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Conformational Analysis of Peptides Containing Enantiomerically Pure α-Methylasparagine: Correspondence Between Computed and Solid State Structures

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Abstract—Asparagine has a high frequency of occurrence in β-turns. We have recently completed an asymmetric synthesis of α-methylasparagine suitably protected for incorporation into polypeptides, and report herein the synthesis and structure of dipeptide Ac-Ala-(Me)Asn-NHMe (**1**). The synthesis proceeds through the corresponding α-methylasparagine succinimide derivative. The three-dimensional structure of the dipeptide around the α,α-dialkylated α-amino acid is compared to that predicted for Ac-(Me)Asn-NHMe. © 2000 Elsevier Science Ltd. All rights reserved.

The replacement of proteinogenic amino acids with unusual but structurally related compounds has developed into a well-respected method both for the study of local conformational states on biological activity and for the preparation of new drug candidates. Of the many approaches to peptidomimetic design,[1] the use of α,α-dialkylated α-amino acids has been pursued vigorously for the obvious advantages of increased hydrolytic stability and predictable, and restricted, conformational properties.

Some years ago we began to explore the chemistry of the amino acid asparagine (Asn). While our initial work focused, among other uses, on the synthesis of β-amino acids,[2] our more recent efforts have centered on the bioorganic chemistry of Asn derivatives. For example, we have investigated synthetic routes to asparagine-derived heterocyclic systems that act both as protection for asparagine during incorporation into polypeptides[3] and as proline mimics.[4] Most recently, the synthesis of enantiomerically pure α-methylasparagine methyl ester has been completed.[5,6]

We wished to probe the effect of the more conformationally restricted α-methylasparagine on peptides. It is well established that asparagine has a high frequency of participation in turn conformations.[7] The formation of β-hairpin loops around the Asn-Gly dipeptide in proteins has been employed extensively in the design and study of small β-sheet structures.[8] The requirement of an Asx turn[9] for glycosylation of asparagine[10] provides additional impetus for asparagine conformational studies. Herein, we report the synthesis and solid state structure of Ac-Ala-(Me)Asn-NHMe. In addition, we present computer-based conformational analysis of a suitably protected derivative of α-methylasparagine for comparison.

The conversion of Fmoc-Ala-(Me)Asn-OMe **2**[5] to *N*-acetyl dipeptide **3** was achieved in good yield by removal of the Fmoc group with 2 M methylamine in THF and subsequent acylation of the free amine with Ac_2O in acetonitrile. Diketopiperazine **4** made up about 15–35% of the product mixture. This undesired side product was easily filtered away from the desired dipeptide.

We had hoped that a suitable method for conversion of ester **3** to the corresponding carboxylic acid would be available so that further elaboration of the C-terminus of the polypeptide would be facilitated. At the same time, we were mindful of the ease with which succinimide derivatives of asparagine can be formed.[11] In the event, attempts to convert methyl ester **3** into the carboxylic acid under basic conditions (0.5 M KOH/MeOH) afforded a high yield of succinimide **5**, as expected from literature precedent.[12] Equally unsuccessful attempts to obtain this carboxylic acid came from treatment of **3** with NaSMe[13] (rapid formation of **5**) and acid (amide hydrolysis) (Scheme 1).

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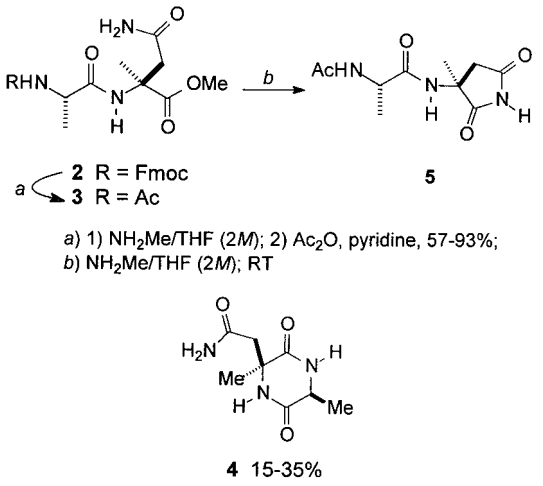


Scheme 1.

