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A shortened, protecting group free, synthesis of the anti-wrinkle venom analogue Syn-Ake® exploiting an optimized Hofmann-type rearrangement

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ABSTRACT

An unusual five-step synthesis of H-β-Ala-Pro-DabNHBz diacetate (a muscular nicotinic acetylcholine receptor antagonist) has been delineated through Hofmann-type rearrangement as a final step to build the target skeleton. The synthesis has been carried out using a protecting group free strategy and employed readily available reagents as the starting materials.

H-β-Ala-Pro-DabNHBz diacetate (Syn-Ake®) (**1**) is a synthetic tripeptide (Fig. 1), with a mode of action similar to that of Waglerin 1, a polypeptide that is found in the venom of the Temple viper (*Tropidolaemus wagleri*).[1]

The peptide **1** is an antagonist of the muscular nicotinic acetylcholine membrane receptor (mnAChR). When mnAChR is blocked, the ion channel remains closed. There is no uptake of Na^+ and the muscle cells stay relaxed.

A traditional peptide approach to the synthesis of **1** from the corresponding amino acids (β-alanine, L-proline, and L-2,4-diaminobutyric acid) involves a 10-step synthesis including the installation of protecting groups, condensation, and finally deprotection.[2] Also, in the case of a L-2,4-diaminobutyric acid (L-DAB), selective protection of the amino groups is required and this amino acid is not a commercially available low cost reagent.

As part of our ongoing research aimed at developing a new, facile, and short synthesis of **1**, we found that the target tripeptide could be obtained from readily available and inexpensive starting reagents. Our synthetic approach for the synthesis of **1** was envisioned via an unusual route, without the use of protecting groups/deprotection strategies, and relies on a Hofmann-type rearrangement as the final step as shown in Scheme 1.

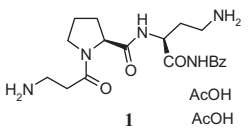

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Figure 1. Structure of Syn-Ake®.

No information on the acylation of L-proline with 3-chloropropionyl chloride has been reported in the literature up to now. Unfortunately, direct acylation provided not only *N*-(3-chloropropionyl)proline (**6**), but also significant quantities of a dehydrochlorination by-product—*N*-acryloylproline (**7**) (20–50% according to LSMS data). After several attempts using both organic and inorganic bases, we found that bases such as Et_3N, pyridine, and K_2CO_3 gave poor yields of **6**. The use of an additional one equivalent of L-proline was chosen as an optimum base—the acylation product was recovered without any dehydrochlorination occurring. Thus, the reaction of 3-chloropropionyl chloride with two equivalents of L-proline in methylene chloride at −10 °C gave **6** in 74% yield. Although there was no need to isolate **6**, the pure product could be obtained as white crystals by removal of methylene chloride under low pressure and crystallization of the residue from EtOAc.[3]

A solution **6** in methylene chloride was cooled to −5 °C and one equivalent of pentafluorophenol (PfpOH) was added, followed by 1.1 equiv of DCC in methylene chloride. The resulting mixture was stirred at 0 °C for one hour and target **2** was isolated as a mixture with *N*-(3-pentafluorophenoxypropanoyl)proline (**8**) (92:8). Crude **2** was crystallized from Et_2O–hexane to give pure (>98%) **2**

