Synthesis and Structure of Chiral Methoxypyrrole Amino Acids (MOPAS)

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Abstract: A methoxypyrrole amino acid (MOPAS) resembling the structure of H2N-Val-D-Ala-OEt in β-sheet conformation has been prepared by a chiral auxiliary approach. The X-ray structure analysis confirms the absolute configuration of the dipeptide mimic. Standard peptide coupling procedures allow coupling of the chiral MOPAS with natural amino acids or their extension by additional MOPAS units. A tight self-association of bis-MOPAS 13 in CDCl3 and the affinity to Ac-Ala-Ile-OMe dipeptides illustrates the ability of the constrained dipeptide mimic MOPAS to interact with peptides.

Key words: heterocycles, amino acids, peptides, chiral auxiliaries, imines

Compounds with a molecular structure that mimic motifs of natural peptides1 have found wide applications in medicinal chemistry2 and protein recognition studies. Structures complementary to β-sheets are of particular interest, because of their potential to intercept protein–protein interactions,3 inhibit protein aggregation,4 or induce5 or mimic peptide β-sheets.6 We have recently reported a heterocyclic dipeptide mimic based on methoxypyrrole amino acids (MOPAS), which resembles the structure of a H2N-Gly-D-Ala-OEt unit in β-sheet conformation.7,8 We now report the extension of the concept to a chiral dipeptide mimic H2N-Val-D-Ala-OEt,9 which has been prepared using a chiral auxiliary approach.

Our first attempts to prepare a chiral MOPAS unit used γ-amino-β-keto ester 10 as starting material. Condensation with Gly gave amino ester 2, unfortunately this resulted in only trace amounts of the desired cyclization product 3.11

We changed the synthetic strategy to a chiral auxiliary approach and reacted aldehyde 4 as starting material with chiral amines.12 The initial route using (R)-phenyl glycineamide to form the Schiff base followed by addition of an allyl zinc reagent13 gave only a disappointing 42% yield in the addition reaction.

The use of amino alcohols as chiral auxiliaries and Grignard reagents for addition proved to be more efficient. Phenylglycinol and valinol gave imine 9 in quantitative yield. The compounds were characterized by X-ray structure analysis and chiral HPLC. Isopropyl magnesium chloride undergoes clean addition in THF to compound 9-Ph yielding 10-Ph in 77% isolated yield. Deprotection with dihydrogen and Pd/C yields the target compound i-Pr-MOPAS 11 nearly quantitatively.14

Scheme 1 Synthesis of a chiral MOPAS 3 from a natural amino acid derivative

Scheme 2 Synthesis of substituted MOPAS 7 via allyl-zinc bromide addition
Scheme 3  Enantioselective synthesis of i-Pr-MOPAS 11, R = i-Pr, Ph

Chiral HPLC analysis shows a diastereoselectivity of > 99:1 for the addition reaction and high optical purity of the final product. Figure 1 shows a likely mechanism of the 1,2-addition reaction. The imine alcohol is deprotonated by the first equivalent of added Grignard reagent. A six-membered chair-like conformation induced by coordination of the imine nitrogen lone pair to the magnesium alcoholate guides the second i-PrMgCl in its diastereoselective addition to the C=N bond. The mechanism proposes the formation of (S)-i-Pr MOPAS from imines of S-amino alcohols, which corresponds to the natural dipeptide sequence L-Val-D-Ala.

Figure 1  Proposed mechanism of the nucleophilic 1,2-addition to imine 9-Ph

Dipeptide 14 was prepared from 11 and L-Boc-Phe-OH using standard peptide coupling conditions to confirm the predicted absolute stereochemistry. The X-ray structure analysis shows the expected S-configured i-Pr-MOPAS group (see Figure 2). Coupling of 11 with previously prepared 12 gave the constrained tetrapeptide mimic 13.

In chloroform compound 13 shows a strong self-association of \( 5.2 \pm 3.7 \times 10^3 \) L/mol. Titration of 13 with the isomeric dipeptides Ac-Ala-Ile-OMe and Ac-Ile-Ala-OMe was monitored by NMR and revealed a binding constants of \( K_{11} = 293 \pm 35 \) L/mol.

In summary, we have prepared the constrained chiral dipeptide mimic 11, which resembles the structure of H$_2$N-Val-D-Ala-OEt in \( \beta \)-sheet conformation. A chiral auxiliary controlled the stereochemistry during synthesis and an X-ray structure analysis confirms the structure of the final product. Standard peptide chemistry protocols allow coupling of 11 with natural amino acids or extension by additional MOPAS units. The tight self-association observed in chloroform for MOPAS 13 resembles a typical peptide \( \beta \)-sheet property. The binding, although rather weak, to Ac-Ile-Ala-OMe dipeptides illustrates the ability of i-Pr-MOPAS to interact with natural peptides. The constrained dipeptide mimic can replace amino acid residues in peptides or proteins in the investigation of structure–function relationships. The new MOPAS building block

Scheme 4  Synthesis of MOPAS dipeptides
allows issues of stereochemistry and amino acid side chain interactions in β-sheet recognition to be addressed.

