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Evaluation of the β-turn inducing properties of an achiral analogue of aminopiperidinone carboxylates

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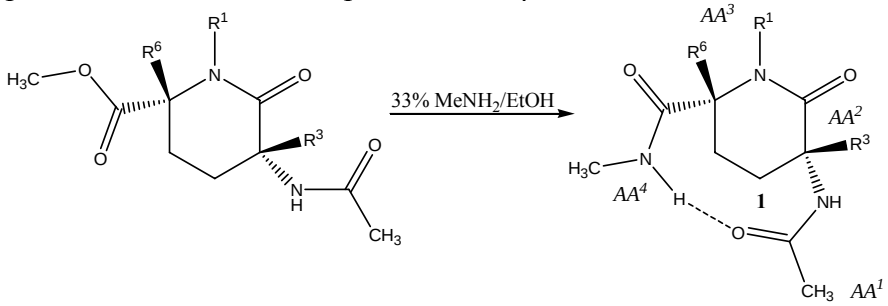
Abstract

c*is*-4-(Acetylamino)-*N*-methylcyclohexane carboxamide has been selected as *all carbon ring* analogue of the previously described 5-aminopiperidinone-2-carboxylate systems. The potential β-turn inducing properties of this model compound are evaluated by means of NMR analysis and molecular modeling.

Keywords: β-turn mimic, intramolecular hydrogen bridge, achiral, aminopiperidinone carboxylate

Introduction

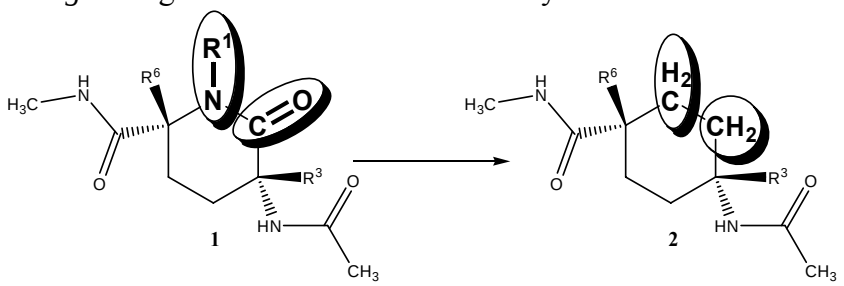
The β-turn inducing potential of 5-aminopiperidone-2-carboxylate (APC) systems has been extensively described by our research group[1] as well as by others[2] via studies on model systems **1** (Scheme 1). These systems are simplified models of conformationally restricted tetrapeptides,[3] in which the presence of a hydrogen bond between the methylamideproton and the *N*-acyl carbonylgroup has been identified via NMR experiments.[4] As a consequence residues *i* (AA^1)and *i+3* (AA^4) are in close enough proximity to induce a β-turn.[5] Molecular modeling results confirm the distance between the α-carbons of residues *i* and *i+3* to be well within the 7Å limit, being the generally accepted criterion to claim the presence of a β-turn.



Scheme 1: Model system of a APC-tetrapeptide **1** and it's precursor

These APC-systems, being the result of the methanolysis of a Diels-Alder adduct of a substituted pyrazinone,[1] are racemic mixtures with a fixed (relative) *cis* geometry between amine and carboxylate function. It is possible to separate the enantiomers via chiral HPLC, but this is not a very practical method since these columns require specific conditions.[6] In this paper we

evaluate a simplified achiral analogue of the known APC systems as a potential beta turn mimic. The *cis*-aminocyclohexane carboxylic acid derivative **2** seemed to be a viable option, considering it to be an *all-carbon ring* analogue of the 'traditional' APC-systems.



