



HAL
open science

Highly stereoselective multigram scale synthesis of (3S,4S)-Statine and (3S,4S)-N-Benzylstatine

Khoubaib Ben Haj Salah, Francesco Terzani, Marine Pietri, Chiara Zanato,
Evelyne Chelain, Julien Pytkowicz

► **To cite this version:**

Khoubaib Ben Haj Salah, Francesco Terzani, Marine Pietri, Chiara Zanato, Evelyne Chelain, et al.. Highly stereoselective multigram scale synthesis of (3S,4S)-Statine and (3S,4S)-N-Benzylstatine. Results in Chemistry, 2022, 4, pp.100333. 10.1016/j.rechem.2022.100333 . hal-04098168

HAL Id: hal-04098168

<https://hal.science/hal-04098168>

Submitted on 15 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Highly stereoselective multigram scale synthesis of (3*S*,4*S*)-Statine and (3*S*,4*S*)-*N*-Benzylstatine

Khoubai Ben Haj Salah^{a,b}, Francesco Terzani^{a,b}, Marine Pietri^{a,b}, Chiara Zanato^{a,b}, Evelyne Chelain^{a,b}, Julien Pytkowicz^{a,b,*}

^a CY Cergy Paris Université, CNRS, BIOCIS, Cergy Pontoise, France

^b Université Paris-Saclay, CNRS, BIOCIS, 92290 Châtenay-Malabry, France

ARTICLE INFO

Keywords:

Statine
β-ketoester reduction
N-alkylated peptides
Aspartic protease inhibitors
L-leucine

ABSTRACT

Statins are essential naturally occurring β-hydroxy-γ-amino acids and (3*S*,4*S*)-Statine, derived from L-Leucine, is found in important aspartic proteases inhibitors like Pepstatin A. We developed a new synthesis of (3*S*,4*S*)-Statine based on the highly diastereoselective reduction of *N,N*-Boc/Benzyl protected β-ketoester intermediate. This Boc/Benzyl protection strategy gives access to either *N*-Fmoc or *N*-Benzyl protected (3*S*,4*S*)-Statine on multigram scale quantities.

Statins are important naturally occurring β-hydroxy-γ-amino acids and statine-containing peptides show aspartic acid protease inhibition properties [1] beneficial against hypertension (renin), malaria (plasmeprins), AIDS (HIV protease), Alzheimer's disease (β-secretase) and cancer [2]. However, their utility as therapeutics often suffers from limitations including poor membrane permeability [3], high susceptibility to metabolism [4] and a lack of specificity for individual protease targets [3,5].

Peptoids or *N*-alkylated peptides (PTA: Peptide Tertiary Amide) were recently developed as a new class of peptidomimetics showing increased proteolytic stability, cell membrane permeability and restrained conformation [6]. However, *N*-alkylation was deeply investigated only for classical amino acids [7] and, consequently, examples of *N*-alkylated β-hydroxy-γ-amino acids (Statins) were limited to the synthesis of *N*-methyl substituted statins (dolastatin and hapalosin) [8]. *N*-methyl statins were employed as Plasmepsin V inhibitors [9]. Nevertheless, to date, only one example of *N*-benzyl substituted statin is present in literature as part of the scaffold of oxazolidinone-based inhibitors of the inositol kinase PI3K [10]. In the context of our research, based on the development of new aspartic protease inhibitors, we aimed to develop a divergent and scalable diastereoselective synthesis of (3*S*,4*S*)-Statine and (3*S*,4*S*)-*N*-Benzylstatine.

