Dipeptide Surrogates Containing Asparagine-Derived Tetrahydropyrimidinones: Preparation, Structure, and Use in Solid Phase Synthesis

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Received November 13, 1996

The amino acid asparagine is the focal point for varied studies of polypeptide and protein structure and function. Asparagine acts as an efficient C-terminal α-helix cap[3] and serves as the linkage point for the oligosaccharide unit of *N*-linked glycopeptides.[4] These ubiquitous cell-surface biomolecules function as recognition elements in cell−cell interactions and are implicated in many biological functions and disease states. However, asparagine-containing polypeptides and proteins require special considerations upon attempted laboratory synthesis. Use of the amino acid with an unprotected side-chain amide can lead, by dehydration, to nitrile and/or succinimide derivatives and, even if the synthesis is successful, general solubility difficulties.[5] An array of protective groups for the primary amide, including trityl and others[5] and, more recently, dimethylcyclopropylmethyl,[6] have been promoted to alleviate, or at least reduce, these difficulties.

Herein we report our initial results on a novel class of protected asparagine building blocks. On one level, these cyclic surrogates function as protected, organic-soluble asparagine residues for peptide synthesis. However, as opposed to more conventional protective groups, the tetrahydropyrimidines employed herein are of known absolute configuration and conformation. Such topological certainty allows for their incorporation into rationally designed polypeptides as stereochemically defined peptidomimetics.[7] Finally, the heterocycle is easily transformed to the natural amino acid within the polypeptide framework.[8] This allows for both the direct comparison between the conformationally restricted and native polypeptide and the observation of the changes that occur in the process.

Imine **3** is obtained in nearly quantitative yield from 4-chloro-3-nitrobenzaldehyde (**4**) and commercially available asparagine *tert*-butyl ester, in accord with the results of Seebach and coworkers.[9] Although no desired product came from the use of more conventional amino acid activated carboxyl residues (including *p*-nitrophenyl ester, anhydride, acyl fluoride, acyl imidazolide), treatment of **3** with Fmoc-protected amino acid chlorides[10] in anhydrous benzene with pyridine as base led to desired heterocycle **5**. Yields of **5** vary from 58−66% for a wide range of amino acid side chains (Table 1), including D-amino acids. Proline can also be employed in this reaction, although in more modest yield.[11]

Compounds **5** are sufficiently (and orthogonally) protected to function in peptide synthesis schemes. Standard treatment with trifluoroacetic acid (TFA) affords corresponding free acids **6** in excellent yield (Table 1). Isolation of free amines **7** after selective removal of the Fmoc group proved somewhat more difficult. Standard treatment with piperidine in DMF results in complete deprotection. Unfortunately, the product amines exhibit enough water solubility to make isolation of pure compounds difficult. Success was achieved with certain of the derivatives through use of Me_2NH as deprotection agent. Under these conditions, excess base is removed by evaporation to leave the desired material contaminated with fluorene residue, which is easily removed by filtration. However, both **5b** and **5d** afforded variable amounts of a largely insoluble material, which has yet to be identified, upon attempted isolation of the desired free amine. While direct acetamide formation proved advantagous in most cases, the yield of **7b** could not be brought to an acceptable level. Fortunately, these problems were not evident in a solid phase peptide synthesis (SPPS) deprotection sequence. For example, compound **6b** was attached to a solid support and deprotected with piperidine/DMF under standard conditions. Acetamide formation and isolation of the product after cleavage from the resin occurs in 77% yield, as opposed to 40% for the solution phase synthesis. Finally, the aminal functionality can be opened with dilute aqueous acid, which also liberates the free asparagine carboxylic acid functionality to afford **8**. Each of the compounds **8a**−**g** is identical with the dipeptide formed by conventional methods.