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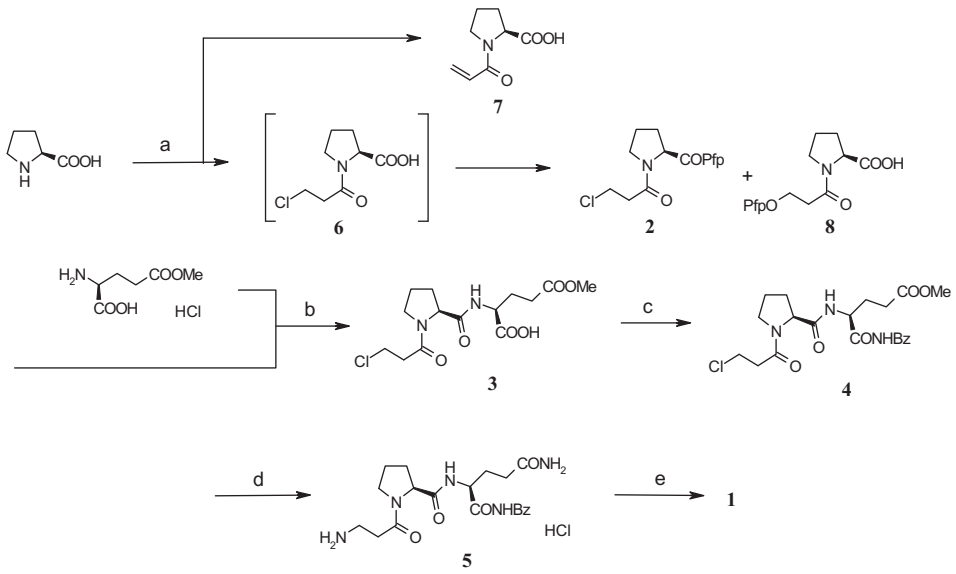

Scheme 1. Reagents and conditions: (a) 3-chloropropionyl chloride, CH_2Cl_2, −10 °C, 0.5 h; −10 °C to 0 °C; 10% aq NaCl; Na_2SO_4. PfpOH, DCC, −5 °C, then 0 °C, 1 h; (b) NMM, CH_2Cl_2, rt, 48 h; (c) $BzNH_2$, HOBt, DCC, DMF, 0 °C, then 5 °C, 18 h; (d) NH_3, MeOH, 10 °C, then rt, 48 h; (e) $PhI(OAc)_2$, 1,4-dioxane–H_2O, Py, 5 °C, 5 h.

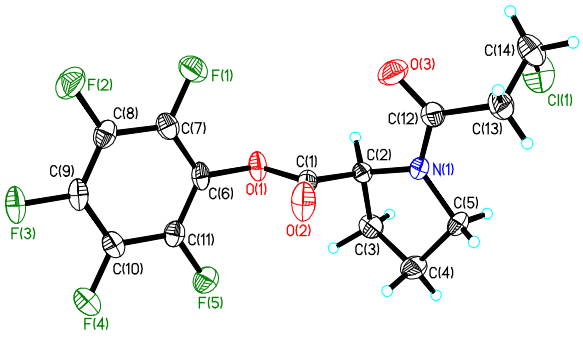

Figure 2. X-ray crystal structure of compound **2**.

in 56% yield as large colorless crystals.[4] The X-ray crystal structure[5] of **2** is shown in Figure 2.

The reaction of 5-methyl ester of L-glutamic acid with pentafluorophenyl ester **2** produced acylated dipeptide **3**. The reaction was carried out under standard peptide synthesis conditions in the presence of NMM at rt for 48 h; methylene chloride was used as the solvent. The yield of pure (96%) **3** was 81%.[6]

Treatment of dipeptide **3** with benzylamine in the presence of the coupling reagents DCC and HOBt at 0–5 °C in DMF afforded benzylamide **4**. Under the optimum conditions: HOBt (1.07 equiv), benzylamine (1.23 equiv) and DCC (1.03 equiv), 18 h, the yield of **4** was 69%.[7]

In the next step, direct ammonolysis of the methyl ester and alkyl chloride resulted in tripeptide **5**. This procedure is conventionally carried out in the presence of methanol as the reaction medium and requires a large excess of ammonia to ensure that further substitution does not take place. Peptide **5** was recovered in 51% yield after purification by flash chromatography on silica gel ($MeOH/CHCl_3$, 1:1).[8]

