Natural Products

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Total Synthesis and Stereochemical Assignment of Baringolin**

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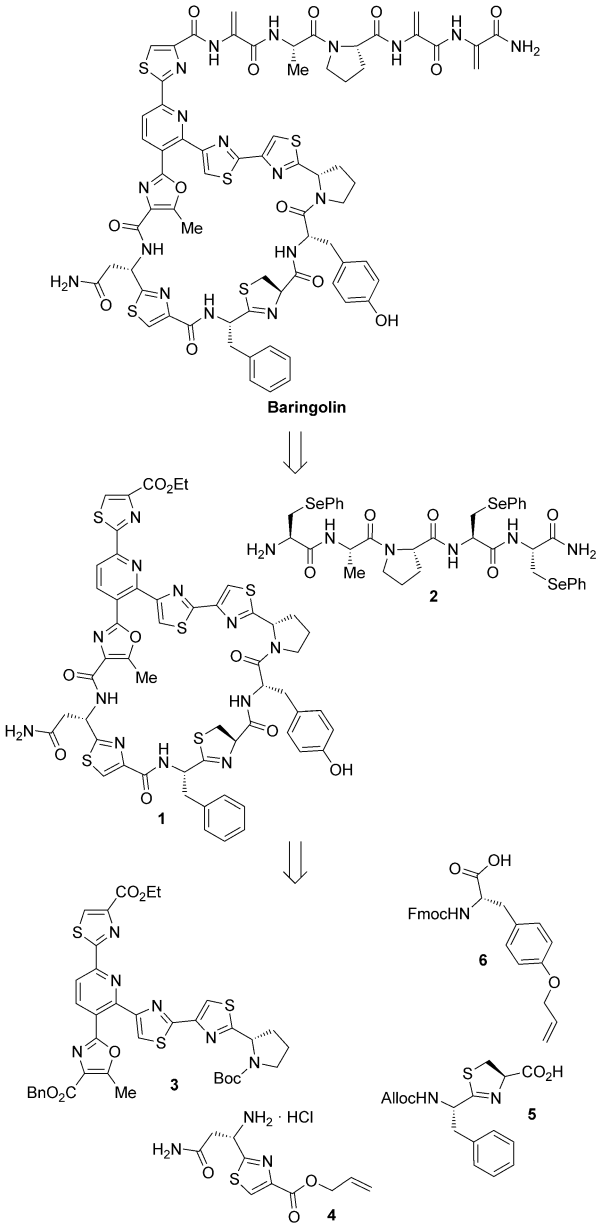
Thiopeptides are a family of naturally occurring, peptide-derived molecules with high sulfur content formed by a central nitrogen-containing six-membered heterocycle decorated with many azoles in a macrocyclic array.[1,2] These natural products have drawn the attention of many research groups mainly owing to their interesting antibiotic activities[3] and their challenging structures.[4] One member of this family, thiostrepton, which is an ingredient of Panolog, has reached the market.

Baringolin is a novel thiopeptide of the *d* series,[1] and thus contains a central 2,3,6-trisubstituted pyridine (for structure see Scheme 1). It was isolated by Biomar SA from fermentation of the marine-derived bacterium *Kucuria sp* MI-67-EC3-038 strain of the Micrococcaceae family, found at the coast of Alicante (southern Spain). Important antibacterial activity at nanomolar concentrations was found in several strains, such as *Staphylococcus aureus*, *Micrococcus luteus, Propionibacterium acnes*, and *Bacillus subtilis*.[5] The structure of baringolin was established by spectroscopic methods.[6] The macrocycle in baringolin contains, in addition to three natural amino acids (Tyr, Phe, and Asn), a pyridine, three thiazoles, a methyloxazole ring, and also some motifs not present in other thiopeptides at the same time, such as a thiazoline with an α-chiral center and a pyrrolidine motif derived from a Pro residue. A long peptidic tail is attached to the pyridine through a fourth thiazole. This tail is a pentapeptide containing three methylidenes resulting from dehydration of Ser.[7] Surprisingly and to our knowledge, this is the longest tail reported to date for this family of antibiotics. Baringolin



Scheme 1. Retrosynthesis of baringolin. Boc = *tert*-butyloxycarbonyl, Alloc = allyloxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl.