NMR spectra: Bruker AC-250, Bruker Avance 300, and Bruker ARX-400. All chemical shifts δ (ppm) relative to TMS as internal standard. All assignment is based on COSY, HMQC, and HSQC spectra. Multiplicity of carbon resonances (+) = CH₃ or CH₂; (–) CH₃; and Cquat = quaternary carbon atom. Mass spectrometry: Variation in Et₂O (20 mL), filtered, and the reaction product was precipitated, then dried over MgSO₄, the solvent was removed under vacuum and the residue was dissolved in H₂O (2 mL), and neutralized by the addition of NaOH. All solvents for synthesis were purified by distillation.

Compounds R-6, R-9-i-Pr, R-9-Ph, and 14 were characterized by X-ray structure analysis. All bond length and distances are typical. Deposition data are available from the Cambridge Structural Database: CCDC 267442–267445.

Ethyl (S)-4-tert-Butoxy carbonylamino-3-ethoxy carbonylmethyl-iminomanoate (2)
A mixture of keto ester (1: 1.27 g, 4.89 mmol) and glycerine ethyl ester (504 mg, 4.89 mmol) was stirred for 14 h at 40 °C. The reaction mixture was dried under vacuum, the viscous residue was dissolved in Et₂O (20 mL), filtered, and the reaction product was precipitated, by the addition of CH₂Cl₂, as a colorless solid. Recrystallization from Et₂O–toluene gave 1.42 g (84%) of 2.

[α]D = 20.5 (c 6.6, CHCl₃).

1H NMR and 13C NMR spectra cannot be assigned. The compound exists in solution as a mixture of tautomeric forms and E/Z-isomers.

MS (CI, NH₃): m/z (% = 345.9 (100) [M + H+], 365.9 (12) [M + Na+], 709.4 (13) [2 M + Na+].


Ethyl 5-{[(Carbamoylphenylmethyl)amino]but-3-enyl}-3-ethoxy carbonyl-methyl-4-phenyl-2-carboxylate (7)
To a suspension of granulated Zn (17.5 mg, 179 mol) and allyl bromide (23.3 μL, 268 mol) in anhyd THF (2 mL) was added allyl bromide (23.3 μL, 268 mol) and the mixture was stirred for 6 h until all Zn had dissolved. Subsequently, this solution of allyl azide was transferred into a solution of 6 (61.4 mg, 179 μmol, non-enantiomerically enriched) in THF (1 mL) at 0 °C and allowed to warm to r.t.; H₂O (2 mL) and EtOAc (5 ml) were added, the mixture was filtered, and the organic phase was extracted with EtOAc (2 × 5 mL). The combined organic phases were dried over MgSO₄, the solvent was removed under vacuum and the crude product recrystallized from EtOAc–PE to give 7 (37.2 mg, 42%) as a colorless solid.

Mp 126–128 °C.

IR (KBr): 3439 (m), 3319 (m), 3192 (m), 2983 (w), 2933 (w), 1675 (m), 1565 (m), 1513 (m), 1471 (s), 1380 (s), 1283 (s), 1111 (s) cm⁻¹.

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IR (KBr): 3439 (m), 3319 (m), 3192 (m), 2983 (w), 2933 (w), 1675 (m), 1565 (m), 1513 (m), 1471 (s), 1380 (s), 1283 (s), 1111 (s) cm⁻¹.

1H NMR (300 MHz, CDCl₃): δ = 1.14 (t, J = 7.14 Hz, 3 H), 2.11 (s, 3 H), 3.86 (s, 3 H), 4.39 (q, J = 7.14 Hz, 2 H), 4.94 (s, 1 H), 5.56 (br s, 1 H), 6.85 (br s, 1 H), 7.27–7.48 (m, 5 H), 8.14 (s, 1 H), 9.26 (s, 1 H).

13C NMR (75 MHz, CDCl₃): δ = 34.9 ( + ), 60.8 ( – ), 64.2 ( + ), 77.2 (+), 114.7 (Cquat), 127.3 (+), 131.8 ( +, 2 C), 132.8 ( +, 2 C), 139.2 (Cquat), 150.7 (Cquat), 151.3 (+), 160.5 (Cquat), 174.2 (Cquat, 2 C).

MS [ESI, CH₂Cl₂–MeOH, NH₄Ac (10 mmol/L)]: m/z (% = 434.9 (100) + H+, 365.9 (12) [M + Na+], 709.4 (13) [2 M + Na+].


