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

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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 β,γ -enol ether family, a γ -substituted subclass of the naturally occurring vinylglycines.¹ It has been initially regarded as a phytotoxin because it induces chlorosis in soybeans.^{2–4}

Figure 1 Rhizobitoxine (**1**)

Rhizobitoxine is a metabolic product secreted by symbiotic bacteria such as *Rhizobium japonicum* (now *Bradyrhizobium elkanii*)^{5–7} or the plant pathogen *Pseudomonas andropogonis* (now *Burkholderia andropogonis*).⁸ Rhizobitoxine, which is a structural analogue of cystathionine, inhibits two pyridoxal phosphate (PLP) dependent enzymes: cystathionine β -lyase,^{5,9} involved in the methionine biosynthesis pathway and 1-aminocyclopropane-1-carboxylate (ACC) synthase¹⁰ involved in ethylene biosynthesis in plants. It inhibits the production of ethylene,¹¹ a gaseous stress phytohormone, and plays a positive role in establishing symbiosis between *B. elkanii* and its host legume by ethylene inhibition.¹² As plant growth regulators or inhibitors of sulfur assimilation, rhizobitoxine and analogues

therefore have potential applications in agronomy and biotechnology.^{13,14}

Synthesis of such unusual amino acids is a challenge, especially because of the enol ether reactivity.^{1,15,16} 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,^{17–21} particularly in the area of peptidomimetics²² and are readily accessible by the Huisgen 1,3-dipolar cycloaddition involving an alkyne and an azide.^{23–26}

In this paper, we describe the synthesis of a new triazole-containing amino acid analogue of rhizobitoxine in protected form (compound **[(1S,2S)-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 β,γ -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.^{27,28}

Figure 2 Protected triazole containing analogue of rhizobitoxine [(1S,2S)-**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'.^{29,30} It is synthesized from D-serine in six steps using Garner aldehyde (**R-5**)^{31,32} 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 Corey–Fuchs strategies.²⁹ 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.³³ In 2002, we described the one-pot synthesis of ethynylglycine synthon **4** in 70% in 72 hours.³⁴ 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 strategy³⁵ 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.³⁵ 80% on 11 mmol scale) (Scheme 1).

Scheme 1 Reagents and conditions: (i) see ref.^{31,32}; (ii) NaH, toluene then 4-acetamidobenzenesulfonyl azide, THF, 72%, see ref.³⁶; (iii) K₂CO₃, MeOH, 83%.

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

Azide (*S*)-**3** was synthesized from protected L-serine (*S*)-**8**.³¹ 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-Gonzales³⁷ to obtain (*S*)-**9a** in 68% yield, and the results were in agreement with the literature (lit.³⁷ 64–69%). Conversion of (*S*)-**8** into methanesulfonate (*S*)-**9b** was performed using the conditions of Shetty et al.³⁸ and allowed (*S*)-**9b** to be obtained in 64% yield after column chromatography purification (lit.³⁸ 81%, crude

Scheme 2 Reagents and conditions: (i) see ref.³¹; (ii) TsCl, Et₃N, 4-DMAP (cat.), Me₃NHCl (cat.), CH₂Cl₂, 0 °C, 2 h, 68%; (iii) MsCl, Et₃N, CH₂Cl₂, 0 °C, 0.5 h, 64%; (iv) NaN₃, 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.³⁸ reported the formation of compound (*S*)-**3** from methanesulfonate (*S*)-**9b** (NaN₃, 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** (NaN₃, DMF, 40 °C, 2 h) in only 18% yield.³⁹ All these observations show that this transformation is somewhat capricious.

Table 1 Results of the NaN₃ Nucleophilic Substitution on (*S*)-**9a** and (*S*)-**9b**^a

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

^a 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.⁴⁰ (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.⁴⁰ 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 β -azido styrenes from cinnamic acid using CAN/NaN_3 and alkynes to form *N*-styryl triazoles.⁴¹

The Huisgen 1,3-dipolar cycloaddition between ethynylglycine synthon (*S*)-**4** and azide (*S*)-**3** was performed using classical conditions⁴² (Scheme 4, path a): L-Ascorbate, 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 methanol⁴³ 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), CuSO_4 (0.1 equiv), *t*-BuOH– H_2O (1:1), 70%, (path a), 65% (path b); (ii) PTSA– H_2O , 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).⁴⁴

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

^a 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>.