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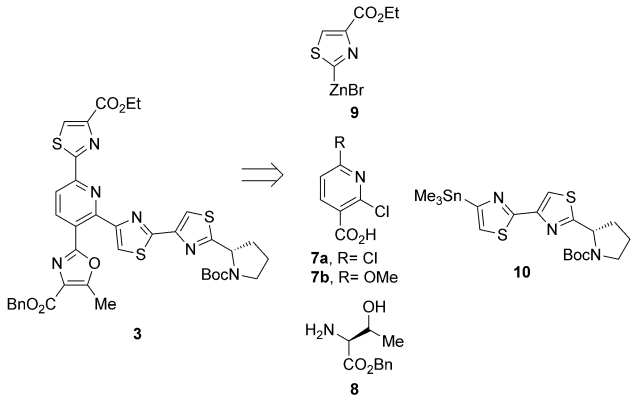
contains seven stereocenters, the configuration of which was considered to be that of natural L-amino acids. A thorough review of literature precedents shows that all thiopeptides of which the stereochemistry has been confirmed to date are made out of L-amino acids; this finding is consistent with their ribosomal origin.[7]

The aim of this work was not only to synthesize this new entity, but also to develop a synthetic strategy that would fulfill our aspiration for an easy construction of closely related new entities to evaluate the structure-activity relationships (SAR) of this interesting family of antibiotics. Moreover, this first synthesis should also serve as the ultimate confirmation of the structure and stereochemical assignment of baringolin. With this premise in mind, the total synthesis of baringolin was designed using only commercially available L-amino acids as the sole source of chirality to confirm if the previous hypothesis was correct.

The retrosynthetic analysis started with the disconnection of the peptidic tail (Scheme 1) to give two synthetic fragments, macrocycle **1** and pentapeptide **2**. In turn, macrocycle **1** could be obtained from trisubstituted pyridine **3** and building blocks **4**–**6**. The concourse of orthogonal protecting groups was key to the success of the synthesis of these complex molecules. This was clearly evidenced in the structure of **3**.

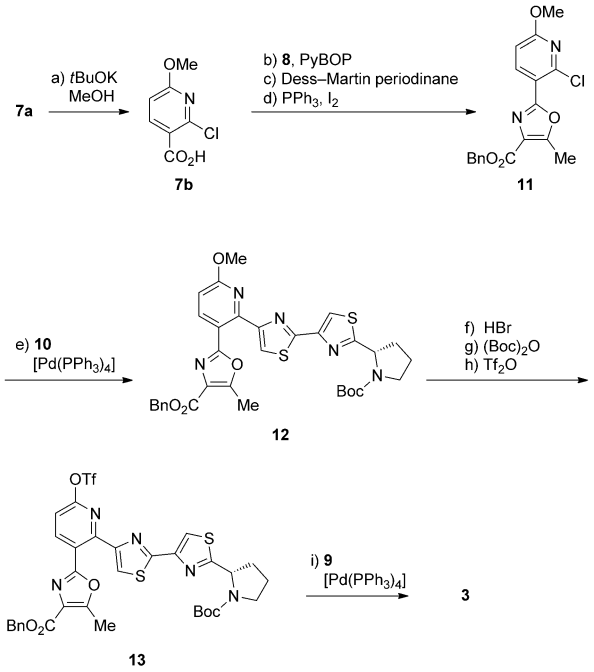
First of all, the synthesis of the central polyheterocyclic core **3** was attempted. A cross-coupling-based strategy[2,8] was chosen, since it would offer a modular approach to the target structure (Scheme 2). The synthetic approach was based on the chemoselective derivatization of commercial 2,6-dichloronicotinic acid (**7a**), which can be easily converted into 2-chloro-6-methoxynicotinic acid (**7b**),[9] which contains two differentiated α-positions along with a carboxylic acid that serves as a precursor of the methyloxazole motif.



Scheme 2. Retrosynthesis of pyridine building block **3**.

The other building blocks for the construction of **3** were benzyl-protected Thr **8**, zinc thiazole **9**,[10] and bithiazole pyrrolidine **10**. The synthesis of the later has been recently reported by us,[11] and it was prepared as a suitable building block for a cross-coupling-based strategy.