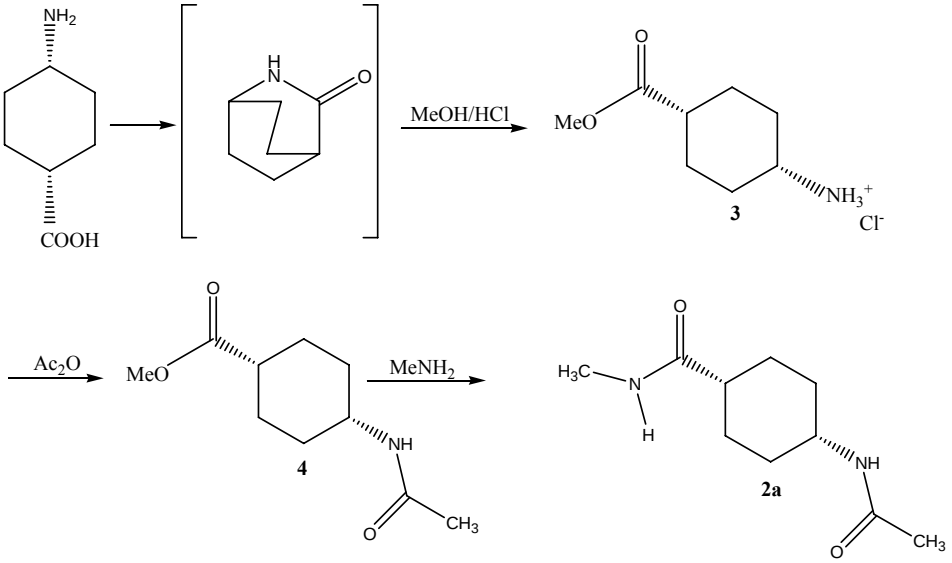
Scheme 2: APC tetrapeptide mimic **1** and corresponding *all-carbon ring* analogue **2**

Since molecule **2** hasn't been described previously as a β-turn mimic, we had to put our hypothesis to the test. This was done via a molecular modeling study and an NMR analysis of model system **2a** (R^3=R^6=H).

Results and Discussion

Synthesis

Esterification of commercially available *cis*-4-aminocyclohexane carboxylic acid with HCl-saturated methanol yields the ammonium chloride salt of the desired product **3** (Scheme 3). Mass spectrometric analysis shows formation of an intermediate bicyclic lactam. After full conversion of this bicyclic lactam to the ammonium salt **3**, it is dissolved in acetic anhydride and triethylamine is added dropwise. The acylated product **4** is purified by column chromatography (silicagel, ethyl acetate:heptane 30:70), producing a yellow oil. The model compound **2a** is formed by treating **4** with a 33% solution of methylamine in ethanol. Evaporation followed by precipitation from a hexane/dichloromethane mixture produces the desired compound **2a** in 20% yield.



Scheme 3: Synthesis of *cis*-4-(acetylamino)-*N*-methyl-cyclohexane carboxamide **2a**

Computational evaluation

To investigate the β-turn inducing and H-bridge forming properties in system **2a**, a molecular modeling analysis was performed. Using the Monte Carlo search function of macromodel,[7] 5000 different conformations of **2a** were energy minimized and duplicates were eliminated from the dataset (AMBER*-force field, vacuum). The 20 conformations with the lowest energy were retained for further analysis. The properties that had to be checked in these systems were the presence or absence of an intramolecular hydrogen bridge[8] and the distance $d_{\alpha C1\text{-}\alpha C4}$. This distance has to be smaller than 7Å in a β-turn (Figure 1).