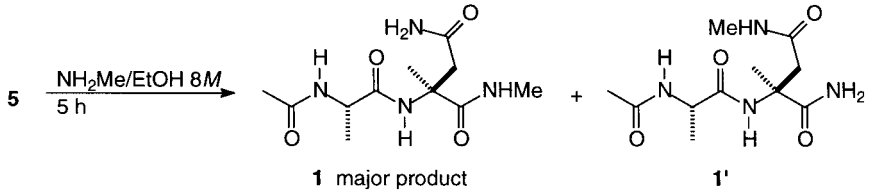
While the more common method of succinimide formation in proteins involves attack on the side chain carbonyl of an asparagine or aspartic acid by the main chain nitrogen of the subsequent amino acid, Nature has also sequestered chemistry similar to the transformation of **3** to **5**. Thus, the repair enzyme L-isoaspartate (D-aspartate) *O*-methyltransferase catalyzes the formation of methyl ester from an isoaspartate residue, which is followed by rapid succinimide formation via side chain amide nitrogen attack.[14] After exhaustive experimentation toward clean ester hydrolysis, it was decided to reverse the strategy, let the natural process prevail, *maximize* the yield of succinimide **5**, and employ **5** as an intermediate toward the formation of the desired product. To this end, the most effective conditions for the synthesis of **5** involved cyclization with 2 M methylamine in THF. Under these conditions the desired product **5** precipitated from the reaction solution, effectively stopping further reactions. By comparison, when **3** was treated with excess dimethylamine in THF (2 M), no precipitate formed and a significant yield of the corresponding dimethyl amide isomers was obtained through opening of the succinimide with this nucleophile. Treatment of **5** with 8 M NH_2Me/EtOH for 5 h resulted in ring opened material as a mixture of regioisomers **1** and **1′** in ~2:1 ratio by ^1H NMR. The mixture of regioisomers was enhanced in the major isomer by dissolution in EtOH and treatment with *tert*-butyl methyl ether, which caused a white solid to precipitate over a 2-day period. Analysis by NMR (HMBC, Table 1, Experimental) indicated that the major isomer was the desired compound **1**, which was confirmed by a single crystal X-ray analysis. Selectivity for main chain carbonyl attack by water on Asx-derived succinimides has been documented previously, but to our knowledge these results are the first example of regioselectivity in amine attack. Similar regioselectivity in hydride reductions of substituted succinimides has been explained by invoking the Bürgi–Dunitz trajectory of the nucleophile. Attack at the main chain carbonyl, which has a relatively unhindered trajectory, is favored over the hindered trajectory of the nucleophile toward the side chain carbonyl (Scheme 2).[15]

Structure of 1

The solid state structure of **1** is shown in Fig. 1B,[16] and depicts a turn conformation. An intramolecular hydrogen bond between O1 and H-N3 is evident. Total distance of the hydrogen bond is 2.99 Å, with a O1-H-N3 angle of 166°, in good correspondence with accepted norms. The ϕ, ψ angles of the $i+1$ (Ala, −58.7, −25.5°) and $i+2$ (α-(Me)Asn, −52.9, −30.5°) residues fall in the range of a type III β turn, a portion of a 3_{10} helix.[17] In addition, the distance of N3 to C11 is 4.46 Å, again in good agreement with the observation of Clark[18] concerning the typical large distance between these two centers in proteins and its effect on succinimide formation.