Since the seminal work of Glover, Castro and Mulzer [11], intense efforts were dedicated to the diastereoselective synthesis of statins [12], including: reduction of β-ketoesters derived from canonical amino acids

[11,13], reduction of tetramic acids [14], organometallic addition on α-substituted aldehydes [15], on Cbz activated imine [16] or on Boc protected aziridine [17] and cyclisation of α,β-unsaturated γ-aminoester [18]. One of the highly diastereoselective methods developed to obtain the (3*S*,4*S*)-Statine **1** (Fig. 1) consists in the borohydride reduction of a hindered *N,N*-dibenzyl protected β-ketoester. However this strategy suffers from a tedious isolation of the intermediate *N,N*-dibenzylated amino acid and an epimerization issue during the saponification of the methyl ester at *C* terminal [13h]. Moreover the dibenzyl protection precludes the synthesis of *N*-Benzylstatine. We hypothesized that the reduction of the Boc/Benzyl protected β-ketoester could be performed with a good diastereoselectivity and could lead, efficiently, to the synthesis of (3*S*,4*S*)-*N*-Benzylstatine **2** (Fig. 1).

The synthesis of **2** started with the reductive amination of the commercial L-leucine methyl ester to give the benzylated amino acid **3**. The following Boc protection led to the Boc/Bn protected compound **4** [19]. After a saponification step which occurred without epimerization (see supporting info), the elongation was successfully performed via the activation of the free carboxylic acid **5** by carbonyl diimidazole (CDI) and the subsequently addition of the in-situ formed monomethyl malonate magnesium salt (Scheme 1). For this last step, we took advantage of the convenient and large-scale amenable method described by Magano et al. [20].

The next step was the reduction of the β-keto ester **6**. It was shown, in literature, that either hindered hydride reagents or amines could give

* Corresponding author at: CY Cergy Paris Université, CNRS, BIOCIS, Cergy Pontoise, France.

E-mail address: Julien.pytkowicz@cyu.fr (J. Pytkowicz).

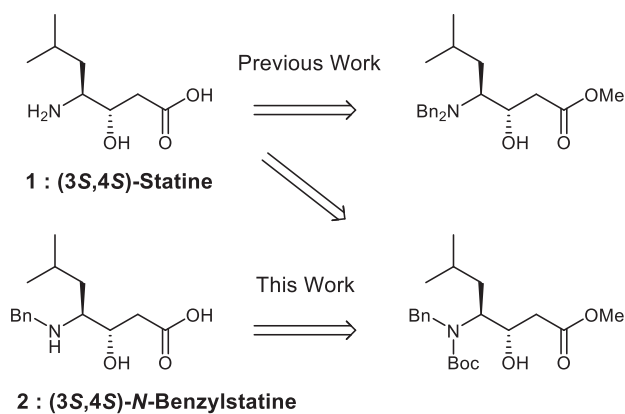
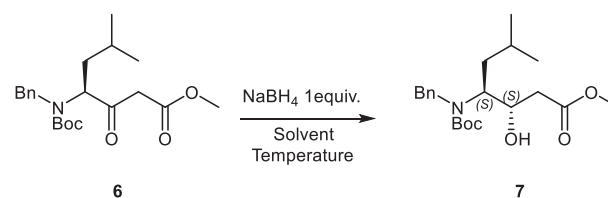


Fig. 1. Compared synthesis of statine 1 and N-Benzylstatine 2.

the desired (3S,4S) diastereomer with a good diastereoselectivity. A first try in ethanol at -10°C using sodium borohydride and the hindered double protected compound **6** only led to an elimination's side product in poor yields. No traces of the desired reduced product were observed. To our great delight, moving from ethanol to methanol, at the same temperature, led to a clean and highly diastereoselective reduction of **6** (Scheme 2, entry 3). When the reduction is conducted in THF, three equivalents of reducing reagent are necessary to reach the complete conversion and the diastereoselectivity of the reaction decreased (entry 2). We also screened different temperature and we concluded that -10°C was the ideal one, since the *de* dropped at lower and higher temperature (entries 5, 6). A sterically hindered, non-chelated transition state that corresponds to a polar Felkin-Anh model, could be involved to explain the excellent selectivity [21].

