5.1 The Asymmetric Synthesis of SPF-32629A (1) and SPF-32629B (2).

We demonstrated the first total synthesis of racemic 1 and 2 in Chapters -2, -3 and -4. In this chapter, we describe an efficient enantioselective synthesis of SPF-32629A and SPF-32629B through one-pot enantioselective reduction and protecting-group-free regioselective O-acylation strategy.

Enantioselective reduction of prochiral ketones to optically active secondary alcohols is one of the most powerful methodologies and occupies a position of prime importance in organic synthesis. The catalytic, asymmetric reduction of ketones may be accomplished through the use of catalytic amounts of an oxazaborolidine catalyst in conjunction with borane or catecholborane as the stoichiometric reducing agent. Chiral borane reductions were first reported by H. C. Brown; since then, a number of effective asymmetric reducing agents including oxazaborolidine chemistry, asymmetric reduction with chiral α-pinene-based reagents and different types of boron chiral reagents have been reported in the literature. Based on these findings, we screened various chiral reducing agents to achieve enantioselective synthesis of SPF-32629A and SPF-32629B through enantioselective reduction of prochiral ketone intermediate. Herein, we report our efforts culminating
in the accomplishment of highly efficient enantioselective synthesis of SPF-32629A and SPF-32629B.

The enantioselective synthesis of SPF-32629A and B was accomplished through a one-pot enantioselective reduction and regioselective acylation approach as depicted in synthetic schemes 8 and 9. The key starting materials 17 and 23 were prepared by following our previously described protocols as illustrated in Chapter-3,33 and -4.45

Scheme 8: Asymmetric Synthesis of SPF-32629A (1).

Reagents and conditions: a) for compound 1, NaBH₄, THF:MeOH (8:2), 0~10 °C, 2 h, 100%; b) for compound 1-(S)-(+)SPF32629A, (-)-DIP-Chloride, THF, -40 ~ -10 °C, 12 h, 80%; c) for compound 1-(R)(-)SPF-
32629A, (+)-DIP-Chloride, THF, -40 ~ -10 °C, 12 h, 80%; d) Isovaleric acid, EDC.HCl, DMAP (cat), THF:DCM (1:2), 0~10 °C to rt, 16 h, 79%.

Scheme 9: Asymmetric Synthesis of SPF-32629B (2).

Reagents and conditions: e) for compound 2, NaBH₄, THF: MeOH (8:2), 0~10 °C, 2h, 100%; f) for compound 2-(S)-(+), (-)-DIP-Chloride, THF, -40~10 °C, 12h, 80%; g) for compound 2-(R)-(-), (+)-DIP-Chloride, THF, -40~10 °C, 12h, 70%; h) Isovaleric acid, EDC.HCl, DMAP (cat), THF:DCM (1:2), 0 °C to rt, 12 h, 87%.

As reported previously, reduction of 17 (Scheme 8), under standard sodium borohydride mediated reduction conditions furnished the requisite crude alcohol 18 in quantitative yield, which upon extractive isolation was acylated without further purification. As expected the resulting crude alcohol 18 underwent coupling with isovaleric acid in
a regioselective manner to produce racemic SPF-32629A in good yield with high purity. Then, we undertook the preparation of the enantiomerically pure isomers via enantioselective reduction of prochiral keto compound 17.

Table 1. Enantioselective reduction of prochiral keto compounds 17 and 23.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents</th>
<th>Reaction conditions</th>
<th>Compound (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>R-(+)-CBS (0.1 eq), Catecholborane (1.2 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>-10 to rt, 12h, then 0 °C to rt, 48h.</td>
<td>No reaction</td>
</tr>
<tr>
<td>2.</td>
<td>R-(-)-CBS (0.1 eq), Catecholborane (1.2 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>-10 to rt, 12h, then 0 °C to rt, 48h.</td>
<td>No reaction</td>
</tr>
<tr>
<td>3.</td>
<td>R-(+)-CBS (0.1 eq), BH₃·THF (1.5 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>-10 to rt, 4h, then 0 °C to rt, 48h.</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>R-(-)-CBS (0.1 eq), BH₃·THF (1.5 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>-10 to rt, 4h, then 0 °C to rt, 48h.</td>
<td>1</td>
</tr>
<tr>
<td>5.</td>
<td>(-)-DIP chloride (1.5 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>0 °C to rt, 12h, then 0 °C to rt, 48h.</td>
<td>1(+) (78.32%)</td>
</tr>
<tr>
<td>6.</td>
<td>(-)-DIP chloride (1.5 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>-40 to -10 °C, 12h, then 0 °C to rt, 48h.</td>
<td>1(+) (96.05%)</td>
</tr>
<tr>
<td>7.</td>
<td>(+)-DIP chloride (1.5 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>0 °C to rt, 12h, then 0 °C to rt, 48h.</td>
<td>1(-) (84.79%)</td>
</tr>
<tr>
<td>8.</td>
<td>(+)-DIP chloride (1.5 eq), Ketone 17, then Isovaleric acid, EDC.HCl.</td>
<td>-40 to -10 °C, 12h, then 0 °C to rt, 48h.</td>
<td>1(-) (96.01%)</td>
</tr>
<tr>
<td>9.</td>
<td>(-)-DIP chloride (1.5 eq), Ketone 23, then Isovaleric acid, EDC.HCl.</td>
<td>-40 to -10 °C, 12h, then 0 °C to rt, 12h.</td>
<td>2(+) (98.28%)</td>
</tr>
<tr>
<td>10.</td>
<td>(+)-DIP chloride (1.5 eq), Ketone 23, then Isovaleric acid, EDC.HCl.</td>
<td>-40 to -10 °C, 12h, then 0 °C to rt, 12h.</td>
<td>2(-) (98.55%)</td>
</tr>
</tbody>
</table>
Table 1 illustrates the enantioselective reduction of prochiral keto compounds 17 and 23 with various chiral reducing agents including (±)-CBS in presence of Catecholborane, (±)-CBS in presence of BH₃-THF and (±)-β-Chlorodiiisopinocampheylborane [(±)-DIP chloride or (±)-Ipc₂BCl].