To better understand the energetics of the system, we undertook a conformational analysis of the basic building block of **1**, namely Ac-^{L}(Me)Asn-NHMe (**6**), with MacroModel[19] version 6.0. The derived Ramachandran plot[20] for **6** is shown in Fig. 2A, which depicts an energy range of −699.6 to −679.0 kJ/mol. Calculations were performed with the Amber 94 force field using the GB/SA solvation model for water. The partial atomic charges were changed to those obtained by use of the semi-empirical AM1 method using the Cramer–Truhlar water model.[21] For comparison purposes, the corresponding Ramachandran plot of Ac-^{L}Asn-NHMe (**7**) is shown in Fig. 2B over a similar energy range (−545.27 to −525.04 kJ/mol) and under identical conditions. Fig. 2B compares well with a plot of ϕ,ψ angle data obtained for asparagine residues found in high resolution crystal structures taken from the Protein Data Base.[7]

The natural amino acid (Fig. 2B) has a broad area of conformational space extending from the α_R-helix region of $-\phi$,$-\psi$ angles through values of $-\phi$,ψ angles found in the polyproline and β sheet regions. Three minima (ϕ,ψ) are visible in this area: −63,−28 (−545.3 kJ/mol), −70,150 (−538.6 kJ/mol), and −139,158 (−537.5 kJ/mol). A fourth, rather shallow, minimum is found at 59,−42 (−530.7 kJ/mol). As expected, much less conformational space is available for **6** (Fig. 2A). There are only two significant minima, and they are much steeper and compact then those found in the compound without a methyl group. The first, at ϕ=−66° ψ=−13° (−699.0 kJ/mol),



Scheme 2.

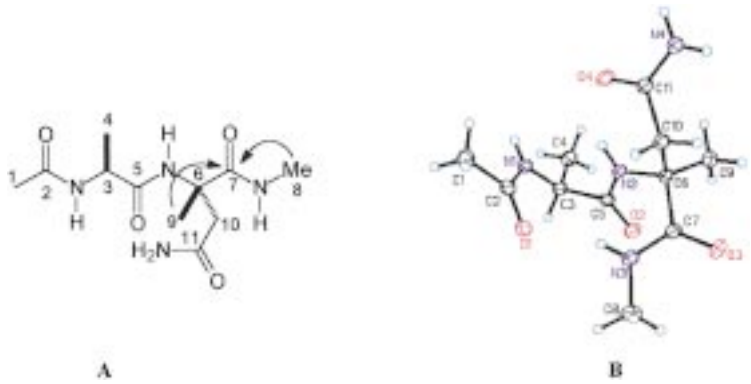


Figure 1. (A) Line drawing of Ac-Ala-^{L}(Me)Asn-NHMe (**1**), showing atom numbering and HMBC data (see Table 1, Experimental). (B) Single crystal X-ray structure of **1**.

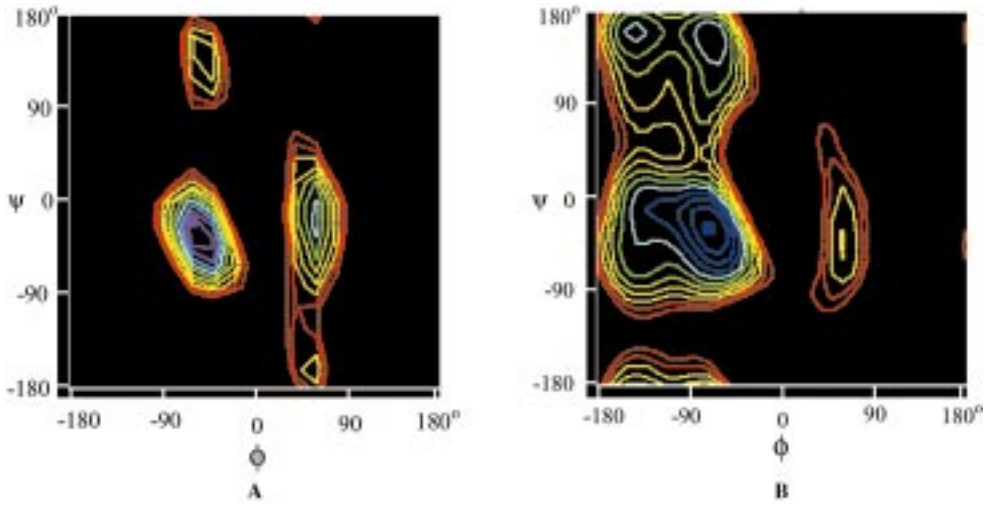


Figure 2. (A) Ramachandran plot of Ac-^{L}(Me)Asn-NHMe (**6**). (B) Ramachandran plot of Ac-^{L}Asn-NHMe (**7**).

