroscopic and optical data with those reported in the literature.

4.3. Preparation of HCN in DIPE

At first, NaCN (10 g) and citric acid (0.1 g) were dissolved in water (100 ml). The solution was cooled in an ice/water bath and extracted with DIPE (50 ml), while acidifying with 33% HCl until pH 5.5. The water layer, which contained a suspension of NaCl, was extracted twice with DIPE (25 ml). The combined DIPE layers were stored in a dark bottle. The above procedure must be performed in a well ventilated fume hood with proper glassware and hardware (gloves).

4.4. (R)-2-((tert-Butyldimethylsilyloxy)-2-phenylacetoinitrile 7

(R)-Mandelonitrile (500 mg, 3.75 mmol) was taken in anhydrous DCM (15 ml) and cooled to 0 °C. Imidazole (281 mg, 4.13 mmol) and DMAP (catalytic) were then added to the reaction mixture followed by the addition of TBS-Cl (620 mg, 4.13 mmol). The reaction mixture was then allowed to warm at room temperature for 6 h, after which water was added to it and the organic layer was diluted with DCM, washed with brine and dried over MgSO₄. Evaporation afforded the TBS-protected compound in 88% yield. The TBS protected cyanohydrin is used in the next step without further purification.

4.5. (R)-2-((tert-Butyldimethylsilyloxy)-2-phenylacetaldehyde 8

The crude TBS protected cyanohydrin (815 mg, 3.3 mmol) was taken in 10 ml of dry DCM under an argon atmosphere. To this solution was added DiBAL-H (1 M in DCM, 3.3 ml), dropwise at –45 °C, and the mixture was warmed up to 0 °C. After stirring for 1 h at this temperature, the reaction mixture was poured into a mixture of ether and saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was washed with brine and dried over MgSO₄. It was then concentrated in a rotary evaporator which yielded the corresponding aldehyde 8 in almost quantitative yield, which was used in the next step without any further purification. 1H NMR (200 MHz, CDCl₃): 7.45–7.39 (m, 5H), 5.07 (d, J = 2.2 Hz, 1H), 0.97 (s, 9H), 0.15 (s, 3H), 0.07 (s, 3H). 13C NMR (50 MHz, CDCl₃): 199.6, 136.8, 128.7, 128.5, 126.9, 80.1, 25.9, 18.2, –4.6.

4.6. (S)-1-Phenylallyloxy)(tert-butyl)dimethylsilane 9

To a suspension of methyltriarylphosphonium iodide (1.5 g, 3.74 mmol) in dry THF was added LiHMDS (3.4 ml) at 0 °C. The yellow mixture was stirred at 0 °C for 15 min. A solution of the aldehyde 8 (800 mg, 3.23 mmol) in 10 ml of THF was added to the reaction mixture. The yellow suspension was then stirred at room temperature for a further 1 h. After completion of the reaction, as indicated by TLC analysis, the reaction was quenched with water and the layers were separated. The organic layer was extracted with 50 ml of ether, washed with brine, and dried over MgSO₄. The solvent was removed in vacuo and the crude residue was purified by flash column chromatography to afford compound 9 (EtOAc/hexane = 1:2) in 82% yield. 1H NMR (200 MHz, CDCl₃): 7.57–7.25 (m, 5H), 6.13–5.96 (m, 1H), 5.44–5.14 (m, 3H), 1.04 (s, 9H), 0.21 (s, 3H), 0.12 (s, 3H). 13C NMR (50 MHz, CDCl₃): 143.9, 141.9, 128.4, 127.2, 126.2, 113.5, 76.1, 26.1, 18.5, –4.4, –4.5. HRMS (ESI) for C₁₃H₂₂NaO₅Si[M+Na]⁺, calc 271.1494, found: 271.1490.

4.7. (S)-3-(tert-Butyldimethylsilyloxy)-3-phenylpropan-1-ol 10

To a cooled (0 °C), stirred solution of 9 (635 mg, 2.56 mmol) in THF (8 ml) was added BH₃·SMES (1.4 ml, 2.8 mmol). The mixture was stirred for an additional 2 h and then quenched with EtOAc (30 ml) followed by the addition of 1 M aqueous NaOH (4.6 ml) and 30% H₂O₂ (4.6 ml) at 0 °C dropwise. The mixture was stirred vigorously for 3.5 h. It was then extracted with EtOAc and washed with brine. The organic solvent was dried (MgSO₄), and concentrated to provide the crude alcohol 10 in 86% yield, which was used in the next step without further purification. 1H NMR (200 MHz, CDCl₃): 7.27–7.25 (m, 5H), 4.93–4.87 (m, 1H), 3.75–3.65 (m, 2H), 1.93–1.84 (m, 2H), 0.85 (s, 9H), 0.01 (s, 3H), –0.19 (s, 3H). 13C NMR (50 MHz, CDCl₃): 144.6, 128.3, 127.3, 125.9, 74.5, 60.3, 42.3, 25.9, 18.2, –4.5, –5.0. HRMS (ESI) for C₁₃H₂₀NaO₂Si[M+Na]⁺, calc 289.1600, found: 289.1606.

