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The first total synthesis of novel human chymase inhibitor SPF32629A

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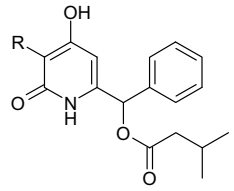
ABSTRACT

The first total synthesis of SPF32629A, a novel human chymase inhibitor, has been accomplished from the readily available precursor 4-nitropyridine N-oxide, following an efficient strategy with a sequence of eight straightforward steps.

Human chymase (EC 3.4.21.39) is a chymotrypsin-like serine protease localized in the secretory granules of mast cells. Several reports demonstrated the involvement of chymase in the progression of dermatitis,[1] chronic inflammation following cardiac[2,3] and pulmonary fibrosis.[4] Therefore, chymase inhibitors are expected to be potential therapeutic agents for the treatment of cardiovascular diseases, allergic inflammation, and fibrotic disorders.[5]

In 2006, Shimatani and coworkers[6a] reported the isolation of two new human chymase inhibitors, SPF-32629A and B, (Fig. 1) from the cultured broth of *Penicillium* sp., SPF-32629. The structural elucidation of these chymase inhibitors was accomplished on the basis of complementary evidence garnered from FAB-MS, HRFAB-MS, IR, and ^{1}H (COSY, HMBC) spectral data. Analysis for optical purity was carried out by four types of chiral HPLC columns; however the absolute configurations of these molecules remain to be established. The specific inhibition of human chymase among four serine proteases—chymase, chymotrypsin, cathepsinG, and Elastase by **1** and **2** constitutes an important lead toward developing new chymase inhibitors. But so far, there is no report of the total synthesis of these molecules. Considering the important and significant biological activities[6a,b] as well as their interesting structures, we initiated a program to develop a flexible strategy for the total synthesis of these molecules. In this preliminary communication, we report the first total synthesis of SPF32629A.

Our synthesis commenced from the commercially available starting material, 4-nitropyridine N-oxide (Scheme 1). Firstly, we wanted to introduce a protected hydroxyl group at the 4-position of the pyridine moiety, and it is well established that the nitro group at the 4-position of pyridine N-oxide can be easily displaced by nucleophilic reagents. Accordingly, displacement of the nitro group with benzyl alcohol in the presence of metallic sodium at 10 °C to rt afforded the corresponding benzyloxy derivative **2** in high yield (81%).[7] Then, the cyano group was introduced at the



1: R = H, SPF-32629A
2: R = COOH, SPF-32629B

Figure 1. Human chymase inhibitors SPF-32629 A and B.

2-position of **2** by reacting it with 1.18 equiv of trimethylsilyl cyanide following a modified Reissert–Henze protocol.[8] This transformation, pyridine N-oxide into the corresponding pyridinecarbonitrile **3,** was done in dichloromethane using 1.2 equiv of diethyl or dimethylcarbamyl chloride as the activating electrophile in 84% yield.

Grignard reaction of nitrile **3** with 1.5 equiv of phenylmagnesium bromide in ether at −30 °C to rt furnished ketone **4** in 76% yield. The pyridine ring in compound **4** was transformed into its corresponding 2-pyridone derivative **6** by employing N-oxidation, followed by reaction with acetic anhydride and hydrolysis protocol. Thus, mCPBA oxidation of compound **4** yielded its N-oxide derivative **5** in DCM at rt in 91% yield, which smoothly rearranged to the corresponding 2-pyridone derivative **6** in 45% yield under the influence of boiling acetic anhydride followed by hydrolysis with aqueous ethanol. Reduction of the keto group in **6** using sodium borohydride in methanolic THF furnished alcohol **7** in 84% yield. Further, coupling of alcohol **7** with isovaleric acid in the presence of EDC·HCl and DMAP in DCM at 10 °C to rt furnished the corresponding isovaleryl ester **9** in 86% yield along with bis-isovaleryl derivative **8** as a by-product in 5–10% yield. Interestingly, when compound **8** was subjected to basic hydrolysis using LiOH·H_2O, selective de-protection of isovaleric ester occurred leading to the formation of compound **9** in 88% yield. Finally, debenzylation

