

Geometrical and Conformational Preferences of the 9-Fluorenylmethoxycarbonyl-amino Moiety

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Abstract: Structural parameters, originating from x-ray crystallographic data, have been compiled for 13 derivatives of amino acids, peptides and related compounds, which contain a total of 14 Fmoc-NH- moieties. For these moieties, molecular geometries and conformations — described by the ω_0 , θ^1 , θ^2 and θ^3 torsion angles — were analysed and compared with the corresponding parameters for the Z-NH- and Boc-NH- moieties (290 and 553, respectively). To gain a deeper insight into the conformational features of the Fmoc-NH- moiety, *ab initio* free molecule calculations were performed for fully relaxed minima. Also the potential energy surface as a function of the torsion angles (θ^3 , θ^2) was generated. The conformational features of the Fmoc-NH- moiety: (i) two possible values for the angle ω_0 ($\sim 180^\circ$ or, rarely, $\sim 0^\circ$) and (ii) the angle $\theta^1 = 180^\circ \pm 15^\circ$, are common to the Z-NH- and Boc-NH- systems. By contrast, the θ^2 and θ^3 angles in the Fmoc, Z and Boc groups differ essentially. In the Fmoc groups θ^2 mostly has values of $180^\circ \pm 30^\circ$ and values up $|115^\circ|$ seem to be forbidden, whereas fewer than half of the Z groups adopt $\theta^2 \sim 180^\circ$ and the remainder have θ^2 in the range of $|90^\circ \pm 20^\circ|$. On the other hand, the Boc methyl groups are staggered. The θ^3 values observed for Fmoc are limited to the regions of $180^\circ \pm 20^\circ$ and $|60^\circ \pm 20^\circ|$, while for the Z group a variety of θ^3 occurs. The orientation of the fluorenyl vs the urethane function is mostly *trans*. Our results suggest a lower conformational flexibility for the Fmoc group compared with that of the Z group. Our calculations confirm that the observed conformational features for the Fmoc-NH- moiety are inherent properties. The Fmoc-NH- moiety in crystals involves the participation of its O=C-NH functionality in hydrogen bonds. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: *ab initio* calculations; Boc amino protection; crystal structure; fluorene; Fmoc amino protection; N-terminally protected peptides; urethane geometry; Z amino protection

INTRODUCTION

In peptides the 9-fluorenylmethoxycarbonyl (Fmoc) group (Figure 1A), along with the *tert*-butoxycarbonyl (Boc) and benzyloxycarbonyl (Z) groups, belongs to the so-called urethane-type protection. They are in

routine use in peptide synthesis, because coupling such N^α-protected amino acids with other amino acids or peptides proceeds without racemization, whatever activation method is employed [1]. Since the early 1970s, the first reports [2,3] of these protecting groups, the Fmoc group has become the most frequently applied in solid-phase peptide synthesis within the well-established Fmoc/*t*Bu chemistry [4–7]. This procedure has been applied more and more extensively in recent years and is now probably the method of choice for the chemical synthesis of peptides. This popularity is due to the relatively mild and versatile Fmoc deprotection with

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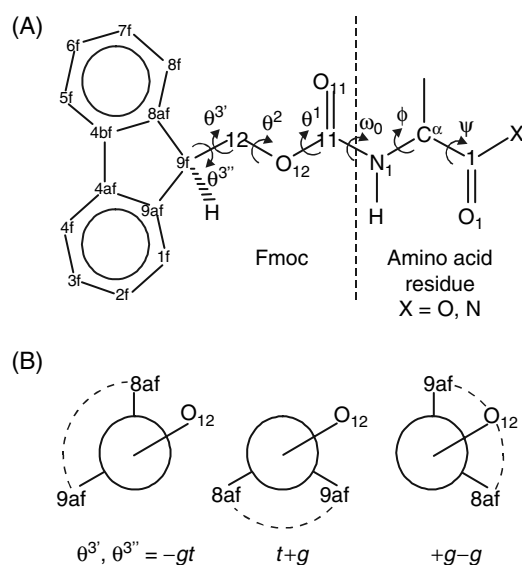


Figure 1 The designations used in this paper. (A) Atom numbering and definition of torsion angles; (B) Conformation about C(12)–C(9f).

amines. In contrast to the more traditional Boc/Bzl chemistry, no repetitive acidolytic steps are required for Fmoc deprotection in the stepwise chain elongation, which are known to cause a progressive loss of the side-chain blockages, i.e. N^w -Z urethanes and carbonates, benzyl esters and benzyl ethers. The use of the Fmoc group is part of a truly orthogonal scheme, thus offering many unique opportunities in bioorganic chemistry. Its utility for the synthesis of peptide conjugates, like glycopeptides, phosphopeptides, pegylated peptides, peptide nucleic acids and peptides with unusual amino acids is well documented [4–9]. Furthermore, the Fmoc group has been patented as a factor increasing the oral absorption of peptide drugs [10]. Short chiral nitroxyl peptides with the N^w -Fmoc protection and a stable secondary structure have been proposed recently as catalysts of enantioselective oxidations [11].

The geometrical and conformational preferences of the Boc-NH– [12] and Z-NH– [13] moieties have already been investigated. However, despite its importance, there is no such analysis for the Fmoc-NH– moiety. Therefore, in this paper, the x-ray crystal structures with the Fmoc-NH– moiety have been surveyed in an effort to reveal patterns of geometry and conformation. In addition, the conformational preferences of the Fmoc-NH– moiety have been calculated by an *ab initio* method for related model molecules.

METHODS

Cambridge Crystallographic Database Survey (November 2002 Release (14))

Compounds having at least one Fmoc-NH– moiety, for which crystallographic structural data ($R < 10\%$) were analysed, are listed in Table 1 [11, 15–24]. This set contains 14 secondary urethane fragments, including the Fmoc derivatives of five amino acids (**1–5**) [15–18], six peptides (**6–11**) [11, 19–22], one amino acid related compound (**12**) [23] and one heterene oligoamide (**13**) (with two Fmoc-NH– moieties) [24]. The Fmoc-NH– moieties were compared with the Z-NH–, Boc-NH– and ester $\text{CH}_2\text{-O-C(O)-C}$ moieties which were retrieved from the CSD. For the N-protecting groups, as in the case of the Fmoc derivatives, only peptides and other amino acid-related compounds were taken into account, and disordered structures were excluded.

