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Synthesis of enantiopure angiotensin II type 2 receptor [AT_2R] antagonist EMA401

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ABSTRACT

We report a facile synthesis of the angiotensin II type 2 receptor antagonist EMA401, which recently passed phase II clinical trials, in high overall yield. The synthesis of the key phenylalanine intermediate involved the formation of an α-nitro cinnamic ester and its reduction followed by a Pictet–Spengler cyclization, which furnished the tetrahydroisoquinoline core structure. Next, EMA401 was separated from its enantiomer EMA402 by four recrystalizations of a diastereomeric salt in 98% *ee*. All steps were performed on gram scale with emphasis on avoiding column purification and using readily available low cost starting materials and reagents.

1. Introduction

Some 12–15 years ago a drug discovery program aimed at identifying drug-like angiotensin II type 2 receptor [AT_2R] agonists was initiated at our department with Prof. Anders Hallberg as the project leader.[1] We applied two different approaches; *a*) starting from non-peptide, non-selective AT_1/AT_2 receptor ligands,[2] and *b*) transforming the native parent peptide angiotensin II, via the AT_2R selective agonistic derivative (*p*-amino-Phe[6])Ang II (Fig. 1),[3] into selective drug like ligands exhibiting agonism at the AT_2R. In the research program, a new class of selective AT_2R antagonists was also discovered, with compound M132/C38 as the most promising example.[4] In fact, the AT_2R antagonist M132/C38 was found to be considerably more effective as an antagonist in the neurite outgrowth model than PD-123,319,[5] which was previously used in most laboratories as the standard selective AT_2R antagonist (Fig. 1).[6]

While angiotensin II type 1 receptor [AT_1R] antagonists such as Losartan (Fig. 1) have been used in the clinic for almost two decades to treat hypertension,[7] the clinical use of AT_2R modulators has been more elusive. The recent successful phase II study of the AT_2R antagonist EMA401 (Fig. 1) for treatment of postherpetic neuralgia has put the spotlight on the therapeutic potential of also the AT_2R.[8] Previous efforts to investigate the biological function of AT_2R have used various peptide derivatives,[3] or preferably metabolically stable compounds such as the selective agonist M24/C21, the non-selective antagonist PD123,319,[5] or the selective antagonist M132/C38.[4] After the clinical results with EMA401, this compound should also become a new important pharmacological tool.

We were curious to study and benchmark the properties exhibited by EMA401 as part of our ongoing research projects to further develop the M132/C38 class of AT_2R antagonists. Synthesis of racemic EMA400 (EMA401/EMA402 1:1) has been reported by several authors[9] and a patent application describing the preparation of EMA400 as well as the partial resolution to the active enantiomer EMA401 have been published.[10] However, these preparative protocols have been difficult to reproduce in our hands, and the problem of preparing the pure enantiomer EMA401 is still unresolved in the scientific literature. The method to produce the pharmaceutical material used in clinic may never be published, and at the time of writing there are no commercial sources.[11] We hereby report a synthetic protocol for preparing EMA401, including a resolution of the enantiomers EMA401 and EMA402 by selective crystallization of diastereomeric salts.

2. Result and discussion

We started the investigation by scouting the first synthetic routes already published for EMA401 by the Hodges team (Scheme 1).[9] The literature strategy involved synthesis of the appropriately substituted phenylalanine derivative from hydantoin **3**, starting from commercially available 2-hydroxy-3-methoxybenzaldehyde.

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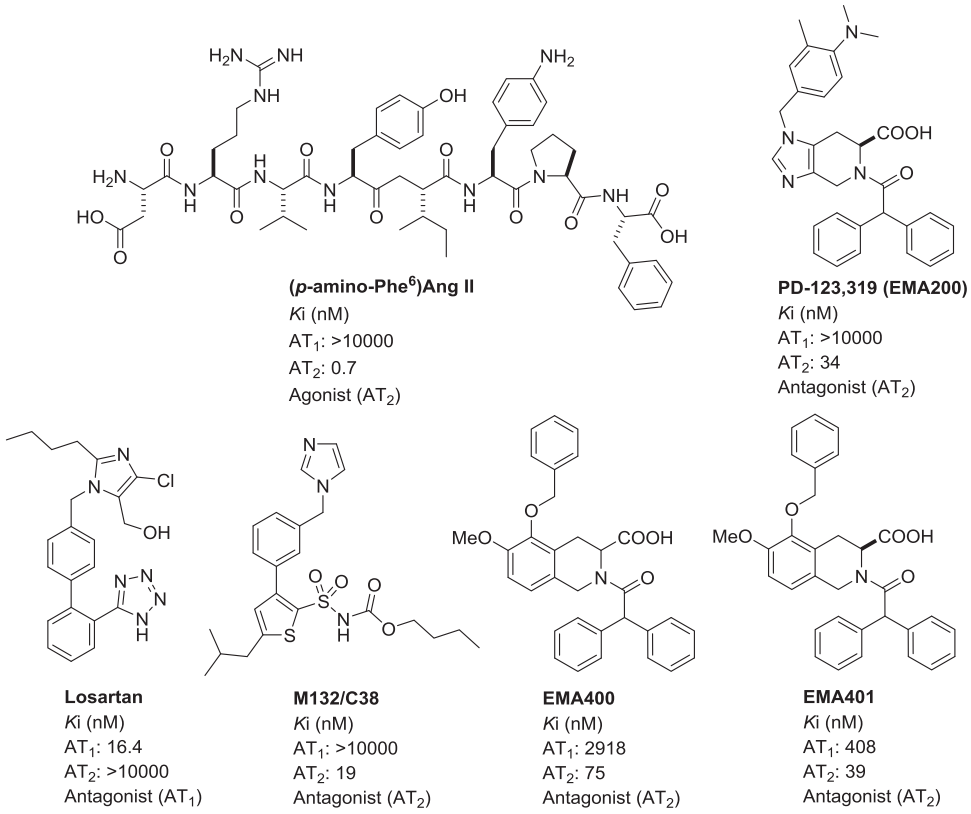
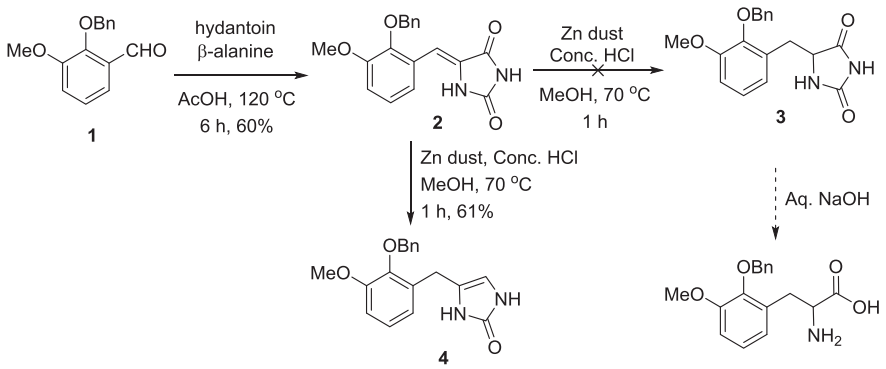