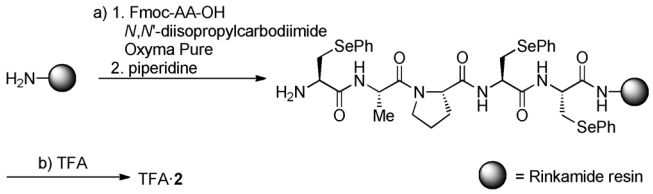
Transformation of pyridine carboxylic acid **7b** into pyridine oxazole **11** (Scheme 3) was performed by condensation with Thr **8**, followed by Dess–Martin oxidation of the side chain into the corresponding methyl ketone and subsequent cyclization to yield the desired biaryl **11**.[12] Stille cross-coupling between chloropyridine **11** and enantiopure trimethyltin derivative **10** rendered methoxypyridine **12**, which could be converted into triflate **13** after acidolysis of the methoxy group. Lastly, **13** was subjected to Negishi cross-coupling conditions with thiazole zinc bromide **9** to render quantitatively the desired central polyheterocyclic core **3**, which was suitably functionalized for subsequent orthogonal deprotections.



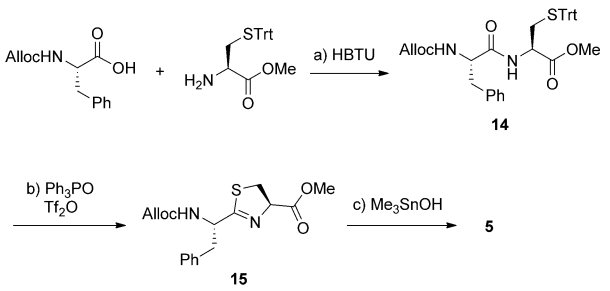
Scheme 3. Synthesis of pyridine building block **3**. Reagents and conditions: a) *t*BuOK, MeOH, 65 °C, 4 days, 85 %; b) **8**, PyBOP, DIPEA, THF, 0 °C, 3 h, 89 %; c) Dess–Martin periodinane, CH_2Cl_2, RT, 6 h, 95 %; d) PPh_3, I_2, NEt_3, CH_2Cl_2, 0 °C to RT, 15 h, 78 %; e) **10**, [Pd(PPh_3)$_4$], 1,4-dioxane, 80 °C, 48 h, 88 %; f) HBr, AcOH, RT, 28 h, 73 %; g) $(Boc)_2O$, NEt_3, CH_2Cl_2, 0 °C, 4 h, 94 %; h) Tf_2O, 2,6-lutidine, DMAP, CH_2Cl_2, 0 °C to RT, 3 h, 88 %; i) **9**, [Pd(PPh_3)$_4$], DMA, 45 °C, 1 h, quant. PyBOP = (1*H*-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate, DIPEA = diisopropylethylamine, DMAP = 4-(dimethylamino)pyridine, DMA = dimethylacetamide.

Construction of the pentapeptide tail **2** was carried out by solid-phase peptide synthesis (SPPS) using Fmoc chemistry and Rinkamide ChemMatrix resin,[13] using L-alanine and L-proline, as well as Fmoc-L-phenylselenocysteine[14] as precursor of dehydroalanine residues (Scheme 4). Condensation of the different Fmoc-protected amino acids (Fmoc-AA-OH) was carried out with *N*,*N*′-diisopropylcarbodiimide and Oxyma Pure[15] as coupling agents. Deprotection before the introduction of a new Fmoc-AA-OH was achieved with piperidine. The final cleavage with trifluoroacetic acid (TFA) afforded pentapeptide **2** with the free amine and a C-terminal amide ready for condensation with the carboxylic acid of the macrocycle in the last steps of the synthetic process.

Thiazole **4** was prepared by protecting-group manipulation of a previously described synthon (see the Supporting



