

Stereoselective Synthesis of Deuterated β -Cyclohexenylserine, a Biosynthetic Intermediate of the Salinosporamides[§]

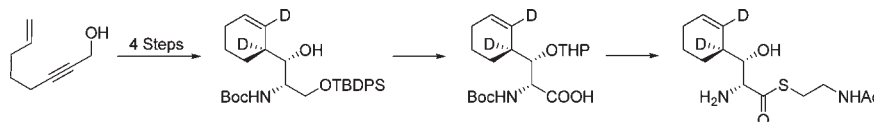
Jan Deska, Saskia Hähn, and Uli Kazmaier*

Universität des Saarlandes, Institut für Organische Chemie, Im Stadtwald, Geb. C4.2,
D-66123 Saarbrücken, Germany

u.kazmaier@mx.uni-saarland.de

Received April 27, 2011

ABSTRACT



A straightforward, highly stereoselective protocol toward the synthesis of deuterium-labeled (2*R*,3*S*,4*S*)- β -cyclohexenylserine has been developed. Key steps are a Nozaki–Hiyama–Kishi reaction generating the stereogenic centers and a ring-closing metathesis for the construction of the cyclohexenyl ring system. The labeled amino acid was further activated as an SNAc-ester for feeding experiments.

The proteasome, a hydrolytically active multienzyme complex, is responsible for the degradation of proteins in the cell, and it is a key player in the regulation of a wide range of cell processes.¹ Proteasome inhibitors are therefore interesting candidates for the development of antitumor agents. Most synthetic inhibitors are peptide-derived small molecules, interacting with a threonine in the active center of the proteasome which is involved in the hydrolysis of the target protein.² Typical inhibitors are peptidic

α -ketoamides,³ α -ketoaldehydes,⁴ epoxyketones,⁵ vinyl-sulfones,⁶ or boronic acids.⁷ Such proteasome inhibitors are also found widespread in nature, produced by a wide range of microorganisms. An important structural class are γ -lactam thioesters and γ -lactam- β -lacton bicycles respectively. (+)-Lactacystin, isolated by Omura from *Streptomyces lactacystineus*,⁸ was found to be a good inhibitor. However, actually, it was not lactacystin itself showing this effect, but its *clasto*-form omuralide (Figure 1).⁹ The strained β -lactone ring interacts with the catalytically active threonine, resulting in a covalent blocking of the enzyme.¹⁰ The same structural motive is also found in the families of the salinosporamides and cinnabaramides.

Salinosporamide was first isolated by Fenical et al. from the marine actinomycete *Salinispora tropica*,¹¹ which also

[§]Dedicated to Prof. Barry M. Trost on the occasion of his 70th birthday.

(1) (a) Goldberg, A. L. *Nature* **2003**, *426*, 895–899. (b) Goldberg, A. L. *Biochem. Soc. Trans.* **2007**, *35*, 12–17.

(2) Borissenko, L.; Groll, M. *Chem. Rev.* **2007**, *107*, 687–717.

(3) Chatterjee, S.; Dunn, D.; Mallya, S.; Ator, M. A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2603–2606.

(4) Lynas, J. F.; Harriott, P.; Healy, A.; McKervey, M. A.; Walker, B. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 373–378.

(5) (a) Meng, L.; Mohan, R.; Kwok, B. H. B.; Eloffson, M.; Sin, N.; Crews, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10403–10408. (b) Meng, L.; Kwok, B. H. B.; Sin, N.; Crews, C. M. *Cancer Res.* **1999**, *59*, 2798–2801.

(6) Bogyo, M.; McMaster, J. S.; Gaczynska, M.; Tortorella, D.; Goldberg, A. L.; Ploegh, H. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6629–6634.

(7) (a) Adams, J.; Behnke, M.; Chen, S.; Cruickshank, A. A.; Dick, L. R.; Grenier, L.; Klunder, J. M.; Ma, Y. T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333–338. (b) Adams, J.; Behnke, M.; Chen, S.; Cruickshank, A. A.; Grenier, L. R.; Klunder, J. M.; Ma, Y.-T.; Plamondon, L.; Stein, R. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 333–338. (c) Palombella, V. J.; Conner, E. M.; Fuseler, J. W.; Destree, A.; Davis, J. M.; Laroux, F. S.; Wolf, R. E.; Huang, J.; Brand, S.; Elliot, P. J.; Lazarus, D.; McCormack, T.; Parent, L.; Stein, R.; Adams, J.; Grisham, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15671–15676.

