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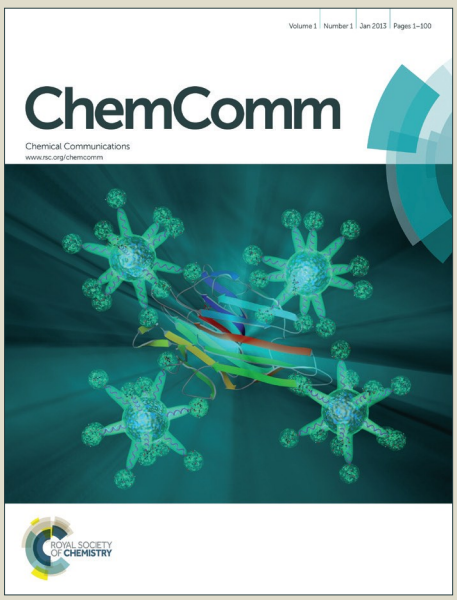

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Facile synthesis of mono- and bis-methylated Fmoc-Dap, -Dab and -Orn amino acids

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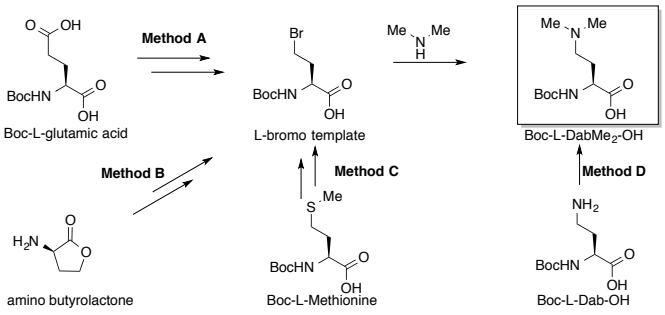
A new methodology for the synthesis of side chain mono- or bis- methylated Fmoc-Dap, -Dab and -Orn amino acids was developed by probing the reactivity of commercially available Fmoc amino acids.

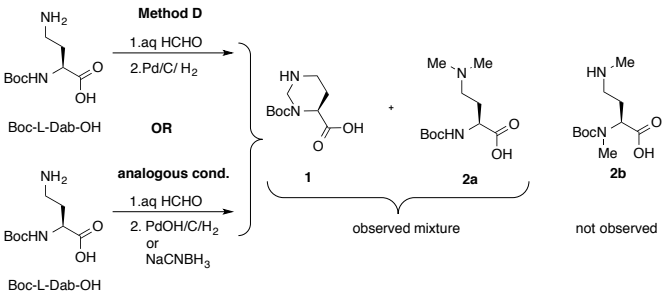
N-methylated amino acid derivatives are often used in peptide drug development due to their effects in modulating the affinity of peptide-receptor interactions, and their potential to enhance bioavailability.[1-4] This has driven the demand for synthetic protocols towards side chain mono-, di- and *N*(α)-methylated lysine derivatives and has led to the development of a vast number of lysine specific synthetic methods.[1-3, 5] However, conversions of the shorter chain analogues of lysine, such as 2,3-diaminopropionic acid (Dap), 2,4-diaminobutyric acid (Dab) and Ornithine (Orn), into *N*-methylated derivatives have been less well studied. In particular, there is no literature precedence for installing mono-methylated species without the prior synthesis of either the tosyl- or nosyl-protected species to facilitate alkylation.[6, 7]

Literature protocols available for the synthesis of Fmoc-Dab(Me_2)-OH were first replicated and scrutinised here. These methods (**A** to **D**) require changing the protecting group from Boc to Fmoc in all cases as a final step to achieve the desired Fmoc derivative. A common first line approach is based on the substitution of Boc-(γ)-bromo propionic acid intermediate with dimethyl amine.[8] The (γ)-bromo amino-acid intermediate is available via multistep syntheses, such as esterification of glutamic acid with 1-hydroxy-2-thiopyridone followed by light induced radical bromination,[9] or reduction of glutamic acid to homo-serine followed by bromination in the presence of PPh_3[10] (method **A**). Alternatively, ring opening of optically pure amino-γ-butyrolactone substrates,[11, 12] commercially available or modified e.g. Boc-methionine,[13] has lead to the desired bromo intermediates (methods **B** and **C**). All these methods (**A-C**) require multiple step syntheses starting from the Boc-protected amino acid precursor as well as additional de-protection / re-protection steps to obtain the required final Fmoc amino acid (Scheme 1). The alternative and more attractive method (**D**) is the Pd/C assisted reductive alkylation of Boc-Dab-OH acid substrate under hydrogen atmosphere and excess aqueous formal aldehyde.[14] However, in our hands method **D** led to a mixture of two products. The main product was not the Boc-Dab(Me_2)-OH analogue **2a** but rather a 6 membered cyclic pyrimidine based analogue **1** (Scheme 2). Similar results when using other reductive reagents such as PdOH/C/H_2 (palladium hydroxide on carbon under H_2 gas) and $NaCNBH_3$ (sodium cyanoborohydride) were observed (Scheme 2).



