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Preparation and reactivity of aminoacyl pyroglutamates. Facile synthesis of 10-membered-ring cyclic dipeptides derived from 1,4-diaminobutyric and glutamic acids

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Abstract: A number of protected proline-containing dipeptides Boc-Xaa-Pro-OBu^t were converted via epimerization-free oxidation with RuO_4 to dipeptides with an internal pyroglutamic acid residue, Boc-Xaa-Glp-OBu^t. The latter were subjected to oxidative Hoffman-type rearrangement induced by $PhI[OC(O)CF_3]_2$ to give *N*-(aminoacyl)-pyroglutamates. The behavior of these derivatives under basic conditions was studied, and for two such a derivatives an aminoacyl incorporation reaction was observed, producing otherwise poorly accessible 10-membered-ring dilactams derived from 1,4-diaminobutyric and glutamic acids in practicable yields. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: ring expansion; pyroglutamic acid; aminoacyl incorporation reaction; dilactams; ruthenium tetroxide; PIFA; Hoffman rearrangement

INTRODUCTION

The formation of a lactam bridge between side-chain amino and carboxyl functions in a dipeptide unit allows the introduction of various conformational constraints into longer peptide molecules [1]. Dilactams of this type contain a medium size ring and their synthesis remains a challenging task. Direct coupling of the above mentioned functions leads to 12-membered rings in dipeptides formed by Glu and Lys [2, 3], but problems were encountered during cyclization of dipeptides formed by Orn and Glu (11-membered ring) [2]. Limited success has been achieved in preparation of the isomeric 11-membered lactam formed between Lys and Asp by Kumar *et al.* [4] in the synthesis of bicyclic antibiotic cairomycin B, and by Heavner *et al.* [5] in the synthesis of bridged thymopentin analogs. The 10-membered dilactam ring corresponding to a Dab-Glu segment has been shown to be inaccessible via direct cyclization [2, 6]. The only successful synthesis of this dilactam (L,D-enantiomer of **16b**), based on an aminoacyl incorporation reaction [7–13] was published by Kemp and Stites [14], and is summarized in Scheme 1. The serious limitation of that approach was ca. 20% racemization during formation of the important intermediate with an internal pyroglutamyl moiety [14].

To overcome this shortcoming of an otherwise very promising approach, a racemization-free synthetic route to peptides with an internal pyroglutamic acid residue was sought. So far, only a few examples of peptides with an internal pyroglutamic acid residue have been described in the literature [15–18]. In this connection, the unique RuO_4-based procedure of Yoshifuji *et al.* [19, 20] for oxidation of some urethane-protected prolines to the related Glp-derivatives attracted our attention. It was decided to apply this reaction to Boc/Bu^t protected dipeptides in order to access the key intermediates for aminoacyl incorporation reaction, as outlined in Scheme 2.

RESULTS AND DISCUSSION

The RuO_4-oxidation of proline residues into pyroglutamic acid residues proceeds without racemization since no chiral carbons are involved [20]. The present work successfully extended this unusually selective reaction to a number of protected proline-containing dipeptides **3a–d**, **6a,b** which were obtained in optically pure form using the standard coupling procedures of solution peptide chemistry, as illustrated in Scheme 3.

It should be noted that direct coupling of preformed ONp esters of Boc-protected Asn and Gln (standard synthons) with H-Pro-OBu^t (Route A) is accompanied by the formation of the respective 5/6-membered cyclic imides [21]. Moreover, in our

Abbreviations: Aad, 2-aminoadipic acid; Dab, 2,4-diaminobutyric acid; Dap, 2,3-diaminopropionic acid; Glp, pyroglutamic acid; hGln, homoglutamine; iAsn, isoasparagine; IBCF, isobutylchloroformate. iGln, isoglutamine; PIFA, [*I,I*-bis(trifluoroacetoxy)iodo]benzene;

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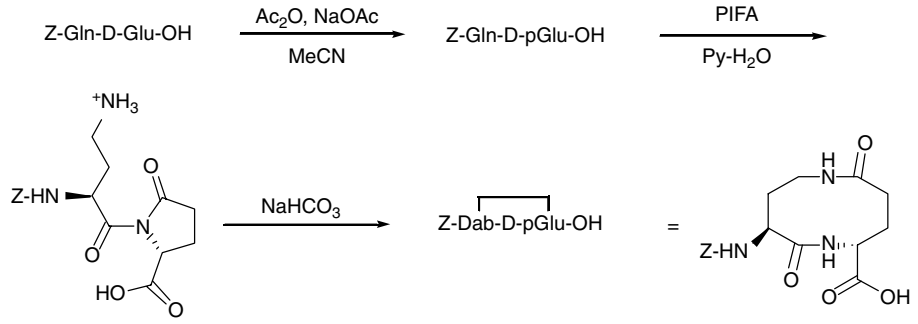
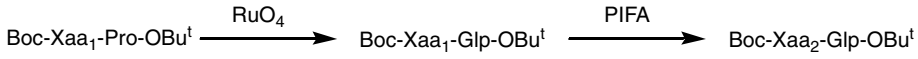
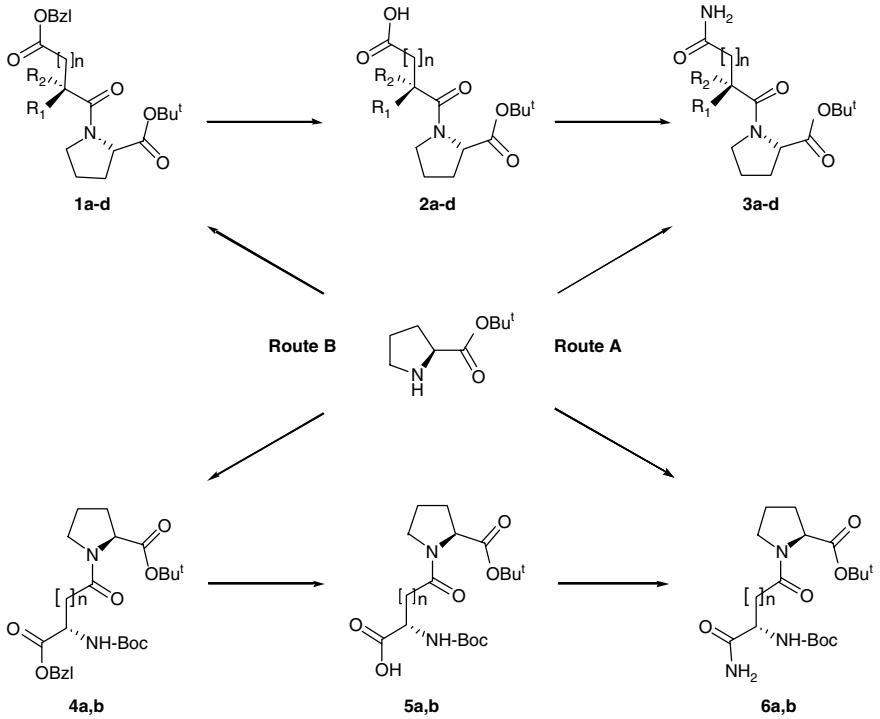


Scheme 1 The aminoacyl incorporation approach to dilactams of Kemp and Stites [14].



Scheme 2 The 'oxidative' approach to dipeptides with internal pyroglutamic acid residues.



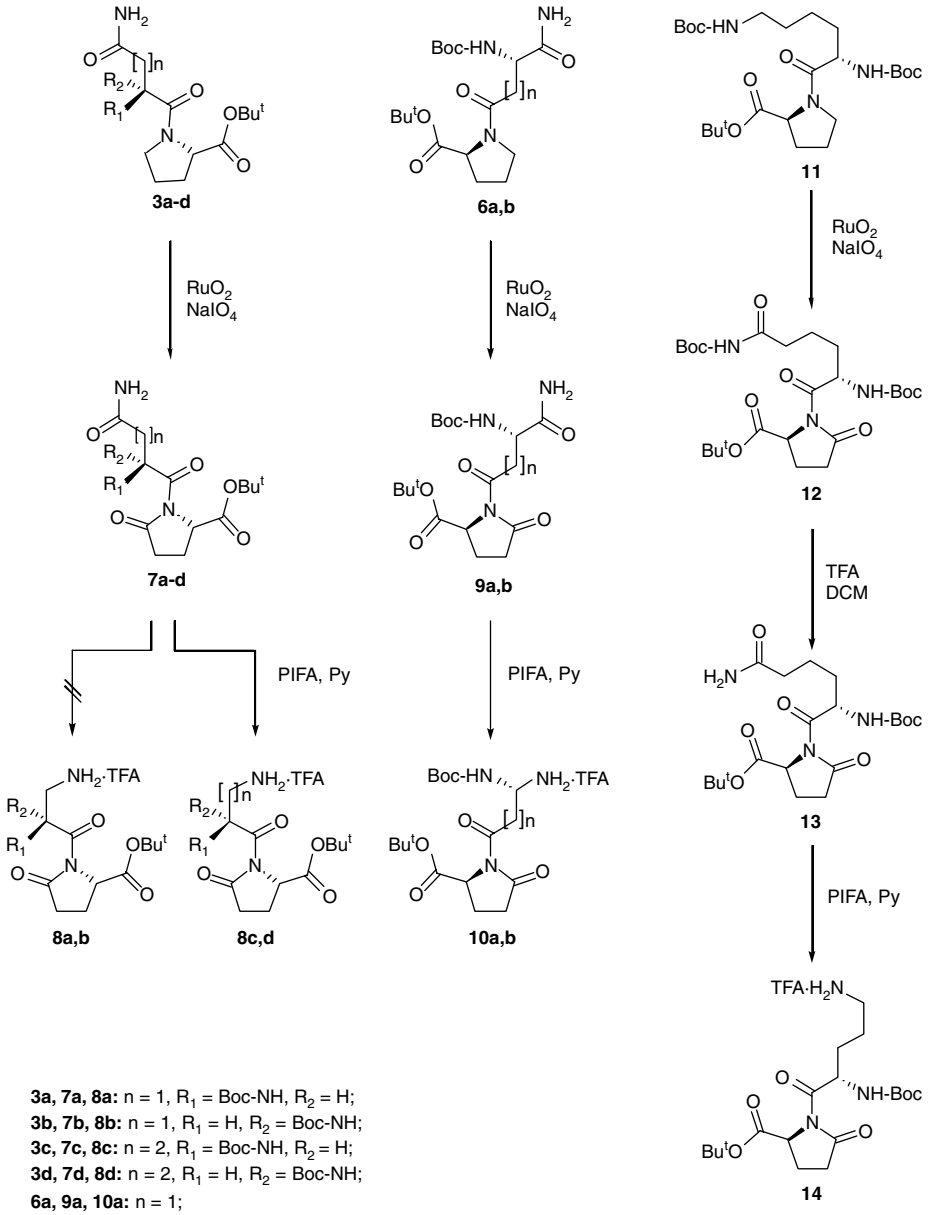
1a, 2a: n = 1, R_1 = Boc-NH, R_2 = H; (Asp);
3a: n = 1, R_1 = Boc-NH, R_2 = H; (Asn);
1b, 2b: n = 1, R_1 = H, R_2 = Boc-NH; (D-Asp);
3b: n = 1, R_1 = H, R_2 = Boc-NH; (D-Asn);
1c, 2c: n = 2, R_1 = Boc-NH, R_2 = H; (Glu);
3c: n = 2, R_1 = Boc-NH, R_2 = H; (Gln);
1d, 2d: n = 2, R_1 = H, R_2 = Boc-NH; (D-Glu);
3d: n = 2, R_1 = H, R_2 = Boc-NH; (D-Gln);
4a, 5a, 6a: n = 1;
4b, 5b, 6b: n = 2.