Fig. 1. Angiotensin II type 1 and 2 receptor ligands.



Scheme 1. First attempt to prepare EMA400.

Thus, 2-hydroxy-3-methoxybenzaldehyde was *O*-benzylated to furnish 2-benzyloxy-3-methoxybenzaldehyde (**1**). In the next step, the obtained *O*-protected **1** was condensed with hydantoin in presence of β-alanine in acetic acid to furnish **2** in 60% isolated yield (Scheme 1). When compound **2** was subjected to reduction using reported conditions[9] using zinc dust and conc. HCl in methanol reflux, we observed formation of compound **4** in 61% yield instead of forming hydantoin **3**.

Under controlled conditions, e.g., lowering the temperature and reducing the equivalents of zinc dust, resulted into incomplete reaction and gave only either over reduced product **4** or unreacted starting **2**. Probably, the reduction of hydantoin **3** to compound **4** is faster than the reduction of compound **2** to **3**, as LCMS was not able to detect any considerable peak for compound **3**.

We therefore decided to switch to an alternative strategy (Scheme 2). A literature survey revealed that there are multiple possibilities to rapidly access the appropriately substituted phenylalanine derivative (intermediate **B**). Since 2-hydroxy-3-methoxybenzaldehyde is commercially available and cheap, we decided to again explore the possibilities to use this benzyl protected derivative as the starting material. The selected route was based on a report by Dauzonne et al.[12] and involved the synthesis of α-nitro cinnamate ester **5** from **1** by condensation with commercially available ethyl nitroacetate and its subsequent reduction to intermediate B (Scheme 2).

Condensation of aldehyde **1** with ethyl nitroacetate in presence of diethyl amine hydrochloride in anhydrous toluene with the removal of formed water by a Dean–Stark apparatus smoothly furnished intermediate **A**. We did not isolate this intermediate and after aqueous work up it was directly subjected for reduction with sodium borohydride in isopropanol–chloroform. The obtained reduced nitro ester **5** was purified by column chromatography to

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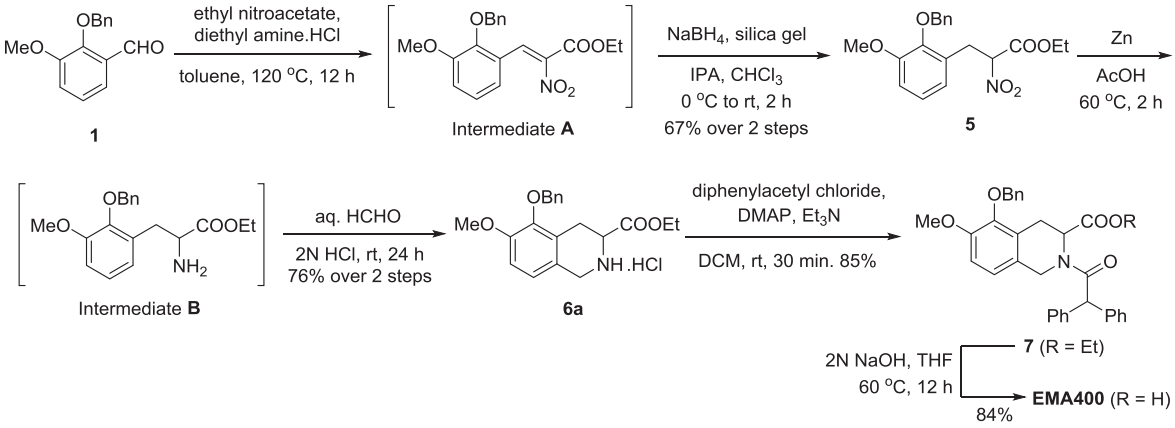
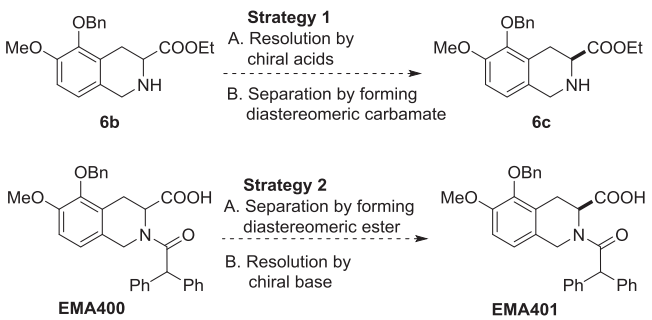