In the final step, conversion of $-CH_2C(O)NH_2$ into $-CH_2NH_2$ was achieved by the aid of a Hofmann-type rearrangement. So far, to the best of our knowledge, there have been no reports on the synthetic application of selective Hofmann-type degradation of bisamides of glutamic residues in peptides. However, Rodionov et al., described the utility of $PhI[OC(O)CF_3]_2$ (PIFA) for the degradation of protected linear dipeptides containing glutamine.[9] Reactions were performed with PIFA (1.5 equiv) in DMF–H_2O (1:1) for three hours at room temperature in the presence of pyridine (2.0 equiv). However, we encountered difficulties which can be ascribed mainly to the presence of two different amide groups (CONHBz and $CONH_2$). A series of compounds including NaOBr, (diacetoxyiodo)benzene [$PhI(OAc)_2$, PIDA], PIFA, and [hydroxy(tosyloxy)iodo]benzene (HTIB) were investigated as reagents for this rearrangement and the results are shown in Table 1.

Table 1
Results of the Hofmann-type rearrangement using various reagents and different conditions

Entry	Reagent (equiv)	Reaction conditions	Yield % (HPLC)
1	NaOBr (1.5)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	<3
2	PIDA (1.5)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	30
3	PIFA (1.5)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	29
4	HTIB (1.5)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	24
5	PIDA (2.0)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	36
6	PIDA (3.0)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	42
7	PIDA (4.0)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	46
8	PIDA (5.0)	1,4-Dioxane–H_2O, Py, 5 °C, 2 h	48
9	PIDA (4.0)	1,4-Dioxane–H_2O, Py, 5 °C, 3 h	49
10	PIDA (4.0)	1,4-Dioxane–H_2O, Py, 5 °C, 5 h	53
11	PIDA (4.0)	1,4-Dioxane–H_2O, Py, 5 °C, 8 h	51
12	PIDA (4.0)	1,4-Dioxane–H_2O, Py, 5 °C, 5 h (2.4 equiv PIDA, then 1.6 equiv after 3 h)	59 (54[a])

[a] Isolated yield.

Among the reagents used (entries 1–4), PIDA gave a slightly better yield than PIFA and HTIB, and subsequent experiments were carried out with PIDA. During the next optimization (entries 5–12), it was found that the significant factors for the success of this reaction were the amount of PIDA, the time of exposure, as well as the fractional addition of the reagent. Thus, with the optimum conditions the isolated yield of target **1** was 54%. The reaction procedure is very simple.[10] A mixture of pyridine and 1,4-dioxane was added to a solution of tripeptide **5** in H_2O. The solution was cooled in an ice bath and then PIDA (2.4 equiv) in 1,4-dioxane was added at 5 °C. After three hours, an additional quantity of PIDA (1.6 equiv) was added and the mixture was stirred for a further two hours. The mixture was diluted with ice water, washed twice with ethyl acetate, and once with ethyl acetate/butanol (4:1). The aqueous layer was evaporated and the residue was chromatographed (HPLC) to give target **1**.

In summary, we have reported a useful and short method for the synthesis of H-β-Ala-Pro-DabNHBz (**1**). The synthesis has been carried out without the use of protecting groups/deprotection strategies, uses readily available reagents, and relies on a Hofmann-type rearrangement as the final step. Hopefully, this new synthetic procedure will receive further attention for the preparation of DAB-containing peptides.