Ab initio Calculations

Small diamide systems YNH-CH(R)-CONHY , most frequently where $\text{Y} = \text{Ac}$ (acetyl) and $\text{R} = \text{Y} = \text{Me}$ [25], show conformational variations that allow these systems to be considered as useful models in conformational studies. In line with this strategy, Fmoc-L-Ala-NHMe was established as the model molecule for calculations of the Fmoc conformational preferences. The starting points for generating the energy-minimized conformers were the possible combinations of the torsion angles ϕ , ψ , ω_0 , θ^1 , θ^2 and $\theta^{3'}$ (Figure 1) that were selected as described below. Calculations were performed on $\omega_0 = 180^\circ$ and 0° , i.e. the *trans* and *cis* form of the urethane amide group, respectively. This latter form, due to the replacement of the peptide C^αH by the smaller urethane O(12) atom, should not be destabilized as much as is the *cis* peptide bond [12]. The minima calculated *ad hoc* for *trans* and *cis* MeOCO-L-Ala-NHMe (Table 2) served as the initial (ϕ , ψ , θ^1) angles. For the rotation about the O(12)–C(11) bond, the *cis* conformation ($\theta^1 \sim 0^\circ$) is unstable just as in the Boc-NH– [12] and Z-NH– moieties [13]. The energy minimization, starting with $\theta^1 = 0^\circ$, always results in a conformation with $\theta^1 \sim 180^\circ$. The starting values for the θ^2 and $\theta^{3'}$ angles were $\pm 60^\circ$ and 180° , i.e. the minima of the torsion energy for the rotation about the $\text{Csp}^3\text{-Osp}^3$ or the $\text{Csp}^3\text{-Csp}^3$ bond. Calculations were performed on the free molecules with the HF/3-21G method using the Gaussian 98 package [26]. The potential energy surface $\Delta E = f(\theta^2, \theta^{3'})$

Table 1 Fmoc-NH- Derivatives

	R	Refcode	Ref.
1		—	[15]
2		CUWKIO	[16]
3		CUWKOU	[16]
4		JAGTAM	[17]
5		MERRIK	[18]
6		SIDWAD	[19]
7		ODULAA ^a	[11]
8		TIBDIR	[20]
9		RATQAE	[21]
10		GOMJUN	[22]
11		ODULEE	[11]
12		WARSUD	[23]
13		ABOMAF	[24]

^a Geometrical constraints used for the atoms of the fluorenyl group.

was generated for molecule Fmoc-L-Ala-NHMe with *trans* amide bonds and in the C_{7eq} conformation for the amino acid residue. The θ^2 , θ^3 , ϕ and ψ variables were fixed at each grid point (30° spacing), and all other degrees of freedom were relaxed. The conformational map was obtained with the Surfer 8 program using the radial basis function as a gridding method.

RESULTS AND DISCUSSION

The Crystal-State Geometry of the Fmoc-NH-Moiety

The selected bond lengths and bond angles of the Fmoc-NH- moiety in compounds **1–13** are reported in Table 3 (atom numbering according to Figure 1). The bond distances in the fluorenyl rings are the same, within the experimental error, as those in the parent unsubstituted fluorene molecule [27,28]. The remaining bond lengths of the Fmoc-NH- moiety are similar to the respective values in Z-NH-, and except for the bond C(12)–O(12) that seems to be shorter, to those in Boc-amino derivatives (Table 4). The mean bond angles about the O(12), C(11) and N(1) atoms compare, in general, with the values in the Z-NH-, Boc-NH- and the ester CH₂–O–C(O)–C groups (Table 4). However, the C(11)–O(12)–C(12) angle has a reduced value with respect to corresponding one in the Boc-amino derivatives. Shortening this bond and decreasing this angle suggest a lower steric hindrance caused by the 9-fluorenylmethyl (and the benzyl) than that caused by the *tert*-butyl group. The angle O(12)–C(11)–N(1) in the Fmoc-NH- moiety, as in other urethane systems [14], amounts to ~110°, i.e. about 6°–7° less than the C^α–C'–N bond angle in peptides [29,30]. This finding results from the reduced repulsion between the O(12) atom and the N(1)-proton compared with the corresponding repulsion involving the peptide C^αH group. The angle C^α–N(1)–C(11) in compounds **4** and **6**, having a *cis* amide arrangement, is somewhat larger than that for the *trans* compounds. This finding is in common with the *cis* peptides [29,30] and the *cis* Boc-urethane amides [14].

The Crystal-State Conformation of the Fmoc-NH-Moiety

The sequence of four torsion angles θ^3 , θ^2 , θ^1 and ω_0 , shown in Figure 1, describes the conformation of the Fmoc-NH- moiety. Their values, as well as the

Table 2 Conformers^a and Relative Energies (kcal mol⁻¹) for the MeOCO-L-Ala-NHMe Molecule as Obtained Using the HF/3-21G Method

Conformation of the		θ^1	ω_0	ϕ	ψ	ΔE
Urethane amide	Ala residue ^b					
<i>trans</i>	C7 _{eq}	-179.1	-175.8	-85.3	66.6	0.00
	C5	180.0	179.7	-168.8	169.4	1.58
	C7 _{ax}	178.6	174.7	74.6	-55.9	2.30
	β_2	-179.8	-172.3	-127.7	24.1	2.89
	α_L	179.8	173.1	65.3	31.0	4.73
	α'	180.0	173.8	178.9	-42.2	6.30
	α_D	179.4	-166.6	65.1	177.8	7.15
<i>cis</i>	C7 _{eq}	-174.4	5.7	-93.0	64.5	3.03
	C5	179.1	0.1	-165.1	167.8	3.06
	C7 _{ax}	175.3	-10.0	82.3	-50.7	6.67
	β_2	179.4	9.7	-130.9	24.1	4.17
	α_L	-177.8	-8.9	61.6	36.5	5.03
	α'	179.1	-3.0	-168.7	-47.2	7.53
	α_D	-162.7	20.5	52.7	179.0	8.58

^a Torsion angles in (°).^b Designation according to ref. [25].

Table 3 Observed Characteristic Bond Lengths (Å) and Bond Angles (°) for the Fmoc Urethane Moiety