Scheme 2. Synthetic route to EMA400.

afford a 67% isolated yield over two steps. The next transformation was chemoselective reduction of the nitro functionality to the corresponding aminoester intermediate **B**. With H_2/Pd–C as the reducing system we observed faster *O*-debenzylation than reduction of nitro group. However, Zn dust in acetic acid provided the desired reduction of the nitro functionality without affecting the benzyl protection group. The reaction was exothermic and the temperature was maintained by controlling the addition of the Zn dust. After aqueous work up, the obtained intermediate **B** was smoothly cyclized with Pictet-Spengler conditions.[13] To our delight the highly pure hydrochloride salt of the isoquinoline (**6a**) precipitated out from the aqueous phase during the reaction (76% isolated yield over two steps). Next, salt **6a** was reacted with diphenylacetyl chloride to furnish ester **7** in high isolated yield (85%). It was found advantageous to have the ester protected isoquinoline as the precursor for this reaction (Scheme 2).[9] Thus, after synthesizing ester **7** in less than five steps, it was hydrolyzed using aqueous NaOH to furnish EMA400 in 84% yield. Although there are no published spectroscopic data available for EMA400 or EMA401, the spectroscopic data concurred well with the obtained product. After accessing the EMA400 in 0.87 g scale, the next task was to separate enantiomers EMA401 and EMA402 since the former is more active and of higher interest as an AT_2R antagonist.

Identified EMA400 resolution strategies are depicted in Scheme 3. Resolution by chiral acid (Strategy 1A) and chiral base (Strategy 2B) appeared to have the best scaling potentials. Since it is advantageous to have the resolution in an early step of the synthesis to improve the atom economy it was decided to first evaluate Strategy 1A. We screened various chiral acid (*R*-mandelic acid, *R*-camphor sulfonic acid, D-tartaric acid, D-dibenzoyl tartarate) and solvent (acetone, toluene, ethanol, ethyl acetate, ethanol–water, ethyl acetate-isohexane, acetonitrile etc.) combinations for the resolution of **6b** but none of the strategy 1A crystallization conditions investigated were able to separate the diastereomeric salts.



Scheme 3. Strategies for enantiomeric separation.

Strategy 1B did not yield sufficient separation of diastereomers with attempted chromatographic and fractional crystallization methods. Strategy 2A gave good separation of the EMA400 menthyl ester diastereomers using preparative HPLC (see Supplementary data, S5 for details). The preparative value of strategies 1B and 2A was, however, limited since diastereomerically enriched carbamates and esters under the examined cleavage conditions underwent racemization (basic) or *O*-debenzylation (acidic). Also, it was observed that free base **6b** was not fully stable as it was turning yellow after 1–2 days storage under air. All the above observations led us to focus on Strategy 2B.

First we decided to investigate Strategy 2B, in an attempt to improve the partial resolution methodology as reported by Klutchko et al.[9] This methodology involved the resolution of EMA400 using *S*-methyl benzylamine as a chiral resolving agent. Unfortunately, we could only obtain poor resolution despite investigating crystallizations in different solvents (ethyl acetate–hexane, ethanol). It has been reported that (1*S*,2*S*)-(+)-thiomicamine is useful for partly resolving EMA401.[10] Screening various crystallization solvents for the resolution using (1*S*,2*S*)-(+)-thiomicamine (Table 1, See Supplementary data, S6), we found that the use of ethanol, ethyl acetate or toluene were not successful, providing only low resolution of EMA401 after single crystallizations. However, when the salt obtained from crystallizations in ethanol was subjected to three additional crystallizations in ethanol; it was observed to generate the EMA401 salt in 93% *ee* according to LC.

When repeating the multiple crystallization strategy in increased scale, we observed high insolubility of the diastereomeric salt after 2–3rd crystallization. This resulted in the need for large amounts of ethanol to dissolve the EMA401/EMA402 salt at 80 °C. The consequence of this was that the crystallization became unpredictable. Thus, it became necessary to identify an alternative solvent in which the salt had higher solubility at an elevated temperature. An acetone solution of EMA400 and (1*S*,2*S*)-(+)-thiomicamine at room temperature was observed to form a precipitate upon addition of water. By the use of an acetone–water (1:1) mixture, we were able to achieve 20% *ee* of EMA401 in the first crystallization (Table 1, Entry 8, See Supplementary data, S6).

This partly resolved material was subjected for repeated crystallizations, providing improved enantiomeric purity of EMA401. The acetone–water solvent system was therefore selected for multiple crystallizations for the generation of enantiopure EMA401.