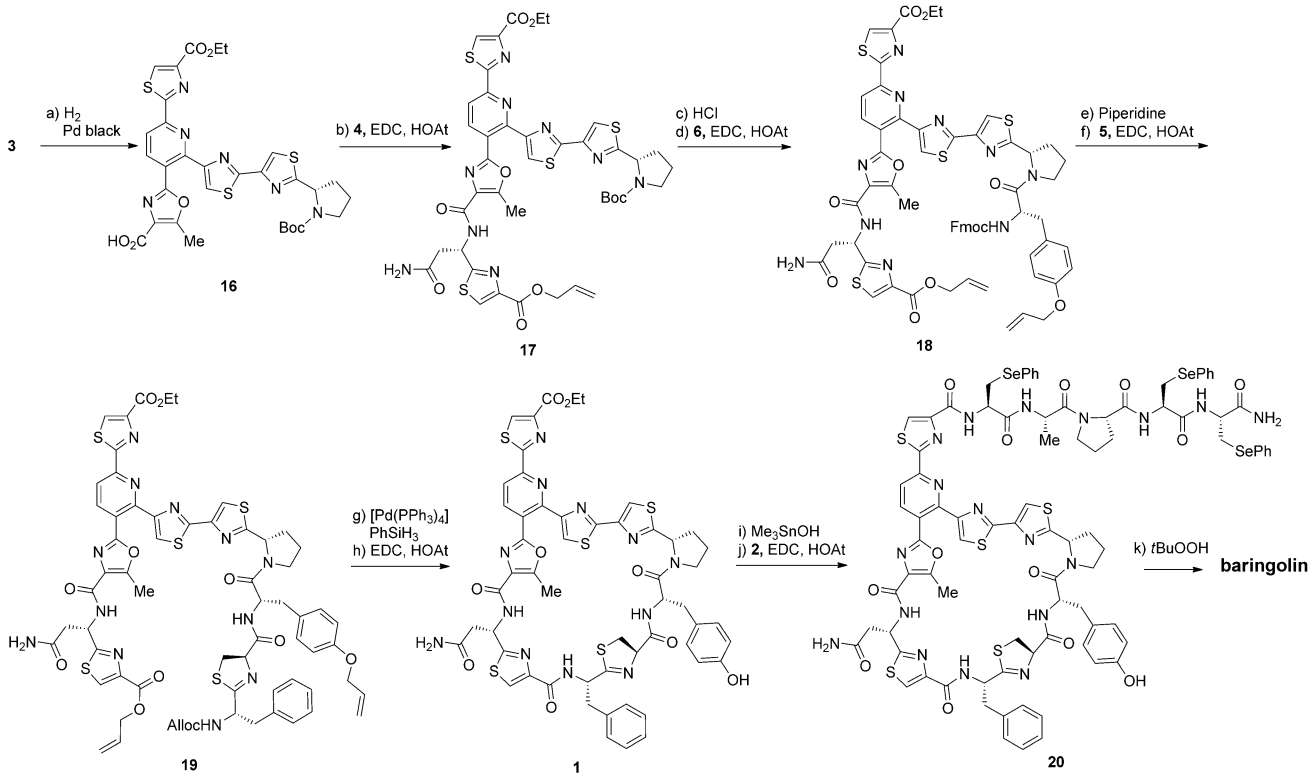
Scheme 4. Synthesis of pentapeptide **2**. Reagents and conditions: a) 1. Fmoc-AA-OH, *N*,*N*′-diisopropylcarbodiimide, Oxyma Pure, DMF, RT, 1.5 h; 2. 20% piperidine in DMF, RT (4 treatments); b) 95% TFA in CH_2Cl_2, RT (4 treatments), 89%.



Scheme 5. Synthesis of thiazoline **5**. Reagents and conditions: a) HBTU, DIPEA, CH_2Cl_2, RT, 1 h, 94%; b) Ph_3PO, Tf_2O, CH_2Cl_2, −20°C, 2 h, 86%; c) Me_3SnOH, CH_2Cl_2, 60°C, 4 h. HBTU = *O*-(1*H*-benzotriazoyl-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, Trt = trityl.

Information).[16] The last building block was the Phe-derived thiazoline **5**, the synthesis of which was addressed by cyclization of the corresponding dipeptide **14** (Scheme 5).[17] Both the condensation and the cyclization steps yielded products **14** and **15** in excellent yields. Further saponification of the methyl ester with trimethyltin hydroxide[18] afforded acid **5** in an excellent diastereomeric ratio (d.r. 96:4).

Next, it was taken into consideration that the thiazoline moiety is prone to epimerization under both basic and acidic conditions, and its manipulation should be limited to as few steps as possible. Deprotection of the carboxylic acid of **3** by hydrogenolysis of the Bn ester was performed with excellent conversion by using Pd black (Scheme 6).[4j,19] Acid **16** was condensed with Asn-derived thiazole **4** to yield **17** by using EDC and HOAt as coupling agents, which would become the reagents of choice for further amide formations. Fmoc-Tyr-OH **6** was introduced next after elimination of the Boc group at the pyrrolidine ring in **17**. Fmoc removal under standard conditions and subsequent condensation with thiazoline **5** rendered **19**, the protected open form of the macrocycle. All allyl-based protecting groups of **19** were simultaneously removed by using catalytic $[Pd(PPh_3)_4]$ and the crude was subjected to macrocyclization conditions in the absence of base, yielding the desired product **1**. Ethyl ester hydrolysis of **1** was carried out by using trimethyltin hydroxide to avoid epimerization of the thiazoline moiety under more common