4.8. (S)-3-(tert-Butyldimethylsilyloxy)-3-phenylpropyl methanesulfonate 11

To a cooled (0 °C), stirred solution of 10 (681 mg, 2.56 mmol) in DCM (8 ml) were added Et₃N (391 μl, 2.81 mmol) and methanesulfonyl chloride (218 μl, 2.81 mmol) and the reaction mixture was stirred at room temperature for 2 h. The reaction was then stopped by adding 5 ml of saturated NH₄Cl. The water phase was extracted with DCM twice. The combined extracts were dried and evaporated to furnish the respective mesylate 11 in 90% yield. 1H NMR (200 MHz, CDCl₃): 7.22–7.20 (m, 5H), 4.74 (t, J = 6.4 Hz, 1H), 4.31–4.26 (m, 1H), 1.95–1.80 (m, 1H), 2.86 (s, 3H), 2.00–1.93 (m, 2H), 0.80 (s, 9H), –0.03 (s, 3H), –0.26 (s, 3H). 13C NMR (50 MHz, CDCl₃): 144.1, 128.3, 127.5,
125.8, 71.0, 66.9, 40.1, 37.3, 25.8, 22.6, 18.1, 14.4, –4.6, –5.1. HRMS (ESI) for C_{13}H_{28}NaO_{4}S [M+Na]^+, calcd 367.1375, found: 367.1381.

4.9. (S)-3-((tert-Butyldimethylsilyloxy)-N-methyl-3-phenylprop-1-amine 12

A solution of (S)-3-((tert-butyldimethylsilyloxy)-3-phenylpropyl methanesulfonate 11 (450 mg, 1.30 mmol) and methylamine (9 ml, 40% in water) in THF (9 ml) was heated at 65 °C for 3 h. After cooling, the solution was diluted with ether, washed with saturated aqueous sodium bicarbonate and brine, and dried over anhydrous potassium carbonate. After concentration, a pale yellow oil was obtained. The residue was then purified by flash chromatography (CH_{2}Cl_{2}/MeOH/NH_{4}OH, 90:5:1) to afford the desired product in 78% yield. 

4.10. (S)-3-(Methylamino)-1-phenylprop-1-ol 5

Compound 12 (279 mg, 1.00 mmol) was taken in dry THF (3 ml) after which TBAF (1 M in THF, 1.2 ml) was added to it, and the reaction mixture was stirred for 3 h at room temperature. After which THF was evaporated and water (1 ml) was added to it. The reaction mixture was extracted with EtOAc (10 ml), washed with NaHCO_{3} and brine, then dried by using CaCO_{3} and concentrated to afford the desired product in 88% yield, which was used in the next step without further purification. 

4.11. Mitsunobu procedure

Triphenylphosphine (100 mg, 0.44 mmol), and diethyl azodicarboxylate (63 μl, 0.44 mmol) were added to a solution of 5 (66 mg, 0.44 mmol) and the properly substituted phenols (0.44 mmol) in THF (2 ml). The mixture was stirred at room temperature overnight until the reaction was completed (TLC). Next, the THF was removed in vacuo and the residue was extract with EtOAc (3 × 5 ml). The combined organic fractions were concentrated, and the residue was purified by flash chromatography (CH_{2}Cl_{2}/MeOH/NH_{4}OH, 97:2:1) to afford the desired product in 60–70% yield.

4.12. (R)-3-(4-(Trifluoromethyl)phenoxy)-N-methyl-3-phenylprop-1-amine 1 (fluoxetine)

4.13. (R)-3-(o-Tolylazo)-N-methyl-3-phenylprop-1-amine 3 (atomoxetine)

4.14. (R)-3-(2-Methoxyphenoxy)-N-methyl-3-phenylprop-1-amine 2 (ninoxetine)

4.15. (S)-2-Hydroxy-2-(thiophen-2-yl)acetophenone 13

4.16. (S)-2-((tert-Butyldimethylsilyloxy)-2-(thiophen-2-yl)acetophenone 14

4.17. (S)-2-((tert-Butyldimethylsilyloxy)-2-(thiophen-2-yl)acetaldheyde 15

4.18. 2-(S)-1-((tert-Butyldimethylsilyloxy)allyl)thiophene 16

4.19. (S)-3-((tert-Butyldimethylsilyloxy)-3-(thiophen-2-yl)prop-1-ol 17
2.3. (R)-N-Methyl-3-(naphthalen-1-yl)-3-(thiophen-2-yl)propion-1-amine 2 (duloxetine)

\[ \Delta \delta = -109.5 \ (c \ 1.2, \ CHCl_3). \]

\[ \Delta \delta = -114.3 \ (c \ 1.0, \ MeOH). \]

1H NMR (400 MHz, CDCl_3): 8.34–8.32 (m, 1H), 7.80–7.77 (m, 1H), 7.52–7.49 (m, 2H), 7.41 (d, J = 8.4 Hz, 1H), 7.29–7.27 (m, 1H), 7.25–7.21 (m, 1H), 7.20–7.09 (m, 1H), 6.92 (dd, J = 6.3 Hz, J = 5.2 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 5.86 (dd, J = 5.2 Hz, J = 7.6 Hz, 1H), 3.27–3.20 (m, 2H), 2.64–2.61 (m, 1H), 2.54 (s, 3H), 2.47–2.41 (m, 1H). 13C NMR (100 MHz CDCl_3): 153.1, 144.9, 134.3, 127.2, 126.3, 126.1, 128.5, 125.0, 124.5, 123.9, 120.3, 106.7, 74.5, 48.0, 38.7, 36.3. HRMS (ESI) for C_{19}H_{19}NaNO_2S: [M+Na]^+ calculated 320.1085, found: 320.1081.

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