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# A novel solid phase approach to Aia-containing peptides

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**A strategy was developed to directly assemble 4-amino-1,2,4,5-tetrahydro-indolo[2,3-*c*]-azepin-3-ones on solid-phase-supported peptide sequences. Fmoc- and Boc-based strategies were investigated. The Fmoc-strategy approach strongly depends on the peptide sequence being synthesized while the Boc-based synthesis leads to excellent results for all the selected peptide analogs. The method was applied to prepare Aia-analogs of several bioactive peptides containing one or more Trp-residues which were shown to be important for biological recognition.** 

**Keywords:** solid phase peptide synthesis; Trp analog; reductive amination; melanocortin; growth-hormone-releasing peptide; feline immunodeficiency virus; conformational constraints; conformational scan

## Introduction

The introduction of conformational constraints into bioactive peptides is a well-established strategy in medicinal chemistry. Literature examples show that it may result in an increase of receptor selectivity, it can convert peptide agonists into antagonists, and it enhances the metabolic stability of the peptide [1,2].

Over the last year, our group has focussed on the azepine-constrained analogs Aba **1a** (4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one), Hba **1b** and Aia **2** (4-amino-1,2,4,5-tetrahydro-indolo[2,3-*c*]-azepin-3-one) (Figure 1) which constrain the $\chi 1$ of Phe, Tyr, and Trp, respectively, in the gauche (+) and the trans-conformation [3,4].

Synthetic routes for Aba **1a** [5–7], Hba **1b** [8,9], and Aia **2** [3] have been developed in solution and they have been applied successfully to many peptides.

The use of Aba **1a** or Hba **1b** has resulted in the development of ACE and neutral endopeptidase (NEP) inhibitors [5,7,10], farnesyl transferase inhibitors [11], tyrosine kinase substrates [9], MHC-II ligands [12], bradykinin analogs [13], selective human melanocortin-3 ligands [14], and selective opioid receptor ligands [4,15,16]. Recently, new somatotropin-release inhibiting factor (SRIF) mimetics were synthesized using the Aia-scaffold. This resulted in highly potent analogs with a spectrum of affinities for the five different sst subtype receptors [17].

The major drawback of the described strategies, however, is that dipeptide analogs with appropriate protecting groups have to be synthesized in solution, prior to their incorporation into peptides. The general applicability of this type of conformational constraint would increase tremendously if the assembly of the heterocycle could be performed directly on a solid-phase-supported peptide sequence. In this paper, a novel solid phase approach to Aia-containing peptides is presented.

## Materials and Methods

### General

Rink amide polystyrene resin (capacity: 0.60 mmol/g), Rink amide HypoGel 200 (capacity: 0.62 mmol/g), and MBHA resin (capacity: 0.95 mmol/g) were purchased from Novabiochem, RAPP Polymere, and Neosystem respectively. DCM and *i*PrOH were provided by Fluka. DMF and DIPEA were purchased from Acros. Triethylsilane and piperidine were obtained from Aldrich.

Tcc **8** [18] and Boc-2′formyl tryptophan [3] were synthesized as described previously.

All the amino acids and reagents used for peptide synthesis were purchased from Novabiochem, Fluka, or Bachem. The side-chain-protected amino acids used for Fmoc-chemistry were Fmoc-His(Trt)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Glu(*t*Bu)-OH, and Fmoc-Asp(*t*Bu)-OH. The side-chain-protected amino acids used for Boc-strategy were Boc-His(Tos)-OH·DCHA, Boc-Glu(Ochx)-OH, Boc-Asp(Ochx)-OH, and Boc-Arg(Tos)-OH. The DESC reagent was provided by LOMAC, University of Leuven [19].

Thin layer chromatography (TLC) was performed on glass plates precoated with silica gel 60F254 from Merck using specified solvent systems. Mass spectrometry (MS) was recorded on a VG Quattro II spectrometer using electrospray (ESP) ionization (positive or negative ion mode). Data collection was done with Masslynx software. Analytical RP-HPLC was performed using an Agilent 1100 Series system with a Supelco Discovery BIO Wide Pore® RP C-18 column (25 cm × 4.6 mm, 5 µm) using UV detection at 215 nm. The mobile phase (water/acetonitrile) contained 0.1% TFA. The gradient consisted of a 20 min run from 3 to 97% acetonitrile at a flow rate of 1 ml/min. Preparative HPLC was performed on a Gilson apparatus and controlled with the software package Unipoint. The reverse phase C18-column (Discovery BIO Wide Pore 25 cm × 21.2 mm, 10 µm) was used under the same conditions as the analytical RP-HPLC, but with a flow rate of 20 ml/min. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded at 250 and 63 MHz, respectively, on a Bruker Avance 250 spectrometer