References and notes

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3. White crystals; purity 97.8% (HPLC); mp 82–84 °C; $[\alpha]_D^{25}$ −74.95 (*c* 5, MeOH); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 1.84–2.27 (m, 4H), 2.76–2.84 (m, 2H), 3.46–3.65 (m, 2H), 3.66–3.83 (m, 2H), 4.42–4.54 (m, 1H), 10.25 (s, 1H); MS (ESI), *m*/*z* (%): 206.5 $[M+H]^+$ (100), 228.2 $[M+Na]^+$ (28.4), 433.3 $[2M+Na]^+$ (94.0).
4. *Compound* **2**: L-Proline (11.50 g, 100 mmol) was suspended in CH_2Cl_2 (100 ml). The suspension was cooled to −10 °C and 3-chloropropanoyl chloride (6.35 g, 50 mmol) was added in one portion. After 30 min, the mixture was heated to 0 °C, washed with brine (2 × 100 ml), and dried over Na_2SO_4. After cooling the organic phase to −5 °C, PfpOH (9.20 g, 50 mmol) and DCC (11.35 g, 55 mmol) in CH_2Cl_2 (50 ml) were added. The mixture was stirred at 0 °C for 1 h. It was then allowed to warm to rt, filtered, and concentrated under reduced pressure. The residue was stirred with Et_2O (10 ml) and hexane (30 ml) and left to stand overnight at 5 °C. The solid residue thus obtained was separated from the solution by filtration and recrystallized from Et_2O–hexane. Yield 10.44 g (56%). Large colorless crystals; purity 98.1% (HPLC); mp 51–53 °C; $[\alpha]_D^{24}$ −78.80 (*c* 5, $CHCl_3$); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 1.98–2.29 (m, 3H), 2.33–2.54 (m, 1H), 2.82 (t, *J* = 6.8 Hz, 2H), 3.57–3.93 (m, 4H), 4.77–4.86 (m, 1H); MS (ESI), *m*/*z* (%): 372.4 $[M+H]^+$ (100), 394.1 $[M+Na]^+$ (4.8).
5. Single crystal X-ray diffraction on a sample of **2** was performed by using a Bruker SMART APEX2 diffractometer. Mercury software was used for the crystal structure plot. Crystals at 150 K, orthorhombic, space group P 21 21 21, *a* = 6.4269(2) Å, *b* = 9.9541(3) Å, *c* = 23.7321(6) Å. Crystallographic data for the structure in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 1016617.
6. *Compound* **3**: Pentafluorophenyl ester **2** (9.29 g, 25 mmol) was dissolved in CH_2Cl_2 (80 ml). To this, 5-methyl ester L-glutamic acid hydrochloride (5.93 g, 30 mmol) and NMM (3.14 g, 31 mmol) were added at rt, and the reaction mixture was stirred for 48 h and washed with 15% citric acid solution (40 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in MeCN (10 ml) and the contents were allowed to stand at 5 °C for 1 d. The resulting precipitate was filtered and dried under reduced pressure. Yield 7.09 g (81%). White amorphous powder; purity 96.2% (HPLC); $[\alpha]_D^{25}$ −66.80 (*c* 5, MeOH); ^{1}H NMR (360.13 MHz, d_6-DMSO): δ 1.70–1.94 (m, 4H), 1.96–2.12 (m, 2H), 2.33–2.41 (m, 2H), 2.66–2.88 (m, 2H), 3.35–3.56 (m, 2H), 3.59 (s, 3H), 3.68–3.81 (m, 2H), 4.14–4.30 (m, 1H), 4.32–4.45 (m, 1H), 8.05 (d, *J* = 8.3 Hz, 0.6H) and 8.33 (d, *J* = 8.3 Hz, 0.4H) (due to rotamers), 12.58 (br s, 1H); MS (ESI), *m*/*z* (%): 349.3 $[M+H]^+$ (100).