EMA400 was next prepared with a modified procedure in multi gram scale, allowing facile purification without column chromatography (Scheme 4). The synthetic sequence involved conversion of the precipitated hydrochloride salt **6a** to amide **7** followed by its hydrolysis to furnish crude EMA400 (Scheme 4, Step 1–2).

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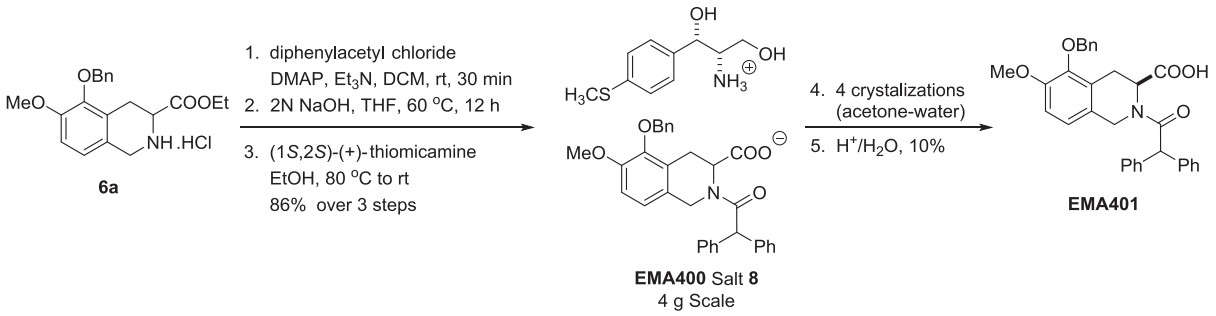


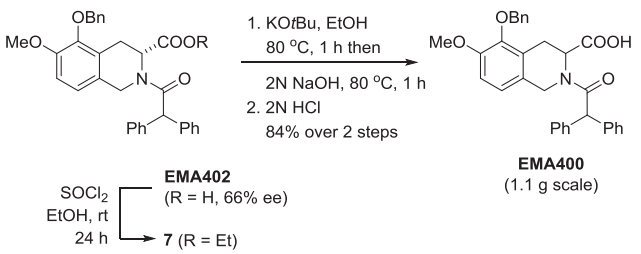
Scheme 4. Multi gram scale procedure for synthesis of racemic EMA400. Resolution of EMA401 using (1*S*,2*S*)-(+)-thiomicamine.

Precipitation of unresolved EMA400 was performed with (1*S*,2*S*)-(+)-thiomicamine in ethanol and ethyl acetate according to Entry 3 and 4, Table 1 (See Supplementary data) providing the racemic material in 4 g scale and in 86% isolated yield over three steps. Minor colored impurities formed in Step 1 and 2 were removed during the filtration of the formed salt.

By employing the resolution condition developed in acetone–water, we were able to obtain enantiopure EMA401 in 4 crystallizations with 10% yield starting from salt **8** (Step 4, Scheme 4). The pure enantiomer EMA401 (>98% *ee*) was separated from (1*S*,2*S*)-(+)-thiomicamine by washing it with aqueous 2N HCl.

Traditional diastereomeric resolution strategies for the separation of enantiomers are extensively used in the pharmaceutical industry today. It is quite often less expensive compared to asymmetric synthesis methods due to the cost of catalyst, ligands etc. The major demerit of classical resolution strategies is the maximum yield of 50%, adding extra cost to the overall synthesis and increasing the chemical waste. Thus, it is highly desirable to develop procedure in which unwanted chiral waste is isolated, racemized and recycled.

During our studies of resolution strategies 1B and 2A (Scheme 3), we observed racemization during hydrolysis of the separated carbamates and esters, indicating the value of the filtrate containing enriched EMA402 (66% *ee*) from Step 4 in Scheme 4. Next, EMA402 was esterified by employing $SOCl_2$ in dry ethanol (Scheme 5). When attempting to racemize ethyl ester **7** using aqueous 2N NaOH, we observed incomplete racemization. Hence compound **7** was treated with KO*t*Bu in ethanol at 80 °C and was thereafter hydrolyzed in the same reaction flask with 2N NaOH. Removal of ethanol and acidification precipitated fully racemized EMA400 in high purity. This combined approach on gram scale furnished racemic EMA400 in 84% overall yield from EMA402.



Scheme 5. Racemization of less active EMA402 to EMA400.

3. Conclusion

In summary, we have achieved the gram-scale preparation of enantiopure EMA401 in 8 steps starting from readily available 2-hydroxy-3-methoxybenzaldehyde and by resolving the racemic EMA400 to EMA401 with (1*S*,2*S*)-(+)-thiomicamine. The efficiency of the synthesis was improved by racemization of EMA402 to EMA400. We hope that this scalable synthetic procedure, with full purification, analysis and characterization data for EMA401 will be helpful to academic and industrial researchers in need of this important AT_2R antagonist.

4. Experimental section

4.1. General information

All purchased chemicals were used as received without further purification. Analytical thin layer chromatography was performed on silica gel 60 F-254 plates and visualized with UV light. Purifications were performed with flash column chromatography by silica gel (60 Å pore size, 230–400 mesh). Analytical HPLC-MS was performed with UV detection (214, 254 and 280 nm) and ion trap MS (ESI− or ESI+), using a C18 column (50×3.0 mm, 2.6 μm particle size, 100 Å pore size) and a flow rate of 1.5 mL/min. A gradient of CH_3CN in 0.05% aqueous HCOOH solution was used. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using DMSO-d_6, acetone-d_6 or chloroform-*d* as a solvent. Chemical shifts for ^1H and ^{13}C are referenced via the residual solvent signal. The HRMS experiment was performed on a 7-T hybrid ion trap (LTQ) FT mass spectrometer modified with a nanospray ion source.