Scheme 3 The synthesis of proline-containing dipeptides.

experience traces of *p*-nitrophenol in the dipeptides obtained via Route A inhibit the subsequent oxidation step significantly. Recourse to the mixed anhydride method for this coupling step did not improve the yields of the desired dipeptides. To avoid both the above complications, the alternative 3-step synthesis of dipeptides **3a–d**, **6a,b** was carried out as shown in Scheme 3, Route B. In the latter approach, mixed anhydride coupling of protected Asp/Glu derivatives followed by mixed anhydride amidation was employed, and dipeptides **3a–d**, **6a,b** were obtained in practicable yields (33%–44%, for three steps) and essentially free

from unwanted by-products. Boc-Lys(Boc)-Pro-OBu^t (**11**), a precursor of the related ornithine-containing dipeptide **14** [22], was also prepared via coupling of pre-formed Boc-Lys(Boc)-OPfp with H-Pro-OBu^t. Boc-Lys(Boc)-OPfp was synthesized starting from TfaOPfp and Boc-Lys(Boc)-OH according to reference [23]. The latter was isolated after t-butyloxycarbonylation of lysine with Boc_2O as a free acid, rather than as the dicyclohexylammoniun salt described in reference [24].

Boc/Bu^t protected dipeptides were subjected to RuO_4-oxidation under the conditions (with minor variations) described for urethane-protected prolines and their homologues by Yoshifuji *et al.* [19] (Scheme 4). Oxidation is carried out in a two-phase system (ethyl acetate — 10% (w/w) aqueous solution of $NaIO_4$) in which RuO_4 is generated continuously from catalytic amounts of RuO_2 and $NaIO_4$ in excess. The very aggressive nature of RuO_4 imposes serious restrictions on the structure of substrates. On one hand, only oxidatively stable protecting groups can be employed. In this study, Boc and Bu^t groups were used, since their stability is absolute, although reasonable resistance to RuO_4 was also claimed for $Z(NO_2)$, Troc and methyl esters [19]. Second, it should be borne in mind that RuO_4 will oxidize any methylene group connected to nitrogen, so that the side-chain functions of any Orn and Lys residues will be transformed into the related carboxamides [22]. This was taken advantage of and Lys-containing dipeptide **11** was oxidized into the corresponding dipeptide **12** containing a homoglutamine

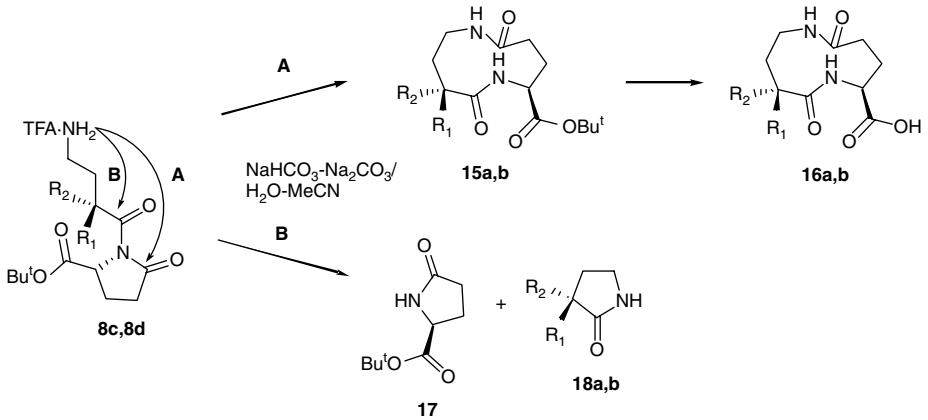


Scheme 4 Synthesis of key intermediates for aminoacyl incorporation reaction.

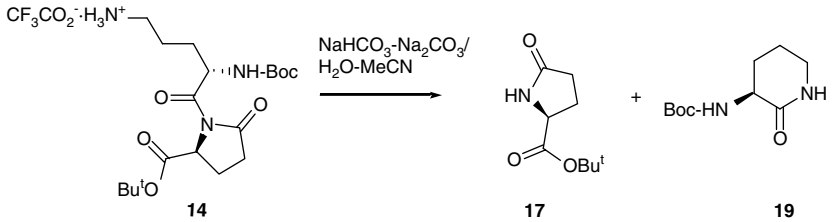
residue, which can be viewed as an ornithine precursor within the framework of this approach (Scheme 4). The bis-Boc protected homoglutamine-containing dipeptide **12** thus obtained was selectively deprotected by 4% TFA in DCM [22] at the side-chain amide functionality prior to reaction with PIFA. The RuO_4 oxidation **3a–d**, **6a,b** and **11** proceeded smoothly, and resulted in practicable yields (54%–84%) of protected aminoacylpyroglutamates **7a–d**, **9a,b** and **12**. These yields indicate that oxidation of $-CH_2N<$ groups in the pyrrolidine ring of proline and of the Lys side chain can be achieved with remarkable selectivity with respect to any $>C_\alpha HN<$, not only in protected prolines [19, 20], but also in simple peptides. Epimerization at proline $C_\alpha H$ is not expected at this step since the absence of racemization has been clearly demonstrated previously [20]. It is believed that the simple and efficient oxidative approach described above will make the hitherto poorly studied peptides with internal pyroglutamic acid more accessible.

In the next step, a Hoffman-type reaction promoted by PIFA as illustrated in the Scheme 4 was used to generate amino groups from the primary amide precursors **7a–d**, **9a,b** and **13**. This highly efficient and selective reaction, developed by Loudon *et al.* [25], was adapted to our cases with minor modifications. The reaction usually proceeds very smoothly, provided freshly prepared PIFA is employed. Indeed, the target amine trifluoroacetates **8c,d**, **10a,b** and **14** were obtained in virtually quantitative yields. However, there were two exceptions. The asparagine-containing dipeptides **7a,b** produced only precipitates containing no peptide material instead of undergoing the normal Hoffman-type amide cleavage. These were tentatively identified as PIFA degradation/self condensation by-product, μ-oxo-*I*,*I*′-bis(trifluoroaceto-O)-*I*,*I*′-diphenyldiiodine(III) [26]. TLC analysis of the supernatant solution revealed only unchanged starting dipeptides **7a,b**. At present, it is not clear why this complication arises in the case of asparagine containing substrates **7a,b** although it does not occur with the isomer **9a**, which reacts in the usual way. This obstacle prompted the development of an alternative synthetic route to 9-membered dilactams [27]. The amine trifluoroacetates **8c,d**, **10a,b** and **14**, prepared according to Scheme 4, were immediately used in the next aminoacyl incorporation step (Schemes 5–7).

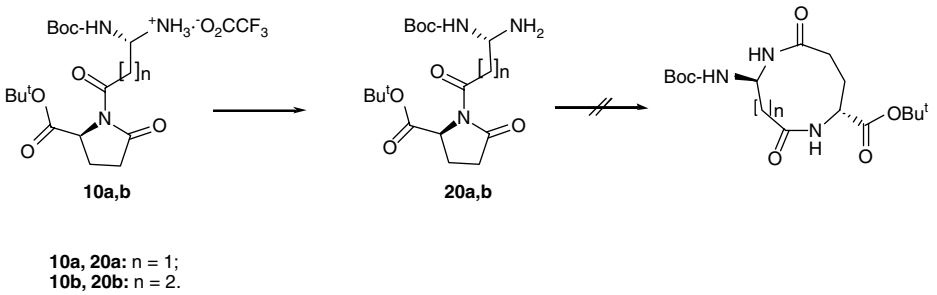
Aminoacyl incorporation reaction was carried out under conditions similar to those described by Stites and Kemp [14] and others [28, 29]. The trifluoroacetates **8c,d**, **10a,b** and **14** were gradually titrated to pH 8.7–9 with $NaHCO_3$–Na_2CO_3 solution in 50% aqueous acetonitrile (2.0–2.5 ml/min). From experience, these



Scheme 5 Aminoacyl incorporation and cleavage side reactions in the synthesis of 10-membered dilactams.



Scheme 6 Cleavage of ornithine-containing pyroglutamate **14**.



10a, 20a: n = 1;
10b, 20b: n = 2.

