



## Asymmetric synthesis of *erythro*- $\beta$ -hydroxyasparagine

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### ABSTRACT

*erythro*-Hydroxyasparagine (eHyAsn, **1**) occurs in a number of naturally occurring peptides and proteins. Previous syntheses have relied on enzyme-catalyzed reactions to produce relevant, optically active intermediates. We report herein a completely chemical synthesis that intercepts Boger's synthesis of the diastereomer (*threo*-HyAsn, **4**), utilizing a Sharpless asymmetric aminohydroxylation reaction to introduce the two stereocenters. Boc-HyAsn(OTBS)-OH (**10**) was coupled effectively with phenylalanine methyl ester using EDC/HOBt.

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$\beta$ -Hydroxy-L-asparagine was identified as a normal constituent in human urine by Tominaga et al. in 1963.<sup>1</sup> Okai and Izumiya subsequently demonstrated that this metabolite was the *erythro* (2*S*,3*R*) diastereomer.<sup>2</sup> *erythro*- $\beta$ -Hydroxy-L-asparagine (**1**, eHyAsn) has subsequently been identified in an integral membrane protein precursor to the epidermal growth factor (EGF),<sup>3</sup> one of the sub-components of the complement protein C1s,<sup>4</sup> and in antifungal peptides, including the theonellamides<sup>5</sup> and the xylocandins.<sup>6</sup>

There have been a number of syntheses of  $\beta$ -hydroxyaspartic acid reported. A couple of approaches have capitalized on the Sharpless asymmetric dihydroxylation reaction followed by manipulations to introduce the amino group with retention<sup>7</sup> or inversion<sup>8</sup> of configuration. Hanessian and Vanasse used internal asymmetric induction to hydroxylate aspartic acid derivatives.<sup>9</sup> Cardillo et al. similarly introduced an iodide at C $\beta$ ; the isolated oxazoline was then hydrolyzed to give free  $\beta$ -hydroxyaspartic acid.<sup>10</sup> Leckta and co-workers described a novel benzoylquinine-catalyzed reaction between acid chlorides and  $\alpha$ -chloroglycine to give various  $\beta$ -substituted aspartic acids.<sup>11</sup> If the two carboxylic acids of  $\beta$ -hydroxyaspartic acid could be differentiated, these syntheses might be applicable to  $\beta$ -HyAsn. However, in most cases, this transformation is not straightforward.

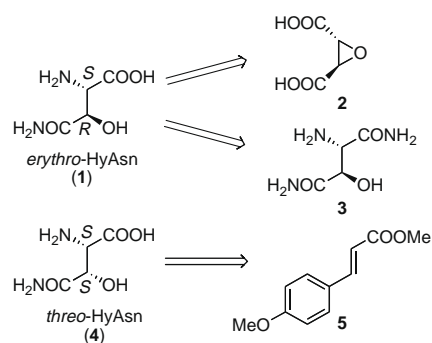
There have been two approaches directed specifically toward eHyAsn (**1**) (Scheme 1). Sendai et al. combined aspects of two earlier reports:<sup>12</sup> their starting material was (–)-*trans*-epoxysuccinic acid (**2**), from the fermentation of glucose by *Aspergillus fumigatus*.<sup>13</sup> The epoxide was ammonolyzed to give *erythro*- $\beta$ -hydroxy-L-aspartic acid and then transformed to **1** according to Singerman and Liwshitz.<sup>14</sup> This approach was also adopted by Tohdo, Hamada, and Shioiri to produce a building block for their theonellamide F synthesis.<sup>15</sup> The second route to eHyAsn involved a regio-

selective hydrolysis of eHyAsp diamide (**3**), using leucine aminopeptidase.<sup>2</sup>

The diastereomeric *threo*- $\beta$ -hydroxy-L-asparagine (*threo*-HyAsn, **4**, Scheme 1) features in ramoplanin and lysobactin, lipoglycopeptide antibiotics that show promise against vancomycin-resistant bacteria.<sup>16</sup> Due to the importance of these antibiotics, the synthesis of *threo*-HyAsn has drawn the attention of the Boger<sup>17</sup> and Van-Nieuwenhze<sup>18</sup> groups. We felt that we could readily intercept Boger's synthesis,<sup>17</sup> invert the  $\beta$ -OH, and rapidly produce a useful eHyAsn building block for peptide synthesis.