7. *Compound* **4**: Acid **3** (6.98 g, 20 mmol) was dissolved in DMF (80 ml) and cooled to 0 °C. $BzNH_2$ (2.46 g, 23 mmol), HOBt monohydrate (3.52 g, 23 mmol), and DCC (4.33 g, 21 mmol) were added successively. After stirring at 5 °C for 18 h, the DCU by-product was filtered off and the filtrate diluted with EtOAc (250 ml). The organic layer was washed with brine (2 × 100 ml), 5% citric acid solution (2 × 70 ml), 1 M $NaHCO_3$ (2 × 70 ml), and H_2O (2 × 70 ml) and dried over Na_2SO_4. On evaporating the EtOAc, an oily residue was obtained, which solidified on triturating with Et_2O (50 ml) and slight warming. Yield 6.05 g (69%). White amorphous powder; purity 95.9% (HPLC); $[\alpha]_D^{25}$ −62.63 (*c* 5, MeOH); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ 1.94–2.18 (m, 6H), 2.35–2.51 (m, 2H), 2.67-2.88 (m, 2H), 3.45-3.76 (m, 4H), 3.62 (s, 3H), 4.24–4.32 (m, 1H), 4.40–4.58 (m, 3H), 7.15–7.36 (m, 6H), 7.56 (d, *J* = 7.7 Hz, 1H); MS (ESI), *m*/*z* (%): 438.4 $[M+H]^+$ (100), 460.0 $[M+Na]^+$ (1.6).
8. *Compound* **5**: Dipeptide **4** (5.69 g, 13 mmol) was dissolved in 20% methanolic NH_3 (200 ml) at 10 °C and kept for 48 h at rt. MeOH was distilled in vacuo and the oily residue triturated with THF (3 × 40 ml) until solidification occurred. Crude **5** was filtered, dissolved in MeOH, and purified by flash chromatography on silica gel (MeOH/$CHCl_3$, 1:1). Yield 2.93 g (51%). White amorphous powder; purity 96.8% (HPLC); $[\alpha]_D^{23}$ −40.70 (*c* 5, MeOH); ^{1}H NMR (360.13 MHz, d_6-DMSO): δ 1.72–2.38 (m, 8H), 2.53–2.75 (m, 2H), 2.91–3.05 (m, 2H), 3.36–3.61 (m, 2H), 4.09–4.44 (m, 4H), 4.87 (br s, 3H), 7.19–7.33 (m, 5H), 7.73 (br s, 2H); ^{13}C NMR (90.56 MHz, D_2O): δ 23.60, 25.94, 28.99, 30.21, 30.54, 34.60, 42.33, 47.18, 53.51, 59.83, 126.60, 126.86, 128.12, 137.73, 170.27, 172.31, 173.72, 177.18; MS (ESI), *m*/*z* (%): 404.3 $[M-Cl]^+$ (100), 426.2 $[M-HCl+Na]^+$ (12.5), 807.5 $[2M-HCl-Cl]^+$ (10.2), 829.4 $[2M-2HCl+Na]^+$ (1.6).
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10. *H-β-Ala-Pro-DabNHBz* (**1**): Tripeptide **5** (2.64 g, 6 mmol) was dissolved in H_2O (60 ml). To this solution were added 1,4-dioxane (30 ml) and Py (8 ml). The mixture was cooled to 5 °C, followed by the addition of PIDA (4.64 g, 14.4 mmol) in 1,4-dioxane (60 ml). After stirring at 5 °C for 3 h, another portion of PIDA (3.09 g, 9.6 mmol) in 1,4-dioxane (40 ml) was added. Stirring was continued for 2 h and the mixture was diluted with ice-H_2O (100 ml) and EtOAc (300 ml). The aqueous layer was washed with EtOAc (2 × 100 ml) and EtOAc/BuOH (4:1) (100 ml) and evaporated under reduced pressure. The residue was chromatographed (HPLC) to give target **1** as yellowish oil. Yield 1.61 g (54%). Purity >98% (HPLC); $[\alpha]_D^{24}$ −81.32 (*c* 1, MeOH); ^{1}H NMR (360.13 MHz, CD_3OD): δ 1.94–2.12 (m, 4H), 2.18–2.29 (m, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 3.05 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 6.3 Hz, 2H), 3.54–3.68 (m, 2H), 4.38–4.43 (m, 3H), 4.48–4.54 (m, 1H), 7.21–7.34 (m, 5H); ^{13}C NMR (90.56 MHz, D_2O): δ 23.65, 28.04, 29.04, 30.34, 34.61, 35.81, 42.43, 47.23, 50.92, 59.47, 126.59, 126.92, 128.16, 137.06, 170.26, 171.43, 177.93; MS (ESI), *m*/*z* (%): 376.2 $[M-AcOH-AcO]^+$ (100), 751.1 $[2M-3AcOH-AcO]^+$ (33.2); IR (ν_{max}, cm^{-1}): 3060, 2212, 1961, 1651, 1557, 1410, 1341, 1276, 1203, 1181, 1137, 1049, 1015, 924, 888, 837, 800, 723, 701, 659, 621.