Scheme 7 Attempted aminoacyl incorporation of gem-diamino derivatives.

conditions were optimal. Identification of reaction products formed under these conditions revealed three different reactivity patterns.

Aminoacylated pyroglutamates **8c,d**, gave the desired monomeric 10-membered dilactams **15a,b** isolated in 33%–40% yields, the rest of the peptide material being mainly two simple lactams, namely Glp-OBu^t (**17**) and Boc-*cyclo*-Dab (**18a**), shown in the Scheme 5. The structures of **17** and **18a,b** were initially assigned on the basis of MS and NMR data and confirmed by independent synthesis starting from Glp or Boc-Dab(Z)-OPfp, respectively. Glp was t-butylated to **17** in the presence of $BF_3 \cdot Et_2O$ using Boc_2O as a convenient source of t-butyl cations. Boc-Dab(Z)-OPfp was subjected to transfer hydrogenolysis with 1,3-cyclohexadiene as donor with the subsequent desired cyclization into **18a**. These exercises led to the conclusion that the principal complication of aminoacyl incorporation is the splitting of aminoacylated pyroglutamates, no oligomerization by-products were formed. Behavior of this kind generally corresponds to the data obtained earlier on simple models [7, 12]. *N*-Aminoacylated lactams can react under basic conditions along two alternative pathways (Scheme 5). On one hand, the free amino group can attack the endocyclic carbonyl group, which results in cyclol formation followed by rearrangement into a dilactam (Scheme 5, Route A), i.e. in an aminoacyl incorporation reaction. On the other hand, the nucleophilic attack at the exocyclic carbonyl gives rise to two simple ring lactams (Scheme 5, Route B). Attempts to suppress the latter unwanted reaction pathway were not successful. To our regret, anhydrous basic conditions (pyridine or tertiary amines in acetonitrile) appeared to favor Route B specifically and to suppress the aminoacyl incorporation reaction.

The higher homolog of **8c**, the ornithine derivative **14**, appeared to react exclusively via Route B (Scheme 6) giving rise to a mixture of Boc-*cyclo*-Orn (**19**) and Glp-OBu^t (**17**). No formation of the target 11-membered dilactam was observed in this case. Again, recourse to different basic conditions (neat pyridine or NMM in anhydrous acetonitrile) did not direct the reaction to Route A, and no dilactam formation was detected under modified conditions. Lactam **19** was identified by comparison with a sample synthesized from Orn in three steps. Ornithine was transformed to its methyl ester which was subsequently cyclized under basic conditions by analogy with *cyclo*-L-lysine [30] into *cyclo*-L-ornithine; the resultant unprotected lactam was treated with Boc_2O.

For the gem-diamino derivatives **10a,b**, no aminoacyl incorporation products were detected after standard treatment with carbonate buffer. Surprisingly, the only isolated products in both these cases were the unreacted free amines **20a** and **20b**. The ring-opened isomeric structures for these compounds were assigned on the basis of their characteristic mass spectra and TLC behavior. Conditions were not found for aminoacyl incorporation with ring enlargement of **10a,b**. It is unclear why the target dilactams were not formed in the case of these pyroglutamates, since their isomers, 9- and 10-membered dilactams, differing only in the position of the urethane-protected NH moiety, were obtained under essentially the same reaction conditions. Perhaps some stereoelectronic effect connected with the geminal Boc-NH group results in reduced nucleophilicity of the adjacent amino group and, consequently, in suppression of the aminoacyl incorporation process.

The behavior of aminoacyl pyroglutamates under basic conditions can be summarized as follows. Gem-diamino derivatives **10a,b** remain unchanged after deprotonation without any sign of aminoacyl incorporation reaction. Nucleophilic attack of the free amino group on the spatially closest exocyclic imide carbonyl followed by splitting of the unusual imide peptide bond (Scheme 6, route B) seems to be the exclusive pathway for compound **14**. For **8c,d** the latter reaction competes seriously with nucleophilic attack on the alternative endocyclic carbonyl, leading to the desired dilactams **15a,b**, i.e. aminoacyl incorporation reaction (Scheme 6, route A). Although the above approach to highly constrained cyclic dipeptides did not meet our expectations in terms of generality, it can be considered as a simple and efficient synthetic method for certain 10-membered cyclic dipeptides which are inaccessible by direct cyclization. Moreover, no oligomerization products are formed in the course of aminoacyl incorporation. The latter is another major

advantage over the direct cyclization technique, which is usually complicated by oligomerization even when carried out at high dilution conditions (10^{-5}M or less) [3]. The reaction work-up is very simple, so that the target medium-ring cycles can be separated from the by-products by repetitive precipitation/recrystallization, i.e. without tedious column chromatography.

A few notes on the physico-chemical behavior of protected dilactams **15a,b** should be made. These substances are prone to self-association and gel formation when dissolved in organic solvents and in this respect their properties are very similar to those described by Kemp and Stites [14] for the Z-protected L-Dab-D-Glu dilactam (Scheme 1). Although the monomeric character of **15a,b** was unambiguously supported by MS data, extensive aggregation in DMSO and $CDCl_3$ precluded interpretation of their ^{1}H-NMR spectra. These observations prompted us to obtain dilactams **15a,b** in deprotected form, **16a,b**, which demonstrated the expected ^{1}H-NMR patterns when recorded in aqueous solutions.

CONCLUSIONS

A simple and convenient method is described for the preparation of *N*-aminoacylated pyroglutamates which is based on two efficient oxidative transformations, of simple proline-containing dipeptides. Transformations of *N*-aminoacylated pyroglutamates under various basic conditions were studied. In two cases, an aminoacyl incorporation reaction was observed which gave access to otherwise poorly available 10-membered ring dilactams derived from Dab and Glu. This approach is not complicated by oligomerization, and is not associated with any danger of epimerization. However, the methodology appeared much less general than initially expected. All attempts to extend the approach to other newly synthesized dipeptides with internal pyroglutamic acid residues were unsuccessful.

MATERIALS AND METHODS

Chemicals

Trifluoroacetic acid, acetic acid, sulfuric acid and all solvents obtained from Reakhim (Moscow, Russia) were purified before use according to standard methods. Ethyl acetate used in RuO_4 oxidations was HPLC grade, L-ornithine was obtained from Sigma Chemical Co. (St Louis, MO, USA). $Ru(OH)Cl_3$, cyclohexene and CrO_3 were supplied by Reakhim (Moscow, Russia), Boc-Gln-OH, Boc-D-Gln-OH, Boc-Glu(OBzl)-OH, Boc-D-Glu(OBzl)-OH, Boc-Glu(OH)-OBzl, Boc-D-Asp(OBzl)-OH and Boc-Asp(OH)-OBzl were obtained from NovaBiochem (Nottingham, UK). Boc_2O, NMM, acetic anhydride, Pd/C, boron trifluoride ethyl etherate, pyroglutamic acid, thionyl chloride, 1,3-cyclohexadiene and trimethyl orthoformate were supplied by Fluka (Buchs, Switzerland). Boc-Glu(OBzl)-ONp, Boc-Asp(OBzl)-OH and HCl·H-Pro-OBu^t were obtained from Reanal (Budapest, Hungary). Boc-Dab(Z)-OH·DCHA was obtained from Bachem (Bubendorf, Switzerland). Pall Boidyne transfer membrane was obtained from Pall Ultrafine Filtration Corp., Glen Cove, NY, USA and BioGel P-6 from Bio-Rad Laboratories, Richmond, CA, USA.

Instrumentation

^{1}H spectra were obtained on a UNITY 600 (Varian, Palo Alto, CA, USA) spectrometer. Peak positions are reported in ppm downfield from tetramethylsilane. Optical rotations were determined on a polarimeter JASCO DIP-360 (JASCO, Tokyo, Japan). Mass spectra were taken on a Finnigan MAT 8430 (Thermo Electron, Bremen, Germany) using the EI technique. TLC was performed on Merck F_{254} silica gel G plates (Merck, Darmstadt, Germany) in solvent systems: (A) $CHCl_3$–MeOH (9 : 1); (B) MeCN–$CHCl_3$–AcOH (8 : 1 : 1). Spots were detected by UV-radiation or, after HBr treatment, by 1% ninhydrin in *n*-butanol.

PEPTIDE SYNTHESIS

(I,I-Diacetoxyiodo)benzene (PIDA)

PIDA was synthesized according to reference [31]. Powdered CrO_3 (3.35 g, 33.5 mmol) was added portion wise to a stirred solution of glacial acetic acid (25 ml) and acetic anhydride (15 ml), so that the temperature was kept below 40 °C. The resulting dark-orange solution was pre-cooled to 10 °C and 5.73 ml (51 mmol) iodobenzene was added, followed by careful dropwise addition of 98% sulfuric acid (6.6 ml, 120 mmol) with vigorous stirring, so that the temperature of the reaction mixture was kept below 30 °C. The stirring was continued for 30 min with heating at 40 °C followed by cooling of the dark-green solution to 5 °C and addition of 20% $AcONH_4$ aqueous solution (100 ml in one portion, pre-cooled in an ice bath). The reaction mixture was kept in the fridge for 3–4 h, the precipitated PIDA was filtered off and washed with cold (5 °C) 10% AcOH (2 × 40 ml) to remove inorganic salts. Crude PIDA was air-dried and dissolved in 100 ml of EtOAc/Ac_2O (9 : 1) under reflux for 5–10 min. The solution was cooled to room temperature and hexane (80 ml) was added. After cooling for 30 min in the fridge the crystals were filtered, washed with ether and hexane and air-dried. The yield was 12.8 g (80%); m.p. 162°–165 °C, (lit. [31], 159°–161 °C).

(I,I-Bis(trifluoroacetoxy)iodo)benzene (PIFA)

The reagent was prepared according to the procedure of Loudon *et al.* [25]. PIDA was dissolved in freshly distilled TFA (2 ml/g PIDA) on moderate heating. The solution was kept for 2 h at room temperature, the crystals were filtered off, dried in a vacuum dessicator protected from sunlight and stored under argon. The yields were 85%–95%. Only freshly prepared material was used in the reactions described below.