4.2. 2-(Benzyloxy)-3-methoxybenzaldehyde 1

To a stirred solution of 2-hydroxy-3-methoxybenzaldehyde (15.2 g, 100.0 mmol) in anhydrous EtOH (150 mL) was added dry K_2CO_3 (34 g, 250.0 mmol) and benzyl bromide (11.9 mL, 100.0 mmol) at room temperature. The reaction mixture was heated at 100 °C under nitrogen atmosphere. After 2 h of heating the reaction mixture was cooled to room temperature, insoluble salts were removed by filtration and was washed by EtOH (50 mL). The combined filtrate was evaporated to dryness. The obtained crude oil was dissolved in diethyl ether (200 mL), washed by 2N aq NaOH (3×40 mL), brine (3×40 mL) and dried over $MgSO_4$. The organic layer was filtered and evaporated to dryness to provide low melting colorless solid (**1**, 22.2 g, 92% yield). Mp 42–44 °C. The obtained product was used in next step without any purification. ^1H NMR (400 MHz, $CDCl_3$) δ 10.24 (d, J=1 Hz, 1H), 7.53–7.29 (m, 6H), 7.23–7.04 (m, 2H), 5.18 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.4, 153.2, 151.2, 136.5, 130.5, 128.8, 128.7, 128.7, 124.4, 119.2, 118.1, 76.5, 56.2.

4.3. 5-(2-(Benzyloxy)-3-methoxybenzylidene)imidazolidine-2,4-dione 2

2-(Benzyloxy)-3-methoxybenzaldehyde (**1**, 5.0 g, 20.0 mmol), hydantoin (2.34 g, 23.0 mmol) and β-alanine (0.38 g, 4.0 mmol) were refluxed in AcOH (50 mL) for 6 h. The reaction mixture was

then cooled to room temperature and poured onto water (200 mL) under stirring. The precipitated product was collected by filtration and washed with water and MeOH. The obtained product was dried under vacuum to provide colorless solid (**2**, 3.80 g, 60%). Mp 190–192 °C. IR (Neat) 1753, 1707, 1650 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 10.35 (s, 1H), 7.44–7.28 (m, 5H), 7.23 (dd, J=7.4, 1.8 Hz, 1H), 7.14–7.05 (m, 2H), 6.62 (s, 1H), 4.95 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.4, 155.6, 152.6, 145.7, 137.1, 128.7, 128.3, 128.3, 128.1, 127.3, 124.5, 120.8, 113.3, 102.8, 74.6, 55.9; HRMS (EI) m/z calcd. For $C_{18}H_{17}N_2O_4$ $[M+H]^+$: 325.1188, found: 325.1193.

4.4. 4-[2-(Benzyloxy)-3-methoxybenzyl]-1,3-dihydro-2*H*-imidazol-2-one 4

To the stirred suspension of compound **2** (3.00 g, 9.0 mmol) and Zn dust (2.40 g, 37.0 mmol) in MeOH (60 mL), conc. HCl (3 mL) was added dropwise under stirring. The reaction mixture was heated at 70 °C for 30 min. During this time the starting material started dissolving slowly. A second portion of conc. HCl (3 mL) was added slowly, dissolving all starting material. After stirring for 30 min the reaction mixture was then cooled to room temperature and the excess unreacted Zn dust was removed by filtration. The filtrate was then poured to water (200 mL) under stirring. The precipitated product was separated by filtration and washed with water. The obtained product was recrystallized from MeOH to provide colorless solid (**4**, 1.74 g, 61%). Mp 155–157 °C. IR (Neat) 1685, 1600 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.71 (br s, 1H), 9.43 (br s, 1H), 7.49–7.42 (m, 2H), 7.42–7.31 (m, 3H), 7.05–6.93 (m, 2H), 6.75 (dd, J=7.5, 1.7 Hz, 1H), 5.83–5.68 (m, 1H), 4.92 (s, 2H), 3.83 (s, 3H), 3.52 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.9, 152.4, 145.2, 137.8, 132.2, 128.3, 128.1, 127.9, 123.9, 121.4, 120.5, 111.3, 104.9, 73.8, 55.8, 25.4; HRMS (EI) m/z calcd. For $C_{18}H_{19}N_2O_3$ $[M+H]^+$: 311.1396, found: 311.1407.

4.5. Ethyl 3-(2-(benzyloxy)-3-methoxyphenyl)-2-nitropropanoate 5

Step I (intermediate **A**): A mixture of 2-(benzyloxy)-3-methoxybenzaldehyde (**1**, 5.00 g, 21.0 mmol), ethyl nitroacetate (3.29 g, 25.0 mmol) and $Et_2NH{\cdot}HCl$ (3.39 g, 31.0 mmol) in anhydrous toluene (100 mL) was heated under nitrogen atmosphere at 130 °C. The formed water was removed using a Dean–Stark assembly. Heating was stopped after 12 h and reaction mixture was cooled to room temperature. The toluene was evaporated to dryness and the obtained residue was dissolved in DCM (50 mL). The organic layer was washed with water (3×50 mL), brine and dried over $MgSO_4$, filtered and evaporated to dryness. The yellow oily compound obtained (8.10 g) was directly subjected for next synthetic step without any purification.

