Design and synthesis of a new class of arginine analogues with an improved anion binding site in the side chain†

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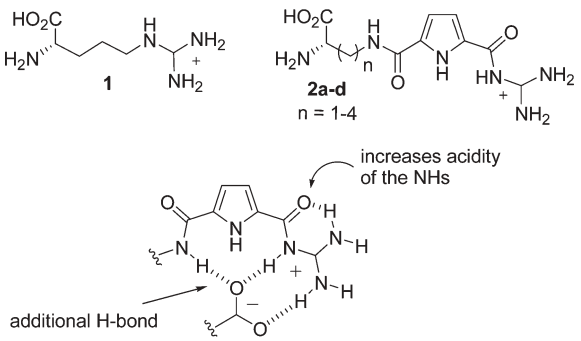
Replacing the guanidinium group in arginine (1) by a guanidiniocarbonyl pyrrole moiety provides a new class of artificial amino acids (2), that can be used as building blocks in standard solid phase peptide synthesis.

The natural amino acid arginine **1** is involved in many physiological and pathophysiological processes.[1–3] Arginine usually exerts biological effects by complexation of negatively charged groups like carboxylate or phosphate. Hence, the development of arginine mimetics[4] with altered binding characteristics for such anions is an interesting probe into the role of arginine in these oligopeptides and proteins.[5] Recently, we have introduced guanidiniocarbonyl pyrroles as a new binding motif for carboxylates which form strong complexes even in aqueous solvents.[6] This guanidiniocarbonyl pyrrole motif binds carboxylates more strongly than a simple guanidinium cation. Therefore, it could exert a tremendous influence on the chemical and biological properties of arginine-containing peptides, if an unnatural analogue with this group in the side chain is implemented as a substitute for the natural amino acid. Herein, we report the successful synthesis of such arginine analogues **2**, which as we demonstrate, can be used as building blocks in standard Fmoc solid phase peptide synthesis.



The general structure of these new arginine mimetics **2** is depicted above. The guanidiniocarbonyl pyrrole group is attached to the ω-amino group in the side chain of either lysine **10** or its smaller homologs. Hence, a series of four new arginine analogues **2a–d** with different size and flexibility was obtained, with the length of the side chain varying from one to four methylene groups.

† Electronic supplementary information (ESI) available: experimental details for the synthesis of **2a–d** together with a list of the abbreviations used in the text. See http://www.rsc.org/suppdata/cc/b4/b415543b/

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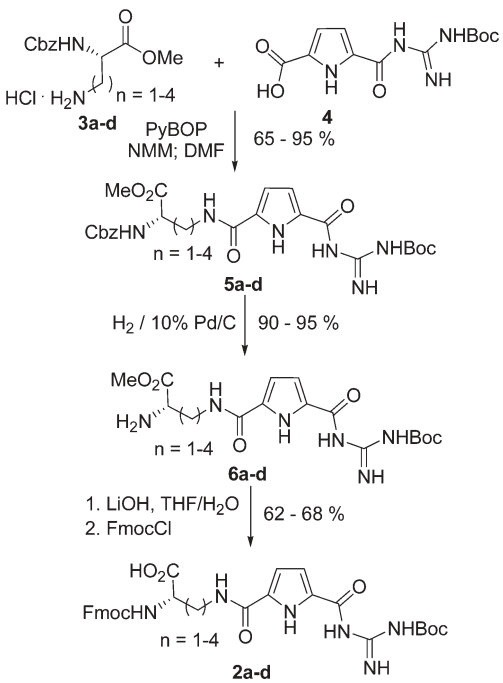
The four Fmoc-protected arginine analogues **2a–d** were synthesized according to Scheme 1.† The key step for the synthesis of **2a–d**, the coupling of the amino acid methyl esters **3a–d** with the Boc-protected guanidiniocarbonyl pyrrole carboxylic acid **4**, was achieved in DMF by activation with PyBOP. In the case of the amino acid **3a** ($n = 1$) the coupling reaction yielded the amide **5a** in a very good yield of 95%. The other amides **5b–d** could be isolated in yields of 65% for amino acid **5b** ($n = 2$) and 80% for the amino acids **5c–d** ($n = 3$–4). For standard solid phase peptide synthesis (SPPS) N-Fmoc protected amino acids are needed. Therefore, the Cbz-group in **5a–d** was cleaved off by a catalytic hydrogenation in methanol affording the corresponding amines **6a–d** in yields of 90–95%. The methyl ester functionalities in **6a–d** were then hydrolyzed with lithium hydroxide in a THF–water mixture and the free α-amino group was finally Fmoc-protected by reaction with Fmoc-chloride in a dioxane–water mixture to provide **2a–d**.