Boc-isoglutamine, Boc-Glu(OH)-NH_2

Method A. 2.0 g (4.36 mmol) Boc-Glu(OBzl)-ONp was dissolved in 10–12 ml of THF and 1.2 ml of aqueous 25% NH_3 was added followed by 2 ml of water and 2 ml of MeCN. The mixture

was stirred vigorously at room temperature and the reaction was monitored to completion by TLC (overnight). Volatiles were removed in vacuum, saturated NaCl solution (20 ml) was added and the mixture was extracted with EtOAc (20 ml). The organic phase was washed with the ice-cold 5% K_2CO_3 (15 ml), 5% $KHCO_3$ (15 ml), 0.1 N HCl (15 ml), water (2×) and dried over $MgSO_4$. A white solid obtained after rotary evaporation of EtOAc was washed with hexane and dried in vacuum to yield Boc-Glu(OBzl)-NH_2: 1.28 g (87%); m.p. 124°–126 °C; $R_f = 0.52$ (A), 0.92 (B). This compound was contaminated with Boc-aminoglutarimide (ca. 5% as estimated from the intensity of the TLC spots).

To a slurry of PdO/C (1.3 g) in 20 ml i-PrOH saturated with argon 0.7 ml (18.25 mmol) of formic acid was added and the mixture was stirred vigorously under argon for 10 min (with intensive gas evolution). A solution of Boc-Glu(OBzl)-NH_2 (1.28 g, 3.8 mmol) in 10 ml of i-PrOH and 0.7 ml (18.25 mmol) of formic acid was added and the reaction was stirred for 2 h at room temperature. The catalyst was filtered off and washed with i-PrOH. The residue obtained after vacuum evaporation of volatiles was recrystallized from Et_2O/hexane. The yield of Boc-Glu(OH)-NH_2 was 0.858 g (92%); $R_f = 0.78$ (B) and contains ca. 3%–7% Boc-aminoglutarimide (TLC estimates).

Method B. Analytically pure Boc-Glu(OBzl)-NH_2 was obtained starting from Boc-Glu(OBzl)-OH (3.37g, 10 mmol) using the amidation approach [28] detailed below for **3c,d**: the yield of Boc-Glu(OBzl)-NH_2 in the amidation step was 3.1 g (91%); m.p. 122°–126 °C (lit. [32], 122°–123 °C; [33], 120°–122 °C; [34], 124°–125 °C). The benzyl group was removed in this case via transfer hydrogenation with cyclohexene as the donor according to the general procedure described for **2d**; the isolated yield of Boc-Glu(OH)-NH_2 was 1.94 g (87%); m.p. 163°–166 °C (lit. [34], 158°–159 °C).

Boc-Gln-Pro-OBu^t (3c) and Boc-D-Gln-Pro-OBu^t (3d)

Method A. Boc-Gln-OH (1.23 g, 5 mmol) was dissolved in THF (15 ml) in the presence of NMM (0.55 ml, 5 mmol), the solution was cooled to −12 °C and IBCF (0.65 ml, 5 mmol). The reaction was stirred for 10 min below −10 °C and a suspension of HCl·H-Pro-OBu^t (5.1 mmol, 1.06 g) in 15 ml of THF pretreated with NMM (0.56 ml, 5.1 mmol) for 3 min and cooled to −12 °C was added in one portion. Vigorous stirring was continued for 30 min at −10 °C followed by 1 h at 0 °C and the reaction was allowed to proceed overnight. The solids were filtered off, the solution was evaporated in vacuum and the residue was taken up in 30 ml of EtOAc. The solution was washed with 0.1 M HCl (2 × 20 ml), 5% aqueous solution of $NaHCO_3$ (2 × 20 ml) and saturated NaCl. The organic solution was dried over $MgSO_4$, filtered and evaporated in vacuum. The residue was crystallized from EtOAc–hexane. The yield of **3c** was 1.53 g (77%); m.p. 154°–155 °C; $R_f = 0.30$ (A). Diastereomeric **3d** was prepared in a similar way. The yield of **3d** was 1.36 g (68%); oil; $R_f = 0.38$ (A). Both dipeptides containing traces of Boc-protected aminoglutarimide were used in the next step without further purification.

Method B. 3.37 g (10 mmol) of Boc-Glu(OBzl)-OH or Boc-D-Glu(OBzl)-OH was activated *in situ* using 30 ml of THF, 1.1 ml (10 mmol) NMM and 1.3 (10 mmol) of IBCF and the mixed anhydride thus obtained was reacted with a suspension of HCl·H-Pro-OBu^t (2.28 g, 11 mmol) in 30 ml THF containing NMM (1.21 ml, 11 mmol) as described above. After the standard work-up (see above) the chromatographically pure dipeptides **1c,d** were obtained as transparent oils and used in the next step without further purification.

The yield of **1c** was 3.0 g (61%); $R_f = 0.73$ (A); and **1d** was 3.1 g (63%); $R_f = 0.76$ (A).

Boc-Glu(OH)-Pro-OBu^t (2c)

3.0 g (6.1 mmol) of **1c** was dissolved in a mixture of 20 ml of MeCN and 20 ml of 1 M aqueous NaOH. After hydrolysis was complete (as judged from TLC), the reaction mixture was neutralized with 1 M HCl to pH 2–3 and concentrated in vacuum to a small volume. The residue was treated with 30 ml of EtOAc and 0.1 N HCl (2 × 15 ml) and the organic layer was dried ($MgSO_4$), filtered and evaporated in vacuum. Recrystallization from EtOAc–Et_2O–hexane yielded chromatographically pure **2c**: the yield was 2.2 g (90%); m.p. 164°–167 °C; $R_f = 0.73$ (B).

Boc-D-Glu(OH)-Pro-OBu^t (2d)

Transfer hydrogenation was performed according to reference [35]. A moderate flow of argon was passed through a slurry of 5 g PdO/C in THF (30 ml), 3 ml of freshly distilled cyclohexene was added and the mixture was refluxed in the argon atmosphere for 5–7 min. After cooling to room temperature 1 ml of cyclohexene was introduced followed by a solution of peptide **1d** (3.1 g, 6.3 mmol) in 15 ml of THF and the reaction mixture was refluxed under argon until the starting material disappeared on TLC (system A). The slurry was filtered through the Celite pad and the solids were thoroughly washed with THF (2×). The THF solutions were evaporated in vacuum and the residue was taken up in 50 ml of EtOAc. The latter solution was washed with 0.1 N HCl, dried over $MgSO_4$ and the oily residue obtained on evaporation was dried in vacuum overnight. Recrystallization from EtOAc–Et_2O–hexane yielded chromatographically pure **2d**: the yield was 2.04 g (81%); m.p. 169°–173 °C; $R_f = 0.73$ (B).

Chromatographically pure dipeptides thus obtained were used in the next step without further purification.

Boc-Gln-Pro-OBu^t (3c) and Boc-D-Gln-Pro-OBu^t (3d) (adapted from (32))

2 g (5 mmol) of **2c** or **2d** dissolved or suspended in 8–10 ml of dioxane was treated with Boc_2O (1.74 g, 8 mmol) and pyridine (0.8 ml, 10 mmol) and after 5 min NH_4HCO_3 (1.19 g, 15 mmol) was added. The reaction could be accelerated by brief heating and was accompanied by the evolution of CO_2. When amidation was complete according to TLC analysis (3–12 h) the reaction mixture was diluted with 2–3 volumes of water and concentrated on a rotary evaporator. The residue was taken up in 35 ml of EtOAc and the organic solution was washed with 0.1 N HCl (2 × 20 ml), water and dried over $MgSO_4$. The solution was filtered, vacuum evaporated and the residue was crystallized from EtOAc/hexane.

The yield of **3c** was 1.75 g (88%); m.p. 154°–155 °C; $R_f = 0.30$ (A); $[\alpha]_D^{20}$ − 39.59 (c 1.5, EtOAc); NMR ^{1}H (600 MHz, $CDCl_3$): δ 1.50–1.40 (m, 9H, t-C_4H_9, Gln), 2.24–2.13 (m, 2H,

$C_\gamma HH'$, Gln), 2.06–1.99, 1.99–1.90 (two m, 2H, $C_\beta HH'$, Gln), 4.55–4.45 (m, 1H, $C_\alpha H$, Gln), 5.42, 6.58 (two br. s, 2H, $CONH_2$, Gln), 5.51 (d, $J = 7.81$ Hz, 1H, HNC_α, Gln); 1.50–1.40 (m, 9H, t-C_4H_9, Pro), 3.73–3.62 (m, 2H, $C_\delta HH'$, Pro), 2.39–2.32, 2.31–2.26 (two m, 2H, $C_\beta HH'$, Pro), 1.99–1.90, 1.88–1.80 (two m, 2H, $C_\gamma HH'$, Pro), 4.44–4.35 (m, 1H, $C_\alpha H$, Pro).

The yield of **3d** was 1.68 g (84%); m.p. 114°–118 °C; $R_f = 0.38$ (A); $[\alpha]_D^{20} - 34.26$ (c 1.5, EtOAc); NMR ^1H (600 MHz, $CDCl_3$): δ 1.50–1.40 (m, 9H, t-C_4H_9, D-Gln), 2.21–2.12, 2.11–2.02 (two m, 2H, $C_\gamma HH'$, D-Gln), 2.11–2.02, 2.01–1.93 (two m, 2H, $C_\beta HH'$, D-Gln), 4.52–4.44 (m, 1H, $C_\alpha H$, D-Gln), 5.37, 6.62 (two br. s, 2H, $CONH_2$, D-Gln), 5.61 (d, $J = 7.32$ Hz, 1H, HNC_α, D-Gln); 1.50–1.40 (m, 9H, t-C_4H_9, Pro), 3.73–3.64, 3.63–3.53 (two m, 2H, $C_\delta HH'$, Pro), 2.01–1.93, 1.81–1.73 (two m, 2H, $C_\gamma HH'$, Pro), 2.40–2.33, 2.32–2.24 (two m, 2H, $C_\beta HH'$, Pro), 4.35–4.28 (m, 1H, $C_\alpha H$, Pro).

