Short and Efficient Synthesis of Statine and Isostatine Derivatives

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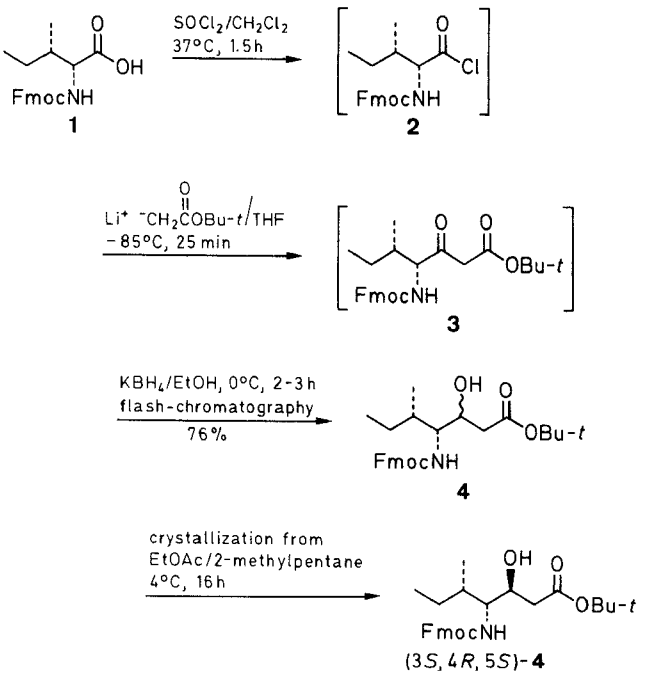
The preparation of Fmoc-(3*R*,4*S*)-statine *tert*-butyl ester [*tert*-butyl (3*R*,4*S*)-4-(9-fluorenylmethoxycarbonylamino)-3-hydroxy-6-methylheptanoate] and the corresponding (3*S*,4*R*,5*S*)-isostatine derivative from Fmoc-leucine and Fmoc-D-alloisoleucine respectively by sequential activation, β-keto ester formation and reduction, is described.

Differentially protected statine (4-amino-3-hydroxy-6-methylheptanoic acid) and isostatine (4-amino-3-hydroxy-5-methylheptanoic acid) derivatives are of common interest for the synthesis of highly active synthetic protesase inhibitors and didemnin analogs.[1-5,12] Many syntheses of statine and its analogs have been reported,[6-12] following different synthetic strategies. However, many have inherent disadvantages, some lack diastereoselectivity, others are laborious or have problems such as unconvenient combinations of protective groups. Since modern peptide chemistry utilizes *N*-terminal 9-fluorenylmethoxycarbonyl protection (Fmoc) with exceptional success especially in the solid phase synthesis of larger peptides, we were looking for a simple straightforward synthesis of orthogonally protected Fmoc-(3*R*,4*S*)-statine and Fmoc-(3*S*,4*R*,5*S*)-isostatine [(3*S*,4*R*,5*S*)-**4**], avoiding a change of protective groups which would be necessary when applying known methods. Following the already known and most simple and successful strategy in statine synthesis: *N*-protected amino acid > carboxyl activated derivative > β-keto ester > diastereoselective reduction[7,10,12], our method is illustrated by the preparation of Fmoc-(3*S*,4*R*,5*S*)-isotatine *tert*-butyl ester (3*S*,4*R*,5*S*)-**4**. Starting from Fmoc-D-alloisoleucine (**1**), we used *in situ* prepared Fmoc amino acid chloride **2**[12,13] as the activated species and coupled it with an excess of lithium *tert*-butoxycarbonylmethanide at −85°C. Without purification of the resulting β-keto ester **3**, reduction with potassium borohydride in ethanol[10] gave the fully protected isostatine derivative **4** which was isolated as a diastereoisomeric mixture after flash-chromatography in 76% yield without racemization at the α-carbon.

Similarly, starting from Fmoc-leucine, Fmoc-statine *tert*-butyl ester was prepared as a diastereoisomeric mixture in 75% yield. In both cases, the yield for each step was 91%.

According to ref.[10], the diastereoselectivity of the reduction depends on the side chain of the substrate and the reducing agent; so potassium borohydride is superior to sodium borohydride giving an de of 90% (95:5) for the protected (3*S*,4*R*,5*S*)-isostatine and 50% (75:25) for the



(3*R*,4*S*)-statine (sodium borohydride reduction: 82% and 42%, respectively). Now the special advantage of Fmoc-protection, the strong tendency of its derivatives to crystallize, allows easy final purification by recrystallisation, leading to diastereoisomerically pure tert-butyl (3*S*,4*R*,5*S*)-4-(9-fluorenylmethoxycarbonylamino)-3-hydroxy-5-methylheptanoate [Fmoc-Ist-OBu-*t*; (3*S*,4*R*,5*S*)-**4**] and *tert*-butyl (3*R*,4*S*)-4-(9-fluorenylmethoxycarbonylamino)-3-hydroxy-6-methylheptanoate (Fmoc-Sta-OBu-*t*).