(8) Omura, S.; Fujimoto, T.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113–116.

(9) Dick, L. R.; Cruickshank, A. A.; Grenier, L.; Melandri, F. D.; Nunes, S. L.; Stein, R. L. *J. Biol. Chem.* **1996**, *271*, 7273–7275.

(10) (a) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, *268*, 726–731. (b) Groll, M.; Ditzel, L.; Löwe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463–471.

(11) Feling, R. H.; Buchanan, G. O.; Minzer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem.* **2003**, *115*, 369–371. *Angew. Chem., Int. Ed.* **2003**, *42*, 355–357.

(12) Reed, K. A.; Manam, R. R.; Mitchell, S. S.; Xu, J.; Teisan, S.; Chao, T. H.; Deyanat-Yazdi, G.; Neuteboom, S. T. C.; Lam, K. S.; Potts, B. C. M. *J. Nat. Prod.* **2007**, *70*, 269–276.

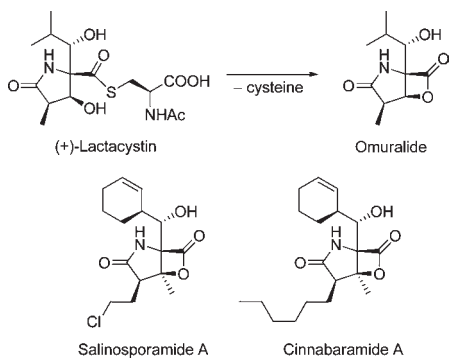


Figure 1. Natural products showing proteasome inhibition activity.

produces a wide range of closely related secondary metabolites.¹² In contrast, the cinnabaramides were found in the terrestrial strain *Streptomyces JS 360*.¹³ Typical representatives such as salinosporamide A and cinnabaramide A inhibit the 20S proteasome in the low nanomolar range^{13,14} and are therefore interesting candidates for antitumor therapies. Salinosporamide is currently in phase I clinical studies for the treatment of multiple myeloma.¹⁵ Therefore, it comes to no surprise that several total syntheses¹⁶ and formal syntheses,¹⁷ especially toward salinosporamide A, have been developed during the past few years.¹⁸ The unusual β -cyclohexenylserine as a central building block was either generated via nucleophilic attack of cyclohexenyl organometallics or of cyclohexanone enolates onto suitable protected serine-derived aldehydes.

(13) Stadler, M.; Bitzer, J.; Mayer-Bartschmid, A.; Müller, H.; Benet-Buchholz, J.; Gantner, F.; Tichy, H.-V.; Reinemer, P.; Bacon, K. B. *J. Nat. Prod.* **2007**, *70*, 246–252.

(14) (a) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, *268*, 726–731. (b) Groll, M.; Ditzel, L.; Löwe, J.; Stock, D.; Bochtler, M.; Bartunik, H. D.; Huber, R. *Nature* **1997**, *386*, 463–471.

(15) Chauhan, D.; Catley, L.; Li, G.; Podar, K.; Hideshima, T.; Velankar, M.; Mitsiades, C.; Mitsiades, N.; Yasui, H.; Letai, A.; Ovaio, H.; Berkers, C.; Nicholson, B.; Chao, T.-H.; Neuteboom, S. T. C.; Richardson, P.; Palladino, M. A.; Anderson, K. C. *Cancer Cell* **2005**, *8*, 407–419.