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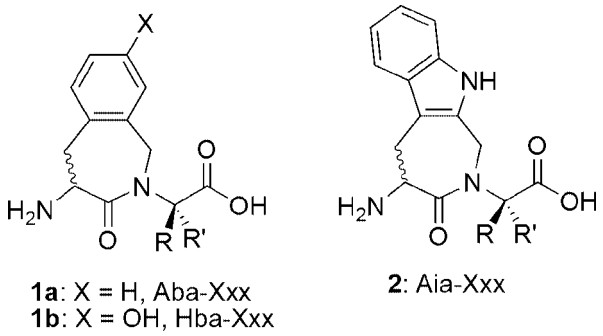



**Figure 1.** Structure of Aba 1a, Hba 1b, and Aia 2.

or at 500 and 125 MHz on a Bruker Avance II 500. Calibration was done with TMS (tetramethylsilane) or residual solvent signals as an internal standard. The solvent used is mentioned in all cases and the abbreviations used are s (singlet), d (doublet), dd (double doublet), t (triplet), and arom (aromatic protons). Optical rotations were measured on a Perkin-Elmer 241 polarimeter or an Optical Activity type AA-5. Infrared spectral data were obtained using an Avatar 370 FT-IR.

### Synthesis of Fmoc-Tcc 9

Tcc **8** (1.50 g, 6.9 mmol) and $Na_2CO_3$ (0.74 g, 6.9 mmol) were dissolved in water : acetone 1 : 1 (18 ml). FmocOSu (2.33 g, 6.9 mmol) was added over a period of 1 h during which the pH was kept at 9 by adding 1 M aq. $Na_2CO_3$. After stirring the reaction mixture overnight, EtOAc (30 ml) was added and the mixture was acidified using 6 M aq. HCl. The phases were separated and the organic layer was washed with water (4 × 5 ml) and dried with $MgSO_4$. After evaporation, the crude product was purified using column chromatography (silicagel, $CHCl_3$: EtOAc gradient).

Yield: 50%

Light yellow powder; IR (neat, $\nu$, $cm^{-1}$) = 3358, 2955, 1697, 1627; $[\alpha]^{20}_D = +58.7^\circ$ (*c* 1, dioxane); $R_f$ (EtOAc) 0.58; HPLC $t_R$ = 17.31 min .; MS 439 $[M + 1]^+$; $^1$H-NMR (250 MHz, $CDCl_3$) $\delta$ 2.91–3.01 (m, 1H, $CH_2\beta$), 3.34 (d, 1H, $J$ = 15.4 Hz, $CH_2\beta'$), 4.34–4.47 (m, 3.4H, CH Fmoc + $CH_2$Fmoc + $CH_2\varepsilon^1$), 4.60 (d, 0.6H, $J$ = 16.2 Hz, $CH_2\varepsilon^2$), 4.80 (d, 0.6H, $J$ = 16.3 Hz, $CH_2\varepsilon^{2'}$), 4.84 (d, 0.4H, $J$ = 16.0 Hz, $CH_2\varepsilon^{1'}$), 5.13 (d, 0.6H, $J$ = 5.2 Hz, $CH\alpha^2$), 5.22 (d, 0.4H, $J$ = 6.4 Hz, $CH\alpha^1$), 6.95–7.92 (m, 12H, Harom), 10.91 (s, 0.4H, $NH^1$ indole), 10.93 (s, 0.6H, $NH^2$ indole), 12.96 (s, 1H, COO*H*); $^{13}$C-NMR (63MHz, $CDCl_3$) $\delta$ 22.8 ($CH_2\beta^1$), 23.1 ($CH_2\beta^1$), 39.1 ($CH_2\varepsilon$), 46.5 (CH Fmoc), 52.8 ($CH\alpha^1$), 53.0 ($CH\alpha^2$), 67.4 ($CH_2$ Fmoc), 104.1–143.8 (C arom + CH arom), 155.3 ($C_1$=O Fmoc), 155.9 ($C_2$=O Fmoc), 172.6 (*C*OOH).