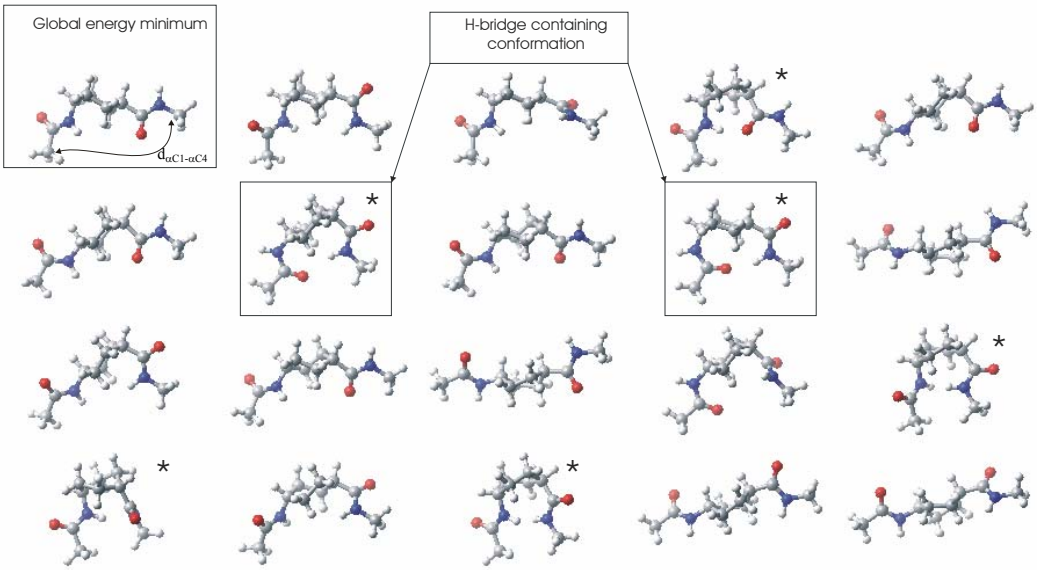


Figure 1: The 20 lowest energy conformations of structure **2a** ranked according to increasing energy (AMBER* force field). Conformations marked with a * fulfill the beta turn criterion.

The data for the 20 selected systems, given in table 1, show that the energetically most favorable conformation (CONF 1) lacks a hydrogen bridge and also has a $d_{\alpha C1\text{-}\alpha C4}$ distance of 8.84 Å. Consequently this conformation can not be considered for β-turn induction. Out of the 20 conformations only conformations 7 and 9 contain a hydrogen bond between (pseudo-)residues *i* and *i*+3. Since they are respectively 29 and 31 kcal/mol higher in potential energy than the global minimum, we can expect that it is energetically very unlikely that these conformations (with hydrogen bridge) are populated.

The lack of a hydrogen bond between residues *i* and *i*+3 alone is inconclusive information to disprove the β-turn properties of this system. The final criterion is the distance αC_1-αC_4. As mentioned above the global minimum does not meet this criterion (nor does it contain a hydrogen bridge). In total there are 6 conformations that do fulfill this requirement (indicated with a * in Figure 1). Out of these 6, conformation 4 has the lowest energy. As this 'open turn' conformation (without intramolecular hydrogen bridge) is 17 kcal/mol higher in energy than the global minimum, it will not be the favored conformation in solution.

Conformation	Potential Energy-AMBER* (kcal/mol)	Relative Potential Energy-AMBER* (kcal/mol)	αC_1-αC_4 distance (Å)	H-Bridge present between residue *i* and *i*+3 ?
CONF 1	-90,148918	0	8,843191	No
CONF 2	-81,127693	9,021225	7,303593	No
CONF 3	-78,871384	11,277534	8,01966	No
CONF 4	-72,870079	17,278839	**6,248838**	No
CONF 5	-72,090256	18,058662	9,362591	No
CONF 6	-71,665833	18,483086	9,440627	No
CONF 7	-60,217384	29,931534	**6,254655**	**Yes**
CONF 8	-58,516373	31,632545	9,262678	No
CONF 9	-58,468124	31,680794	**6,279634**	**Yes**
CONF 10	-57,770046	32,378872	10,322329	No
CONF 11	-57,682434	32,466484	7,843061	No
CONF 12	-57,143272	33,005646	9,291582	No
CONF 13	-56,498245	33,650673	10,321632	No
CONF 14	-56,393692	33,755226	7,128303	No
CONF 15	-52,384823	37,764095	**4,090806**	No
CONF 16	-51,56678	38,582138	**4,229878**	No
CONF 17	-50,293926	39,854992	7,80197	No
CONF 18	-50,058628	40,09029	**3,981442**	No
CONF 19	-49,050415	41,098503	10,164367	No
CONF 20	-49,023705	41,125214	10,189578	No

Table 1: 'molecular modeling' results

NMR-analysis

If the data from the computational analysis are correct, we would not expect to see intramolecular hydrogen bonds in molecule **2a**. The presence of hydrogen bonds can be checked by means of NMR analysis.

If we cannot 'observe' a hydrogen bond between H^B and CO (Figure 2), there is no direct evidence to assume that the system can be used as β-turn mimic.