References and Notes

- Berkowitz, D. B.; Charette, B. D.; Karukurichi, K. R.; McFadden, J. M. *Tetrahedron: Asymmetry* **2006**, 17, 869.
- Erdman, L. W.; Johnson, H. W.; Clark, F. *Plant Dis. Rep.* **1956**, 646.
- Owens, L. D.; Wright, D. A. *Plant Physiol.* **1965**, 40, 927.
- Okazaki, S.; Sugawara, M.; Yuhashi, K.-I.; Minamisawa, K. *Ann. Bot.* **2007**, 100, 55.
- Owens, L. D.; Guggenheim, S.; Hilton, J. L. *Biochim. Biophys. Acta, Gen. Subj.* **1968**, 158, 219.

- (6) Owens, L. D.; Thompson, J. F.; Pitcher, R. G.; Williams, T. J. *Chem. Soc., Chem. Commun.* **1972**, 714.
- (7) Yasuta, T.; Okazaki, S.; Mitsui, H.; Yuhashi, K.-I.; Ezura, H.; Minamisawa, K. *Appl. Environ. Microbiol.* **2001**, *67*, 4999.
- (8) Mitchell, R. E.; Frey, E. J.; Benn, M. H. *Phytochemistry* **1986**, 2711.
- (9) Xiong, K.; Fuhrmann, J. J. *Plant Soil* **1996**, *186*, 53.
- (10) Yasuta, T.; Satoh, S.; Minamisawa, K. *Appl. Environ. Microbiol.* **1999**, *65*, 849.
- (11) Owens, L. D.; Lieberman, M.; Kunishi, A. *Plant Physiol.* **1971**, *48*, 1.
- (12) Sugawara, M.; Okazaki, S.; Nukui, N.; Ezura, H.; Mitsui, H.; Minamisawa, K. *Biotechnol. Adv.* **2006**, *24*, 382.
- (13) Villalobos-Acuña, M.; Mitcham, E. J. *Postharvest Biol. Technol.* **2008**, *49*, 187.
- (14) Hirase, K.; Molin, W. T. *Weed Biol. Manage.* **2003**, *3*, 147.
- (15) Dumas, M.; Vo-Quang, L.; Le Goffic, F. *Tetrahedron* **1992**, *48*, 2373.
- (16) Keith, D. D.; Tortora, J. A.; Ineichen, K.; Leimgruber, W. *Tetrahedron* **1975**, *31*, 2633.
- (17) Agalave, S. G.; Maujan, S. R.; Pore, V. S. *Chem. Asian J.* **2011**, *6*, 2696.
- (18) Hein, C. D.; Liu, X.-M.; Wang, D. *Pharm. Res.* **2008**, *25*, 2216.
- (19) Sahu, J. K.; Ganguly, S.; Kaushik, A. *Chin. J. Nat. Med.* **2013**, *11*, 456.
- (20) Kumar, D.; Reddy, V. B.; Kumar, A.; Mandal, D.; Tiwari, R.; Parang, K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 449.
- (21) Lebeau, A.; Abrioux, C.; Bénimèlis, D.; Benfodda, Z.; Meffre, P. *Med. Chem.* **2016**, *12*, DOI: 10.2174/1573406412666160404125718.
- (22) Valverde, I. E.; Mindt, T. L. *Chimia* **2013**, *67*, 262.
- (23) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565.
- (24) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.
- (25) Kumar, D.; Reddy, V. B.; Varma, R. S. *Tetrahedron Lett.* **2009**, *50*, 2065.
- (26) Totobenazara, J.; Burke, A. J. *Tetrahedron Lett.* **2015**, *56*, 2853.
- (27) Walsh, C. *Tetrahedron* **1982**, *38*, 871.
- (28) Rando, R. R. *Pharmacol. Rev.* **1984**, *36*, 111.
- (29) Benfodda, Z.; Bénimèlis, D.; Reginato, G.; Meffre, P. *Amino Acids* **2015**, *47*, 271.
- (30) Reginato, G.; Meffre, P.; Gaggini, F. *Amino Acids* **2005**, *29*, 81.
- (31) Garner, P.; Park, J. M. *Org. Synth.* **1992**, *70*, 18.
