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Dicyclopropylmethyl Peptide Backbone Protectant†

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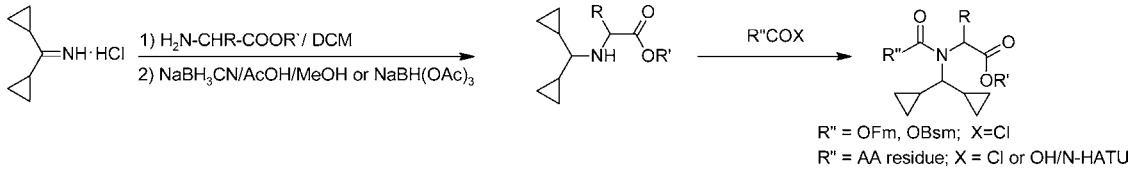
ABSTRACT



The *N*-dicyclopropylmethyl (Dcpm) residue, introduced into amino acids via reaction of dicyclopropylmethanimine hydrochloride with an amino acid ester followed by sodium cyanoborohydride or triacetoxyborohydride reduction, can be used as an amide bond protectant for peptide synthesis. Examples which demonstrate the amelioration of aggregation effects include syntheses of the alanine decapeptide and the prion peptide (106-126). Avoidance of cyclization to the aminosuccinimide followed substitution of Fmoc-(Dcpm)Gly-OH for Fmoc-Gly-OH in the assembly of sequences containing the sensitive Asp-Gly unit.

We describe a new backbone protectant for the synthesis of so-called "difficult peptides", namely, those peptides for which the presence of specific sequences of amino acids, often hydrophobic amino acids, lead to slow deblocking and incomplete coupling processes. Several approaches to overcome these problems have been devised. These include the use of more potent coupling reagents,[1] pseudoproline insertions,[2] the depsipeptide technique,[3] and the use of backbone protection.[4] Recently, dicyclopropylmethyl (Dcpm) and dimethylcyclopropylmethyl (Dmcp) groups have been described as amide protectants for Asn, Gln, and the C-terminal position of linear peptide amides.[5] In view of their utility as amide protectants, consideration has now been given to their possible use as peptide bond protectants. Of these two alkyl residues, the Dcpm group was chosen due to its lesser steric requirements and the fact that the appropriate N-substituted amino acids could be synthesized via standard reductive alkylation techniques. Finally, deblocking of the Dcpm residue from an internal (tertiary amide) position is expected to be enhanced and perhaps as rapid as the removal of the

† Abbreviations used: ACN = acetonitrile; BSA = bistrimethylsilylacetamide; Bsmoc = benzothiophenesulfone-2-methyloxycarbonyl; Dcpm = dicyclopropylmethyl; DIC = diisopropylcarbodiimide; Dmcp = dimethylcyclopropylmethyl; DODT = 3,6-dioxa-1,8-octanedithiol; Fmoc = 9-fluorenemethyloxycarbonyl; N-HATU = 1-[bis(dimethylamino)methylene]-1-*H*-1,2,3-triazolo[4,5-b]pyridinium hexafluorophosphate 3-oxide; N-HBTU = 1-[bis(dimethylamino)methylene]-1-*H*-benzotriazolium hexafluorophosphate 3-oxide; Hmb = 2-hydroxy-4-methoxybenzyl; HOAt = 1-hydroxy-7-azabenzotriazole; Phth = *o*-phthaloyl; TFA = trifluoroacetic acid; TFFH = tetramethyl fluoroformamidinium hexafluorophosphate.

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Dmcp residue from the terminal amide position. This expectation follows from the curious fact recorded in the literature[6] that secondary amide, *N*-*t*-butylbenzamide **1**, is stable toward TFA, whereas the analogous tertiary amide *N*-*t*-butyl-*N*-methylbenzamide **2** loses the *t*-butyl residue upon treatment with the same acid. It has long been recognized that *N*-*t*-butyl amides such as **1** differ from the corresponding oxygen analogues (*t*-butyl esters) in being stable toward acidic deblocking.[7] The sensitivity of **2** could perhaps be explained on the basis of its being a tertiary alkyl-based tertiary amide which might be subject to more facile N- relative to O-protonation with subsequent ready loss of the *t*-butyl cation in TFA.[8]