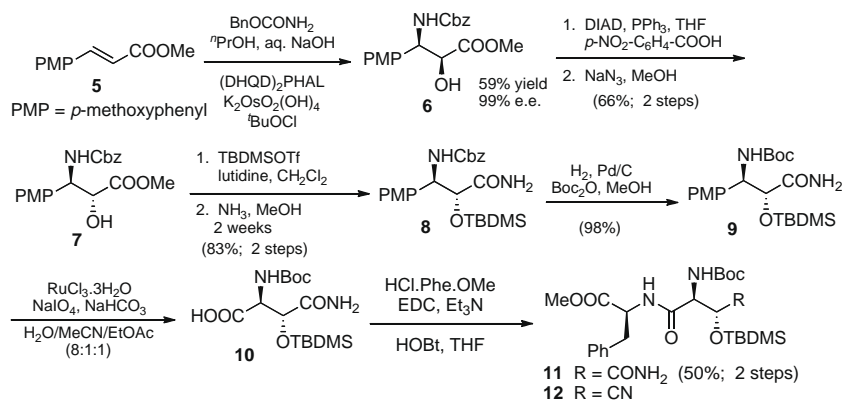
As reported previously,<sup>17</sup> the Sharpless asymmetric aminohydroxylation reaction gave a good yield of protected amino alcohol **6** in excellent enantiomeric excess (Scheme 2). We then conducted a Mitsunobu reaction with *p*-nitrobenzoic acid as the nucleophile.<sup>19</sup> The ester was readily removed by azidolysis to give **7**. The hindered alcohol was protected as its TBDMS ether and the methyl ester converted to the side chain primary amide,<sup>20</sup> giving compound **8**.

Recent reports by Jung and coworkers indicated that it is possible to oxidatively degrade a *p*-methoxyphenyl aromatic ring in

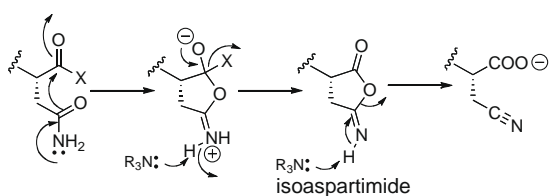


Scheme 1. Previous approaches to hydroxyasparagines.

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Scheme 2. Synthesis of an eHyAsn building block and incorporation into dipeptide **11**.



Scheme 3. Mechanism of  $\beta$ -cyanoalanine formation.

the presence of a benzyl carbamate.<sup>21</sup> We tried unsuccessfully to unmask the  $\alpha$ -COOH from compound **8** and resorted to switching carbamate protecting groups. The oxidative degradation of compound **9** had to be conducted on a reasonable scale (500 mg or more) with a mechanical stirrer. In addition to the issues discussed for conducting the reaction, careful control of pH during workup and exhaustive extraction of the aqueous layer are vital. The optical rotation, while somewhat larger than that reported by Tohdo et al.,<sup>15</sup> was the same sign and of similar magnitude.

We next sought to form a dipeptide, relevant to theonellamide C,<sup>5d</sup> and to test whether or not the side chain amide would require protection during this amide bond formation. Tohdo et al. had coupled **10** with a *p*-bromo-phenylalanine- $\beta$ -alanine dipeptide ester using diethyl-phosphoryl cyanide (DEPC, DMF, DIEA) in high yield.<sup>15</sup> The groups of both Boger and VanNieuwenhze protected the side chain functionality as a tritylamide. The potential side reaction of the unprotected primary amide, upon activation of the carboxylic acid in **10**, is isoaspartimide formation, followed by dehydration to give  $\beta$ -cyanoalanine derivative **12** (Scheme 3).<sup>22</sup> Indeed, this was the major product isolated on coupling with BOP/DIEA. As has been described elsewhere,<sup>23</sup> the most effective conditions to circumvent this side reaction involve a carbodiimide-mediated coupling and inclusion of hydroxybenzotriazole (HOBt) in the reaction mixture. The role of the latter additive is to serve as a superior proton donor, relative to the NH of the isoaspartimide. Thus, formation of the BtO<sup>-</sup> anion occurs in preference to dehydration to generate a nitrile.

The crude acid **10** was subjected directly to the peptide coupling reaction.<sup>24</sup> The isolated yield of dipeptide **11** is 50% over the two steps.

In summary, we have prepared eHyAsn building block **10** in seven steps and 23% overall yield from methyl *p*-methoxycinnamate. We have found conditions to form a dipeptide, and demonstrated that this can be done without protection of the side-chain amide functionality.

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- Compound **7**. *p*-Nitrobenzoic acid (3.94 g, 23.6 mmol, 2.2 equiv) was added to a solution of compound **6** (3.86 g, 10.7 mmol, 1.0 equiv) in dry THF (130 mL). Triphenylphosphine (6.19 g, 23.6 mmol, 2.2 equiv) was added, and then the mixture was cooled to 0 °C under N<sub>2</sub>. Diisopropyl azodicarboxylate (4.65 mL, 4.77 g, 23.6 mmol, 2.2 equiv) was slowly added via syringe. Upon completion of the addition, the ice bath was removed, and the contents were stirred at room temperature for 2 d. The reaction mixture was concentrated and the product was isolated from the residue by flash chromatography (Hex–EtOAc, 3:1) to give **7** as a pale yellow gum (2.56 g, 66%), R<sub>f</sub> 0.32 (1:1 Hex–EtOAc); [α]<sub>D</sub><sup>25</sup> –20.8 (c 0.8, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 2.92 (d, J = 6.5 Hz, 1H), 3.69 (s, 3H), 3.77 (s, 3H), 4.59 (dd, J = 6.5, 3.3 Hz, 1H), 5.05 (d, J = 12.2 Hz, 1H), 5.11 (d, J = 12.2 Hz, 1H), 5.10–5.13 (m, 1H), 5.83 (d, J = 8.6 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H), 7.34 (m, 5H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 52.4, 55.1, 56.4, 66.8, 73.1, 113.8, 128.1, 128.3, 128.4, 128.6, 136.2, 155.5, 159.3, 172.1; HRMS (ESI) calcd for C<sub>19</sub>H<sub>20</sub>NO<sub>6</sub> (M–H)<sup>+</sup> 358.1296, obsd 358.1295.