	C ^α -N(1)	N(1)-C(11)	C(11)-O(12)	C(11)-O(11)	O(12)-C(12)	C(12)-C(9f)	C ^α -N(1)-C(11)	N(1)-C(11)-O(12)	N(1)-C(11)-O(11)	O(12)-C(11)-O(11)	C(11)-O(12)-C(12)	O(12)-C(12)-C(9f)
1	1.470	1.304	1.348	1.223	1.459	1.494	119.4	112.4	125.3	122.2	115.2	109.3
2	1.437	1.341	1.355	1.204	1.438	1.500	119.5	111.1	125.1	123.7	115.3	107.3
3	1.446	1.341	1.321	1.227	1.430	1.492	123.1	111.8	123.7	124.6	118.7	108.5
4^a	1.468	1.379	1.347	1.217	1.468	1.516	125.7	111.2	122.5	126.4	117.9	108.3
5	1.458	1.341	1.335	1.218	1.449	1.517	124.1	111.2	123.7	125.0	117.5	107.6
6^a	1.437	1.349	1.339	1.215	1.440	1.547	127.2	111.9	123.6	124.5	115.4	106.5
7	1.457	1.347	1.336	1.218	1.441	1.514	121.4	110.4	124.6	125.0	118.5	108.8
8	1.439	1.343	1.339	1.223	1.446	1.514	120.7	109.5	127.0	123.5	116.3	108.7
9	1.447	1.376	1.322	1.202	1.455	1.522	120.2	109.0	124.2	126.8	117.0	106.8
10	1.461	1.340	1.357	1.226	1.447	1.528	119.8	111.3	125.4	123.3	114.8	108.0
11	1.469	1.347	1.334	1.209	1.456	1.525	121.1	111.0	124.7	124.3	116.7	106.9
12	1.442	1.350	1.355	1.206	1.443	1.517	122.1	109.7	126.3	124.1	114.7	108.1
13	—	1.369	1.347	1.210	1.449	1.524	—	108.5	126.3	125.2	117.8	106.7
13	—	1.362	1.356	1.216	1.448	1.525	—	109.2	127.0	123.8	116.0	107.6
Mean	1.453	1.349	1.342	1.215	1.448	1.517	122.0	110.6	125.0	124.5	116.6	107.8

^a *cis* Urethane amide.

torsion angles of the amino acid residue following this moiety, observed in crystal structures **1–13**, are collected in Table 5.

The torsion angles ω_0 and θ^1 characterize the conformation of the urethane function. The Fmoc amide bond is most frequently *trans*-arranged,

Table 4 Characteristic Parameters for the Z-NH-, Boc-NH- and Ester Moieties. Mean Values for Bonds (Å) and Bond Angles (°) Calculated from the CSD [14]

Number of moieties	Z-NH- <i>trans</i> 287	Boc-NH- <i>trans</i> 529	Boc-NH- <i>cis</i> 24	Esters 2920
C ^α -N(1) ^a	1.455	1.449	1.453	—
N(1)-C(11)	1.341	1.344	1.344	—
C(11)-O(12)	1.348	1.343	1.333	1.330
C(11)-O(11)	1.214	1.214	1.225	1.197
O(12)-C(12)	1.450	1.472	1.475	1.447
C ^α -N(1)-C(11)	121.4	121.1	125.6	—
O(11)-C(11)-O(12)	124.0	125.6	124.9	123.5
N(1)-C(11)-O(11)	125.6	124.5	123.3	—
N(1)-C(11)-O(12)	110.3	109.9	111.7	—
C(11)-O(12)-C(12)	116.0	121.2	121.7	116.4

^a Atom numbering as in Figure 1.

Table 5 Observed Torsion Angles (°) for the Fmoc-NH- Moiety and the N-Terminal Amino Acid Residue

	$\theta^{3'}$ O(12)-C(12)- C(9f)-C(8af)	$\theta^{3''}$ O(12)-C(12)- C(9f)-C(9af)	θ^2 C(11)-O(12)- C(12)-C(9f)	$(\theta^{3'}, \theta^{3''}, \theta^2)$	θ^1 N(1)-C(11)- O(12)-C(12)	ω_0 C ^α -N(1)- C(11)-O(12)	ϕ C(1)-C ^α - N(1)-C(11)	ψ X-C(1)- C ^α -N(1)
1	-73.1	174.8	168.4	- <i>gtt</i>	172.2	174.8	-84.0	145.9
2	-172.2	72.9	-179.7	<i>t + gt</i>	178.3	179.6	-65.6	150.6
3	-178.2	65.6	-152.8	<i>t + gt</i>	-177.8	172.0	59.3	-158.6
4^a	47.9	-69.2	116.9	<i>+g - g + ac</i>	-172.4	-3.4	-65.5	160.9
5	-176.8	66.8	-134.0	<i>t + g - ac</i>	164.4	177.4	-59.7	-43.1
6^a	62.3	-53.0	164.5	<i>+g - gt</i>	-177.5	1.8	-88.6	21.8
7	64.0	-52.3	-116.7	<i>+g - g - ac</i>	-175.8	-178.4	-59.5	-31.7
8	-60.7	-175.8	-176.8	- <i>gtt</i>	-177.7	178.7	-127.3	152.5
9	52.0	-65.3	117.9	<i>+g - g + ac</i>	-170.3	164.7	-77.9	118.0
10	-67.0	178.8	-175.6	- <i>gtt</i>	-178.0	174.5	-56.3	125.2
11	-73.4	172.6	-152.8	- <i>gtt</i>	178.7	-157.9	-62.9	-31.9
12	54.9	-63.5	172.6	<i>+g - gt</i>	176.8	175.2	129.8	—
13	-162.2	80.3	-142.9	<i>t + g - ac</i>	-174.2	173.2	—	—
13	-171.9	72.3	-162.1	<i>t + gt</i>	-173.3	-179.6	—	—

^a *cis* Urethane amide.

$\omega_0 \sim 180^\circ$, and is usually planar. In a few cases this bond departs from planarity up to 22° [31]. This property is in common with the Z- and Boc-amino protections ($180^\circ \pm 22^\circ$; Figure 2). For **9** and **11**, the ω_0 deviation is the largest, perhaps because of some overcrowding present in these molecules. In two compounds (**4** and **6**), which have a quaternary C^α atom, the *cis* amide arrangement occurs. Of the derivatives with the Z-NH- moiety,

three compounds containing *cis* urethane amides have been reported; however, none of those is amino acid- or peptide-related. The *cis* urethane amide conformation is much more populated among the Boc-amino derivatives. For 24 moieties (including four non-amino acid compounds) the observed ω_0 angle is in the range -17° to 13° . For Fmoc-amino moieties, the angle θ^1 always takes a value $180^\circ \pm 10^\circ$, except for the C^{α,α}-dialkylated glycine

