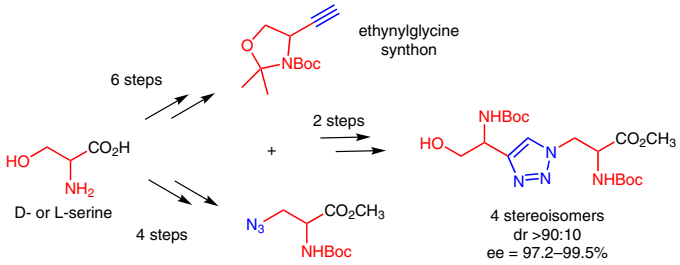
Synlett T. Boibessot et al. Letter

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

Thibaut Boibessot[a]
David Bénimèlis[a]
Marion Jean[b]
Zohra Benfodda[a]
Patrick Meffre*[a]

[a] Université de Nîmes, EA7352 CHROME, Rue du Dr G. Salan, 30021 Nîmes Cedex 1, France
patrick.meffre@unimes.fr
[b] Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, Marseille, France



Received: 10.06.2016
Accepted after revision: 05.08.2016
Published online: 18.08.2016
DOI: 10.1055/s-0036-1588300; Art ID: st-2016-d0381-l

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]

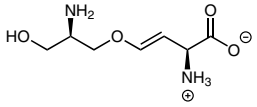


Figure 1 Rhizobitoxine (**1**)

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) synthase[10] 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 peptidomimetics[22] 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]

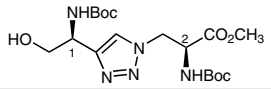
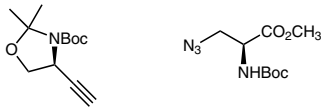


Figure 2 Protected triazole containing analogue of rhizobitoxine [(1*S*,2*S*)-**2**]

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).

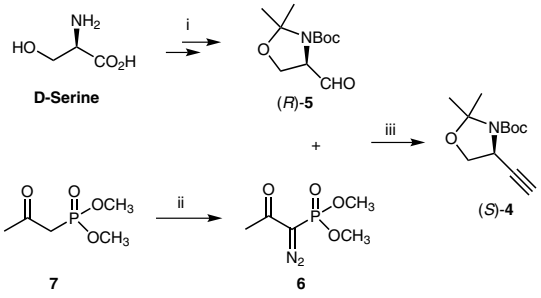
Alkyne (*S*)-**4** is an 'ethynylglycine synthon'.[29,30] It is synthesized from D-serine in six steps using Garner aldehyde (*R*)-**5**[31,32] as a key precursor (Scheme 1). Two principal methods have been described to synthesize alkyne **4** from



(*S*)-**4** (*S*)-**3**

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

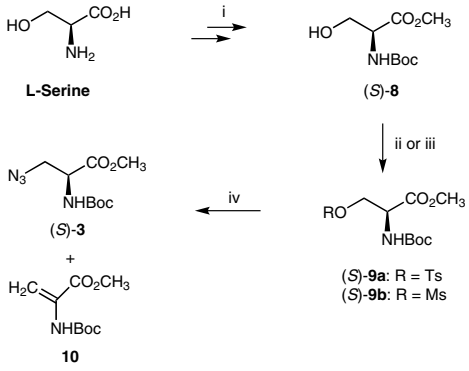
aldehyde **5**: the Bestmann–Ohira and the the Corey–Fuchs strategies.[29] We decided here to use the Bestmann–Ohira strategy using diazophosphonate **6** for this aldehyde-to-alkyne transformation, as this was well-known in our laboratory.[33] In 2002, we described the one-pot synthesis of ethynylglycine 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 strategy[35] 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) K_2CO_3, 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-Gonzales[37] 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, Et_3N, 4-DMAP (cat.), Me_3NHCl (cat.), CH_2Cl_2, 0 °C, 2 h, 68%; (iii) MsCl, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, 64%; (iv) NaN_3, DMF, see Table 1.

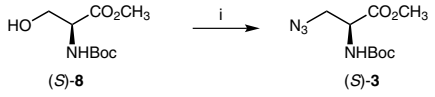
yield). The results of the transformation from (*S*)-**9a** and (*S*)-**9b** to (*S*)-**3** are summarized in Table 1. The best reaction conditions in our hands for nucleophilic substitution were using sodium azide in DMF at 70 °C for a short reaction time (Table 1, entry 2). Under these reaction conditions, 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** (NaN_3, 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_3, 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 NaN_3 Nucleophilic Substitution on (*S*)-**9a** and (*S*)-**9b**[a]

Entry	Starting material	T(°C)	Time (h)	Yield of (*S*)-3 (%)	Yield of 10 (%)
1	(*S*)-**9a**	20	5	33	35
2	(*S*)-**9a**	70	0.17	39	37
3	(*S*)-**9b**	20	24	25	55
4	(*S*)-**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).