The Cbz-protected amino acid esters **3a–d** needed for these couplings were synthesized by two different routes (Scheme 2). The amino acids **3a–b** with spacer length $n = 1$–2 were prepared by a Hofmann-degradation of commercially available Cbz-protected asparagine **7** ($n = 1$) and glutamine **8** ($n = 2$) using either bis(acetoxyiodo)benzene (PIDA) or bis(trifluoroacetoxyiodo)benzene



Scheme 1 Synthesis of the arginine analogues **2a–d**.

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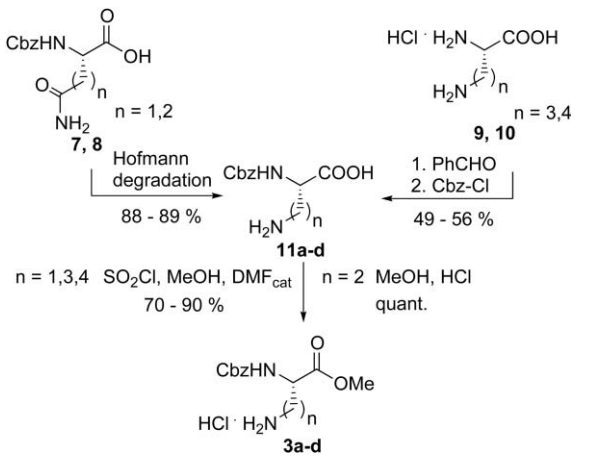
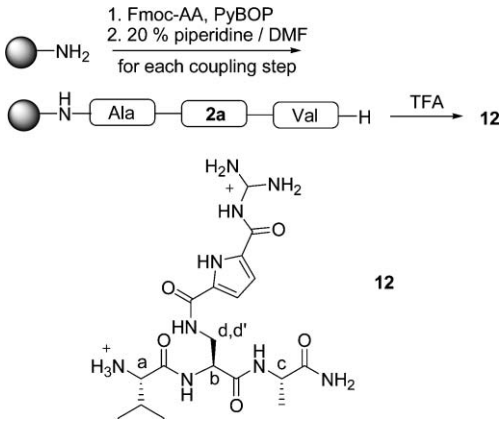


Scheme 2 Synthesis of the Cbz-protected amino acid methyl esters **3a–d**.



Scheme 3 Solid phase synthesis of the tripeptide H-Ala-**2a**-Val-NH_2 *via* standard Fmoc protocol.

(PIFA) in 88–89% yield (Scheme 2).[7] The two amino acids **11c–d**, with spacer length n = 3–4, were prepared *via* selective imine formation of the ω-amino group of ornithine **9** and lysine **10** with benzaldehyde, Cbz-protection of the remaining free α-amino group and subsequent hydrolysis of the benzylidene group.[8] The corresponding methyl esters **3a** and **3c–d** for n = 1, 3, 4 were then easily prepared in good yields of 70–90% by reaction with $SOCl_2$ in methanol.[9] For n = 2 (**3b**) these reaction conditions led only to the formation of the lactam *via* intramolecular cyclisation, so HCl in methanol was used to obtain the desired methyl ester in near quantitative yields.[10]

The hydrolysis of the esters and the work-up must be performed using special precautions. As the Boc-guanidino group can act as a good leaving group under nucleophilic conditions, only a slight excess of lithium hydroxide (1.5 equiv.) was used in the reaction. Additionally, the reaction time was kept at a minimum and the reaction was immediately stopped when the hydrolysis was completed, as monitored by TLC. The reaction mixture was then neutralized to pH = 7 and directly lyophilized. The carboxylic acids were not further isolated, instead the lithium salts were allowed to react with Fmoc-chloride in a mixture of dioxane and aqueous sodium carbonate for 90 minutes. The Fmoc-protected arginine analogues **2a–d** were isolated by column chromatography in good yields of 62–68% over both steps.