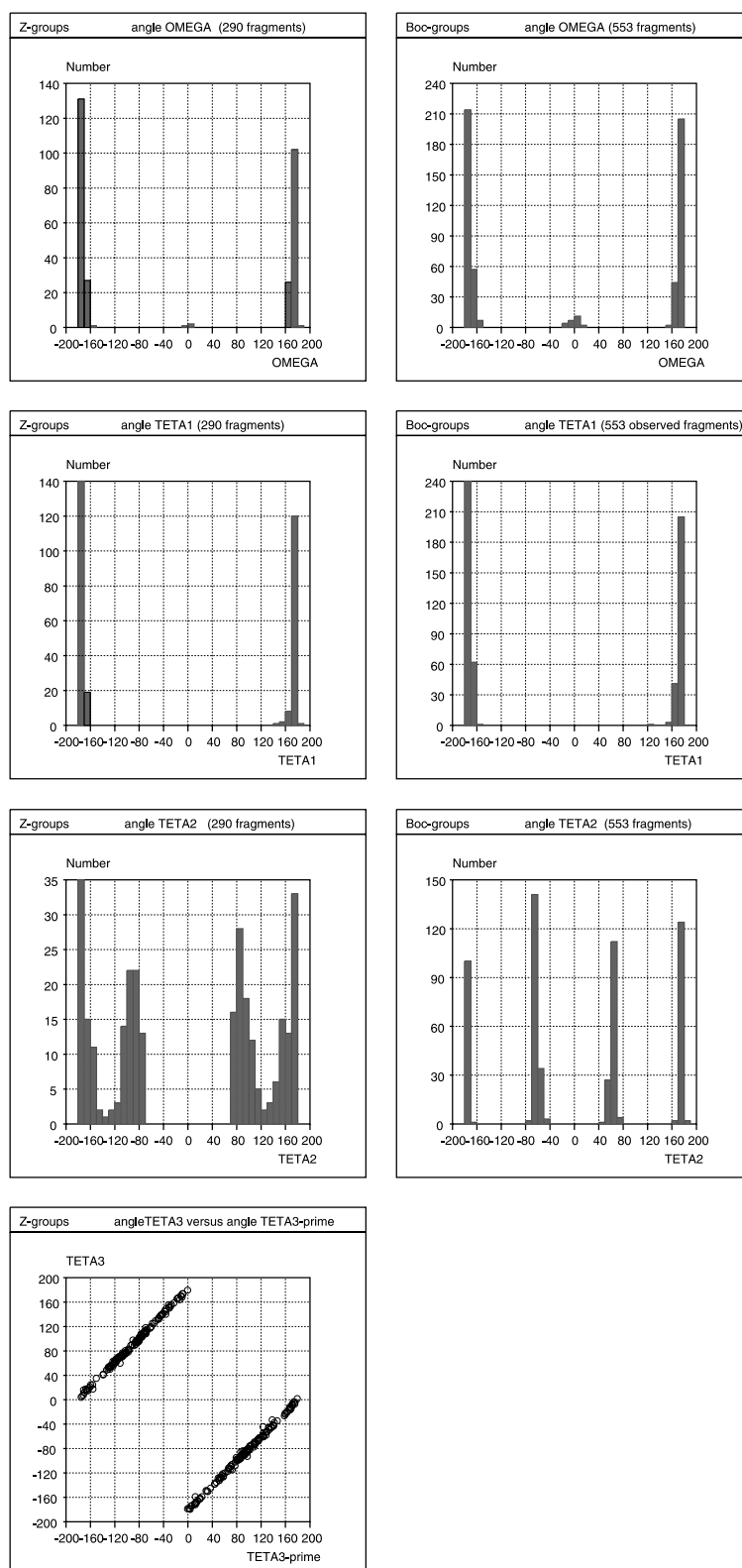


Figure 2 Distribution of torsion angle values (deg) in the Z-NH (left column) and the Boc-NH-moieties (right column). Crystal state conformations taken from the CSD [14].

derivative **5** in which $\theta^1 = 165^\circ$. Thus, the N-atom is always *trans*-oriented with respect to the hydrophobic part of the protecting group. This property constitutes a common feature with the Z-NH- and Boc-NH- systems (Figure 2).

The torsion angles θ^2 and $\theta^{3'}$, at the fixed values of the angle ω_0 and θ^1 , determine the orientation of the fluorenyl vs the urethane moiety, and take different values as is also observed for the Z-NH- and Boc-NH- moieties (Figure 2). Whereas the angles θ^2 in the Boc group have the expected regular distribution (i.e. their values are about 60° , 180° , -60°), indicating a staggered orientation of the methyl groups with respect to the C(11) atom, some restrictions are imposed upon the bigger Z and especially the bulky Fmoc group. Thus, for the crystal-state conformers of Z and Fmoc, forbidden θ^2 regions are $\pm 70^\circ$ and $\pm 115^\circ$, respectively. It seems that at smaller values, it would have come to some steric interaction between the benzyl or fluorenylmethyl and the O(11) atom. The most frequently occurring conformation of Fmoc is *t* (*trans*) with $\theta^2 = 180^\circ \pm 30^\circ$; the remaining conformations are $\pm ac$ (*antichinal*) with the values of $\theta^2 = |130^\circ \pm 15^\circ|$. In sharp contrast, only $\sim 45\%$ of Z-derivatives adopt a θ^2 of about 180° and the remainder adopts a θ^2 in the range $|90^\circ \pm 20^\circ|$. The $\theta^{3'}$ values of the Fmoc group are always rigorously restricted to one of the three regions, viz. $180^\circ \pm 20^\circ$, $60^\circ \pm 20^\circ$ (+*gauche*) and $-60^\circ \pm 20^\circ$ (-*gauche*), with uniform distribution. This finding also differs distinctly from the orientation of the phenyl ring in Z-derivatives, for which an unlimited variety of θ^3 values occur. This difference results from a much larger rotational barrier about the Csp^3-Csp^3 bond in the 9-fluorenylmethyl group than the barrier about the Csp^3-Csp^2 bond in the benzyl one [32]. These data clearly indicate a reduced conformational flexibility of the 9-fluorenylmethyl with respect to that of the benzyl group.

The Fmoc-NH- moiety with the *trans* amide arrangement adopts in seven crystals two out of three $\theta^{3'}$, $\theta^{3''}$, θ^2 angles close to 180° (Table 5; Figure 1B). In addition, the conformations $(\theta^{3'}, \theta^2) = -gt$ or *tt* are preferred. Two molecules, **5** and **13**, adopt the conformation $(\theta^{3'}, \theta^2) = t-ac$, with a rather large θ^2 angle (about -140°). Thus, in the majority of crystals, the conformations of the Fmoc group are extended and there is no steric repulsion between the fluorenyl group and the rest of the molecule. For the remaining compounds, including those with the *cis* amide arrangement, two out of the three $\theta^{3'}$, $\theta^{3''}$, θ^2 angles have \pm

gauche conformations. This property indicates that these structures are bent. By contrast, for each available θ^2 angle in the Z group, various θ^3 angles are adopted.

No relationship between the angles $(\theta^{3'}, \theta^2)$ and the set of peptide backbone torsion angles (ϕ, ψ) has been observed.