The structure obtained from a single crystal X-ray analysis of compound **6b** is depicted in Figure 1[12] and has similarity to related compounds studied both in this laboratory[13] and others.[14,15] In particular, the peptide linkage is maintained in the equatorial plane to allow maximum amide resonance. This comes at the expense of the C2 aryl group, which is stationed as a flagpole substituent on the six-membered ring in a boat conformation. The two flagpole bonds are nearly parallel, with a dihedral angle of only 1.7°, and these substituents are held in close proximity (2.71 Å). The key torsion angle around the amino acid residue (C(Ala)−N1−C6−CO_2H) is 73.2°, somewhat larger than that found in proline (approximately 65°). Intermolecular hydrogen bonds between the free asparagine acid functionality and the adjacent lactam system afford linear arrays of molecules in the solid state. Adjacent linear sequences are held together by edge-to-face π-stacking forces between the fluorene fragments of the Fmoc protection group (see figures in Supporting Information).

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(12) Crystals of **6b** are orthorhombic, $a = 7.654(2)$, $b = 12.190(3)$, $c = 32.529(6)$ Å, space group $P2_12_12_1$, $Z = 4$, $r = 1.337$ g/cm^3 for $C_{29}H_{27}ClN_4O_9$. A total of 2934 independent reflections were measured with nickel-monochromated Cu Kα radiation at 130(2) K on a Siemens P4 diffractometer in the θ range of 2.72 to 56.06°. The structure was solved by using direct methods and refined to a final *R* value of 4.01%. The primary program used was SHELXTL, version 5.03, 1994, by G.M. Sheldrick.

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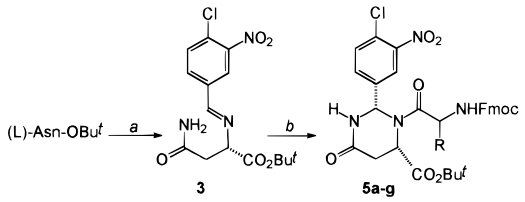
S0002-7863(96)03927-3 CCC: $14.00

Scheme 1[a]



[a] (a) **4**, $(MeO)_3CH$, 98%; (b) Fmoc-Xxx-Cl, C_5H_5N, 58−66%.

Table 1. Yields of Free Acid **6**, Amine **7**, and Native Dipeptide **8** from **5**

Entry	R	% yield 5	% yield 5→6	% yield 5→7	% yield 5→8
a	H	61	88	82[1]	91
b	(*S*)-Me	65	95	40[1] (77[2])	90
c	(*R*)-Me	58	90	90[1]	78
d	(*S*)-$CHMe_2$	66	95	75[1] (80[2])	85
e	(*S*)-CH_2OBn	63	87	87[1]	78
f	(*S*)-$CH_2C_6H_4OBn$	62	89	90	75
g		30	85	85	81

[1] Prepared by treatment with Me_2NH; isolated as the corresponding acetamide by direct treatment with Ac_2O. [2] Prepared by binding corresponding acid **6** to NovaSyn PR-500 resin and treating with piperidine/DMF. Cleavage from resin results in carboxamide, isolated as the corresponding acetamide derivative by direct treatment with Ac_2O.

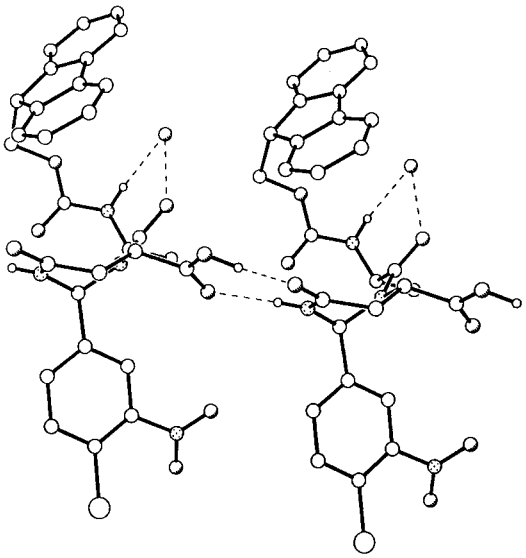
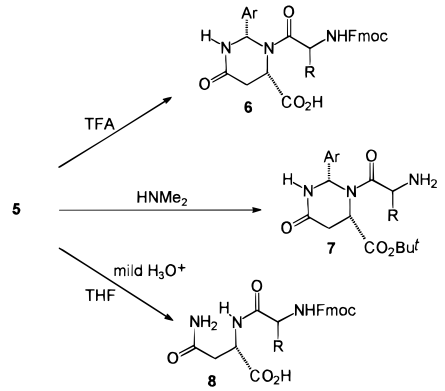


Figure 1. Structure of **6b**. Only hydrogens on heteroatoms are shown.