The entirely orthogonal Fmoc/*tert*-butyl protecting groups could be removed selectively with trifluoroacetic acid giving the crystalline Fmoc-Ist-OH or Fmoc-Sta-OH or by secondary amines under usual conditions resulting in *N*-deprotected derivatives suitable for solid phase synthesis or for classical solution-synthesis of peptides. However, the application of *N*-terminal Fmoc- and *C*-terminal *tert*-butyl-protection for statine- or isostatine-syntheses is already known, but, to our experience, *only* the new combination of both protective groups allows the straightforward and efficient synthesis of Fmoc-(iso-) statine derivatives.

Advantages of this simple and well-reproducible synthesis are: the time-consuming isolation of intermediates[10] or synthesis of starting materials[12] are avoided, as well as chromatographic separations.[10] It could easily be performed in only one working day (beginning with Fmoc-amino-acid, flash-chromatography included). Starting materials are all commercially available reagents, leading, after simple workup procedures, to orthogonally protected crystalline products ensuring a high grade of synthetic flexibility.

***tert*-Butyl (3*S*,4*R*,5*S*)-4-(9-Fluorenylmethoxycarbonylamino)-3-hydroxy-5-methylheptanoate [Fmoc-Ist-OBu-*t* (3*S*,4*R*,5*S*)-4], *tert*-Butyl (3*R*,4*S*)-4-(9-Fluorenylmethoxycarbonylamino)-3-hydroxy-6-methylheptanoate (Fmoc-Sta-OBu-*t*):**

$SOCl_2$ (10 eq, 3.65 mL) and DMF (3 drops) are added to a stirred suspension of Fmoc-D-alle-OH (1.77 g, 5 mmol) or Fmoc-Leu-OH in CH_2Cl_2 (10 mL). After stirring at 35–38 °C for 1.5 h the excess of $SOCl_2$ is removed *in vacuo*; the oily product is kept in high vacuum for 0.5 h where it crystallizes. It is then dissolved in THF (8 mL), cooled to −85 °C and added to a solution of lithium *tert*-butoxycarbonylmethanide (made from commercially available 1.5 M LDA solution in cyclohexane (13.3 mL, 20 mmol) and *tert*-butyl acetate (2.95 mL, 22 mmol) in THF (7 mL) at that temperature. After 25 min, the clear, yellow solution is quenched with 0.5 N HCl (50 mL) and extracted with Et_2O (3 × 50 mL). The organic phase is dried (Na_2SO_4) and evaporated to dryness. To avoid crystallization it is immediately dissolved in dry EtOH (20 mL), cooled to 0 °C, and reduced with KBH_4 (1.08 g, 20 mmol) for 3 h. Then EtOH (20 mL) and glacial AcOH (20 mL) are added with stirring, until all remaining hydride is consumed. Insoluble material is filtered off, the solvent is removed and the residue dissolved in EtOAc/H_2O (2:1, 100 mL). The organic phase is separated and the aqueous phase reextracted with EtOAc (100 mL). The combined organic layers are dried (Na_2SO_4) and concentrated under reduced pressure. After flash-chromatography on silica gel (280 g, EtOAc/Et_2O/2-methylpentane 3:1:7) the combined product fractions are evaporated to dryness, yield and diastereoisomeric composition are determined and the product crystallized overnight from EtOAc/2-methylpentane.

Absolute stereochemistry was determined via proton–proton coupling constants of the cyclic *N,O*-isopropylidene derivatives[10] and by comparing analytical data – mp, optical rotation – of the known Boc-(3*R*,4*S*)-Sta-OH[11] after changing the corresponding protective groups by known methods.

Fmoc-(3S,4R,5S)-Ist-OBu-t; yield: 1.72 g (76%); mp 135–136 °C; $[\alpha]_D^{20}$ −3.6° (*c* = 0.25, EtOAc).

$C_{27}H_{35}NO_5$	calc.	C 71.50	H 7.78	N 3.10
(453.58)	found	71.50	7.73	3.25

MS (FAB): *m/z* = 454.3 (M + H^+).