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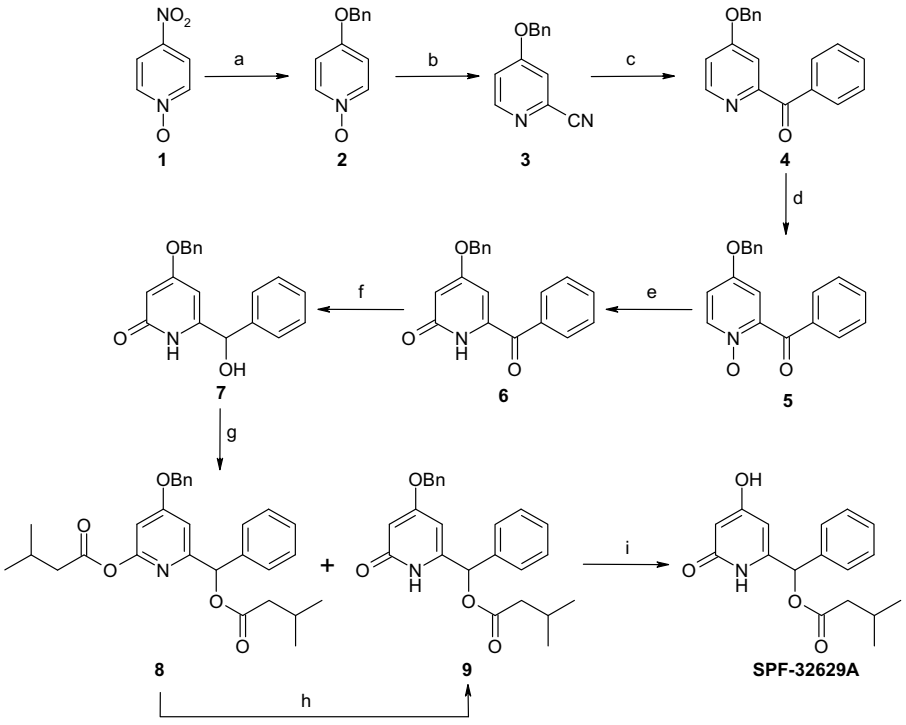


Scheme 1. Total synthesis of SPF-32629A (**1**). Reagents and conditions: (a) BnOH, Na, 10 °C to rt, 12 h, 81%; (b) TMSCN, *N*,*N*-diethylcarbamoyl chloride, DCM, 5 °C to rt, 12 h, 84%; (c) PhMgBr, THF, −30 °C to rt, 5 h, 77%; (d) *m*-CPBA, DCM, rt, 12 h, 91%; (e) $(Ac)_2O$, 145 °C, 4 h, then EtOH, H_2O, 100 °C, 4 h, 40%; (f) $NaBH_4$, THF/MeOH (8:2), rt, 6 h, 85%; (g) isovaleric acid, EDC·HCl, DMAP, DCM, 10 °C to rt, 6 h, 87%; (h) LiOH·H_2O, THF, H_2O, rt, 3 h, 88%; (i) Pd/C (10%), THF/MeOH (8:2), H_2, 50 psi, rt, 30 min (90%).

was accomplished under catalytic hydrogenation using Pd/C at 50 psi in a mixture of methanolic THF to obtain racemic SPF-32629A in 90% yield, which exhibited ^1H and ^{13}C NMR spectral data closely comparable to those reported in the literature.[6a,9] Further confirmation of the structure of synthetic SPF-32629A was accomplished on the basis of ^{13}C DEPT, ^1H–^1H COSY, and gHMBC (Gradient heteronuclear multiple bond coherence) spectral data. The key features obtained from COSY and HMBC are depicted in Figure 2.

