

Synthesis of orthogonally protected *L*-threo- β -ethoxyasparagine

Jan Spengler · Marta Pelay · Judit Tulla-Puche ·
Fernando Albericio

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Abstract Orthogonally protected *L*-threo- β -ethoxyasparagine (Fmoc-EtOAsn(Trt)-OH, **1**) was synthesized from diethyl (2*S*,3*S*)-2-azido-3-hydroxysuccinate **2** in eight steps as a building block for solid-phase peptide synthesis. The starting material is easily available in multi-gram scale from *D*-diethyltartrate. The transformation steps reported here are robust and scalable. Thus, a significant amount of **1** (1.8 g) was obtained in 21% overall yield. The synthesis reported is also expected to be useful for the preparation of other *O*-substituted *L*-threo- β -hydroxyasparagine derivatives.

Keywords Hexafluoroacetone · Tritylamide ·
2,4,6-Trimethoxybenzylamide · Amino acids · Peptide

Abbreviations

ACN	Acetonitrile
DIEA	<i>N,N</i> -Diisopropylethylamine
DMF	<i>N,N</i> -Dimethylformamide
EDC	<i>N</i> -(3-Dimethylaminopropyl)- <i>N'</i> -ethylcarbodiimide
EtOAc	Ethyl acetate
Fmoc-OSu	<i>N</i> -(9-Fluorenylmethoxycarbonyloxy)succinimide
HOAc	Acetic acid
TLC	Thin-layer chromatography

Introduction

L-Threo- β -ethoxyasparagine is structurally closely related to *L*-threo- β -hydroxyasparagine, a non-proteinogenic amino acid present in various antimicrobial peptides. Efficient synthetic routes to derivatives bearing common protecting schemes for solid-phase peptide synthesis (SPPS) have been reported (Boger et al. 2000; Guzmán-Martínez and VanNieuwenhze 2007). However, no information is available for *L*-threo- β -ethoxyasparagine or its diastereomers, despite this compound being a non-proteinogenic amino acid constituent of at least one bioactive peptide (structure not shown). We required a considerable amount of Fmoc-EtOAsn(Trt)-OH **1** to produce this peptide by Fmoc-SPPS. The direct introduction of the additional ethyl ether function in Fmoc- β -OH-Asn(Trt)-OH or in earlier stages of its synthesis calls for additional, orthogonal and temporary carboxylic- or amide protection and this would cause an drop in the yield of the reported multistep synthesis (Guzmán-Martínez and VanNieuwenhze 2007: eight steps, 29% overall yield from *L*-threo- β -hydroxyaspartic

J. Spengler (✉) · M. Pelay · J. Tulla-Puche · F. Albericio (✉)
Institute for Research in Biomedicine,
Barcelona Science Park, Baldiri Reixac 10,
08028 Barcelona, Spain
e-mail: jan.spengler@irbbarcelona.org

F. Albericio
e-mail: fernando.albericio@irbbarcelona.org;
albericio@irbbarcelona.org

J. Spengler · M. Pelay · J. Tulla-Puche · F. Albericio
CIBER-BBN, Networking Centre on Bioengineering
Biomaterials and Nanomedicine,
Barcelona Science Park,
University of Barcelona,
Baldiri Reixac 10, 08028 Barcelona, Spain

F. Albericio
Department of Organic Chemistry,
University of Barcelona,
Martí i Franqués 1-11,
08028 Barcelona, Spain

acid; Boger et al. 2000: nine steps, 11% overall yield from methyl 4-methoxycinnamate). To circumvent this obstacle, we developed an alternative route starting from diethyl (2*S*,3*S*)-2-azido-3-hydroxysuccinate **2**, which is available in multi-gram scale from D-diethyltartrate in two steps and represents a carboxy-protected L-threo- β -hydroxyaspartate scaffold with a masked amino group (Charvillon and Amouroux 1997; Saito et al. 1996). Therefore, we considered this compound perfectly suited for the introduction of substituents at the β -hydroxy function. Although **2** was obtained with ca. 20% of the undesired (2*R*,3*S*)-diastereomer, this contamination was completely removed in later stages (in course of the purification of compound **7**) of the synthesis.