Boc-Asn-Pro-OBu^t (3a) and Boc-D-Asn-Pro-OBu^t (3b)

The aspartyl-precursors of **3a**, **3b**, Boc-Asp(OBzl)-Pro-OBu^t (**1a**): yield 3.71 g (78%); oil; $R_f = 0.79$ (A), Boc-D-Asp(OBzl)-Pro-OBu^t (**1b**): yield 3.29 g (69%); oil; $R_f = 0.76$ (A) were prepared as described above for **1c,d** and were subjected to hydrogenolysis as in the case of **2d** to yield **2a,b**:

Boc-Asp(OH)-Pro-OBu^t (**2a**): yield 2.71 g (90%); oil; $R_f =$ 0.82 (B).

Boc-D-Asp(OH)-Pro-OBu^t (**2b**): yield 2.32 g (87%); oil; $R_f =$ 0.78 (B).

The dipeptides (**2a,b**) were amidated using the Boc_2O-pyridine-NH_4HCO_3 procedure essentially as described above for **2c,d** to give analytically pure **3a,b**.

3a: yield 2.35 g (87%); white crystalline solid; m.p. 135°–139 °C; $R_f = 0.27$ (A); $[\alpha]_D^{20} - 63.99$ (c 1.4, EtOAc); NMR ^1H (600 MHz, DMSO-d_6): δ 1.47–1.34 (m, 9H, t-C_4H_9, Asn), 2.74–2.63, 2.61–2.52 (two m, 2H, $C_\beta HH'$, Asn), 4.79–4.70 (m, 1H, $C_\alpha H$, Asn), 5.28, 6.60 (two br. s, 2H, $CONH_2$, Asn), 5.70 (d, $J = 9.79$ Hz, 1H, HNC_α, Asn); 1.47–1.34 (m, 9H, t-C_4H_9, Pro), 3.75–3.62 (m, 2H, $C_\delta HH'$, Pro), 2.25–2.14, 2.07–1.89 (two m, 2H, $C_\beta HH'$, Pro), 2.07–1.89 (m, 2H, $C_\gamma HH'$, Pro), 4.44–4.37 (m, 1H, $C_\alpha H$, Pro).

3b: crystallization was carried out for 1 week in the fridge at −5 °C. Yield 1.58 g (68%); m.p. 141°–144 °C; $R_f = 0.38$ (A); $[\alpha]_D^{20} - 25.71$ (c 1.4, DMSO); NMR ^1H (600 MHz, DMSO-d_6): δ 1.50–1.31 (m, 9H, t-C_4H_9, D-Asn), 2.77–2.62, 2.61–2.48 (two m, 2H, $C_\beta HH'$, Asn), 4.88–4.76 (m, 1H, $C_\alpha H$, Asn), 5.41, 6.53 (two br. s, 2H, $CONH_2$, D-Asn), 5.62 (br. s, 1H, HNC_α D-Asn); 1.50–1.31 (m, 9H, t-C_4H_9, Pro), 3.80–3.63 (m, 2H, $C_\delta HH'$, Pro), 2.25–2.09, 2.07–1.96 (two m, 2H, $C_\beta HH'$, Pro), 1.97–1.86 (m, 2H, $C_\gamma HH'$, Pro), 4.32–4.20 (m, 1H, $C_\alpha H$, Pro).

Boc-Asp(Pro-OBu^t)-NH_2 (6a)

Boc-Asp(Pro-OBu^t)-OBzl (**4a**) was prepared from Boc-Asp(OH)-OBzl (3.25 g, 10 mmol) which was activated using NMM (1.1 ml, 10 mmol) and IBCF (1.3 ml, 10 mmol) in 30 ml THF and reacted with a suspension of HCl·H-Pro-OBu^t (2.28 g, 11 mmol) in 30 ml THF containing NMM (1.21 ml, 11 mmol) as described above for **1a–d**. Boc-Asp(Pro-OBu^t)-OBzl (**4a**) was obtained in homogeneous form (TLC) as oil: yield 3.1 g (65%); $R_f = 0.8$ (A).

Benzyl ester in **4a** (3.1 g, 6.49 mmol) was removed by transfer hydrogenolysis procedure as described above for (**1a**, **1b**, **1d**) and Boc-Asp(Pro-OBut)-OH (**5a**) [yield 2.16 g (86%); oil; $R_f = 0.7$ (B)] was amidated on mmol scale according to the procedure detailed above for **2a–d**.

6a: white crystals, yield 1.27 g (59%); m.p. 134°–138 °C (from Et_2O/hexane); $R_f = 0.32$ (A); $[\alpha]_D^{20} - 25.99$ (c 1.7, DMSO); NMR ^1H (600 MHz, $CDCl_3$): δ 1.51–1.37 (m, 9H, t-C_4H_9, iAsn), 3.20–3.05, 2.62–2.51 (two m, 2H, $C_\beta HH'$, iAsn), 4.55 (br. s, 1H, $C_\alpha H$, iAsn), 6.95 (br. s, 1H, HNC_α, iAsn), 6.22, 5.37 (two br. s, 2H, $CONH_2$, iAsn); 1.51–1.37 (m, 9H, t-C_4H_9, Pro), 3.66–3.44 (m, 9H, $C_\delta HH'$, Pro), 2.07–1.81. 1.74–1.55 (two m, 2H, $C_\gamma HH'$, Pro), 2.26–2.09, 2.07–1.81 (two m, 2H, $C_\beta HH'$, Pro), 4.43–4.33 (m, 1H, $C_\alpha H$, Pro).

Boc-Glu(Pro-OBu^t)-NH_2 (6b)

The title compound was prepared from Boc-Glu(OH)-NH_2 (1.23 g, 5 mmol) and HCl·Pro-OBu^t (1.06 g, 5.1 mmol) according to the procedure described for **3c**. Recrystallization after standard work-up of the crude product from EtOAc–hexane yielded 1.46 g (73%) of **6b**; m.p. 160°–162 °C; $R_f = 0.4$ (A). The dipeptide **6b** thus obtained is contaminated with Boc-aminoglutarimide; analytically pure **6b** was obtained from Boc-Glu(OH)-OBzl using a three step approach described for **6a**:

Boc-Glu(Pro-OBu^t)-OBzl (**4b**): yield 3.43 g (68%); m.p. 103°–105 °C; $R_f = 0.75$ (A).

Boc-Glu(Pro-OBu^t)-OH (**5b**): yield 2.59 g (92%); m.p. 124°–127 °C; $R_f = 0.70$ (B).

6b: yield 1.44 g (56%); m.p. 160°–162 °C; $R_f = 0.40$ (A); $[\alpha]_D^{20} - 33.53$ (c 1.0, EtOAc); NMR ^1H (600 MHz, $CDCl_3$): δ 1.50–1.39 (m, 9H, t-C_4H_9, iGln), 2.27–2.10 (m, 2H, $C_\gamma HH'$, iGln), 2.09–2.01. 2.0–1.91 (two m, 2H, $C_\beta HH'$, iGln), 4.41–4.35 (m, 1H, $C_\alpha H$, iGln), 7.03 (br. s, 1H, HNC_α, iGln), 5.76, 5.46 (two br. s, 2H, $CONH_2$, iGln); 1.50–1.39 (m, 9H, t-C_4H_9, Pro), 3.72–3.44 (m, 2H, $C_\delta HH'$, Pro), 2.75–2.55, 2.51–2.38 (two m, 2H, $C_\beta HH'$, Pro), 2.00–1.91, 1.88–1.77 (two m, 2H, $C_\gamma HH'$, Pro), 4.22–4.08 (m, 1H, $C_\alpha H$, Pro).

General Procedure for Oxidation of Proline-containing Dipeptides with RuO_4

$RuO_2 \cdot nH_2O$ employed in the reactions below was prepared according to Mills *et al.* [36].

About 60 mg of $RuO_2 \cdot nH_2O$ was dissolved in 30 ml of 10% aqueous solution of $NaIO_4$ and a solution of a proline-containing dipeptide **3a–d**, **6a,b** (3 mmol in 10–12 ml EtOAc) was added and the reaction mixture was stirred vigorously for 12–14 h. In case the color of the emulsion turned black (indicative of RuO_2 precipitation), an extra 5–10 ml of sodium periodate solution was added to regenerate RuO_4 (yellow). Completion of oxidation was checked by TLC (to resolve the reaction products and the starting materials, the plates with samples applied to were treated by ammonia vapors over 25% aqueous ammonia solution for 5–10 min and carefully dried before TLC run). Organic layer was separated and the aqueous phase was extracted with EtOAc (6 × 15 ml) and 4–6 ml of isopropanol was added to the combined organic solutions to precipitate RuO_2, which was filtered off using BIODYNE polyamide membrane (PAL). In case a dark colored filtrate was obtained, RuO_2 precipitation was completed by vigorous stirring for 2–3 min with anhydrous $MgSO_4$ followed by filtration. $MgSO_4$ treatment and filtration were repeated

until a clear yellow solution was obtained. The latter was washed with 0.02 M ascorbic acid solution (2 × 50 ml), water and dried over $MgSO_4$. The residue obtained after evaporation of the volatiles in vacuum was crystallized from Et_2O–hexane and the precipitate was filtered, washed with hexane and dried in vacuum.