### Synthesis of Fmoc-2′-formyl Tryptophan 3

Fmoc-Tcc **9** (1.71 g, 3.76 mmol) was dissolved in dioxane (35 ml). $SeO_2$ (0.54 g, 4.89 mmol) was added and the solution was refluxed for 3 h (monitored with HPLC). After completion of the reaction, dioxane was evaporated and the product was precipitated with $CHCl_3$.

Yield: 49%

Dark yellow powder; IR (neat, $\nu$, $cm^{-1}$) = 3253, 1722, 1693, 1626; $[\alpha]^{20}_D = +104.7^\circ$ (*c* 1, dioxane); $R_f$ (EtOAc : BuOH : H2O : AcOH 1 : 1 : 1 : 1) 0.83; HPLC $t_R$ = 15.96 min; MS 455 $[M+1]^+$, 437 [M-OH] $^1$H-NMR (250 MHz, DMSO) $\delta$ 3.43 (dd, 1H, $J_1$ = 14.0 Hz, $J_2$ = 9.5 Hz, $CH_2\beta$), 3.59 (dd, 1H, $J_1$ = 14.3 Hz, $J_2$ = 4.9 Hz, $CH_2\beta'$), 4.10–4.17 (m, 3H, CH Fmoc + $CH_2$ Fmoc), 4.31–4.33 (m, 1H, $CH\alpha$), 7.09–7.89 (m, 12H, Harom), 8.30 (s, 1H, C*H*O), 10.06 (s, 1H, N*H* indole), 11.73 (s, 1H, COO*H*); $^{13}$C-NMR (63MHz, DMSO) $\delta$ 25.5 ($CH_2\beta$), 46.5 (CH Fmoc), 55.2 ($CH\alpha$), 65.8 ($CH_2$ Fmoc), 112.8–143.7 (C arom + CH arom), 155.9 (C=O Fmoc), 172.6 (*C*OOH), 182.2 (CHO).

### Peptide Synthesis

*General procedure for coupling-deprotection (Boc-strategy)*

Peptides were synthesized manually by the Boc-strategy using MBHA resin (Neosystem) and *N*,*N*′-diisopropylcarbodiimide (DIC)/HOBt as coupling reagents. A threefold excess of amino acid and coupling reagent was used with a standard coupling time of 1.5 h. Boc-deprotections were carried out by treating the resin twice (5 and 15 min) with 0.5% anisole in TFA : DCM 1 : 1.

*General procedure for coupling-deprotection (Fmoc-strategy)*

Peptides were synthesized manually by the Fmoc-strategy using Rink amide PS-resin (Novabiochem) and TBTU/DIPEA as coupling reagents. A threefold excess of amino acid and coupling reagent was used with a standard coupling time of 1.5 h. Fmoc-deprotections were carried out by treating the resin twice (5and 15 min) with 20% piperidine in DMF.

*General procedure for the reductive amination (Boc-strategy)*

The resin was swollen in a solution of 0.5% AcOH in trimethyl orthoformate (TMOF) : DCM 1 : 1 for 30 min 2 eq. of Boc-2′-formyl tryptophan were suspended in 0.5% AcOH in TMOF : DCM 1 : 1 (2 ml/250 mg resin) and this mixture was added to the resin. A solution of 4 eq. of $NaBH_3CN$ in DMF (2 ml/250 mg resin) was added to the reaction vessel and the mixture was shaken for 24 h. The reaction was monitored by the Kaiser test. After completion of the reaction, the resin was washed with DMF (3 × 1 min), *i*PrOH (3 × 1 min), DCM (3 × 1 min).

*General procedure for the reductive amination (Fmoc-strategy)*

The resin was swollen in a solution of 0.5% AcOH in TMOF : DCM 1 : 1 for 30 min. Two eq. of Fmoc-2′-formyl tryptophan were suspended in 0.5% AcOH in TMOF : DCM 1 : 1 (2 ml/250 mg resin) and this mixture was added to the resin. A solution of 4 eq. of $NaBH_3CN$ in DMF (2 ml/250 mg resin) was added to the reaction vessel and the mixture was shaken for 24 h. The reaction was monitored with the Kaiser test and HPLC. Fresh reagents were added until the completion of the reaction was observed. Remaining aldehyde was washed away using a solution of 2 eq. of semicarbazide hydrochloride and 2 eq. of DIPEA in DMF. The resin was finally washed with DMF (3 × 1 min), *i*PrOH (3 × 1 min), DCM (3 × 1 min).