Materials and methods

General remarks

Diethyl (2*S*,3*S*)-2-azido-3-hydroxysuccinate **2** was prepared as mixture of diastereomers from D-diethyltartrate, following reported procedures (Charvillon and Amouroux 1997; Saito et al. 1996). All commercial reagents and solvents were used as received unless otherwise indicated. Solvents were from SDS (Peypin, France). TLC was performed with TLC aluminum sheets coated with silica gel 60 F₂₅₄ (Merck). Spots were observed with UV or after staining and heating as indicated [15 g phosphomolybdic acid in ethanol (100 mL), or with 5 g ninhydrin in acetone (100 mL), or 6 g KMnO₄, 40 g K₂CO₃ and 5 mL NaOH in 600 mL water]. For column chromatography, Silice 60 ACC 35–70 μ m (SDS) was used.

¹H-NMR spectra were acquired at 400.125 MHz, ¹³C at 100.625 MHz with TMS as internal reference, and ¹⁹F at 376.494 MHz) with a Varian Mercury 400 (High-field NMR Unit, Barcelona Science Park). The following abbreviations are used to indicate multiplicity: s, singlet; d, doublet; dd, double doublet; t, triplet; dt, double triplet; m, multiplet, br s, broad signal. Chemical shifts (δ) are expressed in parts per million downfield from tetramethylsilyl chloride. IR spectra were recorded with a Thermo Nicolet spectrometer and with the Omnic 6.0 program from the Thermo Nicolet Corporation. Optical rotation values were measured with a Perkin-Elmer 241 spectrometer (concentrations are expressed in g 100 mL⁻¹). Melting points were determined in a Büchi Melting Point B-540 apparatus and are uncorrected. High-resolution mass spectroscopy (HRMS) was performed with the electrospray (ion spray) (ESI-MS) method with a HRMS LC/MSD-TOF (2006) (Agilent Technologies) in positive or negative mode (as indicated).

Compound synthesis and characterization

Diethyl (2*S*,3*S*)-2-azido-3-ethoxysuccinate (**3**) (as diastereomeric mixture)

A solution of **2** (10.02 g, 43.34 mmol) and iodoethane (35.1 g, 18 mL, 225 mmol) in diethyl ether (200 mL) was placed in a round bottomed flask equipped with a condenser and stirred with a mixture of silver(I)oxide (13 g, 56.1 mmol) and sand (50 g) at 37°C for 15 h (the sand was added to prevent that the silver(I)oxide sticks together). When TLC analysis revealed complete conversion, the solution was filtered and concentrated in vacuo. Product **3** was obtained after column chromatography (hexane/EtOAc, 2:1, *R*_f = 0.5, KMnO₄: yellow) as a yellow oil (all fractions containing product, 10.0 g, 89%). The product was a mixture of diastereomers and some side products were also present. It was used for the next step without further purification. ¹H NMR (CDCl₃): δ = 4.43 (d, *J* = 3.6 Hz, 1H), 4.39–4.17 (m, 14H), 3.91–3.79 (m, ca. 2H), 3.66 (m, ca. 1H), 3.59–3.39 (m, ca. 2H), 1.16–1.40 (m, ca. 24H).

Diethyl (2*S*,3*S*)-2-amino-3-ethoxysuccinate (**4**)