Scheme 1 Common methods found in literature. Protecting group swap from Boc to Fmoc is required as a final reaction, not shown here.

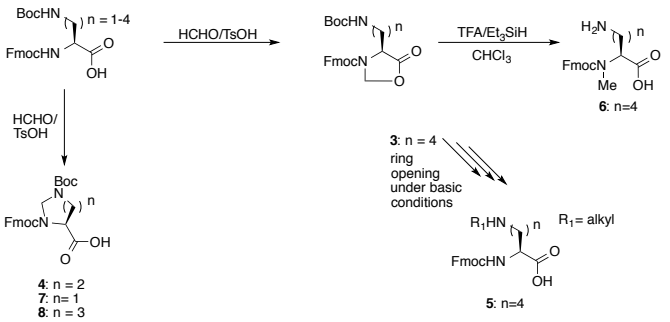
The mixture was found to co-elute in LCMS traces. Careful purification by flash chromatography confirmed two products (**1** and **2a**) in a 60:40 ratio. Further investigative 1D and 2D NMR experiments clearly revealed the lack of the backbone secondary amide proton in the pyrimidine component **1**. These findings, together with the mass analysis identified the structure of **1**. To our knowledge, this has not previously been reported for Dab based amino acids (Scheme 2).



Scheme 2 Observations when performing Method **D** and analogous conditions.

Another Fmoc/Boc-Lys(Boc)-OH protection strategy that allows selective side chain methylation is the formation of an oxazolidinone ring between the alpha amine and the amino-acid carboxylate using formaldehyde under acid catalysis (**3**).[15, 16] This strategy allowed for the selective alkylation of the (de-protected) side chain amine followed by ring opening under basic conditions to restore the amino acid backbone[17] (**5**). Conveniently, this 5 membered oxazolidinone ring **3** can also be reductively ring opened, under mild racemisation-free[18, 19] conditions, using TFA-Et_3SiH in chloroform to produce *N*(α)-methylated derivatives[18, 19] of type **6** (Scheme 3).

Adapting this general protocol[15] for the shorter chain analogue amino acid Fmoc-Dab(Boc)-OH (n=2), the 1D and 2D NMR analysis of products indicated the formation of pyrimidine **4** (the *N*(γ)Boc analogue to **1)** as the major product, with retention of the side chain Boc protection group. Surprisingly, the expected oxazolidinone ring formation was only observed as a minor side product. Similarly, the short chain Dap (n=1) and the longer chain Orn (n=3) cyclized with formaldehyde to yield a 5-membered imidazolidine ring **7,** and a 7-membered diazepane ring **8** respectively. These initial results suggested that the chemistry of short chain homologues of lysine probably was driven by the relatively more reactive side chain amide and the formation of an aminal, rather than the expected 5-membered oxazolidinone ring **3** (Scheme 3).



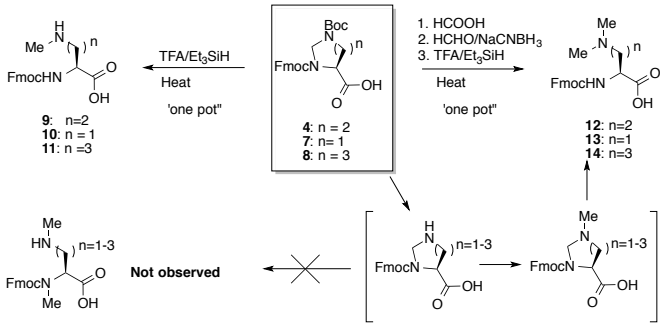
Scheme 3 Side chain length drives the product formation during formaldehyde condensation.

