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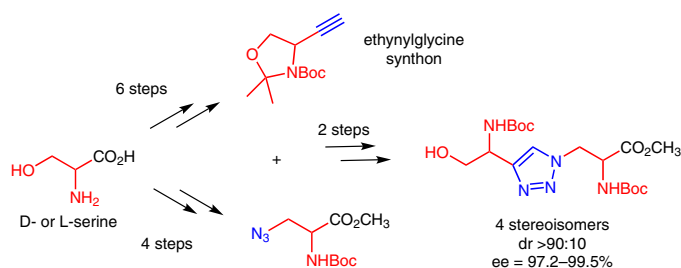
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# Synthesis of a Novel Rhizobitoxine-Like Triazole-Containing Amino Acid

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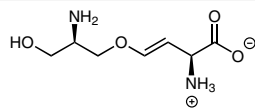
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**Abstract** The synthesis of the four stereoisomers of a new 1,2,3-triazole analogue of rhizobitoxine from serine is described. The key step is a Huisgen 1,3-dipolar cycloaddition on an ethynylglycine synthon.

**Key word** rhizobitoxine, triazole amino acid, Huisgen cycloaddition, ethynylglycine synthon, chiral HPLC

Rhizobitoxine **1** (Figure 1) is an unusual amino acid that belongs to the  $\beta,\gamma$ -enol ether family, a  $\gamma$ -substituted subclass of the naturally occurring vinylglycines.<sup>1</sup> It has been initially regarded as a phytotoxin because it induces chlorosis in soybeans.<sup>2–4</sup>



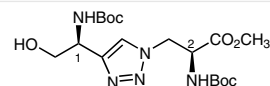
**Figure 1** Rhizobitoxine (**1**)

Rhizobitoxine is a metabolic product secreted by symbiotic bacteria such as *Rhizobium japonicum* (now *Bradyrhizobium elkanii*)<sup>5–7</sup> or the plant pathogen *Pseudomonas andropogonis* (now *Burkholderia andropogonis*).<sup>8</sup> Rhizobitoxine, which is a structural analogue of cystathionine, inhibits two pyridoxal phosphate (PLP) dependent enzymes: cystathionine  $\beta$ -lyase,<sup>5,9</sup> involved in the methionine biosynthesis pathway and 1-aminocyclopropane-1-carboxylate (ACC) synthase<sup>10</sup> involved in ethylene biosynthesis in plants. It inhibits the production of ethylene,<sup>11</sup> a gaseous stress phytohormone, and plays a positive role in establishing symbiosis between *B. elkanii* and its host legume by ethylene inhibition.<sup>12</sup> As plant growth regulators or inhibitors of sulfur assimilation, rhizobitoxine and analogues

therefore have potential applications in agronomy and biotechnology.<sup>13,14</sup>

Synthesis of such unusual amino acids is a challenge, especially because of the enol ether reactivity.<sup>1,15,16</sup> Therefore, there is a need for readily accessible stable structural analogues. 1,2,3-Triazole derivatives have gained a recent interest in medicinal chemistry because they are pharmacophores with good stability and high aqueous solubility,<sup>17–21</sup> particularly in the area of peptidomimetics<sup>22</sup> and are readily accessible by the Huisgen 1,3-dipolar cycloaddition involving an alkyne and an azide.<sup>23–26</sup>

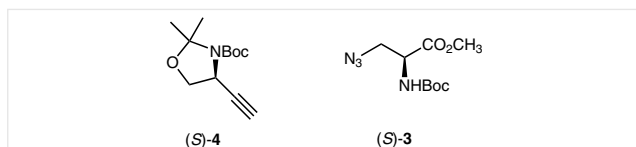
In this paper, we describe the synthesis of a new triazole-containing amino acid analogue of rhizobitoxine in protected form (compound [(1*S*,2*S*)-**2**] from serine (Figure 2) where the central enol ether linkage in rhizobitoxine is replaced by the robust 1,2,3-triazole linker in such a way that there is no longer  $\beta,\gamma$ -unsaturation to the amino acid moiety. This analogue should be a stable analogue compared to unstable vinylglycine derivatives and, based on reported mechanisms of inhibition, such an unusual amino acid could be a potential inhibitor of PLP-dependent enzymes.<sup>27,28</sup>