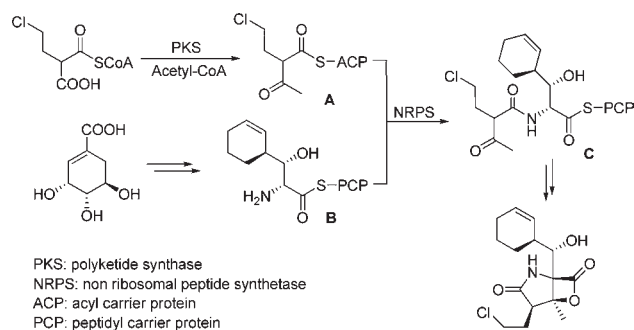
(16) (a) Reddy, L. R.; Saravanan, P.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 6230–6231. (b) Endo, A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 8298–8299. (c) Mulholland, N. P.; Pattenden, G.; Walters, I. A. S. *Org. Biomol. Chem.* **2006**, *4*, 2845–2846. (d) Ma, G.; Nguyen, H.; Romo, D. *Org. Lett.* **2007**, *9*, 2143–2146. (e) Ling, T.; Macherla, V. R.; Manam, R. R.; McArthur, K. A.; Potts, B. C. M. *Org. Lett.* **2007**, *9*, 2289–2292. (f) Mulholland, N. P.; Pattenden, G.; Walters, I. A. S. *Org. Biomol. Chem.* **2008**, *6*, 2782–2789. (g) Takahashi, K.; Midori, M.; Kawano, K.; Ishihara, J.; Hatakeyama, S. *Angew. Chem.* **2008**, *120*, 6340–6342. *Angew. Chem., Int. Ed.* **2008**, *47*, 6244–6246. (h) Fukuda, T.; Sugiyama, K.; Arima, S.; Harigaya, Y.; Nagamitsu, T.; Omura, S. *Org. Lett.* **2008**, *10*, 4239–4242. (i) Nguyen, H.; Ma, G.; Romo, D. *Chem. Commun.* **2010**, *46*, 4803–4805.

(17) (a) Caubert, V.; Langlois, N. *Tetrahedron Lett.* **2006**, *47*, 4473–4475. (b) Caubert, V.; Massé, J.; Retailliau, P.; Langlois, N. *Tetrahedron Lett.* **2007**, *48*, 381–384. (c) Margalef, I. V.; Rupnicki, L.; Lam, H. W. *Tetrahedron Lett.* **2008**, *64*, 7896–7901. (d) Mosey, R. A.; Tepe, J. J. *Tetrahedron Lett.* **2009**, *50*, 295–297. (e) Momose, T.; Kaiya, Y.; Hasegawa, J.-I.; Sato, T.; Chida, N. *Synthesis* **2009**, 2983–2991.

(18) Shibasaki, M.; Kanai, M.; Fukuda, N. *Chem. Asian J.* **2007**, *2*, 20–38.

The developments in this field were excellently covered by a recent review by Gulder and Moore.¹⁹

Scheme 1. Biosynthesis of Salinosporamide A According to Moore²⁰



The group of Moore also investigated the biosynthesis of the salinosporamides (Scheme 1).²⁰ In principle, the molecule can be divided into two major building blocks. The chlorinated activated β -keto thioester **A** is obtained from a tetrose via chloroethylmalonyl-CoA, which is coupled with acetyl-CoA using a polyketide synthase (PKS). The second building block **B**, a β -cyclohexenylserine, is obtained via a modified shikimate pathway. Both building blocks were coupled on a nonribosomal peptide synthetase (NRPS) giving **C**, which undergoes cyclizations toward salinosporamide A. In the original publication, a (2*R*)-configuration was shown for the unusual amino acid **B**.²⁰ Later on, during investigations of the enzymes involved in this biosynthesis, this configuration was assigned to be 2*S*.²¹

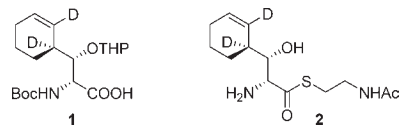


Figure 2. Deuterated β -cyclohexenylserine derivatives.

Our group has been involved in the synthesis of unusual amino acids for several years,²² and for biosynthetic studies we were interested in a stereoselective synthesis of a dideuterated cyclohexenylserine derivative for feeding experiments. We began our investigations shortly after the proposal by Moore²⁰ in 2007. Therefore, our targets were the protected (2*R*,3*S*,4*S*)-configured amino acid **1** and the deprotected activated derivative **2** (Figure 2).

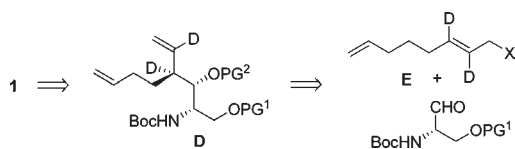
(19) Gulder, T. A. M.; Moore, B. S. *Angew. Chem.* **2010**, *122*, 9534–9556. *Angew. Chem., Int. Ed.* **2010**, *49*, 9346–9367.

(20) Beer, L. L.; Moore, B. S. *Org. Lett.* **2007**, *9*, 845–848.

(21) McGlinchey, R. P.; Nett, M.; Eustáquio, A. S.; Asolkar, R. N.; Fencial, W.; Moore, B. S. *J. Am. Chem. Soc.* **2008**, *130*, 7822–7823.