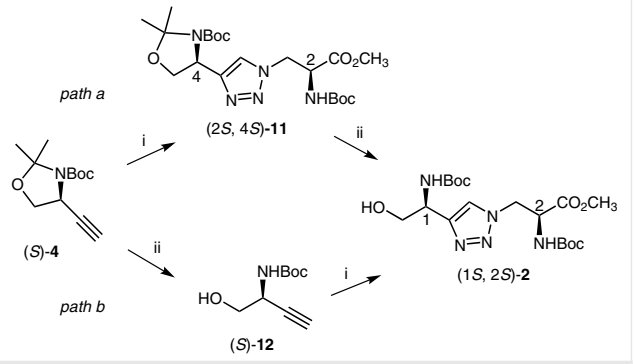
Scheme 3 *Reagents and conditions*: (i) Ph_3P/DIAD/DPPA (1.14/1.28/1.14), THF, 0 °C, 41%.

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 conditions[42] (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 methanol[43] 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*S*,4*R*)-**11**	10:1	99.5	(1*S*,2*S*)-**2**	11:1	99.5
(2*R*,4*S*)-**11**	16:1	96.2	(1*R*,2*R*)-**2**	10:1	99.5
(2*S*,4*S*)-**11**	14:1	99.5	(1*S*,2*R*)-**2**	14:1	99.5
(2*S*,4*R*)-**11**	9:1	99.5	(1*R*,2*S*)-**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

We gratefully thank the French 'Ministère de l'Éducation Nationale, de l'Enseignement Supérieur et de la Recherche' for financial support. Dr Nicolas Vanthuyne (Aix-Marseille Université, Plateforme de Chromatographie Chirale, ISM2 – UMR7313 – Chirosciences) is acknowledged for fruitful collaboration.

Supporting Information

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

References and Notes

(1) Berkowitz, D. B.; Charette, B. D.; Karukurichi, K. R.; McFadden, J. M. *Tetrahedron: Asymmetry* **2006**, *17*, 869.
(2) Erdman, L. W.; Johnson, H. W.; Clark, F. *Plant Dis. Rep.* **1956**, 646.
(3) Owens, L. D.; Wright, D. A. *Plant Physiol.* **1965**, *40*, 927.
(4) Okazaki, S.; Sugawara, M.; Yuhashi, K.-I.; Minamisawa, K. *Ann. Bot.* **2007**, *100*, 55.
(5) 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) Daumas, 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.; Bénimélis, 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) Dondoni, A.; Perrone, D. *Org. Synth.* **2000**, *77*, 64.
(33) Meffre, P.; Gauzy, L.; Perdigues, 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*, 11215.
(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.; Duttagupta, 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}-1*H*-1,2,3-triazol-1-yl)propanoate [(1*S*,2*S*)-2, (Scheme 4, Path a]**

To a solution of (2*S*, 4*S*)-**11** (0.337 g, 0.72 mmol) in MeOH (5 mL) was added PTSA·H_2O (0.137 g, 0.72 mmol). The reaction mixture was stirred for 2 h at room temperature and sat. aq $NaHCO_3$ 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 $NaHCO_3$ solution (40 mL), sat. aq NaCl solution (40 mL), dried over $MgSO_4$, 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 (1*S*, 2*S*)-**2** (0.053 g, 17%) as a white solid and recovered starting material (2*S*, 4*S*)-**11** (0.184 g, 55%).

Analytical Data

R_f = 0.26 (EtOAc); mp 55–57 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 [s, 18 H, $C(CH_3)_3$], 2.89 (br s, 1 H, OH), 3.79 (s, 3 H, CO_2CH_3), 3.84–3.87, 4.10–4.12 (2 m, 2 H, CH_2O), 4.70–4.86 (m, 4 H, 2 CH, CH_2N), 5.43 (br s, 1 H, NH), 5.59 (br s, 1 H, NH), 7.57 (s, 1 H, $CH_{triazole}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.4, 28.5 [2 s, 18 H, $C(CH_3)_3$], 48.2 (CH), 51.5 (CH_2N), 53.4 (CO_2CH_3), 53.9 (CH), 65.0 (CH_2O), 80.1 [$C(CH_3)_3$], 81.0 [$C(CH_3)_3$], 123.9 ($CH_{triazole}$), 147.1 ($C_{triazole}$), 155.2 (NCO_2), 155.8 (NCO_2), 169.5 (CO_2CH_3). $[\alpha]_D^{20}$ +49.9 (*c* 0.91, $CHCl_3$). HRMS (ES^+): *m/z* [M + H]$^+$ calcd for $C_{18}H_{32}N_5O_7$: 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 ^{1}H NMR and ^{13}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}-1*H*-1,2,3-triazol-1-yl)propanoate [(1*S*,2*S*)-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_2O (10 mL, 1:1, v/v). Sodium L-asborbate (0.068 g, 20 mol%) and $CuSO_4{\cdot}5H_2O$ (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_2O (70 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried over $MgSO_4$, 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 (1*S*,2*S*)-**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.