*General procedure for the cyclization (Boc- and Fmoc-strategy)*

The resin was suspended in DMF. A solution of 3 eq. TBTU and 6 eq. of DIPEA in DMF was added. The mixture was shaken for 4 h and the reaction was monitored using the DESC test [19]. After completion of the reaction, the resin was washed with DMF (3 × 1 min), *i*PrOH (3 × 1 min), DCM (3 × 1 min).



*General procedure for the resin cleavage (Boc-strategy)*

The dry resin was placed in a Teflon reaction vessel with a Teflon-coated magnetic stirrer and anisole was added (1 ml anisol/g peptide resin). HF was distilled under vacuum into the cooled reaction vessel (−78 °C) in a ratio of 10 ml HF/g peptide resin. The reaction mixture was stirred for 60 min in an ice-bath. After 1 h, the HF was removed under vacuum and the cleaved peptide and resin were suspended in dry ether. After filtration, the residue was dissolved in acetic acid and the acetic solution was lyophilized to obtain the crude peptide. The peptides were then purified by preparative HPLC (purity ≥99%) and characterized (Table 1).

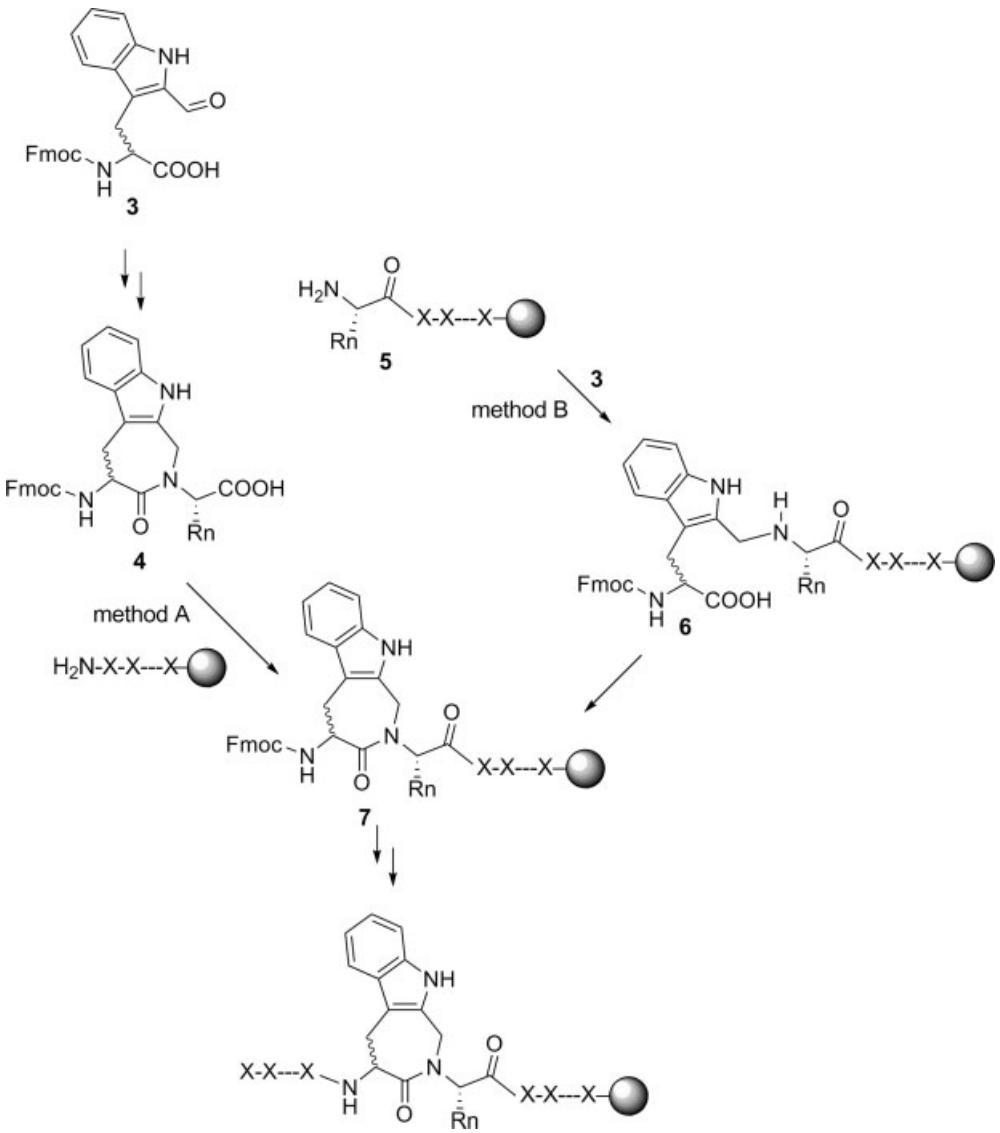
*General procedure for the resin cleavage (Fmoc-strategy)*