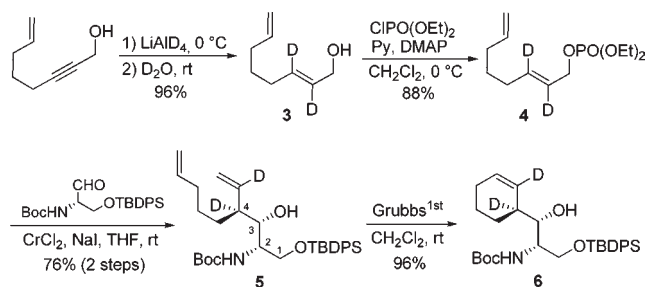
(22) (a) Kazmaier, U. *Amino Acids* **1996**, *11*, 283. (b) Deska, J.; Kazmaier, U. *Curr. Org. Chem.* **2008**, *12*, 355–385. (c) Kazmaier, U. In *Frontiers in Asymmetric Synthesis and Application of α -Amino Acids*; Soloshonok, V. A., Izawa, K., Eds.; American Chemical Society: Washington, DC, 2009; p 157 and references cited therein.

Scheme 2. Retrosynthesis of Deuterated Cyclohexenylserine Derivatives



According to Scheme 2, we planned to obtain the cyclohexenyl ring via a ring-closing metathesis of the deuterated linear precursor **D**, accessible by a stereoselective Nozaki–Hiyama–Kishi (NHK) reaction²³ of deuterated diene **E** and protected serinal.

Scheme 3. Stereoselective Synthesis of Protected Cyclohexenylserinol **6**



The starting point for the synthesis of the dideuterated dienyl substrate was oct-7-en-2-ynol, easily accessible from propargylic alcohol and 5-bromopentene.²⁴ Stereoselective reduction with LiAlH_4 , followed by hydrolysis with D_2O , gave rise to the dideuterated allyl alcohol **3** in almost quantitative yield (Scheme 3). This alcohol was converted into the corresponding phosphate **4** for the subsequent NHK reaction. The phosphate has been chosen because phosphates are very convenient allylation reagents, showing a better stability than the corresponding bromides or even chlorides.²⁵ For the subsequent allylation reaction protected serinal was prepared fresh by Dibal-H reduction of the corresponding methyl ester and was used without further purification. In initial experiments using phosphate **4** alone, only low yields of the allylation product **5** were obtained. The situation changed significantly after addition of NaI, and the NHK reaction provided dideuterated homoallyl alcohol **5** in good yield. The corresponding more reactive allyl iodide is probably formed in situ.

(23) (a) Takai, K.; Kimura, K.; Kuroda, T.; Hiyama, T.; Nozaki, H. *Tetrahedron Lett.* **1983**, *24*, 5281–5284. (b) Jin, H.; Uenishi, J.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644–5646.

(24) (a) Mendès, C.; Renard, S.; Rofoo, M.; Roux, M.-C.; Rousseau, G. *Eur. J. Org. Chem.* **2003**, 463–471. (b) Bagutski, V.; Moszner, N.; Zeuner, F.; Fischer, U. K.; de Meijere, A. *Adv. Synth. Catal.* **2006**, *348*, 2133–2147.

(25) (a) Yanagisawa, A.; Noritake, J.; Nomura, N.; Yamamoto, H. *Synlett* **1991**, 251–253. (b) Yanagisawa, A.; Nomura, N.; Yamamoto, H. *Synlett* **1991**, 513–514. (c) Nowotny, S.; Tucker, C. E.; Jubert, C.; Knochel, P. *J. Org. Chem.* **1995**, *60*, 2762–2772.

Subsequent ring-closing metathesis provided the protected cyclohexenylserinol **6** in almost quantitative yield.

The allylation product **5** was obtained with high 3,4-*anti*-diastereoselectivity (92% ds), which can be explained by a chairlike transition state (Figure 3)²⁶ and is in good agreement with literature precedence.²⁷ Out of the four possible stereoisomers, only two were observed by HPLC. Obviously, the induced diastereoselectivity from the protected (*S*)-serinal was almost perfect.

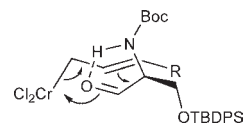


Figure 3. Transition state of the Nozaki–Hiyama–Kishi reaction.