A solution of **3** (as diastereomeric mixture) (10.0 g, 38.6 mmol) in EtOAc (150 mL) was stirred with Pd-C catalyst (5% Pd, 0.5 g) under an atmosphere of H₂ (air pressure) for 15 h. When TLC (hexane/EtOAc, 2:1, *R*_f = 0.5, KMnO₄: starting material yellow) revealed complete conversion, it was filtered and concentrated in vacuo. The diastereomers were partially separated by column chromatography (dichloromethane (DCM)/methanol (MeOH)/HOAc, 10:1:0.5, *R*_f = 0.3 (product), *R*_f = 0.26 (other diastereoisomer), ninhydrin: red brown). The fractions with product were sampled and concentrated in vacuo. HOAc was removed by co-evaporation with toluene to give 7.85 g, 87% of **4** as a yellow oil with ca. 10% of the (2*R*,3*S*)-diastereomer (NMR). ¹H NMR (CDCl₃): δ = 4.33 (d, *J* = 3.0 Hz, 1H), 4.27–4.18 (m, 4H), 3.89 (d, *J* = 3.0 Hz, 1H), 3.76 (m, 1H), 3.40 (m, 1H), 1.29 (m, 6H), 1.17 (m, 3H) ppm; ¹³C NMR (CDCl₃): δ = 170.3, 79.1, 67.0, 61.5, 61.3, 56.8, 14.8, 14.1, 14.1 ppm; [α]_D²⁵ –33 (c 2.0, CHCl₃, diastereomeric mixture); IR (film): 2,980, 1,751, 1,221, 1,164, 1,104 cm⁻¹; HRMS (ES⁺) calc. for C₁₀H₁₉NO₅ *m/z* (M + Na)⁺ 256.1161, found 256.1154.

Diethyl (2*R*,3*S*)-2-amino-3-ethoxysuccinate: ¹H NMR (CDCl₃): δ = 4.14–4.28 (5H), 3.98 (d, *J* = 3.4 Hz, 1H), 3.80 (m, 1H), 3.49 (m, 1H), 1.32–1.19 (9H).

(2*S*,3*S*)-2-amino-3-ethoxysuccinic acid (**5**)

Diethyl (2*S*,3*S*)-2-amino-3-ethoxysuccinate **4** (7.85 g, 33.65 mmol) was refluxed with stirring with 5 N aqueous

HCl (100 mL) for 5 h. When TLC (DCM/MeOH/HOAc, 10:1:0.5, R_f = 0.3 (starting material), ninhydrin: red brown) revealed complete conversion, the solution was concentrated at 70°C. The residue was dissolved in THF (30 mL) and propylene oxide (10 mL) was added drop wise. The supernatant was removed by suction and the white precipitate was dried under vacuo to give 5.01 g (84%) of **5**. Decomp. at 210°C. The ^1H and ^{13}C NMR spectra showed approx. 10% contamination with the (2*R*,3*S*)-diastereomer. ^1H NMR (DMSO- d_6): δ = 3.92 (d, J = 9.2 Hz, 1H), 3.79 (m, 2H), 3.72 (d, J = 9.2 Hz, 1H), 3.42 (m, 1H), 1.13 (m, 3H) ppm; ^{13}C NMR (DMSO- d_6): δ = 170.9, 168.4, 75.2, 66.4, 53.4, 14.9 ppm; (diastereomer 2*R*,3*S*: δ = 170.1, 167.9, 79.7, 65.5, 53.8, 14.7 ppm); IR (KBr): 3,431, 3,112, 1,695, 1,466, 1,138, 1,100 cm^{-1} ; $[\alpha]_D^{25}$ -56 (c 1.5, DMSO, diastereomeric mixture); HRMS (ES $^-$) calc. for $\text{C}_6\text{H}_{11}\text{NO}_5$ m/z (M-H) $^-$ 176.0559, found 176.0562.

(2*S*)-2-Ethoxy-2-[(4*S*)-5-oxo-2,2-bis-trifluoromethyl-oxazolidin-4-yl]-acetic acid (**6**)