The resin was treated with a TFA/triethylsilane 9/1 solution for 3 h. The mixture was filtrated and the filtrate was evaporated. Dry $Et_2O$ was added in order to precipitate the cleaved peptide. The precipitate was filtrated and purified by preparative HPLC (purity ≥97%) and characterized (Table 1).

## Results and Discussion

Our first attempts were directed toward the development of an Fmoc-based approach for the assembly of Aia-containing peptides. As shown in Scheme 1, two strategies toward the synthesis of these peptide analogs can be used. Until now, all peptides containing the Aba, Hba, or Aia conformational constraint were prepared by incorporation of pre-assembled D- or L-dipeptide units. For Aia-containing peptides, this is illustrated by method A in which Fmoc-protected 2′-formyl-Trp **3** is transformed by solution phase synthesis into the dipeptide analog **4** by a reductive amination, cyclization sequence [3]. For each dipeptide sequence in a peptide, a separate synthesis of the constrained dipeptide analog **4** is therefore required. We have now developed method B, in which the reductive amination of **3** is directly performed on the solid-phase-supported peptide sequence **5**, to yield **6** followed by cyclization to **7**. This method is independent of the amino acid succeeding the Aia residue, and therefore more efficient.

There are two crucial steps in this scheme. The reductive amination of a resin-bound peptide **5** with Fmoc-protected




**Scheme 1.** General Fmoc-based approach toward Aia-containing peptides.



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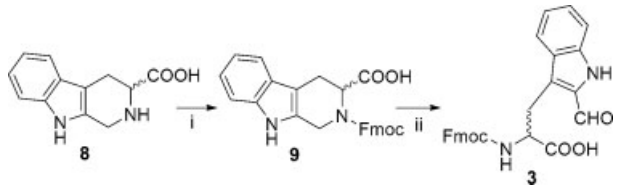



**Scheme 2.** Synthesis of Fmoc-2′-formyl tryptophan 3. (i) FmocOSu, $Na_2CO_3$, $H_2O$ : acetone, 24 h, 50%; (ii) $SeO_2$, dioxane, reflux, 3 h, 48%.

2′-formyl tryptophan **3** leading to secondary amine **6** and the subsequent intramolecular cyclization of **6** to **7**. After that, standard solid phase synthesis can be continued.

L- or D-Fmoc-protected 2′-formyl tryptophan **3** was synthesized analogously to the described procedure for the Boc-2′-formyl tryptophan (Scheme 2) [3].

Tcc **8** [18] was Fmoc-protected using FmocOSu with a yield of 50%. Fmoc-Tcc **9** was then transformed into **3** by oxidation with selenium dioxide (49% yield).

The first tests of the reductive amination/cyclization reactions on solid support were carried out on a model dipeptide, Ala-Phe-$NH_2$, using a Rink amide linker. The reductive amination as well as the cyclization was monitored by cleaving a sample from the resin after each of these reactions and subsequent HPLC/MS analysis. Because of the low solubility of the Fmoc-protected aldehyde **3** in dichloromethane, a DMF/TMOF [20] solvent mixture was used with 2 eq. of aldehyde and 4 eq. of reducing agent $NaBH_3CN$. After 24 h, only a very small amount of dipeptide had reacted. Moreover, the HPLC chromatograms of the samples, which were cleaved from the resin after different reaction times for the reductive amination, always showed the presence of remaining aldehyde **3**, indicating that it could not be removed using the standard protocols (washings with DMF, *i*PrOH, DCM). This points out a possible strong adsorption of this aldehyde to the polystyrene resin. Changing to the more hydrophilic HypoGel resin, which contains an oligoethyleneoxide spacer, did not result in any improvement. This problem could be solved by converting the remaining aldehyde to the corresponding semicarbazone, by reaction with a semicarbazide solution, which then could be efficiently washed away. The low reactivity could however be overcome by a switch of the solvent system to dichloromethane/TMOF (1/1) resulting in 85% conversion after 24 h. The reaction was completed after 48 h despite the fact that the Fmoc-protected aldehyde **3** is only sparingly soluble in this solvent, and is used as a suspension which dissolves during the course of the reaction. The previously observed adsorption of the aldehyde to the resin was not present in this case, which may explain the higher reactivity. More equivalents of aldehyde (3 eq.) or the use of $NaBH(OAc)_3$ as a reducing agent was tested in order to reduce the reaction time, but no improvement was obtained.