It is well documented in the literature that the enantioselective borane reduction of prochiral ketones catalyzed by oxazaborolidine reagents, (±)-CBS and (±)-DIP chloride⁴⁶ is an excellent tool for the synthesis of alcohols in high enantiomeric excess. However, initial experiments using (+) or (-)-CBS⁴⁷ in presence of catecholborane did not afford any reduced alcohol (entry 1-2). On the other hand, reduction of ketone 17 with (+) or (-)-CBS in presence of BH₃-THF followed by subsequent regio-selective acylation led to the formation of racemic 1 (entry 3-4). Gratifyingly, enantioselective reduction of 17 was successfully accomplished using (+) and (-)-DIP-Chloride. Thus, reaction of ketone 17 with 1.5 equivalents of (+) or (-)-DIP-Chloride in THF at 0 °C to room temperature over 12 h followed by subsequent reaction with isovaleric acid afforded desired compounds 1-(S)·(+) and 1-(R)·(-) in 78.32% and 84.79% ee., respectively (entry 5 and 7). High enantiomeric purity (96% ee) of 1-(S)·(+) and 1-(R)·(-) was achieved by performing the reduction at low temperatures -40 to -10 °C followed by subsequent acylation at 0 °C to rt reaction conditions (entry 6 and 8).
Following similar sequence of reactions under aforementioned reaction conditions, the synthesis of enantiomerically pure enantiomers of SPF-32629B (>98% ee, Table 1, entry 9 and 10, respectively) was accomplished starting from compound 23 (Scheme 9). The optical purity of the synthesized pure isomers was determined by chiral HPLC on Chiral PAK AD-H column.

In summary, we have developed a novel and efficient approach for the synthesis of pure enantiomers of SPF-32629A and SPF-32629B by employing direct enantioselective reduction and regioselective acylation as the key step, wherein no protecting group was employed for the amide, acid and phenol functional groups.

5.2. Experimental Section.

5.2.1. Synthesis of 4-hydroxy-6-(hydroxy(phenyl) methyl) pyridin-2(1H)-one (18).

Described in 3.2.4 (Chapter-3)

Spectral data Described in 3.3.4 (Chapter-3)
5.2.2. Synthesis of (S)-(+) -4-Hydroxy-6-(hydroxy(phenyl) methyl) pyridin-2(1H)-one. (18-(S)-(+) ).

To a cooled suspension of compound 17 (1 g, 4.64 mmol) in tetrahydrofuran (10 mL) was portion wise added (-)-DIP-Cl (1.8 g, 5.61 mmol) at -40 °C. The resulting reaction mixture was stirred at -40 to -10 °C for 12 h, quenched with ice cold water and extracted with ethyl acetate. Combined organic layer was washed with brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain crude compound, which was purified by flash column chromatography over silica gel 60 (230–400 mesh) using 8% methanol: DCM as eluent to afford the title compound 18-(S)-(+) as pale yellowish solid (0.8 g, 80%).

$\text{Rf} = 0.25$ (Methanol:Dichloromethane, 10:90).

$\alpha^{20}$ = +57.48 (c 0.21, Methanol).

IR (KBr pellet): $v_{\text{max}}$ 3342, 3247, 1635, 1481, 1454 cm$^{-1}$.

$^1$H NMR (400 MHz, d$_6$-DMSO): $\delta$ 10.75 (1H, brs, D$_2$O exchangeable Pyridine-OH), 10.41 (1H, s, D$_2$O exchangeable CONH), 7.43-7.41 (2H, m), 7.35-7.32 (2H, m), 7.28-7.25 (H, m), 6.124 (1H, d, $J$ 4.4 Hz, D$_2$O exchangeable OH), 5.864 (1H, s), 5.41 (1H, d $J$ 3.6 Hz), 5.34 (1H, s).
**LCMS:** (ES) \( m/z \) calcd for \( \text{C}_{12}\text{H}_9\text{NO}_3 \) [M-H]: 216.21, found: 216.3; Column used: Acquity-BEH-C18 (50x2.1mm) 1.7 µm, Mobile Phase: A: 0.1% Aq.HCOOH, B: 0.1% HCOOH in Acetonitrile, T/%B: 0/3, 0.1/3, 1.5/90, 1.8/90, 2.2/95, 3.2/95, 3.8/03; Flow Rate: 0.4 mL/min, Diluent: Acetonitrile; UV: 265 nm, RT = 1.14, Purity = 98.92%.

**HPLC:** Column used: Symmetry-C18 (4.6×75 mm), 3.5 µ; Mobile Phase: A: 0.01% Aq.Ammonium acetate; B: Acetonitrile; T/%B: 0/10, 3/45, 4/45, 8/90, 12/90, 12.1/10; Flow Rate: 1.0 mL/min, Diluent: Acetonitrile; UV: 215 nm; RT = 1.95; Purity = 98.68%.

**Chiral HPLC:** Column used: Chiral PAK-IC (4.6x250 mm), 5 µ, Mobile Phase: A: n-hexane (0.1% DEA), B: Ethanol (0.1% DEA) Isocratic; A:B (70:30), Isocratic; Flow Rate: 1 mL/min, Diluent: Ethanol, Run time: 30 min, UV: 215 nm, RT = 8.59, Purity = 100%.

5.2.3. **Synthesis of \((R)-(\_)-4\text{-Hydroxy-6-(hydroxy (phenyl) methyl) pyridin-2(1H)-one} \) (18: \((R)-(\_))\).**

To a cooled suspension of compound 17 (1 g, 4.64 mmol) in tetrahydrofuran (10 mL) was portion wise added (+)-DIP-Cl (1.8 g, 5.61 mmol) at -40 °C. The resulting reaction mixture was stirred at -40 to -10
°C for 12 h, quenched with ice cold water and extracted with ethyl acetate. Combined organic layer was washed with brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain crude compound, which was purified by flash column chromatography over silica gel 60 (230–400 mesh) using 8% methanol: DCM as eluent to afford the title compound 18-(R)-(●) as yellowish solid (0.8 g, 80%).

\[ R_f = 0.25 \text{ (Methanol:Dichloromethane, 10:90).} \]

\[ [\alpha]_{20}^{\circ} = -49.78 \text{ (c 0.21, Methanol).} \]

**LCMS:** (ES) \( m/z \) calcd for \( C_{12}H_{11}NO_3 \ [M+H]^+ \): 218.21, found: 218.3; Column used:Acquity-BEH-C18 (50x2.1 mm) 1.7 \( \mu \)m, Mobile Phase: A: 0.1% Aq.HCOOH, B: 0.1% HCOOH in Acetonitrile, T/%B: 0/3, 0.1/3, 1.5/90, 1.8/90, 2.2/95, 3.2/95, 3.8/03; Flow Rate: 0.4 mL/min, Diluent: Acetonitrile; UV: Maxplot, RT = 1.14, Purity = 90.46%.