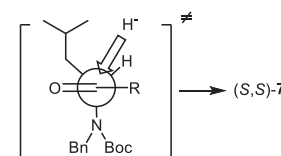
Subsequently, the *N*-Boc/*N*-Bn protection was replaced with a Fmoc protecting group in order to allow the using of the Statine in solid phase peptide synthesis (SPPS). The ester **7** is saponified prior to the cleavage of the Boc moiety with trifluoroacetic acid to give the acid **8** as its TFA salt in good yield. Hydrogenolysis of the benzyl group followed by a Fmoc protection of the free amino acid gives the Fmoc protected (3S,4S)-Statine **9** ready for SPPS (Scheme 3).

The possibility to insert *N*-Benzylstatine in a Peptide was validated coupling the saponified ester **7** with L-leucine methyl ester using classical reagents/conditions. After the cleavage of the Boc protecting group, the *N*-terminal benzylated dipeptide **10** was synthesized in



Entry	Solvent	Temp.	yield (%)	<i>de</i> (%) ^b
1	EtOH	-10°C	-	-
2 ^a	THF	-10°C	86	76
3	MeOH	-10°C	99	97
4 ^a	THF	-78°C	-	-
5	MeOH	-78°C	99	88
6	MeOH	rt	96	69

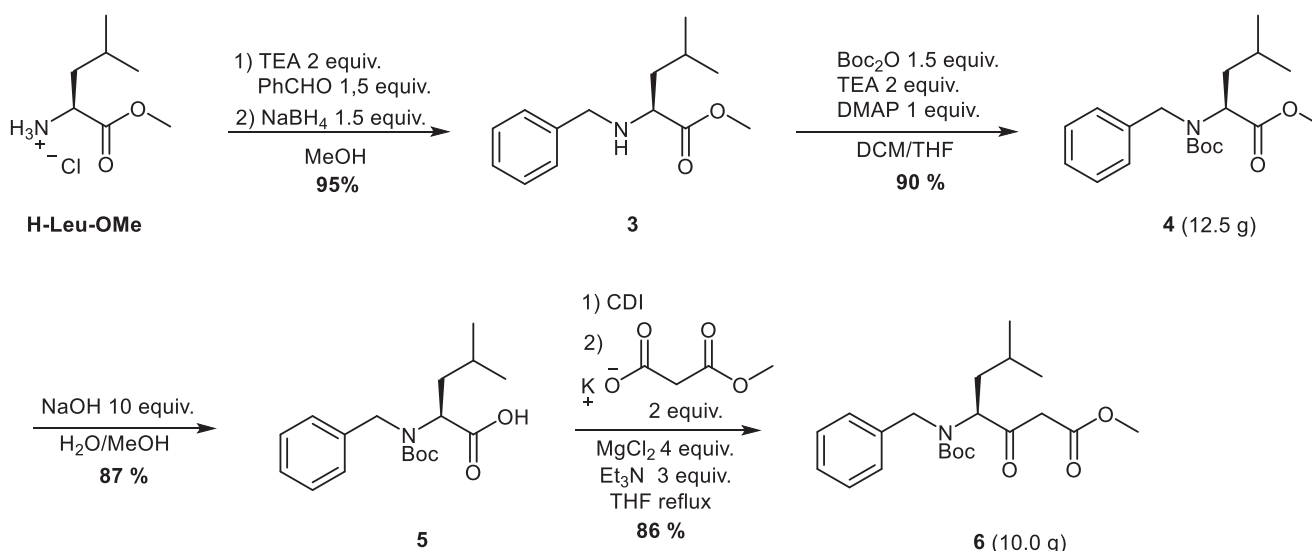
^a: 3 equiv. of NaBH_4 were used; ^b: *de* were measured by HPLC