Calculated Conformers for the Model Derivative Fmoc-L-Ala-NHMe

The relative energies of the energy-minimized conformers of Fmoc-L-Ala-NHMe are collected in Table 6 and the torsion angles thereof are given in Tables 7 and 8. According to Table 2, the list of the conformers has been confined to a level of $8.5 \text{ kcal mol}^{-1}$. The minima for the *trans* Fmoc-L-Ala-NHMe (Table 7) have been found at the same angles (ϕ, ψ) within 2° as those of the *trans* MeOCO-L-Ala-NHMe (Table 2). Therefore, at the angle $\omega_0 = \sim 180^\circ$, the bulkiness of the Fmoc group does not affect the (ϕ, ψ) angles of the energy-minimized conformers of the Ala residue. These angles (ϕ, ψ) also remain constant when the torsion angles $(\theta^{3'}, \theta^2)$ are varied, except for some high-energy conformers. The torsion angles $(\theta^{3'}, \theta^2)$ remain constant too when the angles ϕ, ψ are varied. This finding indicates that there is very little interaction between the Fmoc group and the residue following it, i.e. the conformations of the Fmoc group and the amino acid residue are essentially independent of each other. The *trans* amide group is close to planarity. The departure of ω_0 from the planarity in the majority of conformers (ϕ, ψ) does not exceed 8° , but it can reach 12° – 18° for some conformers. This result is in good agreement with experimental distribution. The energies of *cis* urethane amide structures (Table 8) are higher than those of the *trans* amides (Table 7). Although many conformers fall within the range $2.8 < \Delta E < 8.3 \text{ kcal mol}^{-1}$, some unfavourable steric interaction takes place in most cases between the fluorene and either the Ala C^α -methyl group or the C-terminal methyl amide. Therefore, departures of the angles (ϕ, ψ) from the corresponding values in *cis*-MeOCO-L-Ala-NHMe (Table 2) and an influence of the conformation of the Fmoc group on the amino acid residue are observed. Moreover, the *cis* urethane amide bond, more frequently than the *trans* one, can be non-planar with a deviation which can reach 23° . These inherent features of the *cis* arrangement reasonably explain its limited occurrence in crystal structures. So far, *cis* urethane amides have only been found

Table 6 Relative Energies^a, ΔE (kcal mol⁻¹), of the Energy-minimized Conformers of Fmoc-L-Ala-NHMe

Conformation of the		Conformation of the Fmoc group (θ^3, θ^2)								
Urethane amide (ω_0)	Ala residue	$-g-g$	$+g+ac$	$+g-ac$	$-g+ac$	$t-ac$	$t+g$	tt	$+gt$	$-gt$
<i>trans</i>	C7 _{eq}	0.00	2.94	2.85	0.85	b	0.03	0.89	3.83	0.85
	C5	1.20	4.90	4.67	b	b	1.16	2.07	b	2.06
	C7 _{ax}	2.39	4.87	5.13	b	b	2.32	3.21	6.02	3.21
	β_2	2.92	4.17	5.12	b	b	2.95	3.91	b	3.91
	α_L	4.69	7.14	6.61	b	b	4.73	5.62	b	5.81
	α'	6.27	c	8.33	b	b	6.32	7.23	b	7.30
	α_D	4.21	c	b	b	6.56	6.76	8.28	b	7.77
<i>cis</i>	C7 _{eq}	3.78	4.81	5.18	b	5.81	3.22	b	4.99	4.87
	C5	2.83	5.32	5.16	5.85	b	3.65	4.45	5.73	4.13
	C7 _{ax}	7.83	c	c	b	b	7.12	c	c	b
	β_2	3.02	6.18	5.46	b	b	4.26	4.39	b	8.33 ^d
	α_L	6.43	c	c	6.94	7.61	5.69	3.37	c	5.51
	α'	7.83	c	c	b	b	c	c	b	3.76 ^d
	α_D	7.99	c	c	8.00	b	5.87	c	c	b

^a $\Delta E = E - E_0$, where $E_0 = -1058.78336982$ hartree mol⁻¹ is the energy of the global minimum. Only conformations with $\Delta E < 8.5$ kcal mol⁻¹ are shown.

^b No minima were obtained in this conformational region.

^c High-energy minimum ($\Delta E > 8.5$ kcal mol⁻¹).

^d Minimum $-g-ac$.

connected with a C ^{α,α'} -cycloalkyl glyceryl residue in compounds **4** and **6**.

In most cases optimization of the *trans* urethane Fmoc-L-Ala-NHMe geometry, when starting with either of the *gauche* conformers about the C(12)-O(12) bond ($\theta^2 = \pm 60^\circ$), gave an increase in the value of the θ^2 angle to $\pm(105^\circ-130^\circ)$. Thus, starting with the (θ^3, θ^2) values: $+g+g$, $+g-g$, $t-g$ or $-g+g$, when either of the fluorenyl benzene rings and the O(11) atom are involved in a steric interaction, leads to the optimized geometries: $+g+ac$, $+g-ac$, $t-ac$ and $-g+ac$, respectively. The increase in the θ^2 angle results in a decreased steric overcrowding, although at the cost of the torsion energy. Of nine potential conformers of the Fmoc group that were calculated, only two kept the *gauche* conformation about the C(12)-O(12) bond, $-g-g$ and $t+g$, with an optimized θ^2 angle = $\pm(73-80^\circ)$, i.e. again larger than the initial $\pm 60^\circ$. These two are the most stable conformers (θ^3, θ^2) for each energy-minimized conformer (ϕ, ψ) of the Ala residue. Their stability, despite unfavourable steric interactions, can derive from the weak intramolecular C-H...O hydrogen bonds [33] operating in

tandem: [C(9f)...O(11) and C(8f)...O(12)] in conformer $-g-g$ and [C(9f)...O(11) and C(1f)...O(12)] in conformer $t+g$ (Figure 3). However, neither of these conformers has been found in the crystal state, possibly because of the few published x-ray crystal structures. Another reason may be that the necessary stabilizing couple of intramolecular C-H...O=C hydrogen bonds cannot compete in the crystal structure with the much stronger intermolecular hydrogen bonds (Table 9).

Optimizations of the *cis* urethane Fmoc-L-Ala-NHMe geometry gives similar results, i.e. the values of the θ^2 angle are in the range $\pm(105^\circ-145^\circ)$ in most cases and two *gauche* conformers, $-g-g$ and $t+g$, with an optimized θ^2 angle = $\pm(74^\circ-82^\circ)$, are the most stable. In the case of the *cis* urethane amide arrangement combined with $\theta^2 = t$, steric repulsions take place between the fluorenyl and C-terminal amide groups of the Fmoc-L-Ala-NHMe molecule. In general, in conformers with starting values for the (θ^3, θ^2) angles = tt , $+gt$ and $-gt$, these interactions cause a decrease of the θ^2 angle which sometimes can be as low as $\approx -140^\circ$.