**HPLC:** Column used: Symmetry-C18 (4.6×75 mm), 3.5 \( \mu \); Mobile Phase: A: 0.01% Aq.Ammonium acetate; B: Acetonitrile; T/%B: 0/10, 3/45, 4/45, 8/90, 12/90, 12.1/10; Flow Rate: 1.0 mL/min, Diluent: Acetonitrile; UV: 215 nm; RT = 1.77; Purity = 97.62%.

**Chiral HPLC:** Column used: Chiral PAK-IC (4.6x250 mm), 5 \( \mu \), Mobile Phase: A: n-hexane (0.1%DEA) B: Ethanol (0.1%DEA) Isocratic; A: B (70:30), Isocratic; Flow Rate: 1 mL/min, Diluent: Ethanol, Run time: 30 min, UV: 215 nm, RT = 9.30, Purity = 97.70%.
5.2.4. Synthesis of (4-Hydroxy-6-oxo-1, 6-dihydropyridin-2-yl) (phenyl) methyl 3-methylbutanoate (±)-SPF-32629A (1).

Described in 2.2.10 (Chapter-2)

Spectral data Described in 2.3.9 (Chapter-2)

5.2.5. Synthesis of (S)-(+)-(4-hydroxy-6-oxo-1, 6-dihydropyridin-2-yl) (phenyl) methyl 3-methylbutanoate (1: (S)-(+)).

To a cooled solution of compound 18-(S)- (+) (1.0 g, 4.6 mmol) in a mixture of tetrahydrofuran:dichloromethane (1:2, 30 mL) was sequentially added isovaleric acid (0.5 g, 4.9 mmol), EDC.HCl (1.06 g, 5.55 mmol) and 4-(dimethylamino)pyridine (10 mg) at 10 °C and stirred at room temperature for 16 h. The reaction mixture was quenched with
ice cold water, separated organic layer and again extracted aqueous layer with dichloromethane. Combined organic layer was washed with brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain crude solid. Obtained crude solid was purified by flash column chromatography over silica gel 60 (230–400 mesh) using 6% methanol:dichloromethane as eluent to afford 1.1 g (79.13%) of 1-(S)-(+) as colorless solid.

**Rf** = 0.5 (Methanol:Dichloromethane, 10:90).

**mp** = 128-132 °C.

**IR** (KBr pellet): $\nu_{\text{max}}$ 3394 (br), 1748, 1645, 1612 cm$^{-1}$.

[$\alpha$]$^{23\degree C} = +24.834$ (c 0.5879, Chloroform).

**$^1$H NMR** (400 MHz, CDCl$_3$): $\delta$ 9.5 (brs, D$_2$O exchangeable CONH and OH), 7.37-7.31 (5H, m), 6.61 (1H, s), 5.96 (1H, s), 5.87 (1H, s, D$_2$O exchangeable pyridine 3-CH), 2.30 (2H, d $J$ 1 Hz, 7.2 Hz), 2.14-2.07 (1H, m), 0.92 (3H, d, $J$ 1 2 Hz), 0.90 (3H, d, $J$ 1 2Hz).

**$^1$H NMR** (400 MHz, d$_6$-DMSO): $\delta$ 11.23 (1H, brs, D$_2$O exchangeable OH), 10.55 (1H, s, D$_2$O exchangeable CONH), 7.44–7.33 (5H, m), 6.43 (1H, s), 5.77 (1H, s), 5.42 (1H, s, D$_2$O exchangeable pyridine 3-CH), 2.32 (2H, d, $J$ 7.2 Hz), 2.06-1.99 (1H, m), 0.88 (6H, d, $J$ 6.8 Hz).

**$^{13}$C NMR** (100 MHz, CDCl$_3$): $\delta$ 171.87 (C), 170.01 (C), 165.71 (C), 146.03 (C), 135.88 (C), 129.16 (CH), 128.93 (2CH), 127.19 (2CH), 101.83 (CH), 98.44 (CH), 72.78 (CH), 43.1 (CH$_2$), 25.64 (CH), 22.28 (2CH$_3$).
**LCMS:** (ES) m/z calcd for C_{17}H_{18}NO_{4}, [M-H]: 300.33, found: 300.2;
Column used: Develosil ODS MG-3 (4.6x33 mm), Mobile Phase: A: 0.1% Aq.HCOOH, B: 0.1% HCOOH (Acetonitrile:MeOH (50:50)), T/%B: 0/30, 4/90, 8/90, 8.1/30; Flow Rate: 1 mL/min, Diluent: Acetonitrile; UV: 284 nm, RT = 3.62, Purity = 99.59%.

**HPLC:** Column used: Eclipse-XDB-C18 (4.6×150mm), 5 µ; Mobile Phase: A: 0.1% Aq.HCOOH; B: methanol; T/%B: 0/30, 8/90, 15/90, 15.1/30; Flow Rate: 1.0 mL/min, Diluent: Acetonitrile; UV: 286 nm; RT = 8.73; Purity = 99.68%.

**Chiral HPLC:** Column used: Chiral PAK-AD-H (4.6x250 mm), 5 µ, Mobile Phase: A: B: n-hexanes: isopropanol (90:10), Isocratic; Flow rate: 0.7 mL/min, Diluent: Ethanol, Run time: 25 min, UV: 286 nm, RT = 10.27, Purity = 99.77%.

### 5.2.6 Synthesis of (R)- (-) (4-hydroxy-6-oxo-1, 6-dihydropyridin-2-yl) (phenyl) methyl 3-methylbutanoate (1: (R)-(−)).

