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

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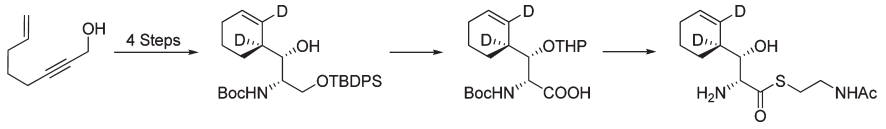
ABSTRACT



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] vinylsulfones,[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 actimomycete *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.; Elofsson, 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.; Cruikshank, 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.

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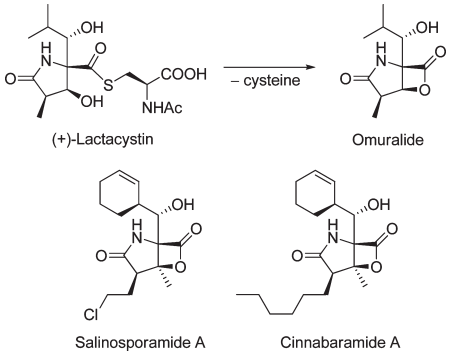
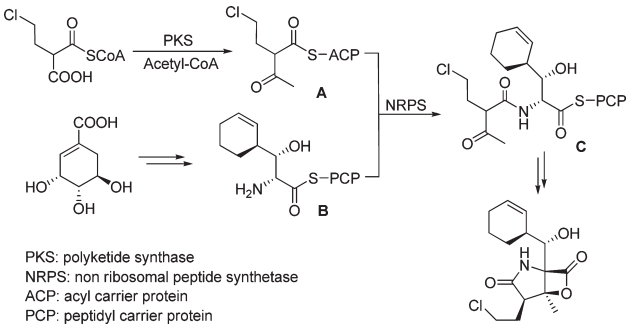


Figure 1. Natural products showing proteasome inhibition activity.

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 syntheses[16] 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. 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 Moore[20]



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]

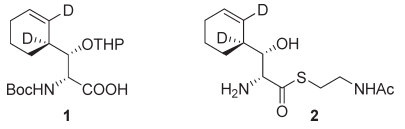


Figure 2. Deuterated β-cyclohexenylserine derivatives.

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 Moore[20] 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).

(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.; Ovao, 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.; Retailleau, 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.

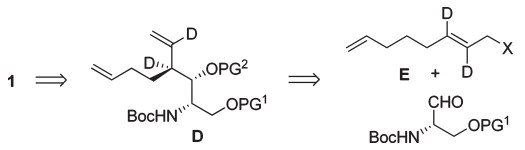
(19) Gulder, T. A. M.; Moore, B. S. *Angew. Chem.* **2010**, *122*, 9534–9556. *Angew. Chem., Int. Ed.* **2010**, *49*, 9346–9367.

(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.

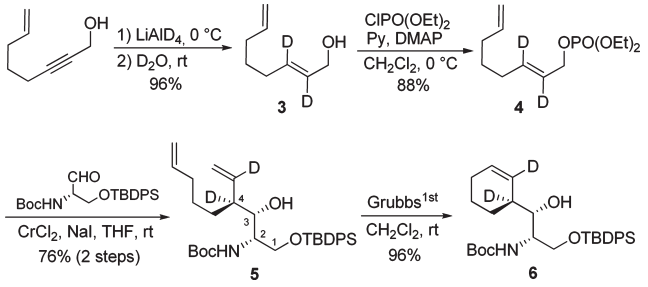
(22) (a) Kazmaier, U. *Amino Acids* **1996**, *11*, 283. (b) Deska, J.; Kazmaier, U. *Curr. Org. Chem.* **2008**, *12*, 355–385. (c) Kazmaier, U. In *Frontiers in Asymmetric Synthesis and Application of α-Amino Acids*; Soloshonok, V. A., Izawa, K., Eds.; American Chemical Society: Washington, DC, 2009; 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) reaction[23] 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. 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.

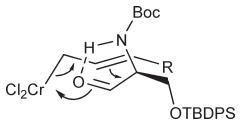


Figure 3. Transition state of the Nozaki–Hiyama–Kishi reaction.

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).

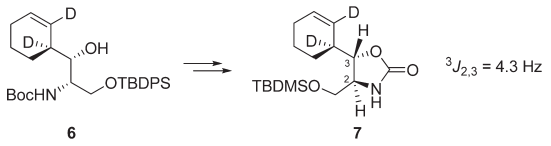


Figure 4. Determination of configuration.

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

(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.

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

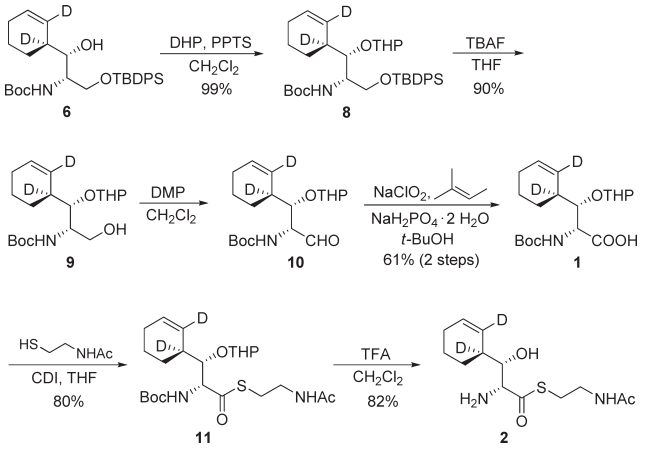
(27) For general reviews on reactions of crotylmetal reagents with carbonyl, see: (a) Hoffmann, R. W. *Angew. Chem.* **1982**, *94*, 569–580. (b) Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207–2293.

(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 periodinane[29] gave rise to aldehyde **10**, which was further oxidized to **1** with $NaClO_2$ 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.

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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.