***Boc-Gln-Glp*-OBu^t (7c).** Yield 893 mg (72%); m.p. 168°–170 °C; $R_f = 0.56$ (A); $[\alpha]_D^{20} - 47.19$ (c 1.5, EtOAc); NMR ^{1}H (500 MHz, DMSO-d_6): δ 1.43 (br. s, 9H, t-C_4H_9, Gln), 2.12–2.01. 2.23–2.12 (two m, 2H, $C_\gamma HH'$, Gln), 1.68–1.55, 1.86–1.72 (two m, 2H, $C_\beta HH'$, Gln), 5.07 (br. d, $J = 7.5$ Hz, 1H, $C_\alpha H$, Gln), 6.97 (br. d, $J = 8.9$ Hz, 1H, HNC_α, Gln), 6.70, 7.23 (two br. s, 2H, $CONH_2$, Gln); 1.47 (br. s, 9H, t-C_4H_9, Glp), 2.68–2.45 (m, 2H, $C_\gamma HH'$, Glp), 1.95–1.86, 2.38–2.27 (two m, 2H, $C_\beta HH'$, Glp), 4.46 (d, $J = 9.5$ Hz, 1H, $C_\alpha H$, Glp).

***Boc-D-Gln-Glp*-OBu^t (7d).** Yield 856 mg (69%); m.p. 78°–81 °C; $R_f = 0.46$ (A); $[\alpha]_D^{20} - 31.69$ (c 1.3, EtOAc); NMR ^{1}H (600 MHz, DMSO-d_6): δ 1.42–1.38 (m, 9H, t-C_4H_9, D-Gln), 2.18–2.08, 2.28–2.18 (two m, 2H, $C_\gamma HH'$, D-Gln), δ 1.71–1.60. 1.89–1.81 (two m, 2H, $C_\beta HH'$, D-Gln), 5.10 (d.t, 1H, $C_\alpha H$, D-Gln), 7.01 (br. d, $J = 8.52$ Hz, 1H, HNC_α, D-Gln), 6.73, 7.23 (two br. s, 2H, $CONH_2$, D-Gln); 1.42–1.38 (m, 9H, t-C_4H_9, Glp), 2.73–2.56 (m, 2H, $C_\gamma HH'$, Glp), 1.98–1.90. 2.41–2.30 (two m, 2H, $C_\beta HH'$, Glp), 4.45 (d, $J = 9.35$ Hz, 1H, $C_\alpha H$, Glp).

***Boc-Asn-Glp*-OBu^t (7a).** Yield 731 mg (61%); m.p. 179°–183 °C; $R_f = 0.65$ (B); $[\alpha]_D^{20} - 53.53$ (c1.3, EtOAc); NMR ^{1}H (600 MHz, $CDCl_3$) δ 1.42 (br. s, 9H, t-C_4H_9, Asn), 2.83–2.77 (m, 2H, $C_\beta HH'$, Asn), 5.31 (br. s, 1H, $C_\alpha H$, Asn), 6.46 (br. s, 1H, HNC_α, Asn), 5.70–5.64, 5.60–5.53 (two m, 2H, $CONH_2$, Asn); 1.46 (br. s, 9H, t-C_4H_9, Glp), 2.65–2.56 (m, 2H, $C_\gamma HH'$, Glp), 2.50–2.35 (m, 2H, $C_\beta HH'$, Glp), 4.79–4.72 (m, 1H, $C_\alpha H$, Glp).

***Boc-D-Asn-Glp*-OBu^t (7b).** Yield 647 mg (54%); m.p. 169°–172 °C; $R_f = 0.59$ (B); $[\alpha]_D^{20} - 16.76$ (c1.7, MeOH); NMR ^{1}H (600 MHz, DMSO-d_6) δ 1.35 (br. s, 9H, t-C_4H_9, D-Asn), 2.69–2.58 (m, 2H, $C_\beta HH'$, D-Asn), 5.40–5.30 (m, 1H, $C_\alpha H$, D-Asn), 6.76 (d, $J = 8.44$ Hz, 1H, HNC_α, D-Asn), 6.86, 6.96 (two br. s, 2H, $CONH_2$, D-Asn); 1.41 (br. s, 9H, t-C_4H_9, Glp), 2.58–2.53, 2.46–2.40 (two m, 2H, $C_\gamma HH'$, Glp), 1.99–1.84 (m, 2H, $C_\beta HH'$, Glp), 4.37 (d, $J = 8, 81$ Hz, 1H, $C_\alpha H$, Glp).

***Boc-Asp(Glp-OBu^t)-NH$_2$ (9a).** Yield 695 mg (58%); m.p. 108°–111 °C; $R_f = 0.62$ (A); $[\alpha]_D^{20} - 17.99$ (c1.1, EtOAc); NMR ^{1}H (600 MHz, DMSO-d_6) δ 1.37 (br. s, 9H, t-C_4H_9, i-Asn), 3.26–3.17, 3.10–2.99 (two m, 2H, $C_\beta HH'$, iAsn), 4.33–4.22 (m, 1H, $C_\alpha H$, iAsn), 6.79 (d, $J = 7.59$ Hz, 1H, HNC_α, iAsn), 7.24, 6.99 (two br. s, 2H, $CONH_2$, iAsn); 1.40 (br. s, 9H, t-C_4H_9, Glp), 2.65–2.54 (m, 2H, $C_\gamma HH'$, Glp), 2.37–2.25, 1.96–1.85 (m, 2H, $C_\beta HH'$, Glp), 4.49 (dd, $J = 2.67, 9.51$ Hz, 1H, $C_\alpha H$, Glp).

***Boc-Glu(Glp-OBu^t)-NH$_2$ (9b).** Yield 1.04 g (84%); m.p. 124°–127 °C; $R_f = 0.59$ (A); $[\alpha]_D^{20} - 25.07$ (c 1.3, EtOAc); NMR ^{1}H (600 MHz, DMSO-d_6); δ 1.37 (br. s, 9H, t-C_4H_9, iGln), 2.90–2.80 (m, 2H, $C_\gamma HH'$, iGln), 1.94–1.82 (m, 2H, $C_\beta HH'$, iGln), 3.90–3.82 (m, 1H, $C_\alpha H$, iGln), 6.76 (br. d, $J = 8.23$ Hz, 1H, HNC_α, i-Gln), 7.19, 6.97 (two br. s, 2H, $CONH_2$, iGln); 1.40 (br. s, 9H, t-C_4H_9, Glp), 2.61–2.52 (m, 2H, $C_\gamma HH'$, Glp), 2.37–2.24, 1.78–1.67 (two m, 2H, $C_\beta HH'$, Glp), 4.51 (dd, $J = 2.89, 9.51$ Hz, 1H, $C_\alpha H$, Glp).

Hoffman-type Rearrangement Induced by PIFA for 7c,d

1 mmol of dipeptide (**7c,d**, **9a,b**) was dissolved in 8 ml of DMF–water (1 : 1, v/v) in a light-protected flask and freshly prepared PIFA (615 mg, 1.5 mmol) was added with vigorous stirring. Pyridine (0.160 ml, 2 mmol) was added after 15 min and stirring was continued for 4 h. About 32 mg of polyacrylamide (BioGel P-6) was added to destroy excess PIFA and after efficient stirring for 20 min the reaction mixture was filtered and concentrated in vacuum to a volume of 5–6 ml. The trifluoroacetates **8c,d** thus obtained were immediately used in the next step without further purification. These were treated with a standard solution of $NaHCO_3$–Na_2CO_3 in aqueous acetonitrile (80–100 ml) that was added dropwise (2.0–2.5 ml/min).

Aminoacyl Incorporation Reaction for 8c,d. Boc-*cyclo*(Dab-Glu)-OBu^t (15a) and Boc-*cyclo*(D-Dab-Glu)-OBu^t (15b)

To a residue containing about 1 mmol of trifluoroacetate **8c,d** a buffer solution (obtained from 8.4 g (0.1 mol) $NaHCO_3$, 10.6 g (0.1 mol) Na_2CO_3 dissolved in 1 l of water and diluted with 1 l of MeCN) was added dropwise over the period 30–40 min to pH 8.3–8.5 with efficient stirring and the reaction was left to stand overnight (ca. 80–100 ml of buffer/mmol of the peptide are required). Acetonitrile was removed in vacuum, the residue was extracted with EtOAc (4 × 25 ml), the organic solution was thoroughly washed with 5% K_2CO_3 (2 × 50 ml), 5% $KHCO_3$ (2 × 50 ml), 0.1 N HCl (20 ml) and water, dried over $MgSO_4$, evaporated and dry residue was triturated with 2 ml of acetonitrile, filtered, washed with acetonitrile (2 × 1 ml) and dried to give analytically pure **15a,b**.

***Boc-cyclo(Dab-Glu)*-OBu^t (15a) and *Boc-cyclo(D-Dab-Glu)*-OBu^t (15b).** L-L isomer (**15a**): yield 142 mg (40%); m.p. 135°–137 °C; $R_f = 0.55$ (B); $[\alpha]_D^{20} + 3.3$ (c 1.5, DMSO); MS 385 ($[M]^+$, 4), 341 ($[M\text{-}CO_2]^+$, 20), 329 ($[M\text{-}Bu^t]^+$, 3), 285 ($[M\text{-Boc}]^+$, 9, 79), 227 ($[M\text{-Boc-}Bu^t]^+$, 22), 183 ($[M\text{-Boc-}Bu^t\text{-}CO_2]^+$, 9);

D-L isomer (**15b**): yield 126 mg (33%); m.p. 230°–232 °C; $R_f = 0.62$ (B); $[\alpha]_D^{20} - 37.0$ (c 1.5, DMSO); MS 385 ($[M]^+$, 6.00), 341 ($[M\text{-}CO_2]^+$, 32), 329 ($[M\text{-}Bu^t]^+$, 4), 285 ($[M\text{-Boc}]^+$, 11), 227 ($[M\text{-Boc-}Bu^t]^+$, 23), 183 ($[M\text{-Boc-}Bu^t\text{-}CO_2]^+$, 18).

H-cyclo(Dab-Glu))-OH (16a) and H-cyclo(D-Dab-Glu)-OH (16b). 15 mg (0.039 mmol) of protected dilactam **15a** or **15b** was dissolved in 1.5 ml of TFA, after 20 min the volatiles were removed in vacuum and treatment with TFA (1.5 ml) was repeated. The residue obtained after vacuum evaporation and re-evaporation with water was dissolved in 10 ml of water, neutralized with 5% aqueous ammonia to pH 6.5 and lyophilized.