To a cooled solution of compound 18- (R)- (-) (1.0 g, 4.6 mmol) in a mixture of tetrahydrofuran:dichloromethane (1:2, 30 mL) was
sequentially added isovaleric acid (0.5 g, 4.9 mmol), EDC.HCl (1.06 g, 5.55 mmol) and 4-(dimethylamino)pyridine (10 mg) at 10 °C and stirred at room temperature for 16 h. The reaction mixture was quenched with ice cold water, separated organic layer and again extracted aqueous layer with dichloromethane. Combined organic layer was washed with brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain crude solid. The crude solid was purified by flash column chromatography over silica gel 60 (230–400 mesh) using 6% methanol: dichloromethane as eluent to afford 1.0 g (71.94%) of 1-(R)- as colorless solid.

\[ R_f = 0.5 \text{ (Methanol:Dichloromethane, 10:90).} \]

\[ m_p = 128-133 \degree \text{C.} \]

\[ \text{IR (KBr pellet): } \nu_{\text{max}} 3401 (\text{br}), 1748, 1650, 1613 \text{ cm}^{-1}. \]

\[ [\alpha]_{23 \degree \text{C}} = -25.043 \text{ (c 0.5870, Chloroform).} \]

\[ ^1\text{H NMR (400 MHz, CDCl}_3\text{): } \delta 9.0 \text{ (brs, D}_2\text{O exchangeable CONH and OH), 7.38-7.33 (5H, m), 6.62 (1H, s), 5.96 (1H, d, J 1 2 Hz), 5.86 (1H, s, D}_2\text{O exchangeable pyridine 3-CH), 2.31 (2H, d J 1 Hz, 7.2 Hz), 2.14-2.07 (1H, m), 0.92 (3H, d, J 1 2 Hz), 0.91 (3H, d, J 1 2Hz).} \]

\[ ^1\text{H NMR (400 MHz, d}_6\text{-DMSO): } \delta 11.2 \text{ (2H, brs, D}_2\text{O exchangeable OH and CONH), 7.44–7.32 (5H, m), 6.43 (1H, s), 5.77 (1H, s), 5.41 (1H, s, D}_2\text{O exchangeable pyridine 3-CH), 2.31 (2H, d, J 7.2 Hz), 2.06-1.99 (1H, m), 0.88 (6H, d, J 6.8 Hz).} \]
$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 171.86 (C), 169.99 (C), 165.76 (C), 146.09 (C), 135.91 (C), 129.14 (CH), 128.92 (2CH), 127.19 (2CH), 101.8 (CH), 98.45 (CH), 72.79 (CH), 43.1 (CH$_2$), 25.64 (CH), 22.29 (CH$_3$), 22.28 (CH$_3$).

LCMS: (ES) m/z calcd. for C$_{17}$H$_{18}$NO$_4$, [M-H]: 300.33, found: 300.2;
Column used: Develosil ODS MG-3 (4.6x33 mm), Mobile Phase: A: 0.1% Aq.HCOOH, B: 0.1% HCOOH (Acetonitrile:MeOH (50:50)), T/%B: 0/30, 4/90, 8/90, 8.1/30;Flow Rate: 1 mL/min, Diluent: Acetonitrile; UV: 283 nm, RT = 3.63, Purity = 99.57%.

HPLC: Column used: Eclipse-XDB-C18 (4.6×150 mm), 5 µ; Mobile Phase: A: 0.1% Aq.HCOOH; B: methanol; T/%B: 0/30, 8/90, 15/90, 15.1/30; Flow Rate: 1.0 mL/min, Diluent: Acetonitrile; UV: 286 nm; RT = 8.74; Purity = 99.72%

Chiral HPLC: Column used: Chiral PAK-AD-H (4.6x250 mm), 5 µ, Mobile Phase: A: B: n- hexane:isopropanol (90:10) Isocratic, Flow Rate: 0.7 mL/min, Diluent: Ethanol, Run time: 25 min, UV: 286 nm, RT = 12.96, Purity = 99.02%.

5.2.7. Synthesis of 4-Hydroxy-6-(hydroxy (phenyl) methyl)-2-oxo-1, 2-dihydropyridine-3-carboxylic acid (24).
5.2.8. (S)-(+)-4-hydroxy-6-(hydroxyl (phenyl) methyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid (24: (S)-(+)).

To a cooled suspension of compound 23 (1 g, 3.85 mmol) in tetrahydrofuran (15 mL) was portion wise added (-)-DIP-Cl (1.48 g, 4.62 mmol) at -40 °C. The resulting reaction mixture was stirred at -40 to -10 °C for 12 h, quenched with ice cold water and extracted with ethyl acetate. Combined organic layer was washed with brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain crude compound 24-(S)-(+) , which was purified by flash column chromatography over silica gel 60 (230–400 mesh) using 8% methanol:DCM as eluent to afford the title compound 24-(S)-(+) as yellowish solid (0.8 g, 80%).

Rf = 0.25 (Methanol:Dichloromethane, 10:90).

[α]20°C = +61.57 (c 0.21, Methanol).

LCMS: (ES) m/z calcd for C19H10NO5, [M-H]+: 260.23 found: 260.3;
Column used: Acquity-BEH-C18 (50x2.1mm) 1.7 µm, Mobile Phase: A:
0.1% Aq.HCOOH, B: 0.1% HCOOH in Acetonitrile, T/%B: 0/3, 0.1/3, 1.5/90, 1.8/90, 2.2/95, 3.2/95, 3.8/3; Flow Rate: 0.4 mL/min, Diluent: Acetonitrile; UV: 302 nm, RT = 1.39, Purity = 99.88%.

**Chiral HPLC**: Column used: Chiral PAK-IA (4.6x250 mm), 5 μ, Mobile Phase: A: n-hexane, B: Ethanol, C: TFA, Isocratic (60:40: 0.1% TFA), Flow Rate: 1 mL/min, Diluent: Ethanol, Run time: 15 min, UV: 215 nm, RT = 5.79, Purity = 100%.

**5.2.9.** (R)-(-)-4-hydroxy-6-(hydroxy (phenyl) methyl)-2-oxo-1, 2-dihydropyridine-3-carboxylic acid (24: (R)-(-)).

To a cooled suspension of compound 23 (1 g, 3.85 mmol) in tetrahydrofuran (15 mL) was portion wise added (+)-DIP-Cl (1.48 g, 4.62 mmol) at -40 °C. The resulting reaction mixture was stirred at -40 to -10 °C for 12 h, quenched with ice cold water and extracted with ethyl acetate. Combined organic layer was washed with brine solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain crude compound 24-(R)-(-), which was purified by flash column chromatography over silica gel 60 (230–400 mesh) using
8% methanol: DCM as eluent to afford the title compound 24-\((R)\)-(\-) as yellowish solid (0.7 g, 70%).

\( \text{RF} = 0.25 \) (Methanol: Dichloromethane, 10:90).

\([\alpha]^{20}_D = -70.95 \) (c 0.21, Methanol).

\( \text{IR} \) (KBr pellet): \( \nu_{\text{max}} \) 3381, 3152, 1665, 1629, 1452 cm\(^{-1}\).