- (32) Dononi, A.; Perrone, D. *Org. Synth.* **2000**, *77*, 64.
- (33) Meffre, P.; Gauzy, L.; Perdigue, C.; Desanges-Levecque, F.; Branquet, E.; Durand, P.; Le Goffic, F. *Tetrahedron Lett.* **1995**, *36*, 877.
- (34) Meffre, P.; Hermann, S.; Durand, P.; Reginato, G.; Riu, A. *Tetrahedron* **2002**, *58*, 5159.
- (35) Meffre, P.; Gauzy, L.; Branquet, E.; Durand, P.; Le Goffic, F. *Tetrahedron* **1996**, *52*, 1215.
- (36) Pietruszka, J.; Witt, A. *Synthesis* **2006**, 4266.
- (37) Jackson, R. F. W.; Perez-Gonzalez, M. *Org. Synth.* **2005**, *81*, 77.
- (38) Shetty, D.; Jeong, J. M.; Ju, C. H.; Kim, Y. J.; Lee, J.-Y.; Lee, Y.-S.; Lee, D. S.; Chung, J.-K.; Lee, M. C. *Bioorg. Med. Chem.* **2010**, *18*, 7338.
- (39) Friscourt, F.; Fahrni, C. J.; Boons, G.-J. *J. Am. Chem. Soc.* **2012**, *134*, 18809.
- (40) Stanley, N. J.; Pedersen, D. S.; Nielsen, B.; Kvist, T.; Mathiesen, J. M.; Bräuner-Osborne, H.; Taylor, D. K.; Abell, A. D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7512.
- (41) Kavitha, M.; Mahipal, B.; Mainkar, P. S.; Chandrasekhar, S. *Tetrahedron Lett.* **2011**, *52*, 1658.
- (42) Gajewski, M.; Seaver, B.; Esslinger, C. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4163.
- (43) Goswami, K.; Duttgupta, I.; Sinha, S. J. *Org. Chem.* **2012**, *77*, 7081.
- (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-H₂O (0.137 g, 0.72 mmol). The reaction mixture was stirred for 2 h at room temperature and sat. aq NaHCO₃ 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 NaHCO₃ solution (40 mL), sat. aq NaCl solution (40 mL), dried over MgSO₄, 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*_f = 0.26 (EtOAc); mp 55–57 °C. ¹H NMR (300 MHz, CDCl₃): δ = 1.42 [s, 18 H, C(CH₃)₃], 2.89 (br s, 1 H, OH), 3.79 (s, 3 H, CO₂CH₃), 3.84–3.87, 4.10–4.12 (2 m, 2 H, CH₂O), 4.70–4.86 (m, 4 H, 2 CH₂N), 5.43 (br s, 1 H, NH), 5.59 (br s, 1 H, NH), 7.57 (s, 1 H, CH_{triazole}). ¹³C NMR (75 MHz, CDCl₃): δ = 28.4, 28.5 [2 s, 18 H, C(CH₃)₃], 48.2 (CH), 51.5 (CH₂N), 53.4 (CO₂CH₃), 53.9 (CH), 65.0 (CH₂O), 80.1 [C(CH₃)₃], 81.0 [C(CH₃)₃], 123.9 (CH_{triazole}), 147.1 (C_{triazole}), 155.2 (NCO₂), 155.8 (NCO₂), 169.5 (CO₂CH₃). [α]_D²⁰ +49.9 (c 0.91, CHCl₃). HRMS (ES⁺): *m/z* [M + H]⁺ calcd for C₁₈H₃₂N₅O₇: 430.2302; found: 430.2303. HPLC: purity = 99.6%, *t*_R = 12.43 min. IR: 3358, 2362, 2338, 1742, 1683 cm⁻¹. 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 ¹H NMR and ¹³C 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-H₂O (10 mL, 1:1, v/v). Sodium L-asorbate (0.068 g, 20 mol%) and CuSO₄·5H₂O (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 H₂O (70 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried over MgSO₄, 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.