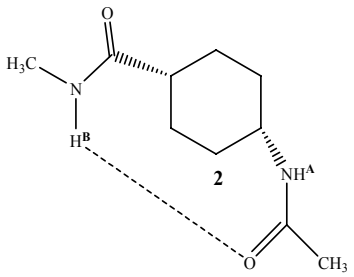


Figure 2: *cis*-4-(acetylamino)-*N*-methylcyclohexane carboxamide **2a** with the potential hydrogen bridge indicated by a dotted line

Spectral analysis of the system focusses on the resonance behavior of the two amide protons H^A en H^B. In theory we expect easy assessment of these protons based on their respective couplings with the neighbouring protons: a doublet is expected for H^A whereas H^B should be seen as a quartet. However these coupling patterns were not resolved in the NMR spectrum. The 2D-COSY spectrum could be used to solve this problem because there was a clear correlation between the methyl group doublet at 2.80 ppm and the amide amide proton H^B at 5.73 ppm (*N*-Me amide proton B). Additional evidence can be drawn from correlation between the cyclohexane proton H^4 at 4.04 ppm and the second amide proton H^A at 5.89 ppm (*N*-acyl amide proton A).

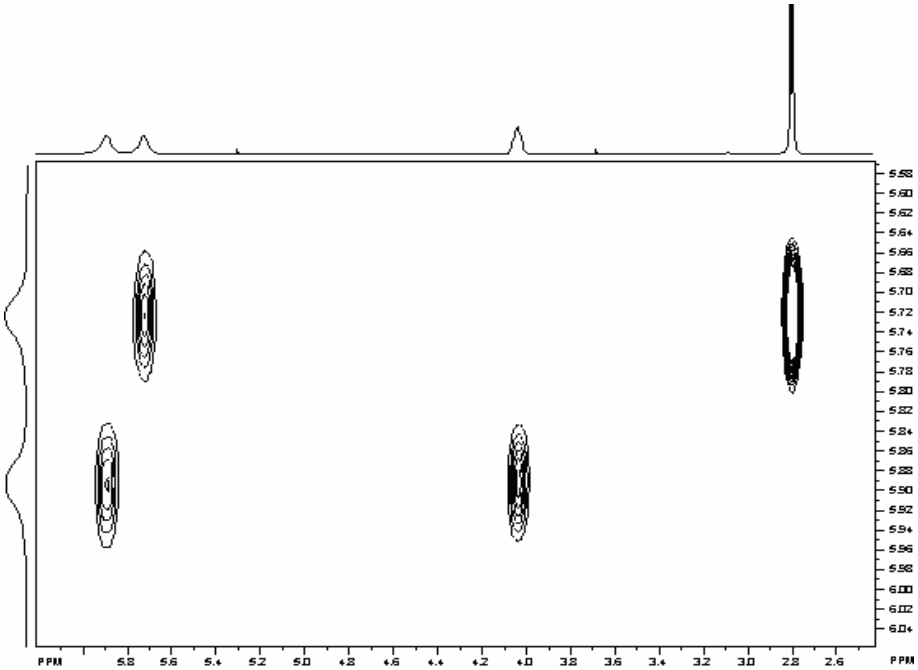


Figure 3: Detail of model system **2a**'s COSY spectrum

Upon heating the sample, amide protons involved in hydrogen bonding show a smaller change in chemical shift than solvent exposed protons: an intramolecular hydrogen bridge shields the amide proton from the solvent and thus this proton will hardly be affected by changes in the bulk solution. In general, shift changes from 0 to -3 ppb/°C are characteristic for hydrogen bonded protons; while shift changes smaller than -7ppb/°C indicate the proton is exposed to the solvent. These shift changes can be determined by gradually heating the product dissolved in

$CDCl_3$, and taking an ^{1}H-NMR spectrum at regular temperature intervals. Heating causes an upfield shift for both protons H^A and H^B. Linear regression on the acquired data points, indicates that both of the protons lack a hydrogen bond as the deduced temperature coefficients of respectively -8.4ppb/°C (in the case of the acylamide proton) and -6.3ppb/°C (for the *N*-Me amide proton) fall well out of the region for hydrogen bonding.