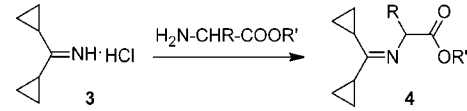
In the present work, the high acid sensitivity of the internal Dcpm residue was verified, thus allowing for its use as a backbone protectant. Regarding the susceptibility of an *N*-Dcpm-substituted amino acid or amino acid ester to coupling reactions, it is well-known that coupling to *N*-methyl amino acids is difficult[9] and coupling to *N*-Dcpm amino acids is expected to be even less facile. However, the steric requirements of the Dcpm group may be unique since in its coupling chemistry the Dcpm-bearing model amino acid α,α-dicyclopropylgycine resembles its simple α,α-dimethyl analogue, H-Aib-OH, more than its extremely hindered analogue, α,α-diisopropylglycine.[10] Since H-Aib-OH is an easily handled amino acid for peptide incorporation, similar effects are to be expected for the *N*-Dcpm residue.[10] Indeed, as shown in the present work, for the relatively unhindered amino acids glycine and alanine, coupling to the *N*-Dcpm derivatives is readily achievable. On the other hand, for the more hindered amino acids such as valine, isoleucine, and threonine, special acylating techniques may be required.

The currently most commonly used backbone protectant, the Hmb group,[4] differs from the Dcpm residue in being acylated via prior reaction at the *o*-hydroxyl group followed by an O → N acyl shift.[11]

Prior to preparation of appropriate amino acid derivatives, a model experiment was carried out with the previously described imine *N*-*n*-propyl dicyclopropyl-ketimine.[13] Reduction and benzoylation gave the corresponding tertiary amide which was tested for its stability toward TFA under conditions normally used for the final deprotection step during Fmoc/*t*-Bu-based solid phase peptide assembly. As expected, the Dcpm residue was readily removed with the formation of *N*-(*n*-propyl)benzamide.

A general method for the synthesis of *N*-Dcpm amino acids was then developed. A key intermediate **3**, obtainable by treatment of the commercially available, or easily synthesized dicyclopropyl ketone, with ammonia in the presence of titanium tetrachloride, has been described in the patent literature.[14] Reaction of **3** with an amino acid ester gave imine **4** (Scheme 1) which upon reduction with sodium

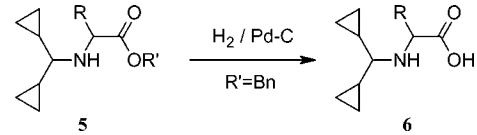
Scheme 1



3 **4**

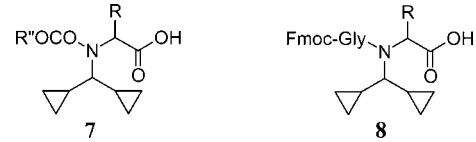
cyanoborohydride or triacetoxyborohydride in the presence of acetic acid gave the amino acid derivative **5** which in the case of the benzyl ester (**5**, R′ = Bn) underwent standard catalytic hydrogenolysis to give the free amino acid **6** (Scheme 2).

Scheme 2



5 **6**

For relatively unhindered amino acids (e.g., **6**, R = H, Me) the amino function is readily attacked by an appropriate acylating agent. Thus, the Fmoc[15] and Bsmoc[16] derivatives **7** (R″ = Fm, Bsm) are readily obtained via the corresponding chloroformates using the Bolin technique.[17] Analogously, treatment with Fmoc-Gly-Cl[18] or Fmoc-Gly-F[19] yields the corresponding protected dipeptide **8** (R = H, Me).



7 **8**

It was demonstrated that in the synthesis of **5** (R = Me, R′ = Bn) via **3** no significant loss of configuration

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(11) To make it more suitable for use with hindered amino acids, Miranda and Alewood and co-workers[12] have modified the Hmb residue by the introduction of a nitro group into the benzylic ring. While this change has the desired effect, the resulting protectant cannot be removed by acid-catalyzed deblocking.

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occurred at the alanine residue as shown by conversion to **7** (R = Me, R′′ = Fm). Coupling of the Fmoc-protected acid to proline atached to a Rink amide resin by means of N-HATU/DIEA in DMF gave the corresponding dipeptide resin which was Dcpm-deblocked and cleaved from the resin by treatment with TFA to give Fmoc-Ala-Pro-NH_2[20] which was contaminated by only 0.95% of the DL-isomer showing that no more than this amount of contamination could have been present in the sample **5** used or could have been formed during the coupling process. Further work will be required to determine the exact source of the contamination, but the amount is small enough not to impinge upon the present work.