For analytical purposes and to determine the relative 2,3-stereochemistry, we converted **6** into the corresponding oxazolidinone **7** by treatment with NaH. The TBDPS protecting group was not stable under these reaction conditions, and therefore, it was replaced by the TBDMS group. The ring protons of *anti*-disubstituted oxazolidinones typically show coupling constants J of 4–6 Hz, while $J = 7–9$ Hz are found in the corresponding *syn* derivatives.²⁸ A coupling constant of $J = 4.3$ Hz for **7** clearly indicates an *anti*-configuration at C_2 and C_3 of **7**, corresponding to a *syn* substitution pattern of **6**. (Figure 4).

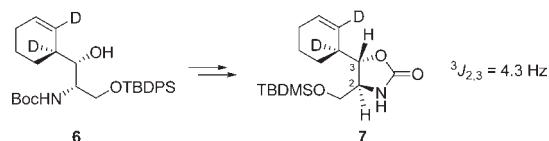


Figure 4. Determination of configuration.

The formation of this stereoisomer can be explained by a hydrogen bond in the preferred conformation of the serinal derivative, resulting in a *syn* periplanar orientation of the amide and the carbonyl functionality and a nucleophilic attack of the chromium reagent from the *Si*-face (Figure 3). Obviously this attack is highly favored because only the

(26) Ciapetti, P.; Falorni, M.; Taddei, M. *Tetrahedron* **1996**, *52*, 7379–7390.

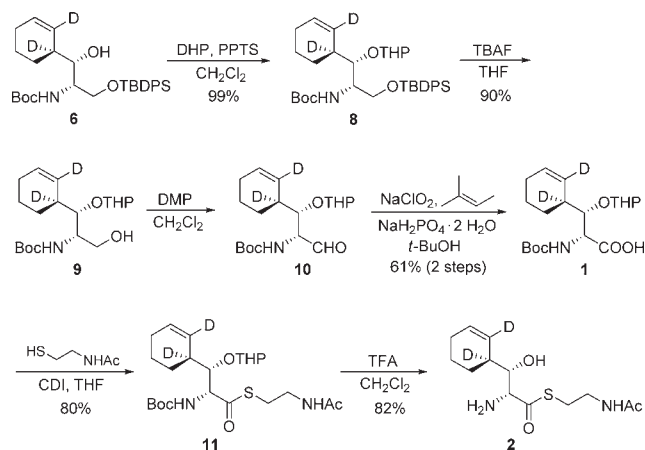
(27) For general reviews on reactions of crotylmethyl reagents with carbonyl, see: (a) Hoffmann, R. W. *Angew. Chem.* **1982**, *94*, 569–580. (b) Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207–2293.

(28) Futagawa, S.; Inui, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3308–3310.

(29) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.

(30) Bal, B. S.; Childers, W. E.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091–2096.

Scheme 4. Synthesis of Protected Amino Acid **1** and SNAc-ester **2**



formation of the *anti*-oxazolidinone was observed by NMR.

To complete the syntheses of **1** and **2** the free OH-group of **6** was protected with a THP group (Scheme 4). Two diastereomers of **8** were formed as a 1:1 mixture, which are separable by flash chromatography. Deprotection of the silyl protecting group with TBAF gave rise to the primary alcohol **9**, which could be oxidized to the required amino acid **1**. In principle, PDC oxidation gave the expected

product in good yield, but it was difficult to remove the chromium completely.

Because **1** should be applied to biological systems, we tried to avoid contamination with traces of toxic heavy metal. Therefore, we decided to use a metal-free two step oxidation protocol. Oxidation with Dess–Martin periodinane²⁹ gave rise to aldehyde **10**, which was further oxidized to **1** with NaClO₂ and 2-methyl-2-butene according to Pinnick.³⁰

For the synthesis of **2**, amino acid **1** was coupled with *N*-acetylcysteamine to **11** prior to the simultaneous removal of the remaining protecting groups by trifluoroacetic acid (TFA).

In conclusion, we have shown that the Nozaki–Hiyama–Kishi reaction is a highly suitable tool for the stereoselective synthesis of cyclohexenylserine derivatives, including deuterium-labeled ones. Further investigations of the (2*S*)-configured isomer and other deuterium-labeled biosynthetic intermediates are currently in progress.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

Supporting Information Available. Analytical and spectroscopic data of all new products. This material is available free of charge via the Internet at <http://pubs.acs.org>.