\( \text{\(^1\)H NMR} \) (400 MHz, \( d_6 \)-DMSO): \( \delta \) 15.5 (1H, brs, \( D_2 \)O exchangeable COOH), 13.35 (2H, brs, \( D_2 \)O exchangeable Pyridine-OH and CONH), 12.75 (1H, s, \( D_2 \)O exchangeable CONH), 7.48-7.46 (2H, m), 7.38-7.28 (3H, m), 6.48 (1H, d, \( J \) 3.6 Hz, \( D_2 \)O exchangeable OH), 6.42 (1H, s), 5.575 (1H, d, \( J \) 4 Hz).

\( \text{LCMS} \): (ES) \( m/z \) calcd for \( C_{19}H_{10}NO_5 \), [M-H]+: 260.23 found: 260.3;

Column used: Acquity-\( \text{BEH-C18} \) (50x2.1 mm) 1.7 µm, Mobile Phase: A: 0.1% Aq.HCOOH, B: 0.1% HCOOH in Acetonitrile, T/%B: 0/3, 0.1/3, 1.5/90,1.8/90, 2.2/95, 3.2/95, 3.8/3; Flow Rate: 0.4 mL/min, Diluent: Acetonitrile; UV: 302 nm, RT = 1.39, Purity = 99.42%.

\( \text{Chiral HPLC} \): Column used: Chiral PAK-IA (4.6x250 mm), 5 µ, Mobile Phase: A: n-hexane, B: Ethanol, C: TFA, Isocratic (60:40: 0.1% TFA), Flow Rate: 1 mL/min, Diluent: Ethanol, Run time: 15 min, UV: 215 nm, RT = 5.79, Purity = 98.35%.

\( \textbf{5.2.10}\).4-Hydroxy-6-((3-methylbutanoyloxy) (phenyl)methyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid, (±)-SPF-32629B (\textbf{2}).
5.2.11. (S)-(+) -4-hydroxy-6-((3-methylbutanoyloxy)(phenyl)methyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid. (2:(S)-(+) ).

To a cooled solution of compound 24-(S)-(+) (1 g, 3.82 mmol) in dichloromethane (12 mL) and tetrahydrofuran (8 mL) was sequentially added isovaleric acid (0.47 g, 4.59 mmol), EDC.HCl (1.17 g, 6.11 mmol) and 4-(dimethylamino)pyridine (10 mg) at 10 °C and stirred at room temperature for 12 h. The reaction mixture was quenched with ice cold water, separated organic layer and again extracted aqueous layer with dichloromethane. Combined organic layer was washed with brine.
solution, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to obtain crude solid. Obtained crude solid was purified by flash column chromatography over silica gel 60 (230-400 mesh) using 5% methanol:dichloromethane as eluent to afford 1.1 g (83.2%) of compound 2-(S)-(+) as colorless solid.

$R_f = 0.55$ (5% Methanol:Dichloromethane, 10:90).

$M_p = 106-108\, ^\circ C$.

$IR$ (KBr pellet): $\nu_{\text{max}} 3471, 1744, 1684, 1637, 1484\, \text{cm}^{-1}$.

$[\alpha]^{23}\, ^\circ C = +50.5\, (c\ 0.4,\ \text{Chloroform})$.

$[\alpha]^{24}\, ^\circ C = +24\, (c\ 0.4,\ \text{MeOH})$.

$^1\text{HNMR}$ (400 MHz CDCl$_3$): $\delta$ 14.19 (1H, brs, D$_2$O exchangeable COOH), 13.49 (1H, brs, D$_2$O exchangeable OH), 11.07 (1H, brs, D$_2$O exchangeable CONH), 7.40-7.37 (5H, m), 6.62 (1H, s), 6.29 (1H, s), 2.34 (2H, d, $J 6.8\, \text{Hz}$), 2.15-2.09 (1H, m), 0.94 (6H, d, $J 6.8\, \text{Hz}$).

$^{13}\text{CNMR}$ (100 MHz, CDCl$_3$): $\delta$ 174.95 (C), 171.80 (C), 171.35 (C), 165.99 (C), 151.02 (C), 134.65 (C), 129.83 (CH), 129.37 (2CH), 127.199 (2CH) 101.46 (CH), 96.86(C), 72.64 (CH), 42.93 (CH$_2$), 25.70 (CH), 22.26 (2CH$_3$).

$\text{MS}$ (ESI) $m/z\ 346.2(\text{M}+\text{H})^-$.

$\text{LCMS:}$ (ES) $m/z$ calcd for C$_{18}$H$_{20}$NO$_6$ [M+H]$^-$: 346.34, found: 346.2;

Column used: Acquity-BEH-C18 (50x2.1 mm) 1.7 $\mu$m, Mobile Phase: A: 0.1% Aq.HCOOH, B: 0.1% HCOOH in Acetonitrile, T/%B: 0/10, 1.5/100,
2.9/100, 3/10; Flow Rate: 0.5 mL/min, Diluent: Acetonitrile; UV: UV: Maxplot nm, RT = 1.54, Purity = 96.72%.

**HPLC:** Column used: Kromasil C18 (4.6×250 mm), 5.0 μ; Mobile Phase: A: 0.05% Aq.TFA; B: Methanol; T/%B: 0/30, 2/30, 8/90, 16/90, 18/30, 20/30; Flow Rate: 1.0 mL/min, Diluent: Acetonitrile; UV: 210 nm; RT = 9.67; Purity = 97.25%.

**Chiral HPLC:** Column used: Chiral PAK-AD-H (4.6x250 mm), 5 μ, Mobile Phase: A: 0.1% TFA in n-hexanes, B: Ethanol (70:30), Isocratic; Flow rate: 0.7 mL/min, Diluent: Ethanol, Run time: 25 min, UV: 304 nm, RT = 9.26, Purity = 100%.