The methodology described was also applied to the synthesis of the valine derivatives **5** (R = *i*Pr, R′ = Bn) and **6** (R = *i*Pr); however, this more hindered amino acid could not be acylated via Fmoc-Cl or Bsmoc-Cl to give **7** (R = *i*Pr, R′′ = Fm or Bsm). Similarly, the coupling of a simple protected amino acid (e.g., Z-Phe-OH) to **5** (R = *i*Pr, R′ = Bn) did not succeed with a variety of standard coupling reagents (e.g., N-HBTU,[21,22] N-HATU,[22] TFFH,[23] etc.). However, with the more potent acylating agent Phth-Phe-Cl, coupling in the presence of BSA[24] gave the dipeptide in 56−62% yield. Details will be provided in a subsequent publication.

As a first sequence in which to examine the utility of the Dcpm residue as a backbone protectant, the difficult decaalanine sequence built onto arginine was examined.[25] Previously this system has been shown to be subject to the effects of aggregation leading to both deblocking and coupling deficiencies. An automated synthesis of the decaalanine sequence using 4 equiv of Fmoc amino acid with coupling via N-HATU gave material contaminated by several des-Ala units as well as undeblocked Fmoc-containing segments (Figure 1, Supporting Information). An attempt to improve the synthesis was made by substituting Fmoc(Dcpm)-Ala-OH for Fmoc-Ala-OH to introduce the fifth alanine unit and at the same time reduce the effect of aggregation for all subsequent coupling steps. The remainder of the synthesis was carried out in the normal manner, but the only material obtained following workup and removal of the peptide from the resin was the *N*-Dcpm-pentaalanine derivative (Figure 2, Supporting Information) showing that the system shut down following introduction of the Dcpm-Ala unit.

However, since previous studies had suggested that Bsmoc amino acids were more reactive than their Fmoc counterparts,[3c] it was simply necessary to substitute Bsmoc-Ala-OH for the Fmoc analogue in this synthesis, and remarkably, about 50% of the desired decaalanine product was obtained. The 11-mer is accompanied by about 50% of the *N*-Dcpm-substituted deletion sequence which had been the sole product formed in the all Fmoc case (Figure 3, Supporting Information). If HOAt/DIC was used in place of N-HATU and coupling continued for a longer time, the reaction could be pushed toward completion (Figure 8, Supporting Information).

A sequence which is expected to be subject to difficulties due to the presence of the base-sensitive Asp-Gly unit[26] is dodecapeptide **9**, which on attempted synthesis under standard conditions via N-HBTU gave only the aminosuccinimide cyclization product. With Fmoc-(Dcpm)-Gly-OH substituted for Fmoc-Gly-OH under the same conditions

ADGSLDDYNHLV-amide
9

(N-HBTU), the result was still unsatisfactory (only 17% of **9**), but if N-HATU was substituted for N-HBTU, the desired dodecapeptide amide was obtained in a yield of 91% along with only 8% of the des-Asp deletion peptide (see Figure 9a,b, Supporting Information).

A classical "difficult sequence" was then examined, namely, the prion peptide (106-126) **10** which was previously examined by Jobling et al.[27] Using Fmoc chemistry with

106 114 119 126
KTNMKHMAGAAAAGAVVGGLG
10

N-HBTU coupling and introducing the Hmb residue at positions 114 and 119, these workers obtained a low yield of the desired peptide (7.3%) along with a number of deletion sequences. With Fmoc (Dcpm)-Gly-OH introduced at the same two positions and using N-HATU for coupling using normal Fmoc amino acids except for positions 113 and 118 which were introduced by Bsmoc-Ala-OH, a relatively clean sample of the desired peptide was obtained (crude yield 41%, purity 89%) (see Figure 10a,b, Supporting Information). The

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only major byproduct was a small amount of the methionine sulfoxide derivative.

In conclusion, it has been shown that the *N*-dicyclopropylmethyl residue can be used as an amide backbone protectant in the case of relatively unhindered amino acids. *N*-Dcpm amino acids can be readily synthesized via treatment of dicyclopropyl ketone imine hydrochloride with an amino acid ester followed by sodium cyanoborohydride or sodium triacetoxyborohydride reduction.

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Supporting Information Available: Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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