Step II: The above obtained intermediate **A** was dissolved in $CHCl_3$ (150 mL) and IPA (50 mL). The reaction mixture was cooled to 0 °C and then silica gel (50 g, 230–400 mesh) was added under stirring. Subsequently $NaBH_4$ (3.8 g, 103.0 mmol) was added over 5–7 min. The reaction mixture was stirred at room temperature for 2 h and then quenched by adding 5 mL AcOH. The reaction mixture was filtered to remove silica gel, the silica gel was washed with $CHCl_3$ (100 mL) and the combined filtrate evaporated to dryness. The crude residue obtained was dissolved in DCM (100 mL) and washed by water (3×100 mL), brine and dried over $MgSO_4$. The organic layer was filtered, evaporated to dryness and further purified by flash column chromatography with 10% EtOAc in petroleum ether to provide thick oil (**5**, 4.95 g, 67% over two steps). IR (Neat) 1748, 1559, 1269 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 7.51–7.46 (m, 2H), 7.41–7.31 (m, 3H), 7.06–6.98 (m, 2H), 6.78–6.75 (m, 1H), 5.59 (dd, J=9.4, 6.0 Hz, 1H), 5.16 (d, J=11.1 Hz, 1H), 5.08 (d, J=11.1 Hz, 1H), 4.22 (q, J=7.1 Hz, 2H), 3.91 (s, 3H), 3.47–3.33 (m, 2H), 1.20 (t, J=7.1 Hz, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 165.2, 153.7, 147.0, 138.8, 129.2, 129.2, 129.1, 128.9, 125.0, 123.2, 113.4, 88.8, 75.1, 63.5, 56.2, 32.4, 14.1; HRMS (EI) m/z calcd. For $C_{19}H_{22}NO_6$ $[M+H]^+$: 360.1447, found: 360.1444.

4.6. Ethyl 5-(benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate hydrochloride 6a

Step I (intermediate **B**): To a stirred solution of compound **5** (4.5 g, 13.0 mmol) in AcOH (45 mL) at room temperature was added zinc dust (6.55 g, 100.0 mmol). The exotherm was maintained below 60–65 °C by controlled addition of Zn dust. After the completion of addition, the reaction was continued further at 60 °C. LC-MS and TLC after 2 h of stirring confirmed the completion of reaction. The reaction mixture was then cooled to room temperature and filtered. The insoluble inorganic solid was washed by AcOH (20 mL). The combined colorless filtrate was evaporated to dryness. The crude product obtained was dissolved in DCM (100 mL), washed with water (3×50 mL), satd aq $NaHCO_3$ (3×50 mL) and brine, dried over $MgSO_4$, filtered and evaporated to dryness to provide aminoester intermediate **B** (3.90 g) as colorless oil, which was used in next step without any purification.

Step II: The solution of above obtained aminoester intermediate **B** in 2N HCl (aq) (39 mL) was purged with nitrogen gas for 30 min and then 37% aq formaldehyde solution (4 mL, 39.9 mmol, 3 equiv) was added under stirring. The reaction was continued further for 24 h at room temperature under nitrogen atmosphere. The precipitated hydrochloride salt of product was filtered, washed with water (40 mL) and suction dried. The obtained moist product was dried under vacuum to furnish pure compound **6a** (3.6 g, 76% over two steps). Mp 165–167 °C. IR (Neat) 1748, 1452 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 2H), 7.55–7.29 (m, 5H), 7.07 (d, J=8.6 Hz, 1H), 7.01 (d, J=8.6 Hz, 1H), 4.98 (d, J=1.2 Hz, 2H), 4.38 (dd, J=11.0, 5.2 Hz, 1H), 4.32–4.15 (m, 4H), 3.85 (s, 3H), 3.21 (dd, J=17.3, 5.2 Hz, 1H), 2.90 (dd, J=17.3, 11.0 Hz, 1H), 1.24 (t, J=7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.2, 151.2, 144.4, 137.5, 128.4, 128.0, 124.8, 122.2, 121.0, 112.1, 73.7, 62.0, 56.0, 52.8, 43.2, 23.6, 13.9; HRMS (ESI) m/z calcd. For $C_{20}H_{24}NO_4$ $[M+H]^+$: 342.1705, found: 342.1705.