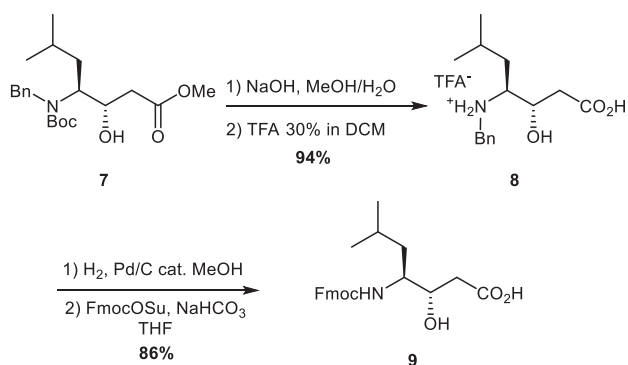
Scheme 2. Diastereoselective reduction of β -keto ester **6**.

excellent yield with no traces of epimerization (Scheme 4). Compound **10** will be tested as an aspartic protease inhibitor and we are currently investigating and optimizing the coupling conditions at the *N*-terminal of the dipeptide.

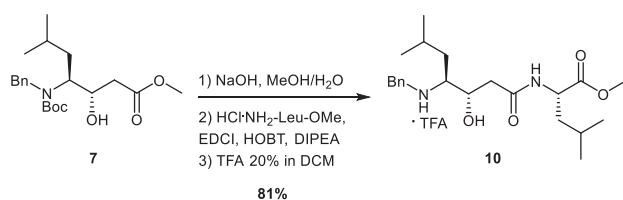
In this paper we described an efficient and divergent synthesis of both (3S,4S)-Statine and (3S,4S)-*N*-Benzylstatine based on a highly diastereoselective reduction of the Boc/Bn di-protected γ -amino- β -keto ester. The Fmoc protected (3S,4S)-Statine **9** is suitably protected for SPPS and C-terminal peptide coupling of Boc protected (3S,4S)-*N*-Benzylstatine **7** was performed in high yield in SPS. *N*-terminal peptide coupling of *N*-benzyl secondary amine and the biological test of *N*-Benzylstatine based peptidomimetics as aspartic protease inhibitors will be reported in due course.



Scheme 1. Synthesis of β -keto ester **6**.



Scheme 3. synthesis of N-Fmoc-Statine 9.



Scheme 4. C-terminal peptide coupling of N-Benzyloxycarbonyl-L-phenylalanyl-L-proline (7).

Funding information

F. Terzani is grateful to French MESR for a PhD grant.

CRedit authorship contribution statement

Khoubuib Ben Haj Salah: Conceptualization, Investigation. **Franco Terzani:** Investigation, Validation. **Marine Pietri:** Validation, Investigation. **Chiara Zanato:** Investigation, Writing – review & editing, Supervision. **Evelyne Chelain:** Validation, Writing – original draft, Supervision. **Julien Pytkowicz:** Visualization, Writing – original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rechem.2022.100333>.