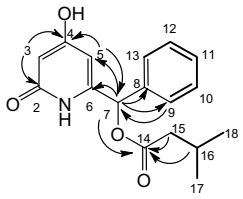
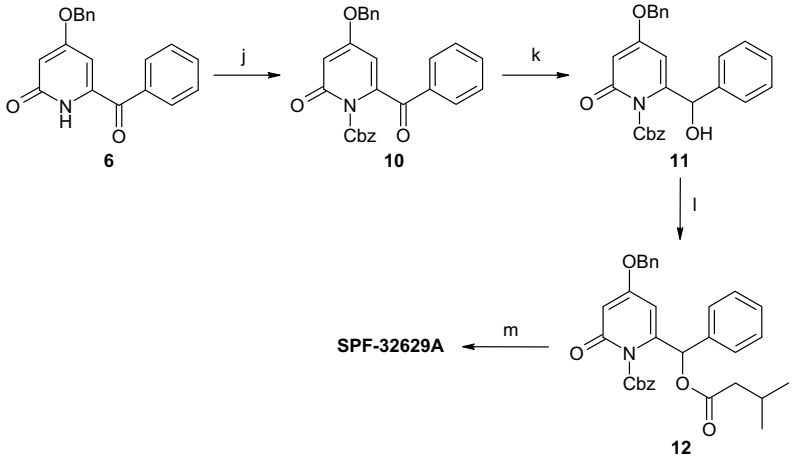


Figure 2. ^1H–^1H COSY (bold lines) and HMBC (arrows) correlations observed for SPF-32629A.

As compound **7** consists of amide and alcohol functionalities, a significant amount of bis-isovaleryl derivative **8** was also observed as a by-product during the coupling reaction of **7** with isovaleric acid, which was again converted to the desired compound **9** under basic conditions. To overcome this difficulty and to further confirm the position of isovaleryl ester at carbon-7, we protected the amide group in compound **6** with Cbz, and followed a similar sequence of reactions as in Scheme 1 to obtain SPF-32629A in satisfactory yields (Scheme 2).[9] Thus, reaction of compound **6** with CbzCl using TEA as base in DCM furnished Cbz protected compound **10** in 91% yield. Compound **10** upon reduction with $NaBH_4$ followed by ester-



Scheme 2. Reagents and conditions: (j) CbzCl, TEA, DCM, 0 °C to rt, 4 h, 91%; (k) $NaBH_4$, THF/MeOH (8:2), rt, 2 h, 92%; (l) isovaleric acid, EDC.HCl, DMAP (cat), DCM, 0 °C to rt, 6 h, 95%; (m) Pd/C (10%), EtOAc/MeOH (8:2), H_2, 50 psi, rt, 30 min, 89%.

ification using isovaleric acid afforded compound **12** in good yield. Finally, de-protection of both Cbz and benzyl groups was achieved in a single step using Pd/C to obtain racemic SPF-32629A in 89% yield, the ^{1}H and ^{13}C NMR spectral data are in agreement with the data obtained from Scheme 1.

In conclusion, we have accomplished the first total synthesis of natural human chymase inhibitor SPF-32629A starting from the readily available 4-nitropyridine N-oxide employing an efficient strategy with a sequence of eight straightforward reactions. Synthetic studies toward another chymase inhibitor SPF-32629B and asymmetric induction during the carbonyl reduction using chiral reducing agents are currently underway in our laboratory, and will be reported in due course.