L-L isomer (16a). Yield 8.7 mg (97%); m.p. 148°–154 °C; $[\alpha]_D^{20} + 22.5$ (c 0.53, H_2O); ^{1}H NMR (600 MHz; $H_2O + D_2O$; H_2O, pH 6.24): δ 2.20–2.08, 2.40–2.26 (two m, 2H, $C_\gamma HH'$, Glu), 2.06–1.95, 2.20–2.08 (two m, 2H, $C_\beta HH'$, Glu), 3.67 (m, 1H, $C_\alpha H$, Glu), 8.51 (br. s, 1H, HNC_α, Glu), 3.08, 2.40–2.26 (two m, 2H, $C_\gamma HH'$, D-Dab), 2.72–2.53 (m, 2H, $C_\beta HH'$, D-Dab), 3.85 (m, 1H, $C_\alpha H$, D-Dab), 7.68 (br. s, 1H, HNC_γ, D-Dab).

D-L-*isomer (16b)*. Yield 8.8 mg (98%); m.p. 118°–120 °C; $[\alpha]_D^{20}$ – 76.3 (c 0.44, H_2O); ^1H NMR (600 MHz; H_2O/D_2O; H_2O, pH 6.37): δ 2.27–2.20, 2.40–2.28 (two m, 2H, $C_\gamma HH'$, Glu); 2.11–1.99 (m, 2H, $C_\beta HH'$, Glu), 3.85 (m, 1H, $C_\alpha H$, Glu), 8.32 (br. d, $J = 9.4$ Hz, 1H, HNC_α, Glu), 3.11.2.44 (two m, 2H, ($C_\gamma HH'$, D-Dab), 2.40–2.28 (m, 2H, $C_\beta HH'$, D-Dab), 4.01 (m, 1H, $C_\alpha H$, D-Dab), 7.81 (br. d, $J = 6.1$ Hz, 1H, HNC_γ, D-Dab).

Attempted Aminoacyl Incorporation in 10a and 10b

Trifluoroacetates **10a,b** were obtained from **9a,b** according to general PIFA-based procedure and treated with carbonate-bicarbonate buffer essentially as described above for the preparation of **8c,d**. The reaction mixture was concentrated in vacuum and extracted with *n*-butanol. TLC analysis (system B) of the crude product did not revealed spots typical of the expected protected dilactams (R_f range 0.5–0.7; ninhydrin negative), while the major product appeared as a low-traveling ninhydrin positive spot with R_f ca. 0.1 which was further analysed by MS-spectrometry.

MS data for **20a**: MS 372 ($[M + 1]^+$, 100), 314 ($[M\text{-}Bu^t]^+$, 78), 258 ($[M + 1 - Bu^t - Bu^t]^+$, 35), 226 ($[M + 1 - OBu^t - OBu^t]^+$, 8), 185 ($[Glp\text{-}OBu^t]^+$, 3), 173 ($[M + 1 - (Glp\text{-}OBu^t) - NH_2]^+$, 3), 129 (Glp-OH, 5).

MS data for **20b**: MS 369 ($[M\text{-}NH_3]^+$, 13), 298 ($[M\text{-}NH_2 - OBu^t]^+$, 7), 241 ($[M + 1 - Boc\text{-}NH\text{-}NH_2]^+$, 7), 188 ($[M + 1 - (Glp\text{-}OBu^t)]^+$, 14), 185 ($[Glp\text{-}OBu^t]^+$, 1), 84 (pyrrolidine-2-one, 88).

Attempted Hoffman-type Rearrangement Induced in PIFA in 7a,b

1 mmol (399 mg) of **7a** or **7b** was dissolved in 8 ml of DMF-water (1 : 1, v/v) in a light-protected flask and freshly prepared PIFA (615 mg, 1.5 mmol) was added with vigorous stirring. After 4–5 min the precipitate was formed. Pyridine (0.160 ml, 2 mmol) was added after 15 min and stirring was continued for 4 h. The precipitate was filtered off and dried in air. The precipitate contains traces of dipeptides. The filtrate was left to stand overnight. The starting dipeptides remained unchanged.

Yield 88–93 mg, m.p. 232°–235 °C; ^1H NMR (600 MHz; DMSO-d_6): δ 7.96 (d, $J = 6.97$ Hz, 4H), 7.61–7.53 (m, 6H); MS 455 ($[M + 1 - 2COCF_3]^+$, 4), 204 (Ph-I, 100), 127 (I, 24), 98 ($[CF_3CO]^+ + 1$, 30), 77 (C_6H_5, 72).

***Boc-Lys(Boc)-OH*.** 3.64 g (20 mmol) of lysine hydrochloride was dissolved in 1 N NaOH (45 ml), 2 g of $NaHCO_3$ was added followed by Boc_2O (9.81 g, 45 mmol) solution in i-PrOH (60 ml) and the reaction was vigorously stirred overnight. Volatiles were removed on a rotary evaporator, the aqueous solution was combined with 5 ml of 1N NaOH, extracted with hexane (30 ml), acidified with 4 M $KHSO_4$ to pH 1.5–2 and extracted with EtOAc (40 ml). The organic extract was washed with 0.1 N HCl (2 × 10 ml), dried over $MgSO_4$, filtered and evaporated in vacuum to give an oily residue which was dried in high vacuum. Yield of Boc-Lys(Boc)-OH was 4.77 g (69%); $R_f = 0.75$ (B).

***Boc-Lys(Boc)-OPfp*.** The title derivative was prepared according to reference [24]. Boc-Lys(Boc)-OH (3.46 g, 10 mmol) was dissolved in DCM (20 ml) and pyridine (0.96 ml, 12 mmol) was added with stirring followed by CF_3CO_2Pfp (1.89 ml, 11 mmol). After 3 h, when TLC indicated that the reaction was complete the mixture was diluted with DCM (20 ml), and washed with 0.1M HCl (2 × 20 ml), 5% $NaHCO_3$ (20 ml), dried over $MgSO_4$, filtered and evaporated in vacuum. Yield 4.45 g (87%); m.p. 100°–103 °C; $R_f = 0.82$ (A).

***Boc-Lys(Boc)-Pro*-OBu^t *(11)*.** A solution of 2.56 g (5 mmol) of Boc-Lys(Boc)-OPfp, H-Pro-OBu^t·HCl (1.08 g, 5.2 mmol) and Et_3N (1.5 ml, 11 mmol) in 20 ml of DCM was stirred overnight at room temperature. The reaction mixture was diluted with DCM to 50 ml, washed with 0.1 M HCl (2 × 20 ml), 5% Na_2CO_3 (20 ml), water and dried over $MgSO_4$. After filtration and evaporation 2.05 g (82%) of **11** was obtained in chromatographically pure form and was used in the next step without further purification (oil; $R_f = 0.75$ [A]).

***Boc-Aad(NHBoc)-Glp*-OBu^t *(12)*.** The dipeptide **11** was subjected to RuO_4 oxidation according to the procedure described above using 15–20 ml EtOAc, 10% $NaIO_4$ solution (60 ml) and 100 mg $RuO_2 \cdot nH_2O$. The mixture was vigorously stirred for 40 h and kept yellow by periodic addition of aliquots of fresh 10% $NaIO_4$ solution (ca. 10 ml). After oxidation was complete according to TLC (A), the organic phase was separated and the aqueous phase was thoroughly extracted with EtOAc (6 × 15 ml). To the combined organic extracts i-PrOH (4–6 ml) was added, the solution was cooled in the ice bath for 2 h, filtered and the precipitate was washed with EtOAc. If any dark discoloration of combined organic solutions persisted, the RuO_2 precipitation was completed by vigorous stirring for 2–3 min with anhydrous $MgSO_4$ (15–20 g/100 ml). $MgSO_4$ treatment was repeated until a clear yellow solution was obtained. The combined filtrates were washed with 0.02 M ascorbic acid (2 × 50 ml) to remove traces of iodine, 0.1 M HCl (2 × 20 ml), 5% Na_2CO_3 (20 ml), water, dried over $MgSO_4$, filtered and evaporated in vacuum. The **12** was obtained as oil, 1.33 g (63%); $R_f = 0.48$ (A).

***Boc-Aad*(NH_2)*-Glp*-OBu^t *(13)*.** 1.33 g (2.52 mmol) of **12** was dissolved in 4% TFA-DCM (v : v, 50 ml) pre-cooled to −5 °C, the solution was stirred for 40 min at this temperature followed by stirring for 20–30 min at room temperature until the reaction was close to completion as judged from TLC (system A). The reaction mixture was poured into aqueous 5% Na_2CO_3 saturated with NaCl and stirred vigorously for 1–2 min. The organic phase was washed with 0.1 M HCl, dried over $MgSO_4$, filtered and evaporated in vacuum. The oily residue was triturated with Et_2O (30 ml) with cooling in an ice bath, the precipitate was filtered off, washed with cold hexane and dried in vacuum. Yield of **13** was 552 mg (51%); m.p. 168°–172 °C; $R_f = 0.40$ (A); $[\alpha]_D^{20}$ – 49.59 (c 1.5, EtOAc); NMR ^1H (600 MHz, DMSO-d_6): δ 1.35 (br. s, 9H, t-C_4H_9, hGln), 1.68–1.56 (m, 2H, $C_\gamma HH'$, hGln), 2.09–1.96 (m, 2H, $C_\beta HH'$, hGln), 2.64–2.56 (m, 2H, $C_\delta HH'$, hGln), 5.13 (m, 1H, $C_\alpha H$, hGln), 7.09 (br. d, $J = 8.06$ Hz, 1H, HNC_α, hGln), 6.65, 7.21 (two br. s, 1H, $CONH_2$, hGln); 1.37 (br. s, 9H, t-C_4H_9, Glp), 2.53–2.46, 2.42–2.30 (two m, 2H, $C_\gamma HH'$, Glp), 1.95–1.82, 1.56–1.47 (two m, 2H, $C_\beta HH'$, Glp), 4.55 (dd, $J = 4.03$, 9.54 Hz, 1H, $C_\alpha H$, Glp).