The cyclization of **6** to **7** was carried out in a 1 : 1 mixture of dichloromethane and DMF with TBTU as a coupling reagent. The reaction was completed overnight.

These promising results on the dipeptide model led us to apply the method to the synthesis of Aia-analogs of some larger Trp-containing peptides. For this purpose some peptide sequences were selected in which the Trp residue is known to be important for receptor recognition. A series of multiple Trp-containing peptides was prepared to demonstrate the use of the new method for an 'Aia conformational scan'. Therefore we selected the Ac-His-D-Phe-Arg-Trp-$NH_2$ and Ac-His-D-Phe-Arg-Trp-Gly-$NH_2$ sequences, which contain the core tetrapeptide of melanocortin peptide analogs [21,22], the growth hormone secretagogue hexapeptide GHRP-6 His-D-Trp-Ala-Trp-D-Phe-Lys-$NH_2$ and the related tetrapeptide analog Ac-His-D-Trp-Ala-Gly-$NH_2$ [23], and the antiviral fusion inhibitory (FIV) peptide C8: Ac-Trp-Glu-Asp-Trp-Val-Gly-Trp-Ile-$NH_2$ and its simplified analog Ac-Trp-Ape-Trp-Ape-Trp-Ile-$NH_2$ [24]. Each Trp residue in these peptides was replaced by its constrained Aia analog using the Fmoc-based procedure as described above. As shown in Table 2, the results were rather disappointing.

The reaction rate and the degree of conversion in the reductive amination were observed to be strongly dependent on the nature of the peptide sequence. Reaction times varied from 24 h to several days and in some cases, the reductive amination could not be driven to completion at all. This resulted in the formation of several side products. In the case of peptide **10**, some of the side products were identified in the HPLC/MS chromatogram (Scheme 3).

In cases where the reductive amination of the solid-phase-supported peptide **5**–**6** is incomplete, there is a competitive interstrand reaction during the cyclization step to **22**, resulting in the formation of **23**. The secondary amine function in **23** and remaining primary amine can be capped by acetylation in order to eliminate further reactivity. However, after Fmoc-deprotection of

**Table 1.** Characterization of peptides **10**–**25**

| Code | Peptide | Rt (min) | $(M + H)^+$ calculated | MS $(M + H)^+$ found |
|---|---|---|---|---|
| GHRP1 | Ac-His-D-Aia-Ala-Trp-$NH_2$ **10** | 10.78 | 652.30 | 652.30 |
| GHRP2 | His-D-Aia-Ala-Trp-D-Phe-Lys-$NH_2$ **11** | 10.11 | 885.45 | 885.50 |
| GHRP3 | His-D-Trp-Ala-Aia-D-Phe-Lys-$NH_2$ **12** | 10.56 | 885.45 | 885.36 |
| MC1 | Ac-His-D-Phe-Arg-Aia **13** | 9.26 | 698.35 | 698.26 |
| MC2 | His-D-Phe-Arg-Aia-Gly-$NH_2$ **14** | 8.34 | 713.36 | 713.28 |
| FIV1 | Ac-Aia-Glu-Asp-Trp-Val-Gly-Trp-Ile-$NH_2$ **15** | 12.61 | 1143.53 | 1143.19 |
| FIV2 | Ac-Trp-Glu-Asp-Aia-Val-Gly-Trp-Ile-$NH_2$ **16** | 12.64 | 1143.53 | 1143.27 |
| FIV3 | Ac-Trp-Glu-Asp-Trp-Val-Gly-Aia-Ile-$NH_2$ **17** | 12.75 | 1143.53 | 1143.29 |
| FIV4 | Ac-Aia-Ape-Trp-Ape-Trp-Ile-$NH_2$ **18** | 13.14 | 941.50 | 941.43 |
| FIV5 | Ac-Trp-Ape-Aia-Ape-Trp-Ile-$NH_2$ **19** | 13.99 | 941.50 | 941.56 |
| FIV6 | Ac-Trp-Ape-Trp-Ape-Aia-Ile-$NH_2$ **20** | 12.95 | 941.50 | 941.41 |
| GHRP4 | Ac-His-D-Trp-Ala-Aia **25** | 10.36 | 652.30 | 652.34 |