Optimisation of the key cyclization reaction forming the pyrimidine ring structure **4** with Fmoc-Dab(Boc)-OH was conducted by employing paraformaldehyde (2-6 eq), two different solvents (MeCN and toluene) and catalysed by either pTsOH or camphorsulphonic acid (CSA). Using elevated temperature (120 °C, 15 min) and toluene as solvent gave 10-15% of the oxazolidinone side product, regardless of the paraformaldehyde amount used. The oxazolidinone formation was supressed further when acetonitrile was used as solvent. Replacing the energy source with a microwave reactor (120 °C) resulted in the cyclic structures **4**, **7** and **8** as exclusive products. This also allowed shortening of the reaction times from 15 min to 2 min, using only 2 eq of paraformaldehyde. Due to the high selectivity and efficiency of the cyclisation reaction for all three products, rapid fast flash chromatography on a silica plug resulted in high purity >95%.

Structures **4**, **7** and **8** provide potential intermediates for the selective synthesis of *N*(β,γ,δ) mono- or bis-methylated Dap, Dab and Orn analogues, if regioselective reductive ring opening is possible.

The optimisation of the ring opening conditions of the model substrate **4**, included various trials of hydride sources such as Pd/C/H_2, $NaBH_4$, $NaCNBH_3$ and TFA-Et_3SiH cocktails, in either MeOH or MeCN as solvent. Only the silane-based cocktail at elevated temperature delivered full conversion to a single *N*(γ)-methylated product (**9**) allowing for minimal purification requirements. These conditions were successfully applied to the other substrates, with the best results achieved when **4**, **7** and **8** were subjected to TFA-Et_3SiH (6 fold excess) in chloroform, with only a major single new product appearing in LCMS traces. Mass spectroscopic evidence of a mass gain of two amu was consistent with 1D and 2D NMR spectra, suggesting that a ring opening had occurred with exclusive formation of the Fmoc side chain mono methylated Dap, Dab and Orn (**9**-**11**) derivatives (Scheme 4).

With the successful conversion of the cyclic intermediates **4**, **7** and **8** into *N*(β,γ,δ) mono-methylated products **9**-**11**, we were interested to determine whether the same intermediates could be used as starting materials for access to the corresponding bis methylated analogues of Dap, Dab and Orn. To achieve this it was necessary to remove the Boc protecting group, alkylate and then ring open to furnish the final product. Using **4** as the model substrate, Boc removal was achieved in neat formic acid followed by concentrating the solution. Reductive amination using $NaCNBH_3$ yielded the *N*(γ) methylated heterocyclic product in quantitative yield. Immediate treatment with TFA-Et_3SiH and heating yielded the desired ring-opened bis-methylated product **12** quantitatively. The key intermediates **7** and **8** followed the same trend giving products **13** and **14**, also quantitatively (Scheme 4).



Scheme 4 Heterocyclic key intermediates **4**, **7** and **8** provide a common building block for synthesis of mono and bis methylated side chain Dap (n=1), Dab (n=2) and Orn (n=3) amino acids.

These findings demonstrate that all Fmoc-Lys(Boc)-OH analogues with shorter side chain length (n=1-3; Dap, Dab, Orn) can be cyclized with formaldehyde under optimised conditions to exclusively give the corresponding aminal ring structures (**4**, **7** and **8**). In contrast, the oxazolidinone **3** is formed as the main product from Fmoc-Lys(Boc)-OH (n=4). The side chain length seemed in all cases to be the major contributing factor in the preference for this reaction pathway. In the lysine case the thermodynamically more stabile oxazolidinone ring was formed[18] rather than the theoretically possible 8 membered aminal-tethered heterocycle. In the case of Dap, Dab and Orn analogues the 5-7 membered N-C-N rings imidazolidine, pyrimidine and diazepine rings (**4**, **7** and **8**), were the respective preferred products, with any preference for the 5-membered ring system offset by the reactivity of the side chain amine in all three cases.

Ring opening of the key ring structures was facilitated under ionic hydrogenation conditions. The most effective system was found to be TFA-Et_3SiH, requiring mild to moderate heating to furnish full conversions. The regioselective preference of the ring opening could possibly be due to the inability of the electron depleted Fmoc-amide nitrogen to collapse the protonated aminal, giving rise to exclusive formation of the side chain mono methylated products **9**-**11**, in a 2

step one pot reaction. Similarly bis-methylated products **12-14** were also obtained from the key intermediates **4**, **7** and **8** via Boc deprotection, *N*-alkylation followed by ring opening, a 4-step in one-pot sequence. Further, this one pot methodology was fully scalable to multi-gram synthesis of the mono methylated Dab derivative **9,** with an overall yield of 95% after Boc protection. In summary, a quick, facile and scalable protocol for the selective synthesis of mono- and bis-methylated Fmoc-Dap/Dab/Orn-OH amino acid building blocks was established, based on commercially available starting materials using standard lab equipment.

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Notes and references

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