Acknowledgments

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9. Yields refer to the isolated pure compounds and the products from all reactions were purified either by flash column chromatography using silica gel 60 (230–400 mesh Kieselgel 60) or by crystallization unless otherwise indicated. All new compounds were fully characterized on the basis of IR, ^{1}H NMR, ^{13}C NMR, and LCMS spectral data and are consistent with their structures. Spectral data for all the synthesized compounds are as follows: *Compound* **2**: Pale yellow solid; mp 179–181 °C (lit. 175–177 °C); IR (KBr pellet): ν_{max} 1622, 1225 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (2H, dd, *J* 5.6 and 2 Hz), 7.43–7.38 (5H, m), 6.87 (2H, dd, *J* 5.2 and 2 Hz), 5.11 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.4 (C), 139.6 (2CH), 134.5 (C), 128.4 (2CH), 128.2 (CH), 127.1 (2CH), 112.2 (2CH), 70.5 (CH_2); MS (APCI) *m*/*z* 202.02 (M+H)$^+$. *Compound* **3**: Colorless solid; mp 90–91 °C; IR (KBr pellet): ν_{max} 2237, 1590 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.5 (1H, d, *J* 6 Hz), 7.44–7.37 (5H, m), 7.27 (1H, d, *J* 2.4 Hz), 7.06 (1H, dd, *J* 5.8 and 2.2 Hz), 5.15 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8 (C), 152.1 (CH), 134.8 (C), 134.3 (C), 128.7 (2CH), 128.6 (CH), 127.4 (2CH), 117.0 (CH), 116.0 (CN), 113.3 (CH), 70.4 (CH_2); MS (ESI) *m*/*z* 211.27 (M+H)$^+$; LCMS (ES) *m*/*z* calcd for $C_{13}H_{11}N_2O$, [M+H]$^+$: 211.09, found: 211.27. *Compound* **4**: Colorless crystalline solid; mp 79–80 °C; IR (KBr pellet): ν_{max} 1661, 1582 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (1H, d, *J* 6 Hz), 8.07 (1H, s), 8.053 (1H, m), 7.64 (1H, d, *J* 2.8 Hz), 7.59–7.55 (1H, m), 7.49–7.36 (7H, m), 7.04 (1H, dd, *J* 6 and 2.8 Hz), 5.19 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.4 (C), 165.4 (C), 156.6 (C), 149.7 (CH), 136.1 (C), 135.1 (C), 132.7 (CH), 130.8 (2CH), 128.6 (2CH), 128.3 (CH), 127.9 (2CH), 127.4 (2CH), 113.1 (CH), 110.6 (CH), 69.9 (CH_2); MS (APCI) *m*/*z* 290.3 (M+H)$^+$; LCMS (ES) *m*/*z* calcd for $C_{19}H_{16}NO_2$, [M+H]$^+$: 290.12, found: 290.30. *Compound* **5**: Pale yellow solid; mp 138–140 °C; IR (KBr pellet): ν_{max} 1673, 1217 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (1H, dd, *J* 5.6 and 2 Hz), 7.85 (1H, s), 7.83 (1H, d, *J* 1.2 Hz), 7.59 (1H, t, *J* 7.6 Hz), 7.48–7.36 (7H, m), 6.99 (2H, dd, *J* 6.4 and 2.8 Hz), 5.11 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.6 (C), 156.5 (C), 147.3 (C), 140.5 (CH), 134.6 (C), 134.4 (C), 134.0 (CH), 129.2 (2CH), 128.66 (2CH), 128.6 (2CH) 128.5 (CH), 127.4 (2CH), 113.9 (CH), 111.0 (CH), 71.0 (CH_2); MS (ESI) *m*/*z* 306 (M+H)$^+$; LCMS (ES) *m*/*z* calcd for $C_{19}H_{16}NO_3$, [M+H]$^+$: 306.11, found: 