5.2.12. **(R)-(-)-4-hydroxy-6-((3-methylbutanoyloxy)(phenyl)methyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid. (2: (R)-(-)).**

![Chemical Structure](image)

To a cooled solution of compound **24-(R)-(-)** (1 g, 3.82 mmol) in dichloromethane (12 mL) and tetrahydrofuran (8 mL) was sequentially added isovaleric acid (0.47 g, 4.59 mmol), EDC.HCl (1.17 g, 6.11 mmol) and 4-(dimethylamino)pyridine (10 mg) at 10 °C and stirred at room temperature for 12 h. The reaction mixture was quenched with ice cold
water, separated organic layer and again extracted aqueous layer with
dichloromethane. Combined organic layer was washed with brine
solution, dried over anhydrous sodium sulfate, filtered and evaporated
under reduced pressure to obtain crude solid. Obtained crude solid was
purified by flash column chromatography over silica gel 60 (230-400
mesh) using 5% methanol:dichloromethane as eluent to afford 1.05 g
(79.54%) of compound 2-(R)-(-) as colorless solid.

\[ R_f = 0.55 \] (5% Methanol:Dichloromethane, 10:90).

\[ mp = 107-110 \degree C. \]

**IR** (KBr pellet): \( \nu_{max} \) 3460, 1743, 1684, 1637, 1484 cm\(^{-1}\).

\[ [\alpha]^{23}_D = -47.4 \text{ (c 0.4, Chloroform).} \]

\[ [\alpha]^{24}_D = -22.6 \text{ (c 0.4, Methanol).} \]

**\(^1H\) NMR** (400 MHz CDCl\(_3\)): \( \delta \) 14.17 (1H, brs, D\(_2\)O exchangeable COOH),
13.48 (1H, brs, D\(_2\)O exchangeable OH), 11.49 (1H, brs, D\(_2\)O exchangeable CONH), 7.39 (5H, s), 6.63 (1H, s), 6.32 (1H, s), 2.34 (2H, d,
J 6.8 Hz), 2.13-2.08 (1H, m), 0.94 (6H, d, J 6.8 Hz).

**\(^{13}C\) NMR** (100MHz, CDCl\(_3\)): \( \delta \) 174.93 (C), 171.76 (C), 171.34 (C), 166.17
(C), 151.22 (C), 134.67 (C), 129.77 (CH), 129.32 (2CH), 127.18 (2CH)
101.43 (CH), 96.82 (C), 72.67 (CH), 42.90 (CH\(_2\)), 25.67 (CH), 22.24
(2CH\(_3\)).

**MS** (ESI) \( m/z \) 346.2(M+H).

**LCMS:** (ES) \( m/z \) calcd for C\(_{18}\)H\(_{20}\)NO\(_6\) [M+H]: 346.34, found: 346.2;
Column used:Acquity-BEH-C18 (50x2.1 mm) 1.7 \( \mu \)m, Mobile Phase: A:
0.1% Aq.HCOOH, B: 0.1% HCOOH in Acetonitrile, T/%B: 0/10, 1.5/100, 2.9/100, 3/10; Flow Rate: 0.5 mL/min, Diluent: Acetonitrile; UV: Maxplot, RT = 1.53, Purity = 99.13%.

**HPLC:** Column used: Kromasil C18 (4.6x250 mm), 5.0 µ; Mobile Phase: A: 0.05% Aq.TFA; B: Methanol; T/%B: 0/30, 2/30, 8/90, 16/90, 18/30, 20/30; Flow Rate: 1.0 mL/min, Diluent: Acetonitrile; UV: 214 nm; RT = 9.62; Purity = 96.25%.

**Chiral HPLC:** Column used: Chiral PAK-AD-H (4.6x250 mm), 5 µ, Mobile Phase: A: 0.1%TFA in n-hexanes,B:Ethanol (70:30), Isocratic; Flow rate: 0.7 mL/min, Diluent: Ethanol, Run time: 35 min, UV: 304 nm, RT = 7.65, Purity = 99.85%.

**5.3 Analytical Spectral data.**
5.3.1. Spectral data of (S)-(+-)4-hydroxy-6-(hydroxy (phenyl) methyl) pyridin-2(1H)-one (18: (S)-(+)).

FT-IR

\[ \text{FT-IR} \]

\[ \text{^1H NMR in} \text{ } d_6\text{-DMSO} \]

\[ \text{^1H NMR in} \text{ } d_6\text{-DMSO} \]
\[ ^1H \text{ NMR in } d_6\text{-DMSO} \]

![H NMR spectrum of compound 18](image1)

\[ ^1H \text{ NMR } D_2O\text{-Exchange} \]

![H NMR spectrum of compound 18](image2)
Column: Acquity BEH C18 (50x2.1 mm) 1.7 μm
Mobile Phase: A: 0.1% HOAc (aq)
B: 0.1% HOAc:ACN
Flow rate: 0.4 ml/min
Diluent: ACN
HPLC

Chromatographic Conditions

Column: Symmetry C18 (4.6x75mm) 3.5 μ
Mobile Phase A: 0.01M Ammonium acetate (AC)
Mobile Phase B: ACN
T%: B: 0/10, 1/10, 3/45, 4/45, 5/90, 9/90, 12/90, 12/1
Flow Rate: 1.0 mL/min
Temp: 25 °C
Diluent: ACN

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.38</td>
<td>2191735</td>
<td>98.68</td>
</tr>
<tr>
<td>2.38</td>
<td>11501</td>
<td>0.52</td>
</tr>
<tr>
<td>4.21</td>
<td>14905</td>
<td>0.67</td>
</tr>
<tr>
<td>4.69</td>
<td>2883</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Chiral HPLC

Chromatographic Conditions

Column: CHIRALPAK IC (4.6X250mm)
Mobile Phase: A: Hexane (0.1% DEA)
Mobile phase: B: Ethanol (0.1% DEA)
Isocratic: A:B (70:30)
Flow Rate: 1.0mL/min
Temp: Ambient
Diluent: Ethanol

Purity Chromatogram

<table>
<thead>
<tr>
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<th>Area</th>
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</tr>
</thead>
<tbody>
<tr>
<td>8.59</td>
<td>20929787</td>
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</tr>
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</table>
5.3.2 Spectral data of (R)-(-)-4-hydroxy-6-(hydroxy (phenyl) methyl) pyridin-2(1H)-one (18: (R)-(-)).