A solution of **5** (3.73 g, 21.05 mmol) in DMF (20 mL) was stirred under an atmosphere of hexafluoroacetone (anhydrous) for 22 h (uptake of 8.8 g). DMF was removed at 1 mm Hg at 55°C and the residue was purified by column chromatography (hexane/EtOAc/HOAc, 1:1:0.05, R_f = 0.3, phosphomolybdic acid: blue). The fractions with product were pooled and concentrated in vacuo. HOAc was removed by co-evaporation with toluene to give 5.65 g (83%) of yellow oil. The ^1H and ^{13}C NMR spectra showed ca. 10% contamination with the other diastereomer. ^1H NMR (acetone- d_6): δ = 5.09 (d, J = 6.8 Hz, 1H), 4.66 (dd, J = 2.4, 7.0 Hz, 1H), 4.32 (d, J = 2.6 Hz, 1H), 3.85 (m, 1H), 3.52 (m, 1H), 1.15 (t, J = 7.0 Hz, 3H) ppm; ^{19}F NMR (acetone- d_6): δ = -80.14 (q, J = 9.0 Hz, 3F), -80.88 (q, J = 9.2 Hz, 3F) ppm; [other diastereomer: -80.31 (m, approx. 0.3F), -80.84 (m, approx. 0.3F) ppm]; ^{13}C NMR (CDCl_3): δ = 174.3, 169.2, 120.99 (q, J = 287.5 Hz), 119.95 (q, J = 285.7 Hz), 89.4 (m), 75.2, 63.3, 58.1, 14.6 ppm (additional signals of the other diastereomer: 173.6, 57.5 ppm); $[\alpha]_D^{25}$ -49 (c 2.5, CHCl_3 , diastereomeric mixture); IR (film): 3,345, 1,831, 1,734, 1,234 cm^{-1} . HRMS (ES $^-$) calc. for $\text{C}_9\text{H}_9\text{F}_6\text{NO}_5$ m/z (M-H) $^-$ 325.0307, found 325.0309.

(2*S*)-2-Ethoxy-2-[(4*S*)-5-oxo-2,2-bis-trifluoromethyl-oxazolidin-4-yl]-*N*-trityl-acetamide (**7**)

Oxazolidinone **6** (2.28 g, 7.01 mmol) was dissolved in oxalyl chloride (10 mL) and five drops of DMF were added. This mixture was stirred for 30 min. The excess of

oxalyl chloride was removed in vacuo and the residue was co-evaporated with dry toluene (20 mL). The acid chloride was directly converted without further purification [^1H NMR (CDCl_3): δ = 4.52 (d, J = 2.0 Hz, 1H), 4.42 (m, 2H), 3.88 (m, 1H), 3.59 (m, 1H), 3.39 (br. d, J = 7.5 Hz, 1H), 1.22 (t, J = 6.7 Hz, 3H) ppm; ^{19}F NMR (CDCl_3): δ = -79.07 (q, J = 8.4 Hz, 3F), -80.78 (q, J = 8.5 Hz, 3F) ppm; diastereomer 2: -79.87 (q, J = 8.5 Hz, approx. 0.3F), -80.53 (q, J = 8.5 Hz, approx. 0.3F) ppm.]. The acid chloride of **6** dissolved in dry toluene (10 mL) was dropped into a solution of triethylamine (3.82 g, 14.72 mmol) in dry toluene (30 mL) at 0°C. After 1 h of stirring at 0°C, the suspension was filtered and the precipitate was washed with toluene (4 $\times$ 10 mL). The unified solutions were concentrated under vacuo and the residue was purified by column chromatography (hexane/EtOAc, 2:1, R_f = 0.55, UV 254 nm: blue). The undesired diastereomer was completely separated and eluted in a mixture with triethylamine (R_f = 0.4, UV 254 nm: blue). The fractions with product were pooled and concentrated in vacuo, then dissolved in ACN/H $_2$ O and lyophilized to give 2.36 g (59%) of diastereomerically pure product **7**. Mp 49–50°C; ^1H NMR (CDCl_3): δ = 8.08 (br. 1H), 7.22–7.33 (18H), 4.48 (dd, J = 1.8, 7.8 Hz, 1H), 4.23 (d, J = 2.1 Hz, 1H), 3.78 (m, 2H), 2.76 (d, J = 7.9 Hz, 1H), 1.26 (t, J = 7.0 Hz, 3H) ppm; ^{19}F NMR (CDCl_3): δ = -80.16 (q, J = 8.6 Hz, 3F), -80.79 (q, J = 8.5 Hz, 3F) ppm; ^{13}C NMR (CDCl_3): δ = 169.2, 168.0, 144.0, 128.4, 128.1, 127.3, 121.0 (q, J = 287.4 Hz), 120.0 (q, J = 284.6 Hz), 89.5 (m), 78.7, 70.4, 69.4, 57.9, 15.2 ppm; IR (KBr): 3,396, 1,836, 1,689, 1,493, 1,239, 1,198, 974, 702 cm^{-1} ; $[\alpha]_D^{25}$ -32 (c 1.6, CHCl_3); HRMS (ES $^+$) calc. for $\text{C}_{28}\text{H}_{24}\text{F}_6\text{N}_2\text{O}_4$ m/z (M + H) $^+$ 567.1719, found 567.1712.