corresponds to the area of the right-handed helices (α- and 3_{10}-). This value is in good agreement with that found in the solid state structure of **1** (see discussion above). The second minimum is found at ϕ=60°, ψ=−16° (−692.7 kJ/mol), close to the area of the left-handed helices. This area of conformational space, with a positive ϕ value and a ψ value that is close to zero, is normally occupied only by glycine, although asparagine is also capable of attaining a left-handed helix conformation.[7] This minimum is quite different from the corresponding shallow minimum in **7** in position, depth, and shape. In addition, this secondary minimum in **6** is only 1.5 kcal/mol higher in energy than the primary minimum in the α_R-helix region. From these calculations, it would appear that **6** is more energetically predisposed to attaining the (rare) left-handed helix conformation than is the parent amino acid. Such a conformation is evidenced by asparagine when, for example, it is functioning as a C-terminal cap to a helix.[22] As expected, the Ramachandran plot obtained for the enantiomeric compound was the mirror image of that obtained above.

In conclusion, the present studies constitute the initial example of α-methylasparagine conformation in a peptide structure. The synthesis proceeds through the corresponding succinimide, which is opened regioselectively to afford the desired material. The selective opening of the succinimide by primary amine nucleophiles suggests that this may be a general approach to the elaboration of the C-terminus of this amino acid. The results of computer-based conformational analysis of **6** correlate well with the solid state structure of dipeptide **1**. The use of α-(Me)Asn in the synthesis of additional polypeptides of defined secondary structure is ongoing research in our laboratory and will be disclosed in due course.

Experimental

Ac-Ala-(Me)Asn-OMe (3). To Fmoc-Ala-(Me)Asn-OMe (**2**, 150 mg, 0.331 mmol) was added $MeNH_2$ (2 M in THF, 4.1 mL, 8.2 mmol). The resulting solution was allowed to react for 15–20 min, at which time TLC analysis indicated complete reaction. The solution was evaporated under reduced pressure at ambient temperature, dissolved in CH_3CN, and again evaporated under reduced pressure at ambient temperature. The solids were triturated with petroleum ether to remove dibenzofulvene (DBF) and DBF-amine adducts. To the free amine (positive ninhydrin test) was added CH_3CN (3 mL) followed by Ac_2O (62.5 μL, 0.662 mmol) and pyridine (52.5 μL, 0.655 mmol) and the

mixture was stirred for 2.5 h when a negative ninhydrin test was obtained. The solids were removed by filtration (diketopiperazine by-products are insoluble) and the eluent was evaporated. The resulting residue was dissolved in CH_2Cl_2 and the dipeptide was extracted into water (3×). The aqueous layer was washed with CH_2Cl_2. The aqueous layer was evaporated to a white foam of adequate purity for further manipulation. Yield 57–93% as a clear oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (d, J=7 Hz, 3H), 1.61 (s, 3H), 1.99 (s, 3H), 2.89 (d, J=15 Hz, 1H), 3.14 (d, J=15 Hz, 1H), 3.74 (s, 3H), 4.75, (m, 1H), 6.19 (s, 1H), 6.33 (s, 1H), 6.84 (d, J=7.5 Hz, 1H), 7.78 (s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.4, 22.9, 23.0, 41.4, 49.1, 52.9, 57.9, 170.2, 172.3, 173.9; IR (thin film) 1660, 1732, 3300 cm^{-1}. HRMS Calcd for $[M+1]^+$ $C_{11}H_{19}N_3O_5$: 274.1403; found 274.1408.