Figure 4 presents the 2D (θ^3, θ^2) conformational energy map for the C7_{eq} conformer of the *trans*

Table 7 Selected Torsion Angles (°) of the Energy-minimized Conformers of Fmoc-L-Ala-NHMe with the *trans* Urethane Amide Bond as Obtained Using the HF/3-21G Method

Conformation	$\theta^{3'}$	$\theta^{3''}$	θ^2	θ^1	ω_0	ϕ	ψ	ΔE (kcal mol ⁻¹)	
$\theta^{3'}, \theta^2$									
C7 _{eq}	<i>-g-g</i>	-68.4	179.3	-79.9	-179.2	-175.7	-85.4	66.7	0.00
	<i>t+g</i>	-179.9	67.8	79.0	179.4	-176.5	-85.6	67.1	0.03
	<i>-gt</i>	-70.6	177.2	177.8	-179.0	-176.1	-85.5	66.9	0.85
	<i>-g+ac</i>	-68.6	179.7	132.6	-175.3	-178.5	-85.1	67.0	0.85
	<i>tt</i>	-177.3	70.4	179.0	-179.4	-175.9	-85.5	66.9	0.89
	<i>+g-ac</i>	52.4	-61.9	-107.6	174.4	-175.6	-86.0	65.7	2.85
	<i>+g+ac</i>	56.0	-58.2	115.1	-179.1	-172.9	-86.8	65.3	2.94
	<i>+gt</i>	57.0	-56.6	-178.0	-178.9	-175.4	-85.5	66.1	3.83
C5	<i>t+g</i>	-179.2	68.3	79.4	179.6	179.3	-168.7	169.4	1.16
	<i>-g-g</i>	-68.2	179.3	-79.2	-179.6	180.0	-168.8	169.8	1.20
	<i>-gt</i>	-69.8	177.7	-177.6	-178.0	179.5	-168.6	169.6	2.06
	<i>tt</i>	-177.8	69.7	177.5	178.8	179.7	-169.2	169.7	2.07
	<i>+g-ac</i>	52.7	-61.6	-106.7	174.7	-179.4	-168.5	168.6	4.67
	<i>+g+ac</i>	62.5	-52.1	107.8	-175.5	179.5	-168.1	169.3	4.90
C7 _{ax}	<i>t+g</i>	-179.3	68.4	79.9	177.6	174.5	75.3	-56.0	2.32
	<i>-g-g</i>	-67.9	179.9	-79.5	-179.6	175.3	75.4	-56.5	2.39
	<i>-gt</i>	-70.4	177.3	-179.7	178.8	174.6	75.4	-56.3	3.21
	<i>tt</i>	-177.1	70.7	-178.7	178.6	174.9	75.3	-56.3	3.21
	<i>+g+ac</i>	61.4	52.6	107.2	-173.4	174.1	76.2	-55.1	4.87
	<i>+g-ac</i>	57.5	-56.5	-112.3	176.5	172.2	76.1	-54.7	5.13
	<i>+gt</i>	56.6	-57.1	178.9	178.3	174.2	75.2	-55.6	6.02
β_2	<i>-g-g</i>	-68.3	179.5	-79.5	-178.4	-172.2	-127.1	24.6	2.92
	<i>t+g</i>	-179.0	68.5	79.8	-180.0	-172.3	128.5	25.7	2.95
	<i>-gt</i>	-69.6	178.1	-178.7	-178.3	-172.5	-127.3	25.4	3.76
	<i>tt</i>	-177.4	70.2	177.3	179.7	-172.3	-128.3	24.9	3.91
	<i>+g+ac</i>	62.8	-51.2	106.7	-170.5	-173.0	-124.5	27.6	4.17
	<i>+g-ac</i>	51.7	-62.3	-106.1	171.5	-171.6	-129.0	22.9	5.12
α_L	<i>-g-g</i>	-68.4	179.1	-79.3	179.6	173.6	64.1	32.4	4.69
	<i>t+g</i>	-179.4	68.4	79.8	178.2	173.2	64.9	31.4	4.73
	<i>tt</i>	-177.9	69.7	178.5	178.4	173.2	70.2	23.4	5.62
	<i>-gt</i>	-70.1	177.4	-174.9	-179.5	173.2	65.3	31.2	5.81
	<i>+g-ac</i>	53.9	-59.9	-114.4	174.0	168.7	70.3	23.3	6.61
	<i>+g+ac</i>	61.2	-52.9	107.8	-173.4	173.4	65.6	31.3	7.14
α'	<i>-g-g</i>	-68.0	179.8	-79.5	-179.0	174.4	-179.9	-42.6	6.27
	<i>t+g</i>	-179.3	68.5	80.0	178.2	173.9	179.6	-43.3	6.32
	<i>tt</i>	-177.6	69.9	176.3	179.2	173.8	179.0	-42.7	7.23
	<i>-gt</i>	-70.3	177.1	-176.7	-179.4	173.9	179.5	-42.6	7.30
	<i>+g-ac</i>	50.5	-63.2	-104.7	163.6	176.0	160.7	-40.5	8.33
α_D	<i>-g-g</i>	-61.8	-172.5	-72.5	-178.1	-161.9	64.4	180.0	4.21
	<i>t-ac</i>	-171.1	76.0	-127.9	-178.6	-165.3	68.5	-177.5	6.56
	<i>t+g</i>	-179.8	67.7	79.5	178.6	-167.4	65.1	177.5	6.76
	<i>-gt</i>	-70.5	176.9	-174.2	179.3	-166.4	65.0	178.4	7.77
	<i>tt</i>	-175.7	71.4	171.0	-179.3	-167.3	65.3	178.3	8.28

Table 8 Selected Torsion Angles (°) of the Energy-minimized Conformers of Fmoc-L-Ala-NHMe with the *cis* Urethane Amide Bond as Obtained Using the HF/3-21G Method