(2*S*,3*S*)-2-Amino-3-ethoxy-*N*-trityl-succinamic acid methyl ester hydrochloride (**8**)

A solution of dry HCl in dioxane (4 M, 60 mL) was added to a solution of **7** (2.98 g, 4.72 mmol) in MeOH (60 mL) at 0°C. This mixture was stirred (closed flask) for 15 h. When HPLC revealed complete conversion, the volatiles were removed in vacuo and the residue was treated with diethyl ether (30 mL). The solid precipitate was dried in vacuo to give 2.1 g of **8** as hygroscopic solid (95%). ^1H NMR (DMSO- d_6): δ = 8.99 (s, 1H), 7.18–7.33 (18H), 4.49 (d, J = 5.9 Hz, 1H), 4.36 (d, J = 5.9 Hz, 1H), 3.70 (s, 3H), 3.51 (m, 2H), 1.08 (t, J = 7.0 Hz, 3H) ppm; ^{13}C NMR (DMSO- d_6): δ = 171.9, 167.9, 144.2, 128.3, 127.6, 126.7, 78.4, 69.5, 66.3, 54.3, 52.4, 14.9 ppm; IR (KBr): 3,396, 1,751, 1,685, 1,491, 1,097, 700 cm^{-1} ; $[\alpha]_D^{25}$ -11 (c 1.8, DMSO); HRMS (ES $^+$) calc. for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_4$ m/z (M + H) $^+$ 433.2127, found 433.2119.

(2*S*,3*S*)-2-(9*H*-Fluoren-9-yl)methoxycarbonylamino-3-ethoxy-*N*-trityl-succinamic acid (**1**)

A solution of KOH (1.22 g, 21.85 mmol) in H₂O (20 mL) was added to a solution of **8** (1.89 g, 4.37 mmol) in dioxane (40 mL) at 0°C. After 1 h of stirring, TLC revealed complete conversion. Glacial HOAc (1.25 mL) was added and the solution was cooled to 0°C. After addition of Fmoc-OSu (2.21 g, 6.55 mmol), DIEA was dropped into the milky suspension until pH 9 was reached (ca. 2 mL). After 1 h of stirring, complete conversion was observed by TLC. The dioxane was removed under vacuo at 30°C, and 1 N aqueous HCl (100 mL) was added. The product was extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over MgSO₄, filtered and concentrated in vacuo.

The residue was purified by column chromatography (hexane/EtOAc, 3:2) until the excess of Fmoc-OSu had eluted, and the product was then eluted with hexane/EtOAc/HOAc, 1:1:0.05, *R*_f = 0.2, UV 254 nm: blue). The fractions with product were pooled and concentrated in vacuo. HOAc was removed by co-evaporation with toluene to give 1.98 g (70%) of **1** as a foamy white solid. Mp 113–116°C; ¹H NMR (DMSO-*d*₆): δ = 8.40 (s, 1H), 7.99 (d, *J* = 9.3 Hz, 1H), 7.86–7.91 (3H), 7.81 (d, *J* = 7.5 Hz, 1H), 7.37–7.44 (2H), 7.33–7.14 (20H), 4.39 (m, 2H), 4.34 (d, *J* = 2.9 Hz, 1H), 4.19 (m, 1H), 4.07 (m, 1H), 3.72 (m, 1H), 3.57 (m, 1H), 1.17 (t, *J* = 7.0 Hz, 3H) ppm; ¹³C NMR (DMSO-*d*₆): δ = 171.3, 167.9, 156.3, 144.3, 143.7, 143.5, 140.6, 140.8, 128.4, 127.6, 127.6, 127.5, 127.0, 126.9, 126.5, 125.8, 125.4, 120.0, 119.9, 80.1, 69.3, 67.1, 66.2, 55.9, 46.5, 15.0 (some signals are doubled) ppm; IR (KBr): 3,397, 1,726, 1,696, 1,492, 700 cm⁻¹; [α]_D²⁵ 13 (c 1.75, DMSO); HPLC (70–100% ACN, 8 min): *t*_r 4.97 min (98%); HRMS (ES⁺) calc. for C₄₀H₃₆N₂O₆ *m/z* (*M* + *H*)⁺ 641.2652, found 641.2649.