References

- [1] (a) B.M. Dunn, *Chem. Rev.* 102 (2002) 4431; (b) C. Dash, A. Kulkarni, B. Dunn, M. Rao, *Crit. Rev. Biochem. Mol. Biol.* 38 (2003) 89.
- [2] (a) J. Roger, L.S. Payne, D.S. Perlow, N.S. Lohr, M. Poe, E.H. Blaine, M. Kawai, D. H. Rich, L.S. Payne, D.S. Perlow, N.S. Lohr, E.H. Blaine, E.H. Ulm, T.W. Schorn, B. I. LaMont, D.F. Veber, M. Poe, T.Y. Lin, *J. Med. Chem.* 28 (1985) 1779; (b) P.O. Johansson, Y. Chen, A.K. Belfrage, M.J. Blackman, I. Kvarnström, K. Jansson, L. Vrang, E. Hamelink, A. Hallberg, Å. Rosenquist, B. Samuelsson, *J. Med. Chem.* 47 (2004) 3353; (c) D. Scholz, A. Billich, B. Charpiot, P. Etmayer, P. Lehr, B. Rosenwirth, E. Schreiner, H. Gstach, *J. Med. Chem.* 37 (1994) 3079; (d) A. Barazza, M. Götz, S.A. Cadamuro, P. Goettig, M. Willem, H. Steuber, T. Kohler, A. Jestel, P. Reinemer, C. Renner, W. Bode, L. Moroder, *ChemBioChem* 8 (2007) 2078; (e) L.L. Vezenkov, C.A. Sanchez, V. Bellet, V. Martin, M. Maynadier, N. Bettache, V. Lisowski, J. Martinez, M. Garcia, M. Amblard, J.F. Hernandez, *ChemMedChem* 11 (2016) 302.
- [3] (a) A.T. Bockus, K.W. Lexa, C.R. Pye, A.S. Kalgutkar, J.W. Gardner, K.C.R. Hund, W.M. Hewitt, J.A. Schwachert, E. Glassey, D.A. Price, A.M. Mathiowetz, S. Liras, M. P. Jacobson, R.S. Lokey, *J. Med. Chem.* 58 (2015) 4581; (b) N. Zaidi, T. Burster, V. Sommandas, T. Herrmann, B.O. Boehm, C. Driessen, W. Voelter, H. Kalbacher, *Biochem. Biophys. Res. Commun.* 364 (2007) 243.
- [4] F. Li, J. Lu, X. Ma, *Drug Metab. Dispos.* 42 (2014) 213.
- [5] (a) P. Free, C.A. Hurley, T. Kageyama, B.M. Chain, A.B. Tabor, *Org. Biomol. Chem.* 2006 (1817) 4; (b) C. Binkert, M. Frigerio, A. Jones, S. Meyer, C. Pesenti, L. Prade, F. Viani, M. Zanda, *ChemBioChem* 7 (2006) 181.
- [6] (a) R. Kaminker, A. Anastasaki, W.R. Gutekunst, Y. Luo, S.H. Lee, C.J. Hawker, *Chem. Commun.* 54 (2018) 9631; (b) A. Muppidi, K. Doi, S. Edwardraja, E.J. Drake, A.M. Gulick, H.G. Wang, Q. Lin, *J. Am. Chem. Soc.* 134 (2012) 14734; (c) J. Morimoto, Y. Fukuda, D. Kuroda, T. Watanabe, F. Yoshida, M. Asada, T. Nakamura, A. Senoo, S. Nagatoishi, K. Tsumoto, S. Sando, *J. Am. Chem. Soc.* 141 (2019) 14612; (d) R. Kaminker, I. Kaminker, W.R. Gutekunst, Y. Luo, S. Lee, J. Niu, S. Han, C. J. Hawker, *Chem. Commun.* 54 (2018) 5237–5240; (e) Y. Gao, *Kodadek Chem. Biol.* 20 (2013) 360.
- [7] K. Pels, T. Kodadek, *ACS Comb. Sci.* 17 (2015) 152.
- [8] (a) M.E. Maier, C. Hermann, *Tetrahedron* 56 (2000) 557–561; (b) C. Palomo, M. Oiarbide, J.M. Garcia, A. Gonzalez, R. Pazos, J.M. Odriozola, P. Bañuelos, M. Tello, A. Linden, *J. Org. Chem.* 69 (2004) 4126.