**Figure 2** Protected triazole containing analogue of rhizobitoxine [(1*S*,2*S*)-**2**]

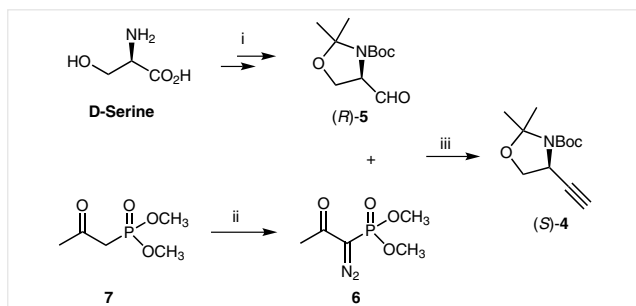
Retrosynthetic analysis shows that this compound should be accessible using a Huisgen 1,3-dipolar cycloaddition of azide (*S*)-**3** with alkyne (*S*)-**4** (Figure 3).

Alkyne (*S*)-**4** is an 'ethynylglycine synthon'.<sup>29,30</sup> It is synthesized from D-serine in six steps using Garner aldehyde (*R*)-**5**<sup>31,32</sup> as a key precursor (Scheme 1). Two principal methods have been described to synthesize alkyne **4** from



**Figure 3** Precursors of the Huisgen 1,3-dipolar cycloaddition for triazole formation in (1*S*,2*S*)-**2**

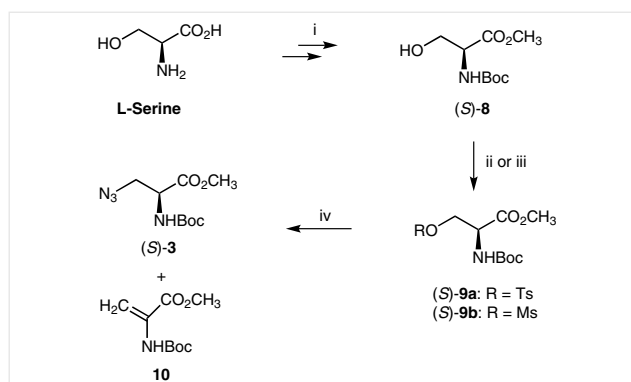
aldehyde **5**: the Bestmann–Ohira and the the Corey–Fuchs strategies.<sup>29</sup> We decided here to use the Bestmann–Ohira strategy using diazophosphonate **6** for this aldehyde-to-alkyne transformation, as this was well-known in our laboratory.<sup>33</sup> In 2002, we described the one-pot synthesis of ethynylglycine synthon **4** in 70% in 72 hours.<sup>34</sup> This procedure involving in situ formation of diazophosphonate **6** is convenient on a small scale (0.95 mmol of aldehyde **5**) but we noticed a dramatic increase of the reaction time when performed on a larger scale. Therefore, we decided to return to the original strategy<sup>35</sup> with preparation of diazophosphonate **6** prior to the homologation. After flash chromatography, the ethynylglycine synthon **4** was obtained in 83% yield on 22 mmol scale (lit.<sup>35</sup> 80% on 11 mmol scale) (Scheme 1).



**Scheme 1** Reagents and conditions: (i) see ref.<sup>31,32</sup>; (ii) NaH, toluene then 4-acetamidobenzenesulfonyl azide, THF, 72%, see ref.<sup>36</sup>; (iii) K<sub>2</sub>CO<sub>3</sub>, MeOH, 83%.

The diazophosphonate **6** was synthesized from phosphonate **7** with minor modifications of the procedure described by Pietruszka and Witt.<sup>36</sup> It was obtained in 72% yield (lit.<sup>36</sup> 77%) (Scheme 1).

Azide (*S*)-**3** was synthesized from protected L-serine (*S*)-**8**.<sup>31</sup> We first envisaged synthesizing azide **3** by a nucleophilic substitution on reactive sulfonic ester derivatives of alcohol **8** (Scheme 2). The conversion of alcohol (*S*)-**8** into *p*-toluenesulfonate (*S*)-**9a** was performed using the conditions described by Jackson and Perez-Gonzales<sup>37</sup> to obtain (*S*)-**9a** in 68% yield, and the results were in agreement with the literature (lit.<sup>37</sup> 64–69%). Conversion of (*S*)-**8** into methanesulfonate (*S*)-**9b** was performed using the conditions of Shetty et al.<sup>38</sup> and allowed (*S*)-**9b** to be obtained in 64% yield after column chromatography purification (lit.<sup>38</sup> 81%, crude



**Scheme 2** Reagents and conditions: (i) see ref.<sup>31</sup>; (ii) TsCl, Et<sub>3</sub>N, 4-DMAP (cat.), Me<sub>3</sub>NHCl (cat.), CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 2 h, 68%; (iii) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 0.5 h, 64%; (iv) NaN<sub>3</sub>, DMF, see Table 1.