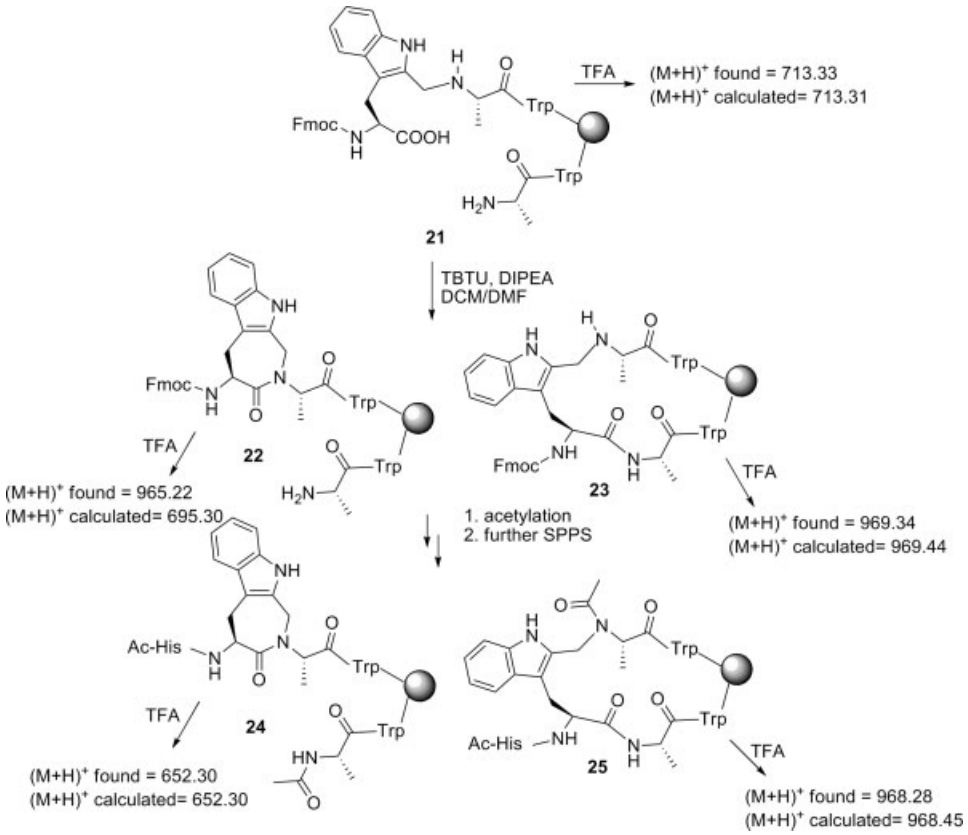


**Table 2.** Peptides synthesized via the Fmoc-strategy

| Code | Sequence | Purity[a] of crude peptide (%) | Yield after HPLC purification (purity[a]) (%) |
|---|---|---|---|
| GHRP1 | Ac-His-D-Aia-Ala-Trp-$NH_2$ **10** | 33 | No HPLC purification possible |
| GHRP2 | His-D-Aia-Ala-Trp-D-Phe-Lys-$NH_2$ **11** | 52 | 15 (99) |
| GHRP3 | His-D-Trp-Ala-Aia-D-Phe-Lys-$NH_2$ **12** | 71 | 16 (>99) |
| MC1 | Ac-His-D-Phe-Arg-Aia **13** | 39 | No HPLC purification possible |
| MC2 | His-D-Phe-Arg-Aia-Gly-$NH_2$ **14** | 26 | <1 (97) |
| FIV1 | Ac-Aia-Glu-Asp-Trp-Val-Gly-Trp-Ile-$NH_2$ **15** | 72 | 6 (98) |
| FIV2 | Ac-Trp-Glu-Asp-Aia-Val-Gly-Trp-Ile-$NH_2$ **16** | 68 | 8 (97) |
| FIV3 | Ac-Trp-Glu-Asp-Trp-Val-Gly-Aia-Ile-$NH_2$ **17** | 16 | No HPLC purification possible |
| FIV4 | Ac-Aia-Ape-Trp-Ape-Trp-Ile-$NH_2$ **18** | 65 | 2 (97) |
| FIV5 | Ac-Trp-Ape-Aia-Ape-Trp-Ile-$NH_2$ **19** | 17 | No HPLC purification possible |
| FIV6 | Ac-Trp-Ape-Trp-Ape-Aia-Ile-$NH_2$ **20** | 43 | 1 (>99) |

[a] Purity as determined by integration of the HPLC chromatogram with UV detection at 215 nm.




