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# Synthesis and Peptide Binding Properties of Methoxypyrrole Amino Acids (MOPAS)

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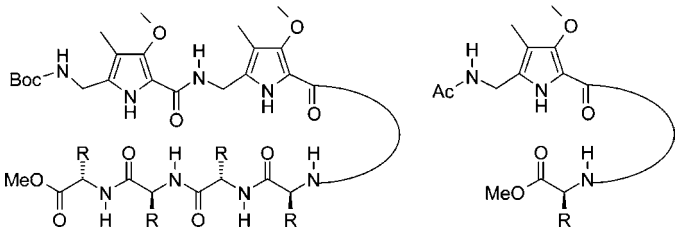
## ABSTRACT




**Methoxypyrrole amino acids (MOPAS) have been prepared and were introduced into small peptides with hairpin structures. The intra- and intermolecular binding properties of this heterocyclic amino acid mimicking a dipeptido β-strand was investigated by NMR titration and X-ray crystal structure analysis. The data reveal a hydrogen bonding pattern that is complementary to a peptide β-sheet.**

The recognition of β-sheet structures is important for protein−protein interactions[1] as illustrated by many examples.[2] Small organic molecules with a hydrogen bonding pattern and structure complementary to β-sheets are promising for interception of protein−protein interaction, for stopping protein aggregation,[3] or stabilization[4] or mimicking β-sheet structures.[5] The work on β-sheet recognition and peptidomimetics[6] has been extensively reviewed. Recently, Chakraborty et al. introduced 5-(aminomethyl)pyrrole-2-

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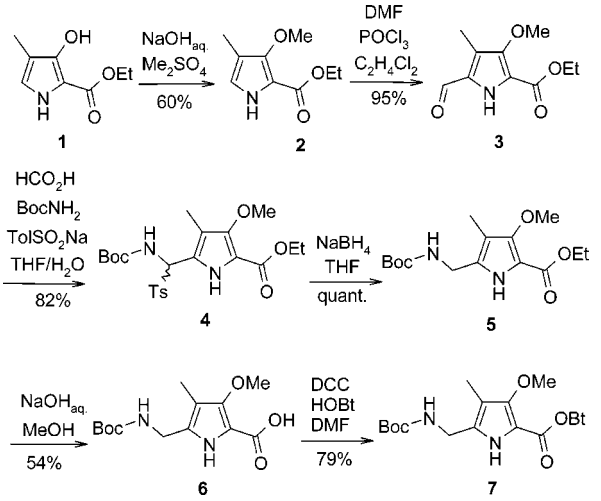
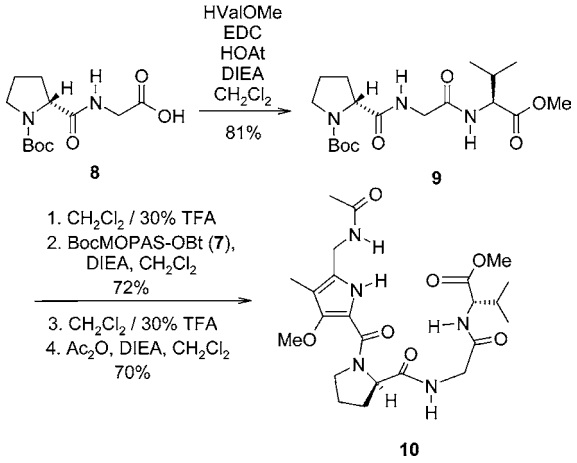
**Scheme 1.** Synthesis of MOPAS **6** and Its Active Ester **7**.




**Scheme 2.** Synthesis $\beta$-Turn Structure **10**.




carboxylic acid as a constrained surrogate of Gly-ΔAla.[7] We have now further elaborated the pyrrole amino acid building block and report the synthesis of methoxypyrrole amino acids (MOPAS), their facile introduction into peptide structures, and their intra- and intermolecular peptide binding properties as determined by NMR and X-ray crystallography.

Oligoamides of pyrrole amino acids must adopt a linear conformation to be complementary to peptide $\beta$-sheets in their hydrogen bonding pattern. A methoxy substituent was therefore introduced in the 3-position to allow the formation of an intramolecular hydrogen bond. This should keep the pyrrole rings of MOPAS oligoamides in one plane. This strategy has been used previously by Nowick et al. in oligomers of methoxy-substituted hydrazino benzoic acids.

Ethyl 3-hydroxy-4-methyl-pyrrole-2-carboxylate (**1**) as starting material for the synthesis of MOPAS was prepared according to a literature procedure.[8] After methylation of the hydroxyl group, Vilsmeier Haack formylation gave pyrrole aldehyde **3**. For compounds **1**−**3**, X-ray crystal structure analyses have been performed (see Supporting Information). The reaction of **3** with *t*-butylcarbamate, sodium *p*-toluenesulfinate, and formic acid gave sulfone **4** in good yield. Quantitative reduction and ester hydrolysis completes the synthesis of Boc-protected MOPAS **6**. The X-ray crystal structure analysis of **5** confirms the connectivity (see Supporting Information). The bond length and angles are typical. The OBt active ester **7** of MOPAS **6** was prepared using standard conditions. Compound **7**, a fine white powder, which decomposes at temperatures exceeding 158 °C, is stable at ambient temperature and can be stored for longer periods of time.