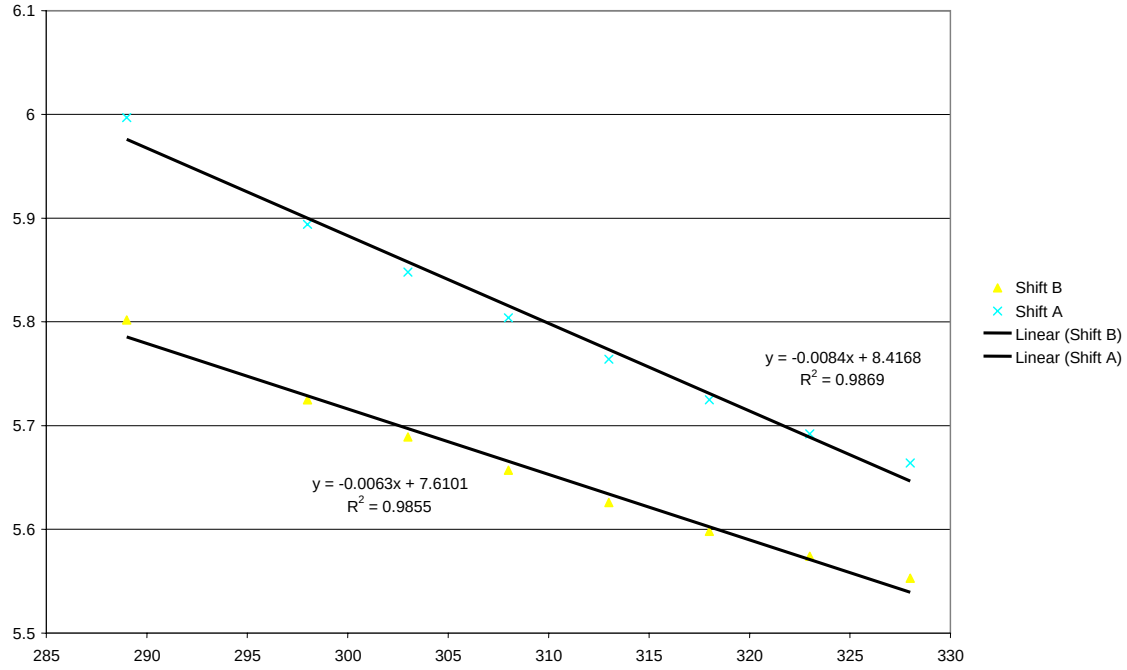


Figure 4: Chemical shifts (ppm) of the amide protons correlated with the temperature (K) (spectra taken in $CDCl_3$ on a 400 MHz spectrometer)

An alternative criterion to investigate the hydrogen bridge presence is by quantitating the solvent dependence of the chemical shifts of the amide protons.[9] By changing the solvent from deuterated DMSO to deuterated chloroform the change in chemical shift is very pronounced (up to 2 ppm) for a solvent exposed proton. Ergo this solvent will engage the free NH protons in an *inter*molecular hydrogen bond. The chemical shifts of the amideprotons of molecule **2a**, recorded at 25°C in DMSO and $CDCl_3$ are shown in table 2. These shift changes, both being close to 2 ppm, confirm the absence of a hydrogen bridge in the model system.

δ_{NHMe} DMSO	δ_{NHMe} $CDCl_3$	$\mathbf{\Delta\delta_{NHMe}}$	δ_{NHCO} DMSO	δ_{NHCO} $CDCl_3$	$\mathbf{\Delta\delta_{NHCO}}$
7.61 ppm	5.73 ppm	**1.88 ppm**	7.74 ppm	5.89 ppm	**1.85 ppm**

Table 2: Temperature dependence of the chemical shifts of the amide protons in molecule **2a**

Based on the results of the computational study and the NMR analysis, we can conclude that the simplified system proposed in this paper is not suitable as a beta turn inducing scaffold. Apparently, the planar lactam function in the native APC systems is extremely important to impose a correct conformation for beta turn induction.

Experimental Section

General Procedures. Melting points were taken using an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1600 Fourier transform spectrometer. Mass spectra were run using a Hewlett Packard MS-Engine 5989A apparatus for EI and CI spectra, and a Kratos MS50TC instrument for exact mass measurements performed in the EI mode. For the NMR spectra (δ, ppm) a bruker AMX 400 and a Bruker Avance 300 spectrometer were used. Analytical and preparative thin layer chromatography was carried out using Merck silica gel 60 PF-224, for column chromatography 70–230 mesh silica gel 60 (E.M. Merck) was used as the stationary phase.

Compound characterization.