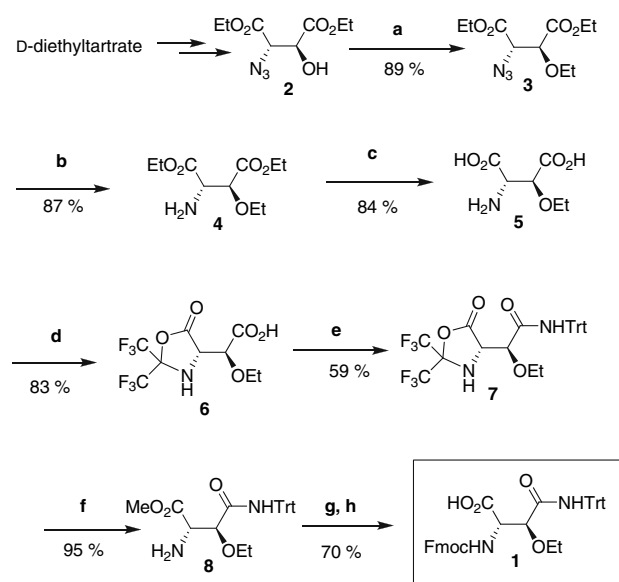
Results and discussion

Highly efficient procedures for the formation of ethyl ethers from hydroxy carboxylic acids by base-induced OH-deprotonation and reaction with iodoethane have been described (Aikins et al. 2005). However, the risk of epimerization under basic conditions must be taken into consideration because the starting material diethyl (2*S*,3*S*)-2-azido-3-hydroxysuccinate **2** contains significant amounts of the (2*R*,3*S*)-diastereomer and epimerization at the (3*S*)-carbon of the diastereomeric contamination would produce the (2*R*,3*R*)-enantiomer of our target molecule. Therefore, we used the mild, non-basic Ag₂O/iodoethane protocol, which is reported to be useful for the formation of ethyl ethers of hydroxy carboxylic acids (Parmenon et al. 2008) and gives 89% of diethyl 2-azido-3-ethoxysuccinate **3** as a

diastereomeric mixture. After Pd-C-catalyzed hydrogenation of the azido function to diethyl 2-amino-3-ethoxysuccinate **4**, the ethyl ester was hydrolyzed in boiling aqueous HCl and the 2-amino-3-ethoxysuccinic acid **5** was precipitated from propylene oxide/THF.

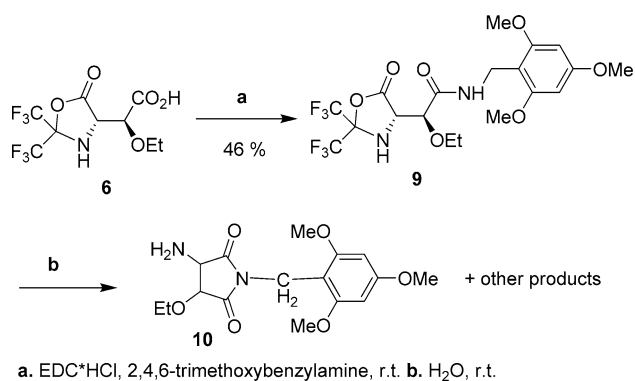
The differentiation between the two carboxyl functions of **5** was accomplished by the use of the bidentate protecting/activating reagent hexafluoroacetone (HFA). HFA undergoes a cyclocondensation with the α-amino carboxylic acid unit to give 2,2-bis(trifluoromethyl)-1,3-oxazolidin-4-ones. This reaction proceeds site selectively in the presence of further carboxylic acid residues in the molecule (Spengler et al. 2006). Thus, **5** was reacted with HFA in DMF to give the oxazolidinone **6** in 83% yield (Scheme 1).