LC-MS

Spectral data for compound 18, showing characteristic peaks at retention times 1.14, 1.38, 1.40, and 2.19. The compound contains a pyridine ring with hydroxy substituents and a (phenyl) methyl group.
HPLC

Chromatographic Conditions

Column: Symmetry C18 (4.6X75mm) 3.5 μ
Mobile Phase A: 0.01M Ammonium acetate (AC)
Mobile Phase B: ACN
T:%A 0:10, 1:10, 3:45, 4:45, 8:90, 12:90, 12:10
Flow Rate: 1.0 mL/min
Temp: 25 °C
Diluent: ACN

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.77</td>
<td>9155229</td>
<td>97.62</td>
</tr>
<tr>
<td>2.19</td>
<td>21439</td>
<td>0.94</td>
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<tr>
<td>2.82</td>
<td>4694</td>
<td>0.07</td>
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<td>3.57</td>
<td>4400</td>
<td>0.07</td>
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<tr>
<td>3.75</td>
<td>15536</td>
<td>0.25</td>
</tr>
<tr>
<td>3.96</td>
<td>15433</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Chromatographic Conditions

Column: CHIRALPAK IC (4.6x250mm)
Mobile Phase: A: Hexane (0.1% DEA)
Mobile Phase: B: Ethanol (0.1% DEA)
Isocratic: A:B (70:30)
Flow Rate: 1.0 ml/min
Temp: Ambient
Diluent: Ethanol

Purity Chromatogram

<table>
<thead>
<tr>
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<tr>
<td>3</td>
<td>10.34</td>
<td>160058</td>
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</table>
5.3.3. (S)-(+)-(4-Hydroxy-6-oxo-1, 6-dihydropyridin-2-yl) (Phenyl) methyl 3-methylbutanoate (1: (S)-(+)).

FT-IR

$^1$H NMR in CDCl$_3$
$^{1}H$ NMR D$_{2}$O-Exchange

$^{13}$C NMR in CDCl$_{3}$
Analysis Result

I. Specific Optical Rotation $[\alpha]_{23^\circ C}^2$: +24.834
   Solvent : Chloroform
   Concentration : 0.5879%

II. Specific Optical Rotation $[\alpha]_{23^\circ C}^2$: +0.681
   Solvent : Methanol
   Concentration : 0.5873%

III. Melting Range : 128-132 °C
Column: DEVELOSIL ODS MG-3 (4.6 x 250 mm)
MP: A: 0.1% HCOOH(Aq)
B: 0.1% HCOOH/ACN-MeOH(50:50)
Flow rate 1.0 M/min
Diluent ACN

1: (S)-(+)

<table>
<thead>
<tr>
<th>Time</th>
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<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
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<td>177</td>
<td>16.82</td>
<td>0.11</td>
</tr>
<tr>
<td>3.62</td>
<td>137248</td>
<td>15141.82</td>
<td>99.59</td>
</tr>
<tr>
<td>4.05</td>
<td>143</td>
<td>14.97</td>
<td>0.10</td>
</tr>
<tr>
<td>5.67</td>
<td>287</td>
<td>30.75</td>
<td>0.20</td>
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</tbody>
</table>

Range: 1.378e-1
LC-MS

1: (S)-(+)

$\text{(m/z)}$ chart with peaks at 170.2, 216.2, 258.2, 302.2, 369.1, 414.4, 500.0, 601.3, 600.8, and 602.2.
**HPLC**

**Chromatographic Conditions**

- **Column:** Eclipse-XDB-C18 (4.6×150mm)5.0 μ
- **Mobile Phase A:** 0.1 % Formic Acid (Aq)
- **Mobile Phase B:** Methanol
- **Flow Rate:** 1.0 mL/min
- **Temp:** 25 °C
- **UV:** 286 nm
- **Diluent:** ACN

**Purity Chromatogram**

<table>
<thead>
<tr>
<th>RT</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>2419</td>
</tr>
<tr>
<td>2</td>
<td>6.74</td>
<td>2899304</td>
</tr>
<tr>
<td>3</td>
<td>11.28</td>
<td>5144</td>
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</table>
Chiral HPLC

Chromatographic Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
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</thead>
<tbody>
<tr>
<td>Column</td>
<td>CHIRAL PAK AD-H 4.6x250mm, 5µm</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Acetonitrile/Hexane 1:10</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.7 mL/min</td>
</tr>
<tr>
<td>Column Temp</td>
<td>-</td>
</tr>
<tr>
<td>Diluent</td>
<td>ETOH</td>
</tr>
</tbody>
</table>

Purity Chromatogram

Peak Results

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT</th>
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<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
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<td>354704</td>
<td>4446024</td>
<td>99.77</td>
</tr>
<tr>
<td>2</td>
<td>12.949</td>
<td>642</td>
<td>16400</td>
<td>0.23</td>
</tr>
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5.3.4. (R) - (-)-(4-Hydroxy-6-oxo-1, 6-dihydropyridin-2-yl) (Phenyl) methyl 3-methylbutanoate (1: (R)-(-)).
$^1$H NMR in CDCl$_3$ D2O Exchange

$^1$H NMR in $d_6$-DMSO
$^1$H NMR in $d_6$-DMSO $D_2O$-Exchange

$^{13}$C NMR in CDCl$_3$
**Analysis Result**

I. Specific Optical Rotation $[\alpha]^{23\circ}$: $-25.043$
   Solvent : Chloroform
   Concentration : 0.5879%

II. Specific Optical Rotation $[\alpha]^{23\circ}$: $-0.845$
   Solvent : Methanol
   Concentration : 0.5873%

III. Melting Range : $128-133\,^\circ$C
Column: DEVELOSIL CDS MG-3(4.6 X 33 mm)
Mobile Phase: A: 0.1% HCOOH/HAc
B: 0.1% HCOOH/ACN/MeOH (60:30)
Flow rate: 1.0 mL/min
Detector: ACN

2: Scan ESI-TIC
1.50e6

1: (R)-

<table>
<thead>
<tr>
<th>Time (min)</th>
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<th>Area (%)</th>
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</thead>
<tbody>
<tr>
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<td>221</td>
<td>20.03</td>
</tr>
<tr>
<td>3.65</td>
<td>14522</td>
<td>16049.96</td>
</tr>
<tr>
<td>4.06</td>
<td>224</td>
<td>20.18</td>
</tr>
<tr>
<td>5.68</td>
<td>248</td>
<td>22.52</td>
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</table>

Range: 1.458e-1

[Graph showing a chromatogram with peaks at 3.65 min, 5.59, 6.54, 6.78, 7.06, 5.9, and 7.87.]