- [9] M. Gazdik, M.T. O'Neill, S. Lopaticki, K.N. Lowes, B.J. Smith, A.F. Cowman, J. A. Boddey, B.E. Sleeb, *MedChemComm* 6 (2015) 437.
- [10] G. Caravatti, R.A. Fairhurst, P. Furet, F. Stauffer, F.H. Seiler, H. Rueeger, C. McCarthy, *WO Patent* 2013124826A1, 2013.
- [11] (a) W.S. Liu, S.C. Smith, G.I. Glover, *J. Med. Chem.* 22 (1979) 577; (b) M.N. Dufour, P. Jouin, J. Poncet, A. Pantaloni, B. Castro, *J. Chem. Soc. Perkin Trans. 1* (1895) 1986; (c) J. Mulzer, B. Buttelmann, W. Munch, *Liebigs Ann. der Chemie* (1988) 445.
- [12] (a) T. Yokomatsu, Y. Yuasa, S. Shibuya, *Heterocycles* 33 (1992) 1051; (b) M. Ordóñez, C. Catiuela, *Tetrahedron Asymmetry* 18 (2007) 3; (c) W.C. Zhang, *J. Org. Chem.* 32 (2012) 2203.
- [13] (a) Reetz, M. T.; Drewes, M. W.; Matthews, B. R.; Lennick, K. A. *J. Chem. Soc., Chem. Commun.* 1989, 19, 1474. (b) Nishi, T.; Kitamura, M.; Okhuma, T.; Noyori, R. *Tetrahedron Lett.* 1988, 29, 6327. (c) Mordant, C.; Dünkemann, P.; Ratovelomanana-Vidal, V.; Genet, J. P. *Chem. Commun.* 2004, 4, 1296. (d) Konno, H.; Kubo, K.; Makabe, H.; Toshiro, E.; Hinoda, N.; Nosaka, K.; Akaji, K. *Tetrahedron* 2007, 63, 9502. (e) Li, X.; Li, Y. L.; Chen, Y.; Zou, Y.; Zhuo, X. Bin; Wu, Q. Y.; Zhao, Q. J.; Hu, H. G. *RSC Adv.* 2015, 5, 94654. (f) Alemany, C.; Bach, J.; Farràs, J.; Garcia, J. *Org. Lett.* 1999, 1, 1831. (g) Malik, A.; Kumar, M. G.; Bandyopadhyay, A.; Gopi, H. N. *Biopolymers* 2017, 108, 30. (h) Hoffman, R. V.; Tao, J. J. *Org. Chem.* 1997, 62, 2292.
- [14] (a) Jouin, P.; Castro, B.; Nisato, D. *J. Chem. Soc. Perkin Trans. 1* 1987, 8, 1177. (b) Ma, D.; Ma, J.; Ding, W.; Dai, L. *Tetrahedron Asymmetry* 1996, 7, 2365. (c) Cadicamo, C. D.; Asante, V.; Ammar, M. A.; Borelli, C.; Korting, H. C.; Koksche, B. *J. Pept. Sci.* 2009, 15, 272.
- [15] (a) M. Ganesh Kumar, S.M. Mali, K.M.P. Raja, H.N. Gopi, *Org. Lett.* 17 (2015) 230; (b) C. Gennari, G. Pain, D. Moresca, *J. Org. Chem.* 60 (1995) 6248; (c) G. Veerasha, A. Datta, *Tetrahedron Lett.* 38 (1997) 5223.
- [16] C. Pesenti, P. Bravo, E. Corradi, M. Frigerio, S.V. Meille, W. Panzeri, F. Viani, M. Zanda, *J. Org. Chem.* 66 (2001) 5637.
- [17] S.J. Kwon, S.Y. Ko, *Tetrahedron Lett.* 43 (2002) 639.
- [18] (a) D. Misi, G. Zappia, *Tetrahedron Asymmetry* 31 (1990) 7359; (b) D. Yoo, J.S. Oh, Y.G. Kim, *Org. Lett.* 4 (2002) 1213.
- [19] V. Veeraswamy, G. Goswami, S. Mukherjee, K. Ghosh, M.L. Saha, A. Sengupta, M. K. Ghorai, *J. Org. Chem.* 83 (2018) 1106.
- [20] J. Magano, T.N. Nanninga, D.D. Winkle, *Tetrahedron Lett.* 49 (2008) 2956.
- [21] (a) M.T. Reetz, *Angew. Chem. Int. Ed. Engl.* 23 (1984) 556; (b) V.J. Cee, C.J. Cramer, D.A. Evans, *J. Am. Chem. Soc.* 128 (2006) 2920.