Conformation		$\theta^{3'}$	$\theta^{3''}$	θ^2	θ^1	ω_0	ϕ	ψ	ΔE (kcal mol ⁻¹)
$\theta^{3'}, \theta^2$									
C7 _{eq}	<i>t</i> + <i>g</i>	179.9	68.2	76.6	-177.8	1.8	-91.4	66.8	3.22
	- <i>g</i> - <i>g</i>	-66.4	-177.3	-74.8	-165.1	13.7	-96.1	69.7	3.78
	+ <i>g</i> + <i>ac</i>	61.0	-52.5	105.5	-164.9	10.5	-96.3	64.5	4.81
	- <i>gt</i>	-72.9	174.9	175.7	-177.3	3.9	-91.9	63.9	4.87
		-68.8	179.5	-150.1	-168.5	10.5	-95.6	55.8	4.13
	+ <i>gt</i>	58.8	-54.9	162.1	-171.9	5.5	-94.5	68.8	4.99
	+ <i>g</i> - <i>ac</i>	51.4	-65.4	-113.1	-170.0	7.7	-98.7	64.5	5.18
	<i>t</i> - <i>ac</i>	-174.6	73.4	-123.8	-173.3	12.3	-95.5	71.4	5.81
C5	- <i>g</i> - <i>g</i>	-67.9	-179.8	-78.4	-177.1	3.3	-166.9	167.8	2.83
	+ <i>t</i> + <i>g</i>	179.6	68.3	76.6	169.1	-7.1	-160.6	168.0	3.65
	<i>tt</i>	-174.6	72.6	178.8	-178.4	1.8	-167.4	168.2	4.45
	+ <i>g</i> - <i>ac</i>	52.6	-61.6	-108.3	171.6	-4.5	-161.7	167.4	5.16
	+ <i>g</i> + <i>ac</i>	64.7	-50.8	109.9	-179.8	1.5	-164.6	167.7	5.32
	+ <i>gt</i>	57.0	-56.9	-171.9	178.3	-0.3	-163.5	167.1	5.73
	- <i>g</i> + <i>ac</i>	-76.1	171.6	141.5	170.4	-6.9	-158.9	167.6	5.85
	C7 _{ax}	+ <i>t</i> + <i>g</i>	177.5	66.4	73.9	168.4	-18.8	84.9	-55.1
- <i>g</i> - <i>g</i>		-70.9	177.8	-76.2	-174.9	0.3	77.5	-55.6	7.83
- <i>g</i> - <i>ac</i>		-74.9	172.8	-143.1	176.0	-13.2	82.0	-39.1	8.33
β_2	- <i>g</i> - <i>g</i>	-69.5	178.1	-81.1	180.0	7.8	-108.0	12.4	3.02
	+ <i>t</i> + <i>g</i>	-177.9	70.0	80.5	175.5	7.0	-127.2	23.4	4.26
	<i>tt</i>	-175.1	72.5	-166.4	-178.3	12.0	-111.0	16.9	4.39
	+ <i>g</i> - <i>ac</i>	51.1	-62.6	-106.3	169.2	4.6	-137.8	36.1	5.46
	- <i>gt</i>	-72.3	175.5	178.8	177.2	8.3	-134.7	25.7	5.51
	+ <i>g</i> + <i>ac</i>	61.6	-52.4	108.7	-173.2	13.5	-122.7	20.6	6.18
α_L	<i>tt</i>	-174.4	74.6	153.2	179.4	-10.6	62.7	37.9	3.37
	- <i>g</i> - <i>ac</i>	-79.1	168.4	-141.3	-178.9	-10.0	62.4	37.4	3.76
	+ <i>t</i> + <i>g</i>	-172.8	74.7	82.0	172.0	-12.2	73.5	27.9	5.69
	- <i>g</i> - <i>g</i>	-69.9	178.8	-76.5	-166.5	1.4	57.5	35.9	6.43
	- <i>g</i> + <i>ac</i>	-75.5	172.7	141.4	170.0	-17.0	69.4	39.2	6.94
	<i>t</i> - <i>ac</i>	-172.2	76.0	-141.5	-166.2	0.4	56.0	38.2	7.61
α'	- <i>g</i> - <i>g</i>	-69.3	178.6	-79.8	-177.9	-1.8	-169.6	-47.9	7.83
α_D	+ <i>t</i> + <i>g</i>	-175.4	73.3	79.4	168.3	8.9	64.5	179.6	5.87
	- <i>g</i> - <i>g</i>	-67.8	-179.8	-82.4	-163.0	23.0	52.5	177.8	7.99
	- <i>g</i> + <i>ac</i>	-80.4	167.0	147.0	168.1	5.7	64.2	-174.5	8.00

urethane Fmoc-L-Ala-NHMe molecule as the most stable structure. The majority of the area with the θ^2 values in the range $\sim -90^\circ$ to $\sim 90^\circ$ is a high-energy area with overcrowded conformers. In this range, however, there are the two lowest energy minima (-*g* - *g*), (*t* + *g*) with the intramolecular hydrogen bonds (Figure 3). For larger $|\theta^2|$ angles no steric interaction takes place between the fluorene group and the O(11) atom. The majority of the area is of low energy and local minima appear at

$\theta^{3'} = \sim \pm 60^\circ$ and $\sim 180^\circ$, i.e. in an expected, staggered orientation for the C(9f)-C(12) bond. As can be seen from the experimental points in Figure 4, the crystal conformers of *trans* urethane Fmoc compounds (**1-3**, **5**, **7-13**) are localized in the vicinity of these minima. The same holds true for the crystal conformers of the *cis* compounds **4** and **6**. Thus, the map in Figure 4 confirms the results of the calculations discussed above for the fully relaxed conformational minima (Tables 7 and 8)

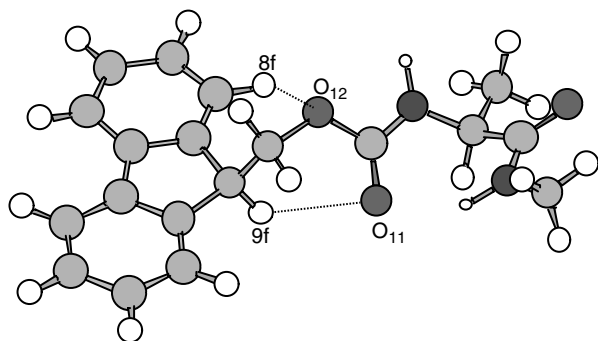
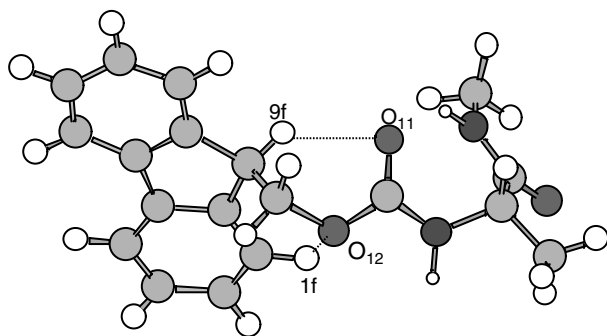
conformer $-g-g$ conformer $t+g$ 

Figure 3 The preferred conformers ($\theta^{3'}$, θ^2) of *trans* Fmoc-L-Ala-NHMe. Intramolecular contacts are marked for $C \cdots O$ distances < 3.2 Å and $H \cdots O$ distances < 2.64 Å.