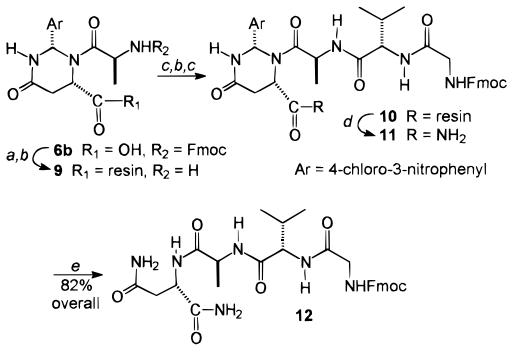
Compounds **5a**−**g** display the full range of cis−trans amide isomer populations, with **5c** and **5g** appearing as single isomers, while **5f,** derived from L-tyrosine benzyl ether, affords two sets of ^1H and ^{13}C NMR ($CDCl_3$) resonances in almost equal amounts. Experiments with **5f** in DMSO-d_6 over the range from room temperature to 100 °C suggest that this cis−trans isomerization around the dipeptide bond has a relatively low barrier, with a coalescence temperature of 70 °C.[16]

It was of interest to determine if these dipeptides could be employed in the SPPS of polypeptides, particularly with the heterocycle at the C-terminal position.[17] To this end, the previously described **6b**-resin adduct was deprotected with piperidine/DMF. Resulting free amine **9** was coupled with Fmoc-Val-OH to give the desired tripeptide, followed by deprotection and coupling with Fmoc-Gly-OH in similar manner to afford **10**. Cleavage from the resin with TFA gave expected tetrapeptide **11** as the Fmoc-protected primary amide in 82% yield from **6b**. Compound **11**, which is readily soluble in common organic solvents, was treated with mild aqueous acid to afford tetrapeptide **12**, identical with material made in the conventional manner in all respects. The solubility of **12** in organic solvents is poor.

Scheme 2



Scheme 3



[a] (a) Diisopropylcarbodiimide, HOBt; (b) 30% piperidine, DMF; (c) Fmoc-Xxx-OH, HATU, DIEA, DMF; (d) 95% TFA; (e) 0.25 M HCl, THF.

These dipeptide surrogates offer attractive scaffolds from which to explore issues of polypeptide synthesis and structure. Incorporation of these building blocks into model polypeptides and the elaboration of their influence on local conformations are the subject of ongoing studies in these laboratories and will be reported in due course.

Acknowledgment. This paper is dedicated to Professor Amos B. Smith, III, recipient of the ACS Award for Creative Work in Synthetic Organic Chemistry, in recognition of his many contributions. Partial support of this work by the American Cancer Society (DHP-79) is gratefully acknowledged. Grants for the purchase of NMR equipment from NSF (BIR-94-19409) and the Elsa U. Pardee Foundation are gratefully acknowledged. We wish to express our sincere thanks to Dr. Nicolay Kulikov (Affymax Research Institute) for many helpful discussions. In addition, we thank both our collaborators in the research group of Professor G. Cardillo (Bologna) for many helpful discussions and NATO for funding this collaboration in the form of a Collaborative Research Grant (CRG 950049).

Supporting Information Available: Experimental procedures and ^1H NMR spectra for key compounds and X-ray data for **6b** (62 pages). See any current masthead page for ordering and Internet access instructions.

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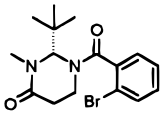
(16) By comparison, Snieckus and co-workers[15] have studied amide restricted rotation in compound **i**, and report overall coalescence at 100 °C.



i

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