Scheme 6. Total synthesis of baringolin. Reagents and conditions: a) H_2 (1 atm), Pd black, CH_2Cl_2/EtOH (1:1), RT, 4 h, quant.; b) **4**, EDC, HOAt, DIPEA, DMF, 0°C, 18 h, 82%; c) HCl, dioxane, RT, 7 h; d) **6**, EDC, HOAt, DIPEA, DMF, 0°C, 7 h, 71% (2 steps); e) piperidine, CH_2Cl_2, RT, 3 h, 87%; f) **5**, EDC, HOAt, DIPEA, DMF, 0°C, 3 h, 68% (2 steps); g) $[Pd(PPh_3)_4]$, $PhSiH_3$, CH_2Cl_2, RT, 7 h; h) EDC, HOAt, DMF (1 mM), 0°C to RT, 21 h, 30% (2 steps); i) Me_3SnOH, $ClCH_2CH_2Cl$, 60°C, 19 h; j) **2**, EDC, HOAt, DIPEA, DMF, 0°C, 3 h, 81% (2 steps); k) *t*BuOOH, CH_2Cl_2, RT, 12 h, 66%. EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, HOAt = 1-hydroxy-7-azabenzotriazole.

and drastic basic aqueous conditions.[18] Condensation of the resulting acid with pentapeptide **2** yielded **20** in excellent yield. Finally, oxidation with *tert*-butyl hydroperoxide and in situ elimination of the resulting phenylselenide oxide groups at room temperature rendered baringolin.

Coelution with a natural product sample of baringolin and comparison of their NMR spectra showed that both compounds are identical (see the Supporting Information). Biological assessment against different strains of methicillin-resistant *S. aureus* (MRSA) showed a minimum inhibitory concentration (MIC) in the nanomolar range for both the natural and synthetic compounds. These results confirm that the structure of baringolin is based only on L-amino acids, as other precedent thiopeptides are.

In conclusion, the total synthesis of baringolin was achieved by a convergent strategy with a good overall yield. The developed convergent synthetic procedure is especially suitable for the preparation of baringolin analogues for SAR-studies, which are currently ongoing. Furthermore, it could also be applied to the preparation of other complex peptides of the same family.

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[1] M. C. Bagley, J. W. Dale, E. Merritt, X. Xiong, *Chem. Rev.* **2005**, *105*, 685 – 714.

[2] R. A. Hughes, C. J. Moody, *Angew. Chem.* **2007**, *119*, 8076 – 8101; *Angew. Chem. Int. Ed.* **2007**, *46*, 7930 – 7954.

[3] a) F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, *Angew. Chem.* **2006**, *118*, 5194 – 5254; *Angew. Chem. Int. Ed.* **2006**, *45*, 5072 – 5129; b) A. L. Demain, *Med. Res. Rev.* **2009**, *29*, 821 – 842.

[4] For a selection of landmark thiopeptides central core constructions and total syntheses, see: a) T. R. Kelly, A. Echevarren, N. S. Chandrakumar, Y. Köksal, *Tetrahedron Lett.* **1984**, *25*, 2127 – 2130; b) T. R. Kelly, C. T. Jagoe, Z. Gu, *Tetrahedron Lett.* **1991**, *32*, 4263 – 4266; c) T. R. Kelly, F. Lang, *Tetrahedron Lett.* **1995**, *36*, 5319 – 5322; d) M. Ciufolini, Y.-C. Shen, *J. Org. Chem.* **1997**, *62*, 3804 – 3805; e) M. C. Bagley, K. E. Bashford, C. L. Hesketh, C. J. Moody, *J. Am. Chem. Soc.* **2000**, *122*, 3301 – 3313; f) C. J. Moody, R. A. Hughes, S. P. Thompson, L. Alcaraz, *Chem. Commun.* **2002**, 1760 – 1761; g) K. C. Nicolaou, B. S. Safina, M. Zak, A. A. Estrada, S. H. Lee, *Angew. Chem.* **2004**, *116*, 5197 – 5202; *Angew. Chem. Int. Ed.* **2004**, *43*, 5087 – 5092; h) K. C. Nicolaou, M. Zak, B. S. Safina, S. H. Lee, A. A. Estrada, *Angew. Chem.* **2004**, *116*, 5202 – 5207; *Angew. Chem. Int. Ed.* **2004**, *43*, 5092 – 5097; i) G. Heckmann, T. Bach, *Angew. Chem.* **2005**, *117*, 1223 – 1226; *Angew. Chem. Int. Ed.* **2005**, *44*, 1199 – 1201; j) R. A. Hughes, S. P. Thompson, L. Alcaraz, C. J. Moody, *J. Am. Chem. Soc.* **2005**, *127*, 15644 – 15651; k) M. C. Bagley, K. Chapaneri, J. W. Dale, X. Xiong, J. Bower, *J. Org. Chem.* **2005**, *70*, 1389 – 1399; l) J.-Y. Lu, H.-D. Arndt, *J. Org. Chem.* **2007**, *72*, 4205 – 4212; m) H. M. Müller, O. Delgado, T. Bach, *Angew. Chem.* **2007**, *119*, 4855 – 4858; *Angew. Chem. Int. Ed.* **2007**, *46*, 4771 – 4774; n) D. Lefranc, M. Ciufolini, *Angew. Chem.* **2009**, *121*, 4262 – 4265; *Angew. Chem. Int. Ed.* **2009**, *48*, 4198 – 4201.