These Fmoc-derivatives **2a–d** should be suitable building blocks for a standard Fmoc solid phase peptide synthesis. Due to the higher acidity of our guanidiniocarbonyl pyrrole moiety (pK_a = 6–7 compared to 13.5 for guanidine),[6b] it is necessary to have the guanidinio group semi-permanently protected during the amide coupling. We chose the acid labile Boc-protection group at the guanidino group which is orthogonal to the Fmoc group on the α-amino group. To test the applicability of our new arginine analogues, we synthesized the tripeptide H-Ala-**2a**-Val-NH_2 (**12**) (Scheme 3). The synthesis was performed on Rink Amide resin by a standard Fmoc protocol using 2.5 equiv. of the Fmoc-protected amino acid and 2.5 equiv. PyBOP in DMF with 3% NMM (reaction time 3.5 h).[11] The completeness of every coupling reaction was controlled by a Kaiser-test. For the next coupling step, the N-terminal Fmoc group was removed by treatment with 20% piperidine in DMF. The tripeptide was subsequently cleaved from the solid support by shaking the resin with CH_2Cl_2–TFA mixture (5 : 95). Under this condition the Boc-protecting group on the guanidino group is also removed. The crude trifluoroacetate salt of the tripeptide H-Ala-**2a**-Val-NH_2 (**12**) obtained in this manner was analytically pure as confirmed by ESI-MS and NMR spectroscopy (Fig. 1).

In conclusion, we have synthesized four new arginine analogues **2a–d** *via* multi-step syntheses and an orthogonal protecting group strategy. These unnatural amino acids contain an improved carboxylate binding site in their side chain making them interesting substitutes for natural arginine. The Fmoc-derivatives can be introduced into a peptide by standard solid phase peptide synthesis. This approach now allows the synthesis of artificial analogs of arginine rich peptides to probe the role of carboxylate binding in their biological activity. Such work is currently in progress and will be reported in due course.

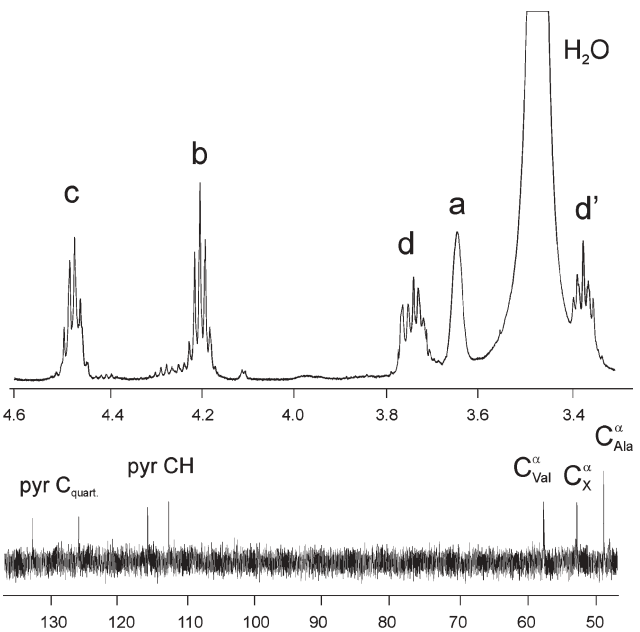


Fig. 1 Parts of the ^1H and ^{13}C NMR spectra of the crude tripeptide H-Ala-**2a**-Val-NH_2 in DMSO-d6 as isolated after cleavage from the solid support (for the proton labeling see Scheme 3).

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