22-May-2009
22:00:20
HPLC

Chromatographic Conditions

Column: Eclipse XDB C18 (4.6x150mm) 5.0 µ
Mobile Phase A: 0.1% Formic Acid (Aq)
Mobile Phase B: Methanol
T/6B: 0/30, 0/90, 1500, 15.1/30
Flow Rate: 1.0 mL/min
Temp: 25 °C
Diluent: ACN

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>5987</td>
</tr>
<tr>
<td>2</td>
<td>8.74</td>
<td>3478593</td>
</tr>
<tr>
<td>3</td>
<td>11.26</td>
<td>4537</td>
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Chromatographic Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Column</td>
<td>CHIRAL PAK AD-H 4.6 X 250mm, 5um</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>n-HEXANE : CIPA</td>
</tr>
<tr>
<td>Isocratic</td>
<td>90:10</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.7 ml/min</td>
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<tr>
<td>Column Temp</td>
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</tr>
<tr>
<td>Diluent</td>
<td>ETHG</td>
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Purity Chromatogram

Peak Results

<table>
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<tr>
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<tr>
<td>2</td>
<td>12.983</td>
<td>229372</td>
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</table>
5.3.5. Spectral data of (S)-(+)\text{-}4\text{-}hydroxy\text{-}6\text{-}(hydroxy (phenyl) methyl)\text{-}2\text{-}oxo\text{-}1, 2\text{-}dihydropyridine\text{-}3\text{-}carboxylic acid (24:\text{(S)-(+)}).
24: (S)(+)

LC-MS
Chiral HPLC

Chromatographic Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
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</thead>
<tbody>
<tr>
<td>Column</td>
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<tr>
<td>Mobile Phase</td>
<td>A:Hexane, B: Ethanol, CITFA</td>
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<tr>
<td>Isocratic</td>
<td>(60:40:0.1% TFA)</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0 ml/min</td>
</tr>
<tr>
<td>Diluent</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Temp</td>
<td>Ambient</td>
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</table>

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT</th>
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<tbody>
<tr>
<td>7.54</td>
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</table>
5.3.6. Spectral data of (R)-(-)-4-hydroxy-6-(hydroxy (phenyl) methyl)-2-oxo-1, 2-dihydropyridine-3-carboxylic acid (24:(R)-(-)).
Expansion of $^1$H NMR

Data Collected on:
gd-rotom0-nmr04010
Archive Directory:
/measure/data/2011/02/22
Sample Description:
VSM-NMR-01
Profile: VSM-NMR-D_022011
Pulse Sequence: PROTON (adjpro)
Solvent: D$_2$O
Data collected on: Feb 22 2011

Temp: 25.0 C / 0.1 K
Sample #, Operator: unstud
Pulse delay 1.000 sec
Pulse 90.0 degrees
Avg. time 4.000 sec
Wait 3.000 2.0
8 repetitions

Chemical shift 8 ppm
Line broadening 0.5 Hz
F1 scan 65536
Total time 9 min 41 sec

$^1$H NMR in D$_2$O-Exchange

Chemical structure 24: (R)(-)
LC-MS

Column: Acquity BEH-C18-50 X 2.1 mm (1.7 μm)
Mobile Phase: A: 0.1% HCOOH (aq)
B: 0.1% HCOOH (ACN)
T:% B: 0.00, 0.3, 0.5, 0.8, 1.2, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0
Flow rate: 0.4 ml/min
Diluent: ACN

Time | Height | Area | Area% |
-----|--------|------|-------|
1.38 | 861.96 | 18887.38 | 99.42 |
1.48 | 4440.00 | 10683.77 | 59.48 |

24: (R){-}
Chiral HPLC

Chromatographic Conditions

Column: CHIRALPAK IA (4.6X250mm)
Mobile Phase: A: Hexane, B: Ethanol, C: TFA
Isocratic: (80:20:0.1% TFA)
Flow Rate: 1.0 ml/min
Diluent: Ethanol
Temp: Ambient

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
</table>
| 5.79| 2084270 | 99.35%
| 7.02| 3456   | 1.65%  |

24: (R)-(-)
5.3.7. (S)-(+)-4-hydroxy-6-((3-methylbutanoyloxy) (phenyl methyl)-2-oxo-1, 2-dihydropyridine-3-carboxylic acid (2: (S)- (+)).
NOE

$^{13}$C NMR in CDCl$_3$
LC-MS

Column: Acquity BEH-C18 (50 X 2.1mm) 1.7um
Mobile phase: 0.1% HCOOH/ACN
T: 0.3/0.5/0.7/1.0/1.2/1.5/1.7/2.0
Flow rate: 0.5 mL/min
Diluent: ACN

2: (S)-(+)

25-May-2010
08:40:32
TIC
6.23x107
Chromatographic Conditions

Column: Kromosil C18 (4.6 x 250) 5 µ
Mobile Phase: A: 0.05% TFA (aq)
Mobile Phase B: MeOH
T,%B: 0/30,2/30,8/90,16/90,18/30,20/30.
Flow Rate: 1.0 mL/min
UV: 210 nm
Temp: 25 °C
Diluent: acn

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.431</td>
<td>62.26</td>
</tr>
<tr>
<td>2</td>
<td>4008</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>5008</td>
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</tr>
<tr>
<td>4</td>
<td>31803</td>
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Chiral HPLC

Chromatographic Conditions

Column: CHIRALPAK AD-H (4.6x250mm)5u
Mobile Phase: A. Hexane (0.1% TFA) B. EtOH (0.1% TFA)
A:B (50:50)
Temp: Ambient
Flow Rate: 0.7ml/min

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
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<tr>
<td>2</td>
<td>10.23</td>
<td>0.07</td>
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</table>
5.3.8. (R)-(-)-4-hydroxy-6-((3-methylbutanoyloxy) (phenyl) methyl)-2-oxo-1, 2-dihydropyridine-3-carboxylic acid (2):(R)-(-)).
$^1$H NMR $\text{D}_2\text{O}$ Exchange

$^{13}$C NMR in $\text{CDCl}_3$
HPLC

Chromatographic Conditions

Column: Kromasil C18 (4.6 x 250) 5 µ
Mobile Phase: A: 0.05% TFA (aq)
Mobile Phase: B: MeOH
T%B: 0/30/2/30,8/90,16/90,18/30,20/30.
Flow Rate: 1.0 mL/min
Temp: 25 °C, Diluent: acn

Purity Chromatogram

<table>
<thead>
<tr>
<th>RT (min)</th>
<th>Area</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
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<td>2</td>
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<td>7</td>
<td>39823</td>
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</table>
Chiral HPLC

Chromatographic Conditions

Column: CHIRALPAK AD-H (4.6x250mm) 5μ
Mobile Phase: A: Hexane (0.1% TFA) B: EtOH (0.1% TFA)
A:B (ISO): (70:30)
Temp: Ambient
Flow Rate: 0.7ml/min

Purity Chromatogram

---End for Chapter-5---