Ethyl 4-Methyl-3-methoxy-5-[(D-phenylglycineamidimethyl)-1H-pyrrole-2-carboxylate (7)
To a suspension of granulated Zn (17.5 mg, 179 μmol) in anhyd THF (2 mL) was added allyl bromide (23.3 μL, 268 μmol) and the mixture was stirred for 6 h until all Zn had dissolved. Subsequently, this solution of allyl azide was transferred into a solution of 6 (61.4 mg, 179 μmol, non-enantiomerically enriched) in THF (1 mL) at 0 °C and allowed to warm to r.t.; H₂O (2 mL) and EtOAc (5 ml) were added, the mixture was filtered, and the organic phase was extracted with EtOAc (2 × 5 mL). The combined organic phases were dried over MgSO₄, the solvent was removed under vacuum and the crude product recrystallized from EtOAc–PE to give 7 (37.2 mg, 42%) as a colorless solid.

Mp 126–128 °C.

IR (KBr): 3439 (m), 3319 (m), 3192 (m), 2983 (w), 2933 (w), 1675 (m), 1565 (m), 1513 (m), 1471 (s), 1380 (s), 1283 (s), 1111 (s) cm⁻¹.
5.37 Hz, 1 H), 3.38 (dd, J = 10.57, J = 8.10 Hz, 1 H), 3.59 (dd, J = 10.57, J = 4.12, 1 H), 3.74 (s, 3 H), 4.25 (q, J = 7.00 Hz, 2 H), 4.46 (br s, 1 H), 8.15 (s, 1 H).

13C NMR (75 MHz, CDC13): δ = 56.6 (+), 15.9 (+), 18.5 (+), 29.7 (+), 59.3 (+), 61.2 (+), 76.2 (+), 77.7 (+), 112.9 (Cquat), 117.0 (Cquat), 124.6 (Cquat), 149.3 (Cquat), 150.7 (+), 158.9 (Cquat).

MS (Cl, NH3): m/z (%) = 297 (100) [M + H]+.

Anal. Calcd for C13H22N2O4, C, 61.40; H, 8.72; N, 11.01.

Found: C, 61.50; H, 8.56; N, 10.84.

Ethyl 5-[(S)-2-Hydroxy-1-phenylethylamino]-2-methyl-1H-pyrrole-2-carboxylate (S,9-Ph)

A solution of S-9-Ph (59.8 mg, 181 μmol) in THF (2 mL) was cooled to −5 °C and i-PrMgCl (400 μL, 2 M in Et2O) was added via cannula. The reaction mixture was stirred for 1.5 h at 0 to −5 °C (a blue color slowly developed), and subsequently was allowed to warm to r.t. and then stirred overnight. Aq NH4Cl (1 mL) and 1 N HCl (1 mL) were added to quench the reaction. The solution was diluted with EtOAc (2 mL), and the organic phase was separated and the aqueous phase was adjusted to >12 by addition of 2 N NaOH and then extracted with CH2Cl2 (20 mL). The combined organic phases were dried over MgSO4 and the solvent was removed under vacuum to give S-9-Ph (54.2 mg, 77%) as a colorless oil.

[a]D20 = +34 (c 2.1, CHCl3).

IR (neat): 3385 (s), 2926 (s), 2937 (sh), 2871 (sh), 1683 (s), 1513 (w), 1474 (s), 1274 (s) cm−1.

HRMS: m/z calcd for C13H22N2O4, 374.2206 [M + H]+; found, 374.2207.

Boc-MOPAS-(S)-i-PrMOPAS-OEt (S-13)

A solution of S-11 (100 mg, 393 μmol), Boc-MOPAS-OEt (158 mg, 393 μmol), and i-Pr2EtNH (82.0 μL, 60.7 mg, 469 μmol) in CH2Cl2 (5 mL) was stirred for 2 h at r.t. The solution was diluted with EtOAc (3 × 2 mL), and the organic phase was extracted with EtOAc (3 × 2 mL), the combined organic phases were dried over MgSO4 and the solvent removed under vacuum to give 69 mg of the crude product, which was purified by column chromatography (SiO2, CHCl3–EtOAc, 80:20 → 50:50) to give S-10-Ph (54.2 mg, 77%) as a colorless oil.

[a]D20 = −71 (c 2.9, MeCN); Rf 0.46 (CHCl3–EtOAc, 50:50).

IR (neat): 3485 (s), 3323 (s), 2960 (s), 2933 (sh), 2871 (sh), 1675 (s), 1463 (s), 1270 (s) cm−1.