Compound **4** was obtained in 15–35% yield as a white solid: mp 206°C (decompose); $[\alpha]_D^{25}=-40.9$, [c=2.09, DMSO]; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.28 (s, 3H), 1.30 (d, J=6.5 Hz, 3H), 2.42 (d, J=15.5 Hz, 1H), 2.59 (d, J=15.5 Hz, 1H), 3.91, (q, J=6.5 Hz, 1H), 6.86 (s, 1H), 7.28 (s, 1H), 7.88 (s, 1H), 7.92 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.9, 27.5, 43.2, 49.9, 56.2, 167.4, 169.4, 171.2; IR (KBr) 3372, 3213, 1656 cm^{-1}. HRMS Calcd for $[M]^+$ $C_8H_{13}N_3O_3$: 199.0957; found 199.0953.

Ac-Ala-(Me)Asn-succinimide (5). To a small round bottom flask fitted with a ground glass stopper was added **3** (210 mg, 0.769 mmol) and $MeNH_2$ (2 M in THF, 3.0 mL, 6.0 mmol). The mixture was allowed to stir 15–24 h; a white precipitate begins to form after ~60 min. The solution was evaporated (may also be filtered). The yield is quantitative (186 mg). mp 212–222°C; $[\alpha]_D^{25}=-54.4$ [c=2.85, MeOH]; ^{1}H NMR (500 MHz, MeOH-d_4) δ 1.29 (d, J=7.5 Hz, 3H), 1.47 (s, 3H), 1.95 (s, 3H), 2.63 (d, J=17.7 Hz, 1H), 2.93 (d, J=17.7 Hz, 1H), 4.36 (q, J=7.5 Hz, 1H); ^{13}C NMR (62.5 MHz, MeOH-d_4) δ 18.4, 22.7, 24.0, 43.8, 50.0, 58.3, 173.1, 175.0, 177.6, 181.7; IR (thin film) 1654, 1718, 3296 cm^{-1}. HRMS Calcd for $[M+1]^+$ $C_{10}H_{15}N_3O_4$: 242.1141; found 242.1143.

Ac-Ala-(Me)Asn-NHMe (1). To a small flask with a ground glass stopper was added **5** (83 mg, 0.344 mmol), followed by $MeNH_2$ (8 M in EtOH, 1.2 mL, 9.6 mmol). The resulting solution was allowed to react for 5 h at room temperature. The solution was evaporated and the residue was dissolved in a small amount of absolute EtOH and *tert*-butyl methyl ether. A precipitate formed after two days. The mother liquors were decanted and the solids were washed with 2-propanol. The mother liquors were evaporated under reduced pressure and a second crop of solid was obtained from EtOH-*tert*-butyl methyl ether as above. The combined crystallized yield of diastereomerically enhanced (approx. 9:1) material was 53% (49.4 mg). The product was again recrystallized with hot EtOH in order to obtain analytical data, including X-ray structural data. mp 193–195°C; $[\alpha]_D^{25}=-92.6$ [c=0.7, MeOH]; ^{1}H NMR (500 MHz, D_2O) see Table 1; ^{13}C NMR (125 MHz, D_2O, dioxane as internal std.) see Table 1; IR (KBr) 3356, 3299, 1654 cm^{-1}.

Table 1. Data from HMBC experiment

Carbon #	^{13}C δ	^{1}H δ	Mult (J, Hz)	HMBC
1	21.5	2.18	s	174.4
2	174.4			
3	50.1	4.36	q (7.0)	16.1, 174.7
4	16.1	1.49	d (7)	50.1, 174.7
5	174.7			
6	57.9			
7	175.5			
8	26.2	2.86	s	175.5
9	23.2	1.71	s	40.4, 57.9, 175.5
10	40.4	2.84, 3.13	d (14), d (14)	23.2, 57.9, 175.5, 174.9
11	174.9			

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