**Scheme 3.** Side products observed for peptide 10.

**22** and **23**, both molecules react in further peptide synthesis. This leads to very similar compounds **24** and **25.** Such impurities are often very difficult to remove from the desired peptide analog by preparative HPLC because of their small differences in retention times, which can explain the low yields of the isolated peptides reported in Table 2.

A change of the solvent system from DCM to DMF or DCM : DMF 1 : 1 after partial completion of the reductive amination, and continuation of the reaction did not result in any improvement. Therefore, reactions to selectively acylate the remaining primary amine in the presence of secondary amine in **21** were tested. Reaction with the hindered pivalic acid and DIC/HOBt acylated both amines. Reaction with ethyl trifluoroacetate resulted in selective acylation of the primary amine; however, the reaction could not be completed [25].

Because the observed side products and the poor reproducibility originate from an incomplete reductive amination reaction, possibly because of a limited accessibility of the aldehyde, we decided to switch to a Boc-strategy-based approach, which worked well in solution [3].



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**Table 3.** Peptides synthesized via Boc-strategy

| Code | Sequence | Yield (%) | Purity (%) |
|---|---|---|---|
| GHRP1 | Ac-His-D-Aia-Ala-Trp-$NH_2$ **10** | Crude: 67 | Crude: 98 |
| | | Pure: 34 | Pure: >99 |
| MC1 | Ac-His-D-Phe-Arg-Aia **13** | Crude: 83 | Crude: 90 |
| | | Pure: 48 | Pure: >99 |
| FIV3 | Ac-Trp-Glu-Asp-Trp-Val-Gly-Aia-Ile-$NH_2$ **17** | Crude: 88 | Crude: 99 |
| | | Pure: 42 | Pure: >99 |
| FIV5 | Ac-Trp-Ape-Aia-Ape-Trp-Ile-$NH_2$ **19** | Crude: 83 | Crude: 89 |
| | | Pure: 35 | Pure: >99 |
| GHRP4 | Ac-His-D-Trp-Ala-Aia **26** | Crude: 72 | Crude: 98 |
| | | Pure: 20 | Pure: >99 |

The synthesis of those sequences **10, 13, 17**, and **19** that resulted in highly impure crude reaction mixtures in the Fmoc-based approach was repeated using the Boc-strategy, using MBHA resin. Boc-2′-formyl tryptophan, 0.5% AcOH in DMF : DCM : TMOF 2 : 1 : 1, and $NaBH_3CN$ were used in the reductive amination. The completion of this reaction was confirmed by the Kaiser test. Cyclizations were carried out using TBTU/DIPEA in DMF and monitored with the DESC test, which gives a strong coloration with secondary amines [19]. The results are shown in Table 3.

As can be seen in Table 3, the crude purities of the peptide analogs are very high. This shows that there is a complete conversion during the reductive amination. Consequently during the subsequent cyclization reaction no side compounds are formed. The excellent purities obtained for the MC4 analog **13** and for the GHS4 analog **26** indicate that the heterocycle can be easily constructed even when bound directly to the resin linker.

## Conclusions

A strategy was developed to directly synthesize Aia-containing peptides on solid phase. Two crucial steps are present in this strategy: a reductive amination using protected-2′formyl tryptophan and $NaBH_3CN$, and the subsequent intramolecular cyclization using TBTU/DIPEA. The Fmoc-based strategy turned out to be irreproducible and too highly dependent on the peptide sequence. In contrast, the Boc-strategy resulted in excellent purities of the crude peptides. The generality of the method has been demonstrated for some selected peptide sequences. The presented synthetic strategy can be applied to a variety of peptide sequences, and should allow an 'Aia-conformational scan' of Trp-containing peptides, similar to a previously proposed lactam-scan [26,27]. The effect on the biological properties of the Aia-containing peptide analogs is under investigation and will be reported elsewhere.

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