***cis*-Methyl 4-(acetylamino)cyclohexane carboxylate (4)**
An ice-cooled suspension of 1.85 mmol *cis*-4-aminocyclohexane carboxylic acid in methanol is purged with gaseous hydrochloric acid for 15 min and stirred overnight at 50°C under inert atmosphere. After evaporation of the solvent, the pale green solid is dissolved in acetic anhydride. Triethylamine is added dropwise till precipate is formed and the reaction mixture is stirred for 24h at ambient temperature. The precipitate is removed by filtration and the solvent is removed under reduced pressure. This residue is purified by column chromatography (silicagel; ethyl acetate:heptane 30:70); Yield: 58%; Yellow oil; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): 5.65 (s, 1H, NH), 3.93 (m, 1H, H^4), 3.68 (s, 3H, OCH_3), 2.50 (m, 1H, H^1), 1.97 (s, 3H, CH_3), 1.86 (m, 2H, CH_2), 1.70 (m, 4H, CH_2), 1.55 (m, 2H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm): 175.5 ($\underline{C}OOCH_3$), 169.3 (CO), 51.53 (OCH_3), 46.0 (C^4), 40.0 (C^1), 29.2 (CH_2), 25.0 (CH_2), 23.4 (CH_3); HRMS: calcd for $C_{10}H_{17}NO_3$: 199.1208; found : 199.1210; m/z (EI, %) : 199 (M^+, 39), 156 (M^+-$COCH_3$, 91); IR (KBr, cm^{-1}): 3418.2 (amide), 1641.5 (amide)

***cis*-4-(Acetylamino)-*N*-methylcyclohexane carboxamide (2a)**
30 mg of compound **4** is dissolved in a 33% solution of methylamine in ethanol and stirred at ambient temperature for 5 days. After removal of the solvent, the product is precipitated out of dichloromethane-hexane. The white solid is separated from the solvent in a centrifuge (5min, 2700 RPM) and washed several times with the dichloromethane-hexane mixture to yield the pure product; Yield: 20%; white solid; Melting point: 185°C (dichloromethane/hexane); ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): 5.98 (br m, 1H, $N\underline{H}CH_3$), 5.79 (s, 1H, $N\underline{H}COCH_3$), 4.03 (m, 1H, H^4), 2.79 (d, 3H, *J*= 4.9 Hz, $NHC\underline{H}_3$), 2.19 (m, 1H, H^1), 1.98 (s, 3H, CH_3), 1.80-1.55 (m, 8H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm): 176.0 ($\underline{C}ONHCH_3$), 169.6 ($\underline{C}OCH_3$), 45.0 (C^4), 42.9 (C^1), 29.3 (C^3), 26.4 ($NH\underline{C}H_3$), 25.1 (C^2), 23.6 (CH_3); HRMS: calcd for $C_{10}H_{18}N_2O_2$: 198.1368; found: 198.1369: m/z (EI, %) : 198 (M^+, 34), 155 (M^+-$COCH_3$, 48); IR (KBr, cm^{-1}): 3290.6 (CONH), 1636.5 (amide), 1546.3 (amide)

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References and Footnotes

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3. Residue *i* is represented by it's α-C with carbonyl function; residues *i+1* and *i+2* by the APC-system and *i+3* by a representative amine function with α-C; the conformational restriction is implied by the ethylene bridge, that forces the amide bond in the *cis*-conformation.
4. Furthermore these NMR-analyses showed that the preferred conformation is half chair.
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6. APC systems can be separated in their enantiomers (on an analytical scale) by means of HPLC using a chiral stationary phase (Daicel Chiralpack series). This method implies some disadvantages. Firstly the nature of the stationary phase limits the eluent to a mixture of alchohols with hexane. Chlorinated solvents dissolve the stationary phase, thus destroying the column. Taking into account the low solubility of certain products in the acceptable mixtures, only minor amounts of product can be purified per injection.
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8. (a) The presence of an intramolecular hydrogen bridge between residue *i* and *i*+3 is assumed when 3 criteria are fulfilled: (I) The distance CO-HN must be smaller than 2.5 Å; (II) The NH-O(*i*) angle must be at least 120°; (III) The H(*i*+3)-OC(*i*) angle must be larger than 90°. (b) Belvisi, L.; Bernardi, A.; Manzoni, L.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **2000**, 2563-2569.
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