20. Boger and co-workers previously explored and discussed other approaches to primary amide formation.<sup>17b</sup> Ammonolysis, while slow due to steric hindrance, proved to be the most satisfactory route.
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24. **Dipeptide 11.** (a) **Acid 10.** Sodium periodate (5.504 g, 26 mmol, 18.1 equiv) in H<sub>2</sub>O (128 mL) was added to a solution of compound **9** (604 mg, 1.4 mmol, 1.0 equiv) in EtOAc–CH<sub>3</sub>CN (1:1, 32 mL). The resulting solution was stirred mechanically at rt for 30 min. Ruthenium trichloride trihydrate (60 mg, 0.3 mmol, 20 mol%) was added, followed by NaHCO<sub>3</sub> (464 mg, 5.5 mmol, 3.9 equiv). The reaction mixture was stirred mechanically at rt overnight. The yellow solution was diluted with satd aqueous NaHCO<sub>3</sub> (240 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (160 mL). The organic layer was washed with satd aqueous NaHCO<sub>3</sub> (240 mL) again. The combined aqueous layers were acidified with 10% aqueous HCl to pH 2.5 at 0 °C and extracted with EtOAc (6 × 300 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered, and concentrated to give compound **10** as a brown foam (376 mg, 73%). This material was used without purification. R<sub>f</sub> 0.64 (6:4:1 CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O); [α]<sub>D</sub><sup>24.0</sup> +51.1 (c 1.0, MeOH), lit.<sup>15</sup> [α]<sub>D</sub><sup>24.0</sup> +40.9 (c 1.0, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 0.15 (s, 3H), 0.16 (s, 3H), 0.94 (s, 9H), 1.45 (s, 9H), 3.83 (s, 1H), 4.57 (d, J = 9.7 Hz, 1H), <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) δ –5.1, –4.9, 19.0, 26.2, 28.7, 58.7, 75.3, 81.0, 157.1, 171.7, 175.9; HRMS (ESI) calcd for C<sub>15</sub>H<sub>31</sub>N<sub>2</sub>O<sub>6</sub>Si (M+H)<sup>+</sup> 363.1945, obsd 363.1955. (b) **Dipeptide 11.** A portion of acid **10** (266 mg, ~0.7 mmol, 1.0 equiv) was dissolved in anhydrous THF (15 mL) and the resulting solution was cooled to 0 °C. L-Phenylalanine methyl ester hydrochloride (158 mg, 0.7 mmol, 1.0 equiv) was added and the solution was stirred at 0 °C for 15 min. Triethylamine (204 μL, 149 mg, 1.5 mmol, 2.0 equiv) was added and the solution was stirred at 0 °C for 10 min. 1-(3-Dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride (148 mg, 0.8 mmol, 1.05 equiv) was added, followed by HOBt hydrate (149 mg, 1.1 mmol, 1.5 equiv) at 0 °C. The reaction mixture was allowed to stir for 20 min at 0 °C and then warmed to rt and stirred overnight. The solvent was removed and the product was isolated from the residue by flash chromatography (Hex–EtOAc 4:1 → Hex–EtOAc 1:1) to give a colorless foam (262 mg, 68%; 50% over two steps). R<sub>f</sub> 0.33 (1:1 Hex–EtOAc); mp 49–50 °C; [α]<sub>D</sub><sup>25.0</sup> +48.6 (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.10 (s, 3H), 0.13 (s, 3H), 0.90 (s, 9H), 1.44 (s, 9H), 3.08 (d, J = 5.4 Hz, 2H), 3.65 (s, 3H), 4.60–4.70 (m, 2H), 4.80 (d, J = 6.5 Hz, 1H), 5.44 (s, 1H), 5.98 (s, 1H), 6.54 (s, 1H), 6.60 (d, J = 7.4 Hz, 1H), 7.12–7.30 (m, 5H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ –5.3, –5.1, 18.0, 25.6, 28.3, 37.8, 52.2, 53.5, 57.4, 74.0, 80.1, 127.1, 128.6, 129.3, 135.7, 154.9, 167.5, 171.4, 174.3; HRMS (ESI) calcd for C<sub>25</sub>H<sub>40</sub>N<sub>3</sub>O<sub>7</sub>Si (M–H)<sup>+</sup> 522.2641, obsd 522.2654.