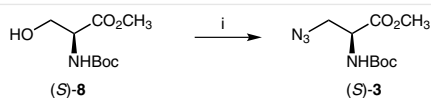
yield). The results of the transformation from (*S*)-**9a** and (*S*)-**9b** to (*S*)-**3** are summarized in Table 1. The best reaction conditions in our hands for nucleophilic substitution were using sodium azide in DMF at 70 °C for a short reaction time (Table 1, entry 2). Under these reaction conditions, azide (*S*)-**3** was obtained from *p*-toluenesulfonate (*S*)-**9a** in 39% yield, together with alkene **10** in 37% yield, resulting from an elimination reaction. Changing from *p*-toluenesulfonate **9a** to methanesulfonate **9b**, or lowering reaction temperature did not improve the yield for **3** (Table 1). We obtained enantiomer (*R*)-**3** under the same conditions (Table 1, entry 2) through (*R*)-**9a** from D-serine with identical yields and opposite specific rotation. It is worth noting that Shelly et al.<sup>38</sup> reported the formation of compound (*S*)-**3** from methanesulfonate (*S*)-**9b** (NaN<sub>3</sub>, DMF, 50 °C, 0.5 h; under the same conditions as Table 1, entry 4) in 56% yield but with a lower specific rotation. Moreover, Friscourt et al. reported the formation of the benzyl ester analogue of **3** (NaN<sub>3</sub>, DMF, 40 °C, 2 h) in only 18% yield.<sup>39</sup> All these observations show that this transformation is somewhat capricious.

**Table 1** Results of the NaN<sub>3</sub> Nucleophilic Substitution on (*S*)-**9a** and (*S*)-**9b**<sup>a</sup>

Entry	Starting material	T(°C)	Time (h)	Yield of ( <i>S</i> )- <b>3</b> (%)	Yield of <b>10</b> (%)
1	( <i>S</i> )- <b>9a</b>	20	5	33	35
2	( <i>S</i> )- <b>9a</b>	70	0.17	39	37
3	( <i>S</i> )- <b>9b</b>	20	24	25	55
4	( <i>S</i> )- <b>9b</b>	50	0.5	25	29

<sup>a</sup> See Scheme 2, reaction conditions (iv).

We then tried the direct formation of azide (*S*)-**3** using a Mitsunobu reaction as described by Stanley et al.<sup>40</sup> (Scheme 3).



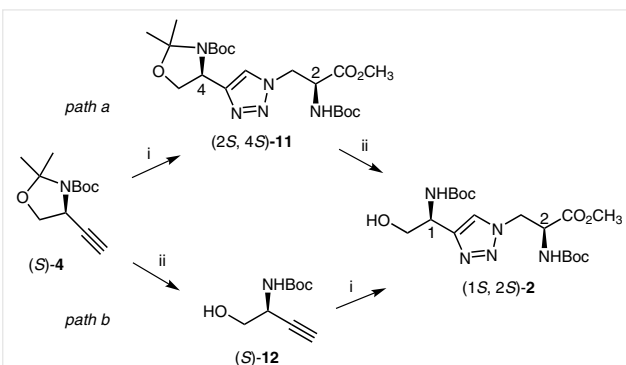
**Scheme 3** Reagents and conditions: (i)  $\text{Ph}_3\text{P}/\text{DIAD}/\text{DPPA}$  (1.14/1.28/1.14), THF, 0 °C, 41%.

Azide (*S*)-**3** was obtained in 41% yield from (*S*)-**8** (lit.<sup>40</sup> 69%) although with a cumbersome purification by column chromatography to isolate a somewhat impure material as observed on the NMR spectrum. We decided therefore to pursue the synthesis using pure compound **3** obtained following conditions described in Scheme 2, Table 1, entry 2.