1H NMR (400 MHz, DMSO-d6): δ = 0.64 (d, J = 6.50 Hz, 3 H, 12-H or 13-H), 1.02 (d, J = 5.60 Hz, 3 H, 12-H or 13-H), 1.26 (t, J = 7.15 Hz, 3 H, 1-H), 1.70 (s, 3 H, 8-H), 1.92 (dsept, J = 8.31, 6.50 Hz, 1 H, 11-H), 2.56 (br s, 1 H, 10-NH or 21-OH), 3.37 (d, J = 8.31 Hz, 1 H, 10-H), 3.46 (m, 2 H, 21-H, 349 (m, 1 H, 14-H), 3.64 (s, 3 H, 6-H), 4.18 (q, J = 7.15 Hz, 2 H, 2-H), 4.59 (br s, 1 H, 21-OH or 10-NH), 7.09–7.21 (m, 5 H, 16–20-H), 10.37 (br s, 1 H, 4-NH).

13C NMR (75 MHz, CDC13): δ = 71.1 (1 C, 8-C), 14.4 (1 C, 1-C), 19.3 (1 C, 12-C or 13-C), 20.4 (1 C, 13-C or 12-C), 32.8 (1 C, 11-C), 58.7 (1 C, 2-C), 59.3 (1 C, 10-C), 61.2 (1 C, 6-C), 63.0 (1 C, 14-C), 65.6 (1 C, 21-C), 108.4 (1 C, 4-C), 109.1 (1 C, 7-C), 126.4 (1 C, 18-C), 127.2 (2 C, 16-C, 20-C or 17-C, 19-C), 127.5 (2 C, 17-C, 19-C or 16-C, 20-C), 135.3 (1 C, 9-C), 142.9 (1 C, 15-C), 150.3 (1 C, 5-C), 159.5 (1 C, 3-C).

MS [ESI, CH3Cl2–MeOH, NH4Ac (10 mmol/L)]: m/z (%) = 375 (100) [M + H]+, 238 (36) [M + H+–C2H6NO]+.

HRMS: m/z calcd for C21H26N2O4, 374.2206 [M]+; found, 374.2207.

A mixture of S-11 (220 mg, 86.5 µmol), S-Boc-Phe-OH (22.9 mg, 86.5 µmol), HATU (32.9 mg, 86.5 µmol), HOAT (11.8 mg, 86.5 µmol), and 1-Pr-NH (14.7 µL, 11.2 mg, 86.5 µmol) in CH2Cl2 (3 mL) was stirred for 6 h at rt. The reaction mixture was diluted with CH2Cl2 (10 mL), washed with aq KHSO3 (5%, 5 × 10 mL), aq NaHCO3 (0.5 M; 3 × 10 mL), and the organic phase was dried over MgSO4, and the solvent was removed under vacuum. The crude product was recrystallized from EtOAc–PE to give S-14 (36.3 mg, 84%) as a colorless solid.

Mp 159.5–161 °C; [α]D20 +90 (c 2.2, MeCN).

1H NMR (300 MHz, CDCl3): δ = 0.69 (m, 3 H), 0.84 (m, 3 H), 1.18–1.46 (m, 12 H), 1.88 (s, 3 H), 1.89–2.06 (m, 1 H), 2.97 (m, 2 H), 3.81 (s, 3 H), 4.15–4.39 (m, 3 H), 4.62 (m, 1 H), 5.29 (m, 1 H), 6.63 (m, 1 H), 6.96–7.17 (m, 5 H), 9.12 (br s, 1 H).

13C NMR (75 MHz, CDCl3): δ = 7.4 (+), 14.6 (+), 19.2 (+), 19.4 (+), 28.3 (+, 3 C), 32.4 (+, 38.1 (–), 52.6 (+), 56.5 (+), 60.1 (–), 62.2 (+), 80.3 (+), 109.7 (+), 110.2 (+), 126.9 (+), 128.6 (+, 2 C), 129.1 (+, 2 C), 132.1 (+), 136.4 (+), 151.2 (+), 155.7 (+), 160.8 (+), 171.0 (+).

MS [ESI, CH2Cl2–MeOH, NH4Ac (10 mmol/L)]: m/z (%) = 502 (100) [M + H]+, 446 (12) [M + H+ –C4H8].

Anal. Calcd for C23H26N4O5 (501.62): C, 64.65; H, 7.84; N, 8.38. Found: C, 64.39; H, 7.27; N, 8.12.

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References


(11) A derivative of 1 bearing a methyl group in position 2 may cyclize more easily. However, our attempts to transform this compound into the Schiff base corresponding to compound 2 were not successful.


(15) The self-association of 10 is one order of magnitude higher if compared to a Boc-(MOPAS)2-OEt missing the isopropyl substituent; see ref. 7a for data.


(17) The self-association of 10 and the dipeptides were included in the binding model.