A short $\beta$-turn fragment was prepared to show the ability of MOPAS to be incorporated into peptide structures and form intramolecular hydrogen bonds. Scheme 2 summarizes the synthesis. Gellman's $\beta$-turn fragment D-Pro-Gly (**8**)[9] was extended by Val and coupled with Boc-MOPAS-OBt **7** using standard peptide coupling conditions. Boc deprotection and acylation gave **10**.

The X-ray structure analysis (Figure 1) of the compound nicely reveals the formation of the expected intramolecular hydrogen bonds of the MOPAS structure to Val. NMR ROE contacts ($CDCl_3$) suggest a similar structure in solution (see Supporting Information for data).

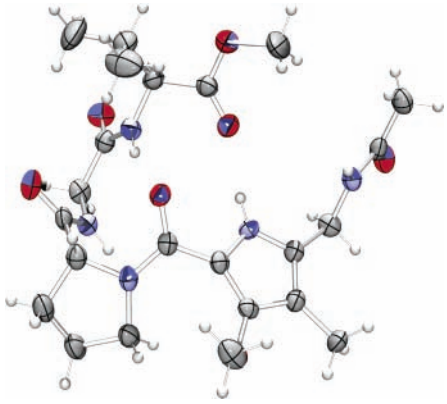


**Figure 1.** Structure of **10** in the crystal.

A more extended peptide structure **16** was prepared to show the complementary structure and binding ability of MOPAS oligomers to peptide $\beta$-sheet structures. The fragment $H_2N$-Phe-Ala-Val-Leu-OMe was coupled with Boc-D-Pro-Gly-OH to give hexapeptide **14**. The coupling of MOPAS

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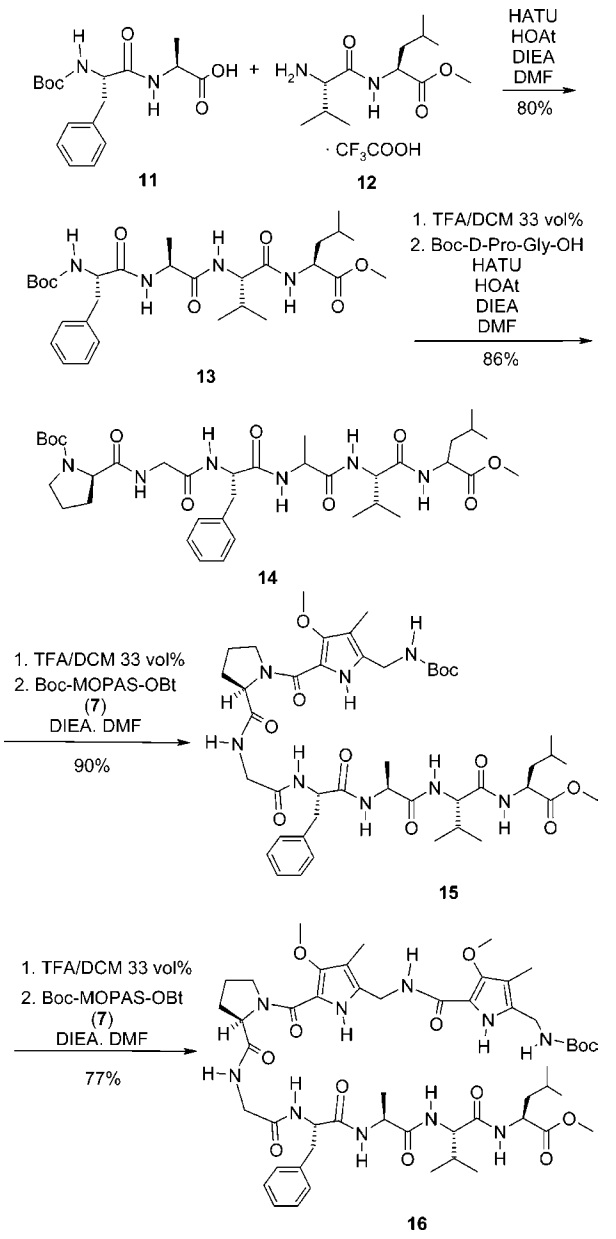
to the N-terminal Pro residue proceeded in good yield using the active ester **7**. A second MOPAS unit was introduced to give compound **16**. The structure of **16** in solution was

**Scheme 3.** Synthesis $\beta$-Turn **16**




investigated by NMR. Analysis of the observed intramolecular ROE contacts in deuterated chloroform clearly support a $\beta$-turn structure as depicted in Figure 2 (see Supporting Information for NMR data). The ROE contacts between the N-terminal MOPAS unit and the C-terminal amino acids show that the interaction of heterocycles and peptide chain propagates from the turn.