***Boc-Orn(H)-Glp*-OBu^t·TFA *(14)*.** In a light-protected flask, 427 mg of **13** (1 mmol) was dissolved in 8 ml of 50% mixture

(v/v) of deionized water and DMF freshly degassed in vacuum and PIFA (615 mg, 1.5 mmol) was added followed by pyridine (0.120 ml, 1.5 mmol) 15 min later. The reaction mixture was stirred to completion (TLC, system A) for ca. 4 h, 80 mg of BioGel P-6 was added and stirring was continued for 20 min to decompose any excess PIFA. The solution was filtered and evaporated to 3 ml volume. The solution of **14** thus obtained was immediately used in the next step without further purification.

Attempted Aminoacyl Incorporation Reaction of 14

To a vigorously stirred solution of **14** obtained as described above the 0.05 M $NaHCO_3$ – 0.05 M Na_2CO_3 buffer in water-MeCN (1 : 1, v/v, ca. 100 ml) was added dropwise (2.0–2.5 ml/min) and stirring was continued for 5 h more. The MeCN was evaporated in vacuum and the residue was extracted with EtOAc (2 × 20 ml). The organic phase was washed with 0.1 M HCl (20 ml), water, dried over $MgSO_4$, filtered and evaporated in vacuum. The crude precipitate obtained on triturating with Et_2O–hexane was dried in vacuum and identified as a mixture of **17** and **19** with the help of proton NMR and TLC analysis in the presence of **17** and **19** obtained independently.

Glp-OBu^t (**17**): $R_f = 0.65$ (A) — spot was visualized using I_2 vapors; NMR ^{1}H (600 MHz, $CDCl_3$): δ 1.50–1.46 (m, 9H, t-C_4H_9), 4.11 (dd, $J = 8.44$, 5.32 Hz, 1H, $C_\alpha H$), 2.22–2.14, 2.45–2.40 (two m, 2H, $C_\gamma HH'$), 2.40–2.28 (m, 2H, $C_\beta HH'$), 6.14 (br. s, 1H, HNC_α); MS 129 ([M + 1-Bu^t]$^+$, 10), 113 ([M + 1-OBu^t]$^+$, 17), 84 ([M-CO_2Bu^t]$^+$, 16), 69 (M-CO_2Bu^t – O]$^+$, 30).

Boc-*cyclo*-Orn (**19**): $R_f = 0.46$ (A); NMR ^{1}H (600 MHz, $CDCl_3$): δ 1.46–1.41 (m, 9H, t-C_4H_9, Boc), 4.03 (m, 1H, $C_\alpha H$), 2.53–2.46, 1.96–1.85 (two m, 2H, $C_\beta HH'$), 1.96–1.85, 1.65–1.55 (two m, 2H, $C_\gamma HH'$), 3.36–3.29 (m, 2H, $C_\delta HH'$), 5.44 (br. s, 1H, HNC_α), 6.09 (br. s, 1H, NHCO); MS 158 ([M + 1-Bu^t]$^+$, 26), 141 ([M-OBu^t]$^+$, 30), 113 ([M-Boc]$^+$, 17), 98 ([M-Boc-NH]$^+$, 3).

Glp-OBu^t ***(17).*** Glp-OH (2.58 g, 20 mmol) was placed into a thick-walled screw-capped bottle (Duran), $Et_2O \cdot BF_3$ (3.27 ml, 26 mmol), and molecular sieves 4A (1.2 g) were added, the mixture was cooled in an ice bath and a solution of Boc_2O (5.45 g, 25 mmol) in DCM (10 ml) was added dropwise accompanied by evolution of CO_2. The reaction mixture was stirred for 20 min at 0 °C followed by stirring for 18 h at room temperature. 2.5 g of $NaHCO_3$ was added, stirring was continued for 15–20 min and the solution was filtered through Celite pad, the solids were washed with DCM and the combined filtrates were evaporated in vacuum. The oily residue thus obtained was dissolved in EtOAc (50 ml), washed with 5% Na_2CO_3 (2 × 30 ml), 0.1 M $NaHCO_3$ (2 × 30 ml), water, dried over $MgSO_4$ and filtered; the solvents were removed in vacuum and the residue was crystallized from EtOAc-hexane, washed with hexane and dried in vacuum. Yield 1.39 g (37%); m.p. 105°–107 °C (lit. [15], 105°–107 °C; [37], 109°–110 °C); $R_f = 0.65$ (A) spot was visualized using I_2 vapors; $[\alpha]_D^{20} + 8.9$ (c 1.0, EtOH); NMR ^{1}H (600 MHz, $CDCl_3$): δ 1.50–1.46 (m, 9H, t-C_4H_9), 4.11 (dd, $J = 8.44$, 5.32 Hz, 1H, $C_\alpha H$), 2.22–2.14, 2.45–2.40 (two m, 2H, $C_\gamma HH'$), 2.40–2.28 (m, 2H, $C_\beta HH'$), 6.14 (br. s, 1H, HNC_α).

Boc-cyclo-Orn, (S)-3-Boc-aminopiperidin-2-one (19). HCl · H-Orn-OH (2.7 g, 16 mmol) was dissolved in 40 ml of MeOH and mixed with trimethyl orthoformate (4 mL, 36.4 mmol), the mixture was cooled to −10 °C, and $SOCl_2$ (2 ml, 27.4 mmol) was added. Stirring was continued for 30 min at −10 °C and 72 h at room temperature. Additional $SOCl_2$ (0.5 ml, 6.8 mmol) was added and the reaction was stirred for an extra 24 h. Volatiles were removed on a rotary evaporator and the residue was dissolved in 20 ml of methanol and re-evaporated. After standing for 3 h at room temperature crystallization of the oil began and was completed by the addition of ether (25 ml). Precipitated H-Orn-OMe · 2HCl was filtered off, washed with ether and dried in vacuum. Yield 3.34 g (95%); m.p. 203°–205 °C.

To a solution of H-Orn-OMe · 2HCl (3.3 g, 15.1 mmol) in dry MeOH (76 ml) 2.2 g of NaOMe was added, the mixture was refluxed for 4 h. Ammonium chloride (250 mg) was added, the mixture was allowed to stand overnight, filtered and evaporated in vacuum. The oily residue was dried and dissolved in 12 ml of 1 M NaOH and treated with solution of Boc_2O (4.5 g, 20.6 mmol) in THF (20 ml). After stirring overnight, the volatiles were removed in vacuum, 1 M NaOH (15 ml) was added to the aqueous solution and the product was extracted into EtOAc (20 ml). The organic layer was washed with 1 M NaOH (20 ml), 0.1 M HCl (2 × 20 ml), water and dried over $MgSO_4$. The residue obtained after filtration and evaporation in vacuum was crystallized from EtOAc–Et_2O–heptane at −5 °C for 48 h. The precipitate was filtered off, washed with heptane and dried. The yield of **19** was 92 mg (2.8%, not optimized); m.p. 112°–115 °C; $R_f = 0.46$ (A); NMR ^{1}H (600 MHz, DMSO-d_6): δ 1.37 (br. s, 9H, t-C_4H_9, Boc), 3.79 (br. s, 1H, $C_\alpha H$), 1.95–1.87, 1.81–1.73 (two m, 2H, $C_\beta HH'$), 1.73–1.56 (m, 2H, $C_\gamma HH'$), 3.09 (br. s, 2H, $C_\delta HH'$), 6.79 (br. s, 1H, HNC_α), 7.45 (br. s, 1H, NHCO).

Boc-cyclo-L-Dab, (S)-3-Boc-aminopyrrolidin-2-one (18a). Boc-Dab(Z)-OH · DCHA (2.66 g, 5 mmol) dissolved in $CHCl_3$ (10 ml) was treated with CF_3CO_2Pfp (0.94 ml, 5.5 mmol), the reaction mixture was stirred for 1 h at room temperature and CF_3CO_2Pfp (0.17 ml, 1 mmol) was added followed by pyridine (0.08 ml, 1 mmol). After 1 h the mixture was diluted with $CHCl_3$ (20 ml), washed with 0.1 N HCl (20 ml), 5% $NaHCO_3$ (20 ml), dried over $MgSO_4$, filtered and evaporated in vacuum. The residue was crystallized from EtOAc-heptane, the solid was washed with heptane and dried. The yield was 2.22 g (85.6%); m.p. 67°–71 °C; $R_f = 0.87$ (A).

Cyclohexadiene (2 ml, 21.3 mmol) was added to a slurry of Pd/C (1.8 g) in THF (20 ml). The mixture was refluxed in argon for 5–7 min and cooled to room temperature. Boc-Dab(Z)-OPfp (1.3 g, 2.5 mmol) and cyclohexadiene (0.2 ml, 2.1 mmol) were added, the mixture was refluxed for 5 min, cooled, filtered through Celite and the solids were washed with THF. The residue obtained after evaporation in vacuum was taken in EtOAc (20 ml), washed with 5% Na_2CO_3 (20 ml), 0.1 M HCl (20 ml), dried over $MgSO_4$, filtered and evaporated to dryness in vacuum. The solid was washed with heptane and dried. The yield was 402 mg (80%); m.p. 168°–171 °C; $R_f = 0.3$ (A); NMR ^{1}H (600 MHz, DMSO-d_6): δ 1.38 (br. s, 9H, t-C_4H_9, Boc), 4.00–3.94 (m, 1H, $C_\alpha H$), 2.27–2.18, 1.86–1.78 (two m, 2H, $C_\beta HH'$), 3.14–3.09 (m, 2H, $C_\gamma HH'$), 6.97 (d, $J = 8.98$ Hz, 1H, HNC_α), 7.67 (br. s, 1H, NHCO).

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