To the best of our knowledge, there is only one precedent describing a Huisgen 1,3-dipolar cycloaddition using an ethynylglycine synthon as substrate. It is one example (with no further application of the product) in a methodology report of a click reaction between in situ generated  $\beta$ -azido styrenes from cinnamic acid using  $\text{CAN}/\text{NaN}_3$  and alkynes to form *N*-styryl triazoles.<sup>41</sup>

The Huisgen 1,3-dipolar cycloaddition between ethynylglycine synthon (*S*)-**4** and azide (*S*)-**3** was performed using classical conditions<sup>42</sup> (Scheme 4, path a): L-Ascorbate,  $\text{CuSO}_4$  in the mixture of *tert*-butanol/water and yielded the desired 1,2,3-triazole (2*S*,4*S*)-**11** in 70% yield. Deprotection of the oxazolidine with APTS monohydrate in methanol<sup>43</sup> furnished the final compound (1*S*,2*S*)-**2** in 17% yield with 55% recovery of starting material. The overall yield from (*S*)-**4** was 12%.

In order to increase the global yield, inversion of the order of the two final steps was examined (Scheme 4, path b). Opening the oxazolidine ring in (*S*)-**4** using the same conditions as before yielded the protected amino alcohol (*S*)-**12** in 48% yield with recovery of starting material (*S*)-**4** in 32% yield. Subsequent Huisgen cycloaddition then led to the same compound (1*S*,2*S*)-**2** in 65% yield, with a 92:8 diastereomeric ratio and an enantiomeric excess higher than 99.5% (*vide infra*). Using this strategy, the overall yield from (*S*)-**4** increased to 31%.



**Scheme 4** Reagents and conditions: (i) azide (*S*)-**3**, L-ascorbate (0.2 equiv),  $\text{CuSO}_4$  (0.1 equiv), *t*-BuOH– $\text{H}_2\text{O}$  (1:1), 70%, (path a), 65% (path b); (ii)  $\text{PTSA}-\text{H}_2\text{O}$ , MeOH, 20 °C, 2 h, 17% (path a), 48% (path b).

The four stereoisomers of compound **2** were synthesized using the same strategy as described above from *D*- and *L*-serine with analogous results (see Supporting Information).<sup>44</sup>

After a screening of several chiral stationary phases by HPLC, Lux-Cellulose-2, and Chiralpak AZ-H were found to be efficient for baseline separation of the mixture of the four stereoisomers of **11** and **2**, respectively, thus allowing the determination of the diastereomeric ratio and the enantiomeric excess of each isomer (Table 2). For all compounds, diastereomeric ratios were found to be greater than 90:10 and the enantiomeric excesses were higher than 96% (see Supporting Information)

**Table 2** Diastereomeric Ratio and Enantiomeric Excess for Stereoisomers of **11** and **2**

Isomer	dr	ee (%)	Isomer	dr	ee (%)
(2 <i>S</i> ,4 <i>R</i> )- <b>11</b>	10:1	99.5	(1 <i>S</i> ,2 <i>S</i> )- <b>2</b>	11:1	99.5
(2 <i>R</i> ,4 <i>S</i> )- <b>11</b>	16:1	96.2	(1 <i>R</i> ,2 <i>R</i> )- <b>2</b>	10:1	99.5
(2 <i>S</i> ,4 <i>S</i> )- <b>11</b>	14:1	99.5	(1 <i>S</i> ,2 <i>R</i> )- <b>2</b>	14:1	99.5
(2 <i>S</i> ,4 <i>R</i> )- <b>11</b>	9:1	99.5	(1 <i>R</i> ,2 <i>S</i> )- <b>2</b>	9:1	97.2

<sup>a</sup> Determined by chiral HPLC.

Deprotection of compounds **2** and biological evaluation of their activity on PLP-dependant enzymes are under investigation in our laboratory, and the results will be reported in due course.

## Acknowledgment

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## Supporting Information

Supporting information for this article is available online at <http://dx.doi.org/10.1055/s-0036-1588300>.