To explore intermolecular MOPAS−peptide $\beta$-sheet interactions, NMR titrations were performed in $CDCl_3$. Binding constants of MOPAS **5** to Ac-Val-Val-OMe (**17**) and MOPAS dimer **18** to peptide **13** were determined. Compound **18** was prepared from compounds **5** and **7**. Self-association

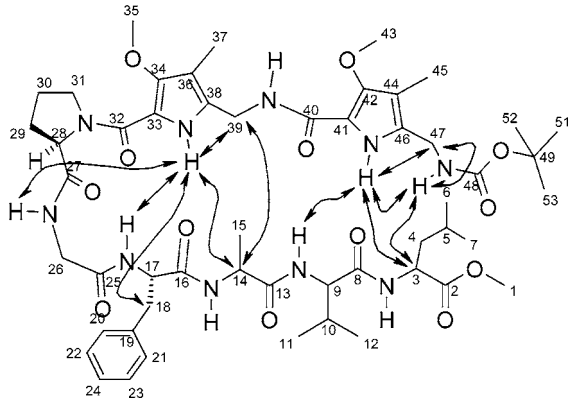


**Figure 2.** ROE analysis of compound **16**.

of all components was measured in dilution experiments and considered in the applied binding model. Chemical-induced shifts (CIS) of several protons of both binding partners were used for determination of the binding constants. CIS of the pyrrole N−Hs are shown as examples in Figure 3. The

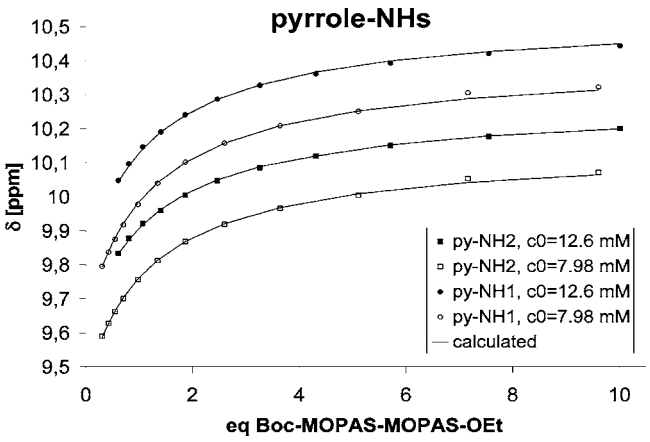
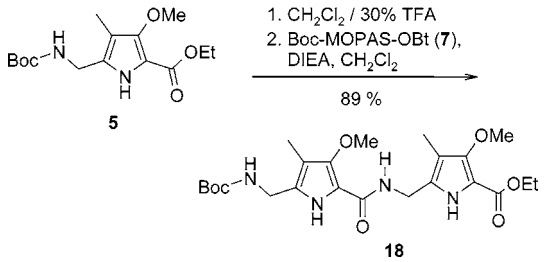



**Figure 3.** NMR titration of tetrapeptide **13** with compound **18**.

binding of **5** to Ac-Val-Val-OMe (**17**) is, with a $K_{11} = 21$ L/mol, significant, but weak. The self-association of **5** has a $K_s = 2$ L/mol. Binding constants and self-association increase with extension of the binding motif. The binding constant of **18** to **13** was determined as $K_{11} = 100$ L/mol, with self-association constants of $K_s = 135$ L/mol for **18** and $K_s = 6$ L/mol for **13**. Although the binding affinities are still rather weak and restricted to nonpolar organic solvents, the results show similar properties for MOPAS and peptides with a tendency to form $\beta$-sheet aggregates. The MOPAS heterocycles resemble the geometry and the binding properties of a dipeptide $\beta$-sheet unit. In summary, we have reported the synthesis of MOPAS **6**, a heterocycle that structurally resembles a dipeptide in $\beta$-strand conformation. The stable

**Scheme 4.** Synthesis of MOPAS Dimer **18**.




Boc-protected active ester **7** allows the facile introduction of the compound into peptide structures using standard peptide coupling conditions. X-ray structure and NMR analyses of MOPAS-containing hairpin peptides show the ability of the heterocycle to form hydrogen bonds that are complementary to peptide $\beta$-sheet structures. NMR titrations reveal intermolecular binding properties. Availability, facile modification, and direct application in standard peptide coupling procedures, including automated solid-phase protocols, make MOPAS heterocycles useful molecules for studies of peptidomimetics and peptide recognition.

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**Supporting Information Available:** Synthesis procedures and characterization for all compounds, NMR data for structure determination, and details of NMR titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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