4.7. Ethyl 5-(benzyloxy)-2-(2,2-diphenylacetyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate 7

To a stirred solution of compound **6a** (1.0 g, 2.64 mmol) in DCM (20 mL) was added diphenylacetyl chloride (0.85 g, 3.70 mmol) under nitrogen atmosphere. After adding catalytic DMAP (16 mg, 0.132 mmol), Et_3N (1.1 mL, 7.92 mmol) was added drop wise at room temperature and reaction was continued. After 30 min when TLC and LC-MS analysis showed complete consumption of the starting material, the reaction mixture was diluted with DCM (20 mL). The organic layer was washed by 2N HCl (3×30 mL), satd aq $NaHCO_3$ (3×30 mL), brine and dried over $MgSO_4$. The organic layer was filtered, evaporated to dryness and further purified by flash column chromatography with 15% EtOAc in petroleum ether to provide a colorless solid (**7**, 1.20 g, 85% yield). Mp 96–98 °C. IR (Neat) 1732, 1639, 1294 cm^{-1}; NMR shows presence of amide rotamers. ^1H NMR (400 MHz, acetone-d_6) δ 7.53–7.43 (m, 2H), 7.43–7.16 (m, 13H), 6.94 (d, J=2.4 Hz, 0.7H), 6.88 (d, J=8.4 Hz, 0.7H), 6.72 (dt, J=8.4, 0.8 Hz, 0.7H), 5.66 (s, 0.7H), 5.57 (s, 0.3H), 5.26 (dd, J=6.2, 4.0 Hz, 0.7H), 5.23 (dd, J=5.9, 2.6 Hz, 0.3H), 5.05 (d, J=11.0 Hz, 0.7H), 5.01 (d, J=11.0 Hz, 0.3H), 4.97–4.83 (m, 2H), 4.55 (d, J=15.1 Hz, 0.7H), 4.41 (d, J=17.1 Hz, 0.3H), 4.09–3.88 (m, 2H), 3.86 (s, 1H), 3.85 (s, 2H), 3.48 (dd, J=16.4, 2.5 Hz, 0.3H), 3.43 (dd, J=16.1, 4.0 Hz, 0.7H), 2.87 (dd, J=16.2, 6.2 Hz, 0.7H), 2.56 (dd, J=16.4, 5.9 Hz, 0.3H), 1.08 (t, J=7.1 Hz, 2H), 1.01 (t, J=7.1 Hz, 1H); ^{13}C NMR

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(101 MHz, acetone-d_6) δ 172.0, 171.6, 171.1, 152.4, 152.0, 146.0, 145.8, 141.2, 141.0, 140.8, 140.7, 139.0, 138.9, 130.3, 130.1, 129.9, 129.3, 129.2, 129.1, 129.1, 129.1, 129.0, 129.0, 128.8, 128.7, 128.7, 127.9, 127.7, 127.5, 127.5, 127.4, 127.0, 127.0, 126.7, 122.8, 122.3, 112.5, 112.1, 75.2, 75.0, 61.8, 61.4, 56.2, 56.2, 55.4, 55.3, 55.2, 52.9, 46.0, 43.7, 26.9, 26.0, 14.4, 14.3; HRMS (ESI) m/z calcd. For $C_{34}H_{34}NO_5$ $[M+H]^+$: 536.2437, found: 536.2432.

4.8. 5-(Benzyloxy)-2-(2,2-diphenylacetyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid EMA400

To a stirred solution of compound **7** (1.1 g, 2.05 mmol) in THF (11 mL) was added 2 N NaOH (11 mL) and biphasic reaction mixture was stirred vigorously at 60 °C for 12 h. Reaction mixture was cooled to room temperature and THF was evaporated to dryness. To the obtained basic aqueous residue, 2N HCl (30 mL) was added slowly. The gummy precipitate obtained was extracted to EtOAc (3×20 mL). The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was filtered and evaporated to dryness. The crude product obtained was purified by flash column with 60% EtOAc in petroleum ether to provide a colorless solid (EMA400, 0.87 g, 84% yield).

4.9. Improved procedure for the synthesis of racemic EMA400 salt 8, Scheme 4

Step I: To a stirred solution of compound **6a** (2.5 g, 6.61 mmol) in DCM (50 mL) was added diphenylacetyl chloride (2.14 g, 9.28 mmol) under nitrogen atmosphere. After adding catalytic DMAP (40 mg, 0.33 mmol), Et_3N (2.8 mL, 19.8 mmol) was added drop wise at room temperature and reaction was continued further. After 30 min of stirring, the reaction mixture was diluted with DCM (50 mL). The organic layer was washed by 2N HCl (3×50 mL), satd aq $NaHCO_3$ (3×50 mL), brine and dried over $MgSO_4$. The organic layer was filtered, evaporated to dryness to provide the crude product **7** (3.50 g), which was used without further purification in the next step.

Step II: To a stirred THF (34 mL) solution of compound **7** obtained in step I was carefully added 2N NaOH (34 mL) and the resulting biphasic reaction mixture was stirred vigorously at 60 °C. After 12 h of stirring, the reaction mixture was cooled to room temperature and the THF was evaporated to dryness. To the obtained aqueous basic residue, 2N HCl (100 mL) was added slowly. The gummy precipitate obtained was extracted to EtOAc (3×30 mL). The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was filtered and evaporated to dryness to provide crude EMA400 (3.20 g), which was used in next step without further purification.

Step III: To the stirred solution of above obtained crude EMA400 in EtOH (40 mL) was added (1*S*,2*S*)-(+)-thiomicamine (1.4 g, 6.61 mmol). After heating the reaction mixture to reflux for 10–20 min, it was cooled to room temperature and stirred for another 3–4 h. The precipitated racemic salt was then filtered, suction dried and washed by EtOH (20 mL). The obtained colorless product was further evaporated to dryness to provide EMA400 salt **8** (4.12 g, 86% over 3 steps).

4.10. Resolution of EMA401

The above obtained salt (**8**, 4.0 g, 5.55 mmol) was dissolved in minimum amount of acetone (40 mL) at 75 °C under stirring. After complete dissolution of salt, water (50 mL) was added dropwise. The solution was then cooled gradually and when the temperature reached 25–30 °C, the crystallized product was filtered, suction dried and further evaporated to dryness to provide the product (2.6 g). Repetition of additional 3 crystallizations in the similar way provided 0.42 g EMA401 salt (10.5% yield).