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- (44) **General Synthetic Procedure for Click-Chemistry Reaction for the Synthesis of 2**  
**(S)-Methyl 2-[(tert-Butoxycarbonyl)amino]-3-(4-[(S)-1-[(tert-butoxycarbonyl)amino]-2-hydroxyethyl]-1H-1,2,3-triazol-1-yl)propanoate [(1S,2S)-2, (Scheme 4, Path a)]**  
 To a solution of (2S,4S)-**11** (0.337 g, 0.72 mmol) in MeOH (5 mL) was added PTSA-H<sub>2</sub>O (0.137 g, 0.72 mmol). The reaction mixture was stirred for 2 h at room temperature and sat. aq NaHCO<sub>3</sub> solution (40 mL) was poured into the solution. The aqueous solution was extracted with EtOAc (3 × 40 mL). The organic phases were combined, washed with sat. aq NaHCO<sub>3</sub> solution (40 mL), sat. aq NaCl solution (40 mL), dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, EtOAc-PE = 0:100, increasing to 100:0, v/v) to give the desired compound (1S,2S)-**2** (0.053 g, 17%) as a white solid and recovered starting material (2S,4S)-**11** (0.184 g, 55%).  
**Analytical Data**  
*R<sub>f</sub>* = 0.26 (EtOAc); mp 55–57 °C. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 1.42 [s, 18 H, C(CH<sub>3</sub>)<sub>3</sub>], 2.89 (br s, 1 H, OH), 3.79 (s, 3 H, CO<sub>2</sub>CH<sub>3</sub>), 3.84–3.87, 4.10–4.12 (2 m, 2 H, CH<sub>2</sub>O), 4.70–4.86 (m, 4 H, 2 CH<sub>2</sub>N), 5.43 (br s, 1 H, NH), 5.59 (br s, 1 H, NH), 7.57 (s, 1 H, CH<sub>triazole</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 28.4, 28.5 [2 s, 18 H, C(CH<sub>3</sub>)<sub>3</sub>], 48.2 (CH), 51.5 (CH<sub>2</sub>N), 53.4 (CO<sub>2</sub>CH<sub>3</sub>), 53.9 (CH), 65.0 (CH<sub>2</sub>O), 80.1 [C(CH<sub>3</sub>)<sub>3</sub>], 81.0 [C(CH<sub>3</sub>)<sub>3</sub>], 123.9 (CH<sub>triazole</sub>), 147.1 (C<sub>triazole</sub>), 155.2 (NCO<sub>2</sub>), 155.8 (NCO<sub>2</sub>), 169.5 (CO<sub>2</sub>CH<sub>3</sub>). [α]<sub>D</sub><sup>20</sup> +49.9 (c 0.91, CHCl<sub>3</sub>). HRMS (ES<sup>+</sup>): *m/z* [M + H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>32</sub>N<sub>5</sub>O<sub>7</sub>: 430.2302; found: 430.2303. HPLC: purity = 99.6%, *t<sub>R</sub>* = 12.43 min. IR: 3358, 2362, 2338, 1742, 1683 cm<sup>-1</sup>. Nitrogen inversion in the oxazolidine ring or slow interconversion of both amide or carbamate conformers of compounds **4**, **11**, and **2** causes considerable line broadening and duplication of signals in the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra (see Supporting Information).
- General Synthetic Procedure for Click-Chemistry Reaction for the Synthesis of 2**  
**(S)-Methyl 2-[(tert-Butoxycarbonyl)amino]-3-(4-[(S)-1-[(tert-butoxycarbonyl)amino]-2-hydroxyethyl]-1H-1,2,3-triazol-1-yl)propanoate [(1S,2S)-2, Scheme 4, Path b]**  
 Azide **3** (0.420 g, 1.72 mmol) and alkyne **12** (0.318 g, 1.72 mmol) were dissolved in a mixture of *t*-BuOH-H<sub>2</sub>O (10 mL, 1:1, v/v). Sodium *L*-asorbate (0.068 g, 20 mol%) and CuSO<sub>4</sub>·5H<sub>2</sub>O (0.041 g, 10 mol%) were added. The reaction mixture was stirred at room temperature for 24 h, the solution was concentrated under vacuum and diluted with H<sub>2</sub>O (70 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, EtOAc-PE = 0:100, increasing to 100:0, v/v) to give the desired compound (1S,2S)-**2** as a white solid (0.480 g, 65% yield). The compound exhibited the same analytical properties as described above. See Supporting Information for the characterization data of other products.