306.28. *Compound* **6**: Cream colored solid; mp 158–160 °C; IR (KBr pellet): ν_{max} 1644, 1620, 1600 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.4 (1H, br s, D_2O exchangeable), 7.77 (2H, d, *J* 7.6 Hz), 7.64 (1H, t, *J* 7.4 Hz), 7.5 (2H, t, *J* 8 Hz), 7.42–7.36 (5H, m), 6.50 (1H, d, *J* 2 Hz), 6.18 (1H, d, *J* 2 Hz), 5.02 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.1 (C), 166.6 (C), 164.3 (C), 139.0 (C), 135 (C), 134.5 (CH), 133.1 (CH), 129.3 (2CH), 128.69 (2CH), 128.61 (2CH), 128.5 (C), 127.7 (2CH), 108.8 (CH), 102.9 (CH), 70.7 (CH_2); MS (APCI) *m*/*z* 306.05 (M+H)$^+$; LCMS (ESI) *m*/*z* calcd for $C_{19}H_{16}NO_3$, [M+H]$^+$: 306.11, found: 306.28. *Compound* **7**: Colorless solid; mp 350 °C (decomposed); IR (KBr pellet): ν_{max} 3429, 3268, 1644, 1628 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 7.44–7.31 (10H, m), 6.39 (1H, s), 6.07 (1H, s), 5.66 (1H, s), 5.17 (2H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9 (C), 166.3 (C), 154.1 (C), 142.1 (C), 136.5 (C), 129.77 (2CH), 129.73 (2CH), 129.53 (2CH), 129.51 (CH), 128.87 (2CH), 127.8 (CH), 101.8 (CH), 96.5 (CH), 73.0 (CH), 72.0 (CH_2); MS (ESI) *m*/*z* 308.08 (M+H)$^+$; LCMS (ES) *m*/*z* calcd for $C_{19}H_{18}NO_3$, [M+H]$^+$: 308.13, found: 308.31. *Compound* **8**: Colorless syrup; IR ($CHCl_3$ film): ν_{max} 1764, 1741, 1602 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (6H, m), 7.33–7.25 (4H, m), 6.92 (1H, d, *J* 2 Hz), 6.75 (1H, s), 6.56 (1H, d, *J* 1.6 Hz), 5.07 (2H, s), 2.44 (2H, d, *J* 7.2 Hz), 2.30 (2H, d, *J* 7.2 Hz), 2.22 (1H, m), 2.14 (1H, m), 1.04 (3H, s), 1.02 (3H, s), 0.95 (3H, s), 0.93 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6 (C), 170.8 (C), 167.8 (C), 159.5 (C), 158.8 (C), 138.6 (C), 135.1 (C), 128.76 (2CH), 128.54 (CH), 128.45 (2CH), 128.15 (CH), 127.7 (2CH), 127.3 (2CH), 106.9 (CH), 101.0 (CH), 76.9 (CH), 70.4 (CH_2), 43.4 (CH_2), 43.3 (CH_2), 25.7 (CH), 25.5 (CH), 22.4 ($2CH_3$), 22.3 ($2CH_3$); MS (ESI) *m*/*z* 476.3 (M+H)$^+$; LCMS (ES) *m*/*z* calcd for $C_{29}H_{34}NO_5$, [M+H]$^+$: 476.24, found: 476.49. *Compound* **9**: Colorless solid; mp 132–134 °C; IR (KBr pellet): ν_{max} 1744, 1646 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.22 (1H, br s, D_2O exchangeable CONH), 7.39–7.26 (10H, m), 6.59 (1H, s), 5.89 (1H, d, *J* 1.6 Hz), 5.85 (1H, d, *J* 2.4 Hz), 4.96 (2H, s), 2.32 (2H, d, *J* 7.2 Hz), 2.12 (1H, m), 0.94 (3H, s), 0.92 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5 (C), 168.2 (C), 166 (C), 146.4 (C), 136.4 (C), 135.1 (C), 128.9 (CH), 128.8 (2CH), 128.7 (2CH), 128.5 (CH), 127.8 (2CH), 127.2 (2CH), 99.4 (CH), 97 (CH), 72.9 (CH), 70.3 (CH_2), 43.1 (CH_2), 25.6 (CH), 22.36 (CH_3), 22.33 (CH_3); MS (ESI) *m*/*z* 392.2 (M+H)$^+$; LCMS (ES) *m*/*z* calcd for $C_{24}H_{26}NO_4$, [M+H]$^+$: 392.19, found: 392.42. *Compound* **10**: Colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (2H, d, *J* 1.6 Hz), 7.59 (2H, m), 7.56–7.33 (12H, m), 6.87 (1H, d, *J* 2.4 Hz), 5.29 (2H, s), 5.27 (2H, s); MS (ESI) *m*/*z* 440.1 (M+H)$^+$, 462.1 (M+Na)$^+$. *Compound* **11**: Colorless solid; mp 142–145 °C; IR (KBr pellet): ν_{max} 3435, 1758, 1649 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.65 (1H, br s, D_2O exchangeable), 7.42–7.32 (15H, m), 6.42 (1H, s), 5.91 (1H, d, *J* 2.4 Hz), 5.84 (1H, d, *J* 2.0 Hz), 5.17 (2H, psq, *J* 12.4 Hz), 4.94 (2H, s); MS (ESI) *m*/*z* 442.2 (M+H)$^+$, 464.2 (M+Na)$^+$. *Compound* **12**: Colorless liquid; IR ($CHCl_3$ film): ν_{max} 1753, 1643, 1602 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.28 (15H, m), 6.98 (1H, d, *J* 2.0 Hz), 6.61 (1H, s), 6.57 (1H, d, *J* 1.6 Hz), 5.16 (2H, d, *J* 1.6 Hz), 5.05 (2H, s), 2.44 (2H, d, *J* 7.2 Hz), 2.22 (1H, m), 1.04 (3H, s), 1.02 (3H, s); MS (ESI) *m*/*z* 526.3 (M+H)$^+$, 548.2 (M+Na)$^+$. SPF32629A: Pale yellow solid; mp 98–102 °C; IR (KBr pellet): ν_{max} 3435 (br), 2960, 1746, 1643, 1617, 1454 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.2 (2H, br s, D_2O exchangeable CONH and OH), 7.35–7.30 (5H, m), 6.62 (1H, s), 5.97 (1H, d, *J* 1.6 Hz), 5.87 (1H, d, *J* 2 Hz, D_2O exchangeable pyridone 3-CH), 2.31 (2H, d, *J* 7.2 Hz), 2.11 (1H, m), 0.92 (3H, d, *J* 0.8 Hz), 0.90 (3H, d, *J* 0.4 Hz); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.22 (1H, br s, D_2O exchangeable CONH), 10.52 (1H, br s, D_2O exchangeable OH), 7.44–7.33 (5H, m), 6.43 (1H, s), 5.75 (1H, br s), 5.41 (1H, br s, D_2O exchangeable pyridone 3-CH), 2.32 (2H, d, *J* 6.8 Hz), 2.04–1.99 (1H, m), 0.89 (3H, s), 0.88 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8 (C), 170.0 (C), 165.7 (C), 146.1 (C), 135.9 (C), 129.1 (CH), 128.9 (2CH), 127.18 (2CH), 101.8 (CH), 98.4 (CH), 72.7 (CH), 43.1 (CH_2), 25.6 (CH), 22.29 (CH_3), 22.27 (CH_3); MS (ESI) *m*/*z* 302.24 (M+H)$^+$; LCMS (ES) *m*/*z* calcd for $C_{17}H_{20}NO_4$, [M+H]$^+$: 302.14, found: 302.3; column used: Develosil ODS MG-3 (4.6 × 50 mm), 2.5 μ, mobile phase: A: 5 mM ammonium bicarbonate, B: acetonitrile, T/%B: 0/10, 4/50, 6/90, 10/90, 10.1/10; flow rate: 1 mL/min, diluent: acetonitrile; UV: 215 nm, rt = 3.60, purity = 97.08%; Chiral HPLC: Method-1: racemic mixture; column used: Chiral PAK-AD-H (4.6 × 250 mm), 5 μ, mobile phase: A: hexane/IPA (90:10) Isocratic, flow rate: 1 mL/min, diluent: mobile phase, run time: 25 min, UV: 286 nm, rt = 5.72 and 7.01. Chiral HPLC: Method-2: racemic mixture; column used: Chiral PAK-IA (4.6 × 250 mm), 5 μ, mobile phase: A: hexane/ethanol (70:30) isocratic, flow rate: 0.8 mL/min, diluent: mobile phase, run time: 25 min, UV: 286 nm, rt = 5.37 and 6.76.