The above obtained colorless salt (0.41 g, 0.57 mmol) was suspended in DCM (20 mL) and 2N HCl (20 mL) was slowly added. The suspension was next stirred for 10–20 min to dissolve the solid material. The organic layer was separated, washed by 2N HCl (2×20 mL), brine and dried over $MgSO_4$. The organic layer was filtered and evaporated to dryness. Thereafter the obtained pure EMA401 was dissolved in acetonitrile and subjected for freeze drying to provide a colorless solid (0.28 g, 98% yield, 98% *ee*). NMR shows presence of amide rotamers. $[\alpha]_D^{20}$ +24° (0.9% in MeOH). Mp 82–84 °C. IR (Neat) 3128–2837 (br s), 1696, 1620 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43–7.26 (m, 11H), 7.25–7.12 (m, 4H), 6.87 (d, J=8.5 Hz, 0.2H), 6.81 (d, J=8.5 Hz, 0.2H), 6.72 (d, J=8.4 Hz, 0.8H), 6.56 (d, J=8.4 Hz, 0.8H), 5.32 (s, 0.8H), 5.17–5.12 (m, 1H), 5.04 (d, J=10.9 Hz, 0.8H), 5.00–4.89 (m, 1.2H), 4.86–4.81 (m, 0.4H), 4.60 (d, J=14.9 Hz, 0.8H), 4.52 (d, J=17.2 Hz, 0.2H), 4.42 (d, J=14.9 Hz, 0.8H), 3.85 (s, 2.4H), 3.84 (s, 0.6H), 3.41 (dd, J=16.3, 2.6 Hz, 0.2H), 3.34 (dd, J=16.2, 5.0 Hz, 0.8H), 2.87 (dd, J=16.2, 6.3 Hz, 0.8H), 2.40 (dd, J=16.4, 6.0 Hz, 0.2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.8, 175.6, 172.6, 172.0, 151.8, 151.1, 145.0, 145.0, 139.2, 139.0, 138.8, 138.3, 137.7, 137.5, 129.5, 129.3, 129.2, 129.1, 128.9, 128.8, 128.71, 128.67 (2 Carbon), 128.6, 128.5, 128.4, 128.3, 128.1, 127.6, 127.3, 127.3, 127.2, 127.1, 125.7, 125.5, 125.3, 122.4, 121.5, 111.8, 111.2, 75.0, 74.9, 56.0 (2 Carbon), 55.8, 55.6, 54.4, 52.4, 45.6, 43.4, 25.9, 25.0; HRMS (ESI) m/z calcd. For $C_{32}H_{30}NO_5$ $[M+H]^+$: 508.2124, found: 508.2102.

4.11. Racemization of EMA402 to EMA400

The filtrates obtained from the first and second crystallizations in the resolution of EMA401 were evaporated to dryness (1.91 g). The obtained colorless salt was suspended in DCM (20 mL) and 2N HCl (20 mL) was added to the stirred suspension to dissolve the salt. The layers were separated and the organic layer was washed by 2N HCl (2×20 mL) and brine, dried over $MgSO_4$, filtered and evaporated to dryness to provide EMA402 (66% *ee*, 1.31 g, 2.58 mmol). EMA402 was dissolved in anhydrous EtOH (20 mL) and $SOCl_2$ (2 mL) was added under nitrogen atmosphere. After stirring the reaction mixture at room temperature for 24 h, it was evaporated to dryness and the obtained crude product was dissolved in EtOAc (20 mL). The organic layer was washed by satd aq $NaHCO_3$ (3×20 mL), brine and dried over $MgSO_4$. Next, the organic layer was filtered and evaporated to dryness. The obtained ester **7** (1.32 g) was dissolved in anhydrous EtOH (20 mL) and KO*t*Bu (0.29 g, 2.58 mmol) was added under nitrogen atmosphere and reaction mixture was stirred at 80 °C. After 1 h, 2N NaOH (10 mL) was carefully added and reaction was further stirred at the same temperature for 1 h. The reaction mixture was then concentrated under reduced pressure to remove the ethanol. The obtained aqueous residue was slowly acidified by 2N HCl (40 mL) to precipitate the EMA400 as white solid (1.1 g, 84% over 2 steps). Chiral purity analysis showed 1:1 enantiomeric ratio.

4.12. Analysis of the enantiomeric excess (*ee*) of EMA401

The diastereomeric salt (**8**, 2–5 mg) was suspended in DCM (2 mL) and washed by 2N HCl (2 mL) in a sample vial. The DCM layer was dried over $MgSO_4$ and filtered. A drop of (*S*)-methyl benzylamine and HBTU (20–30 mg) was added to the filtrate and the reaction vial was shaken. After 5–10 min the formed diastereomers were analyzed for chiral purity by HPLC (Note: The use of EDCI as dehydrating agent shown racemization of the separated enantiomer. Hence HBTU was used instead for the formation of diastereomer.)

4.13. HPLC analysis condition

Reverse phase HPLC (UV-triggered, 214 nm) was performed with C18 column (50×3 mm, 2.6 μm particle size) and a H_2O/

CH_3CN/0.05% HCOOH gradient polarity of 50–56% over 5 min and a flow rate of 1.5 mL/min.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2015.07.018.

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