^{1}H-NMR (270 MHz, DMSO-d_6/TMS): δ = 0.81 (2 d, 6 H, CH_3), 1.00–1.30 (m, 2 H, CH_2), 1.41 (s, 9 H, $C(CH_3)_3$), 1.80 (dq, 1 H, CH), 2.02, 2.31 (2 × dd, 2 H, CH_2), 3.40 (dt, 1 H, CHN), 3.77 (dq, 1 H, CHOH), 4.20 (t, 1 H, Fmoc CH), 4.31 (d, 2 H, Fmoc CH_2), 4.87 (d, 1 H, OH), 6.96 (d, 1 H, NH), 7.88, 7.71, 7.40, 7.30 (respectively d, t, t, t; 8 H_{arom}).

Fmoc-(3R,4S)-Sta-OBu-t; yield: 1.70 g (75%); mp 152–153 °C; $[\alpha]_D^{20}$ −17.2° (*c* = 0.25, EtOAc).

$C_{27}N_{39}NO_5$	calc.	C 71.50	H 7.78	N 3.10
(453.58)	found	70.93	7.74	3.10

MS (FAB): *m/z* = 454.2 (M + H^+).

^{1}H-NMR (270 MHz, DMSO-d_6/TMS): δ = 0.82 (dd, 6 H, CH_3), 1.40 (s, 9 H, $(CH_3)_3$), 1.2–1.6 (m, 3 H, CH_2, CH), 2.07, 2.25 (2 × dd, 2 H, CH_2), 3.38 (m, 1 H, CHN), 3.61 (m, 1 H, CHOH), 4.19 (t, 1 H), 4.33 (d, 2 H) (Fmoc CH, CH_2), 6.98 (d, 1 H, NH), 7.30, 7.40, 7.69, 7.87 (respectively t, t, d, d; 8 H_{arom}).

4-*tert*-Butoxycarbonylamino-3-hydroxy-6-methylheptanoic Acid (Boc-Sta-OH):

Prepared by a known protection-deprotection sequence; mp 134–135 °C (Lit.[11] mp 135–136 °C); $[\alpha]_D^{20}$ −26.5° (*c* = 0.43, MeOH) Lit.[11] $[\alpha]_D^{24}$ −27.6° (*c* = 0.31, MeOH).

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(1) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matzusaki, M.; Hamada, H.; Takeuchi, T. *J. Antibiot.* **1970**, *23*, 259.
(2) Rich, D.H. *J. Med. Chem.* **1985**, *28*, 262.
(3) Rich, D.H., in: *Proteinase Inhibitors*, Barrett und Salwesen, Eds., Elsevier Science Publishers, Amsterdam, 1986, p. 179.
(4) Boger, J.; Payne, L.S.; Perlow, D.S.; Lohr, N.S.; Poe, M.; Blaine, E.H.; Ulm, E.H.; Schorn, T.W.; La Mont, B.I.; Lin, T.Y.; Kawai, M.; Rich, D.H.; Veber, D.F *J. Med. Chem.* **1985**, *28*, 1779.
(5) Rinehart, K.L., in: *Peptides 1987 – Proceedings 10th Amer. Pept. Symp.*, Marshall, G.R., Ed., Escom, Leiden, 1988, p. 626.
(6) Nishi, T.; Kitamura, M.; Ohkuma, T.; Noyori, R. *Tetrahedron Lett.* **1988**, *29*, 6327.
(7) Maibaum, J.; Rich, D.H. *J. Org. Chem.* **1988**, *53*, 869.
(8) Kunieda, T.; Ishizuka, T.; Higuchi, T.; Hirobe, M. *J. Org. Chem.* **1988**, *53*, 3381.
(9) Kano, S.; Yuasa, Y.; Yokomatsu, T.; Shibuya, S. *J. Org. Chem.* **1988**, *53*, 3865.
(10) Harris, B.D.; Joullié, M.M. *Tetrahedron* **1988**, *44*, 3489.
(11) Rich, D.H.; Sun, E.T.; Boparai, A.S. *J. Org. Chem.* **1978**, *43*, 3624.
(12) (a) Schmidt, U.; Kroner, M.; Griesser, H. *Tetrahedron Lett.* **1988**, *29*, 3057.
(b) Schmidt, U.; Kroner, M.; Griesser, H. *Synthesis* **1989**, 832.
(13) Carpino, L.A.; Cohen, B.J.; Stephens, K.E.; Yahya Sadat-Aalaee, S.; Tien, J.-H.; Langridge, D.C. *J. Org. Chem.* **1986**, *31*, 3734.