For the transformation of the unreacted carboxylic acid of **6** into a suitable protected amide group, we reasoned that it would be much easier to obtain the 2,4,6-trimethoxybenzyl-protected amide than the trityl-protected amide, because 2,4,6-trimethoxybenzylamine is less sterically hindered and more nucleophilic than tritylamine. The 2,4,6-trimethoxybenzyl-group is reported to provide efficient amide protection orthogonally to the Fmoc-group and can be removed with acids (Weygand et al. 1968). Indeed, the amide formation was found to be possible by activation of the terminal carboxylic group of **6** with carbodiimide (EDC) and subsequent reaction with 2,4,6-trimethoxybenzylamine. Amide **9** was isolated in 46% yield (Scheme 2). These conditions failed completely when tritylamine was tried to react. Although 2,2-bis(trifluoromethyl)-1,



a. Ag₂O/EtI, Et₂O, reflux. b. H₂, Pd-C, ethyl acetate, r.t. c1. aqu. HCl, reflux, c2. propylene oxide, r.t. d. hexafluoroacetone, DMF, r.t. e1. oxalyl chloride, r.t., e2. tritylamine, toluene, 0 °C. f. MeOH/dry HCl, r.t. g. KOH, dioxane/H₂O, r.t. h. Fmoc-OSu, DIEA, dioxane/H₂O, r.t.

Scheme 1 Synthesis of Fmoc-EtOAsn(Trt)-OH **1** from D-diethyltartrate



Scheme 2 The Tmob-protected amide reacts as nucleophile with the oxazolidinone

3-oxazolidin-4-ones, like **9**, represent carboxy-activated species, their direct use in SPPS is limited (Albericio et al. 2005). Therefore, we envisioned the exchange of the HFA-protecting group for *N*-Fmoc protection. However, mild hydrolysis (room temperature) of the lactone **9** produced only traces of the desired 1-carboxy- α -amino acid, but mainly products such as **10**, originating from an intramolecular cyclization reaction between the (lactone-) activated carboxylic acid and the amide-group (HPLC–MS). This observation led us to question the suitability of 2,4,6-trimethoxybenzyl-(Tmob)-protection for the amide function in our case because the carboxylic group has to be activated for coupling on solid-phase reactions and the observed intramolecular cyclization would then compete with the reaction with the resin-bound peptide.

We reasoned that the steric demanding trityl group could prevent undesired intramolecular cyclizations. Several conditions were tested to generate the trityl-protected amide **7** (Scheme 1). Finally, the best results gave the activation of **6** as acid chloride, and subsequent reaction with 2.1 equiv. of tritylamine. A substitution of the excess of tritylamine for pyridine caused a significant drop in yield. The tritylamide **7** could be completely separated from the remaining amounts of the (2*R*,3*S*)-diastereomer by chromatography on silica and **7** was obtained diastereomerically pure in 59% yield. Also, parts of the tritylamine used as excess were recovered. The hydrolysis of the oxazolidinone **7** proceeded very slowly. Therefore, the methyl ester hydrochloride **8** was prepared as intermediate by stirring in methanol containing dry HCl. After hydrolysis of the methyl ester and amino group protection with Fmoc-OSu under standard conditions, **1** was obtained in 21% yield over eight steps.

Conclusions

Here, we describe a robust and scalable strategy for the preparation of diastereomerically pure (2*S*,3*S*-configuration)

Fmoc-EtOAsn(Trt)-OH **1** in eight steps, which is amenable for SPPS. The starting material is easily available in multi-gram scale from D-diethyltartrate. Contaminations with the undesired (2*R*,3*S*)-diastereomer which was obtained together with the starting material could be removed in step 5. The transformation steps reported here allow the synthesis of the target compound in 21% overall yield. This strategy can be used for the synthesis of other non-natural *O*-substituted L-threo- β -hydroxyasparagine analogs.

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