[5] "Peptides as bioactive compounds": L. M. Canedo Hernandez, F. Romero Millan, A. Fernandez Medarle, R. I. Fernandez Chimeno, J. C. Hidalgo Villar (Instituto Biomar, S.A., Spain), WO 2012062906, May 18, **2012**.

[6] 1D- and 2D- ^{1}H and ^{13}C NMR spectra, as well HRMS of natural sample.

[7] a) H.-D. Arndt, S. Schoof, J. Y. Lu, *Angew. Chem.* **2009**, *121*, 6900 – 6904; *Angew. Chem. Int. Ed.* **2009**, *48*, 6770 – 6773; b) C. Li, W. L. Kelly, *Nat. Prod. Rep.* **2010**, *27*, 153 – 164.

[8] a) T. Bach, S. Heuser, *Angew. Chem.* **2001**, *113*, 3283 – 3284; *Angew. Chem. Int. Ed.* **2001**, *40*, 3184 – 3185; b) T. Bach, S. Heuser, *J. Org. Chem.* **2002**, *67*, 5789 – 5795; c) S. Schröter, C. Stock, T. Bach, *Tetrahedron* **2005**, *61*, 2245 – 2267.

[9] Y. Hirokawa, I. Fujiwara, K. Suzuki, H. Harada, T. Yoshikawa, N. Yoshida, S. Kato, *J. Med. Chem.* **2003**, *46*, 702 – 715.

[10] **9** was prepared following the procedure described in: O. Delgado, H. M. Müller, T. Bach, *Chem. Eur. J.* **2008**, *14*, 2322 – 2329.

[11] X. Just-Baringo, P. Bruno, F. Albericio, M. Álvarez, *Tetrahedron Lett.* **2011**, *52*, 5435 – 5437.

[12] P. Wipf, C. P. Miller, *J. Org. Chem.* **1993**, *58*, 3604 – 3606.

[13] F. García-Martín, M. Quintanar-Audelo, Y. García-Ramos, L. J. Cruz, C. Gravel, R. Furic, S. Côté, J. Tulla-Puche, F. Albericio, *J. Comb. Chem.* **2006**, *8*, 213 – 220.

[14] N. M. Okeley, Y. Zhu, W. A. van der Donk, *Org. Lett.* **2000**, *2*, 3603 – 3606.

[15] R. Subirós-Funosas, R. Prohens, R. Barbas, A. El-Faham, F. Albericio, *Chem. Eur. J.* **2009**, *15*, 9394 – 9403.

[16] P. Bruno, S. Peña, X. Just-Baringo, F. Albericio, M. Álvarez, *Org. Lett.* **2011**, *13*, 4648 – 4651.

[17] S.-L. You, H. Razavi, J. W. Kelly, *Angew. Chem.* **2003**, *115*, 87 – 89; *Angew. Chem. Int. Ed.* **2003**, *42*, 83 – 85.

[18] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew. Chem.* **2005**, *117*, 1402 – 1406; *Angew. Chem. Int. Ed.* **2005**, *44*, 1378 – 1382.

[19] Attempts of hydrogenolysis over palladium on charcoal under different conditions, either heating, working at high pressures or both, only yielded traces of the desired acid.