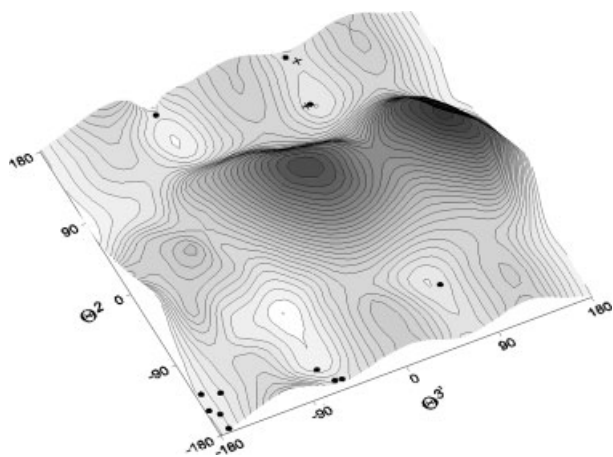


Figure 4 Conformational energy contour map as a function of the θ^2 and $\theta^{3'}$ torsion angles for the *trans* C_7 conformational of Fmoc-L-Ala-NHMe, with fixed torsion angles $(\phi, \psi) = (-88.5^\circ, 66.5^\circ)$ as obtained using the HF/3-21G method. The contours are drawn with 1 kcal mol⁻¹ increments from the energy minimum of $(\theta^{3'}, \theta^2) = (-68^\circ, -80^\circ)$. Symbols (● — *trans* and + — *cis*) denote experimental structures.

Table 9 Inter- and Intramolecular Hydrogen Bonds Involving the Urethane Group in Crystals of the Fmoc Derivatives

	Donor: urethane N-H	Acceptor of N-H	Acceptor: urethane C=O	Donor to C=O
1	Yes	C=O _{urethane}	Yes	NH _{urethane}
2	Yes	COOH	Yes	HOH
3	Yes	C=O _{urethane}	Yes	NH _{urethane}
4 ^a	Yes	COOH	Yes	COOH
5	No	—	Yes	COOH
6 ^a	Yes	CH ₃ OH	Yes	NH _{amide}
7	Yes	C=O _{amide}	Yes ^b	NH _{amide}
8	Yes	COOH	Yes	HOH
9	Yes	HOH	No	—
10	Yes	C ₂ H ₅ OH	Yes ^b	NH _{amide}
11	Yes	N-O	Yes ^b	NH _{amide}
12	Yes	C-O-C	No	—

^a *cis* Urethane amide.

^b Intramolecular hydrogen bond.

and rationalizes the experimental observations (Table 5).

Crystal Structures

The mode of packing of Fmoc-derivatives **1–13** was examined in order to determine the possible hydrogen bonding preferences of the urethane group. Table 9 shows the N-H \cdots O and O \cdots H-O(N) hydrogen bonds involving the urethane N-H and C=O groups. All but one N-H and all but two C=O groups in the crystals **1–12** are engaged in such interactions. Because of the miscellaneous chemical composition of the Fmoc derivatives and their crystals as well, a variety of hydrogen bonding donors/acceptors involving the urethane group has been found. Chain-forming N-H \cdots O=C hydrogen bonds between urethane moieties are observed in two structures (**1**, **3**). As a rule, the N-H groups forms N-H \cdots O hydrogen bonds with an O atom originating from either the carbonyl or the carboxyl, ether and hydroxyl groups (the latter two from solvents of crystallization). The carbonyl O atom is involved in an O \cdots H-N or an O \cdots H-O hydrogen bond with, respectively, an amide or an hydroxyl group (the latter from either a water molecule or a carboxyl group) or in an O \cdots H-C hydrogen bond.

Of the six analysed peptides **6–11**, three (**7**, **10** and **11**) have the potential to produce a β -turn structure with an intramolecular

N-H_{amide}...O=C_{urethane} hydrogen bond, as described by the graph set **S**(10) [34]. Indeed, in the crystals of these compounds, β -turns are observed. This finding is similar to those for crystals of the compounds with the Z/Boc protection and with a β -turn structuring potential. The heterene oligoamide **13** was designed to self-organize into a single-stranded helical structure upon forming intramolecular hydrogen bonds. Its amide -CONH- and the urethane C=O groups of the two Fmoc protections interact with the solvent molecules forming intermolecular hydrogen bonds which, however, do not interfere with the planned hydrogen bonding system. The C=O groups also give intramolecular C-H...O hydrogen bonds, which stabilize the helix.

Crystals **1–13** commonly show hydrophobic interactions between the fluorene systems. Mostly populated are C-H... π contacts [35] in which the interplanar fluorene-fluorene angle is in the wide range of 40°–90°. However, this bulky group does not create any preferred packing pattern. It seems that a crystal net would require solvent molecule(s) of crystallization (Table 1) as a glue connecting the molecules of the Fmoc compounds. This is probably the underlying cause of the significant difficulties experienced in obtaining crystals of Fmoc derivatives, which, in turn, are responsible for the limited number of the structures available for this elaboration. A small amount of the structural data can be also found for the crystals of compounds containing the Fmoc-N = [36–39] and FmO-C(O-) = N- [40] moieties.

CONCLUSIONS

Based on the crystal structural data of compounds listed in Table 1 and *ab initio* calculations, the geometry and conformations (i.e. the torsion angles ω_0 , θ^1 , θ^2 and θ^3) of the Fmoc-NH- moiety were described and compared with those of the Z-NH- and Boc-NH- moieties.

The geometry of the Fmoc groups is rigid, with the fluorenyl ring unchanged with respect to the unsubstituted fluorene molecule, and with the remaining bond lengths and bond angles (Table 3) similar to the corresponding ones in the Z, Boc and ester groups (Table 4). The Fmoc-NH- urethane amide bond occurs in both the *trans* ($\omega_0 \sim 180^\circ$) and the *cis* ($\omega_0 \sim 0^\circ$) arrangement, the former being largely preferred (Table 5). This property is similar to the Boc-NH- urethane amide distribution, while the Z-NH- moiety does not show the tendency to

form a *cis* urethane amide bond (Figure 2). The θ^1 angle is in the range $180^\circ \pm 15^\circ$, which is a common conformation for the Z-NH- and Boc-NH- systems [12–14]. The θ^2 and θ^3 angles in the Fmoc, Z and Boc groups largely differ. In the Fmoc group the θ^2 angle has values of $180^\circ \pm 30^\circ$ in most cases and it is not smaller than $|115^\circ|$. The θ^3 angle falls only in the regions restricted to $180^\circ \pm 20^\circ$, $60^\circ \pm 20^\circ$ and $-60^\circ \pm 20^\circ$. However, less than a half of the Z groups adopts a θ^2 angle of $\sim 180^\circ$, the remainder having $\theta^2 = |90^\circ \pm 20^\circ|$. For this type of protection a variety of values of θ^3 can occur (Figure 2). The Boc groups show a uniform set of θ^2 angle distribution (around $\pm 60^\circ$ and 180°). In most instances the angles (θ^3 , $\theta^{3'}$, θ^2) describing the orientation of the fluorenyl vs the urethane moiety indicate an extended conformation (Table 5). The Fmoc group, compared with the Z and Boc protections, seems to be more rigid. *Ab initio* calculations confirm that the observed conformational features of the Fmoc-NH- moiety are its inherent properties (Tables 2, 6–8 and Figure 4).

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