Prospects in computational molecular medicine: a millennial mega-project on peptide folding

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Abstract

During the second half of the 20th century, *Molecular Computations* have reached to a level that can revolutionize chemistry. The next target will be structural biology, which will be followed soon by *Molecular Medicine*. The present paper outlines where we are at, in this field, at the end of the 20th century, and in what direction the development may take in the new millennium. In view of the gigantic nature of the problem, it is suggested that a suitably designed cooperative *Millennial Mega-project* might accelerate our schedule. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Biomolecular computations; Peptide models; Peptide folding; Molecular medicine

1. Preamble

Every disease starts at the molecular level. Thus, ultimately every cure has to be achieved at the molecular level. This recognition leads us inevitably to the direction of Molecular Medicine.[1]

By the end of the 20th century, a powerful network of new disciplines has been developed which can in fact support molecular medicine. This interdisciplinary approach emerges as a new *mega discipline*. Its translation to practice, i.e. to the drug discovery process, involves at least five major fields, as illustrated in Scheme 1.

Genomics (human genome project).
Structural biochemistry and biology.
Biomolecular computation.
Solid phase supported combinatorial chemistry.
High throughput screening.

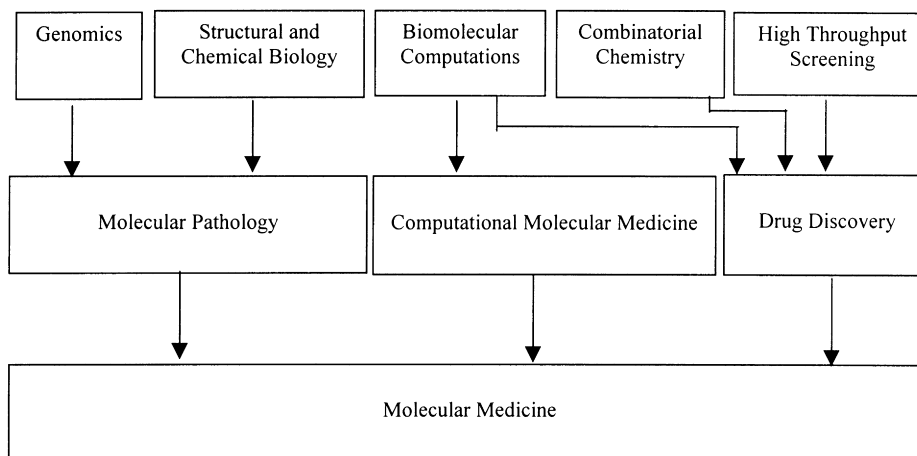Based on the soon-to-be completed human genome project, a massive number of protein sequences are to

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[1] Gene Therapy, which is also part of Molecular Medicine, will not be discussed in this paper.

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│ Genomics │ │ Structural and│ │ Biomolecular │ │ Combinatorial│ │High Throughput│
│          │ │Chemical Biology│ │ Computations │ │  Chemistry  │ │  Screening   │
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│    Molecular Pathology      │ │Computational Molecular Medicine│ │ Drug Discovery │
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│                            Molecular Medicine                                  │
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Scheme 1.

be released; however, the biological functions for some of these proteins are yet to be identified. Using easily available biotechnological methods, a highly diverse protein collection will soon be available for structural studies.

X-ray diffraction and NMR spectroscopy, the two determinative arms of the structural *proteomics,* are developing rapidly for high throughput data collection. For example, synchrotron sources for X-ray studies are currently in use. Furthermore, multi-magnet NMR facilities will become functional in the near future. These NMR installations which contain 10–20 magnets, each in the vicinity of 1 GHz magnitude, are equipped with semi-automatic co-ordinate determination. This development will lead to a high-throughput NMR set-up. Such a complex facility would be able to handle the booming proteomics-depot.

The re-organized Protein Data Bank, supported by all other data banks derived from the high throughput screening, is serving a reliable platform to establish one of the biggest challenges in molecular medicine, the interface between chemistry and biology.

Since the late 1980s, a very important paradigm shift has taken place. There was a move from individually synthesized (and tested) compounds to combinatorially synthesized (and tested) mixtures. These collections of molecules are called *libraries* by the new terminology. This approach has been initiated in peptide chemistry due to its simplicity and the availability of automated

procedures developed on solid phase. The success achieved with peptides [1–5] immediately triggered the development of solid phase organic chemistry [6]. Finally this led to small, pharmacologically relevant compound libraries.

Using robotics, the problem of screening of the emerging number of libraries, containing synthetic compounds, as well as natural products, is now being solved. It has to be emphasized that for successful handling of the above mentioned multi-source data collections, up-to-date data processing software should be used.

In spite of our outstanding and ever developing technologies, as outlined above, we do have conceptual shortcomings. The paramount obstacle to sophisticated drug discovery is our ignorance of the law of protein folding [7,8]. If we could pronounce the law by which proteins fold, we would be able to proceed with unprecedented speed and efficiency. However, this is precisely the basic knowledge that is missing, and we may not find the answer to this burning question for centuries, unless we change our method of investigation.

Until this point, in scientific practice, scientists have always started with folded proteins, of which there are literally billion types in the human body, and tried to find the rules that govern their folding patterns. Although some success has been made, the problem is far from being solved. For example, we cannot even understand what forces determine the

way a short polypeptide, containing for instance 10 amino acids, is folded. How can we then expect to understand why a long polypeptide chain, containing, for instance, 1000 amino acids, is folded in the way it is? Clearly, in order to gain a deeper understanding, we need to launch a project in deciphering the rules of *peptide folding*, yet even this smaller problem will prove to be a *mega-project*.

2. Computational background

Today, most peptide and protein chemists are using one of the varieties of empirical force fields to study peptide and protein conformations. The results of such methods are essentially educated guesses of the potential energy hyper-surface (PEHS) that describes the energetics of folding. The validity of such empirical methods, of course, is dependent on the accuracy of parameterization. The advantages of such computational methods are that they are fast while their disadvantage is that they are not completely reliable. Consequently, there is a calculated risk in their usage, as they could produce misleading results in certain cases but one would not know when this would occur. The method is about a quarter of a century old and no one has had neither the commitment nor the money to polish up the parameterization to the level that is desirable for scientific precision.

In contrast to the above, serious efforts have been made by thousands of university researchers during the past half a century to develop non-empirical or ab initio ("from the beginning") methods to compute PEHS from first principles (i.e. from quantum mechanics). The advantage of these methods is that they are reliable while their disadvantage is that they are computationally intensive. In the 1960s up to four or six atoms could be handled at the ab initio level of theory (for example, molecules such as HCOF [9–11] or even the isoelectronic $HCONH_2$ [12], the latter structure containing one peptide bond). However, all calculations were performed on a fixed structure, without geometry optimization. Nevertheless, by the turn of the millennium, the largest peptide on which ab initio calculations have been carried contains 12 amino acids [13].

The present paper aims to propose a *mega-project*

which will rely on the use of this second method, carrying out computations on relatively small peptides at the reliable ab initio level of theory.

Both methods yield gas phase results, which implies that the intrinsic properties of the molecules are computed in the absence of environmental interactions. Thus, in order to gain an understanding of the role that solvents may play in peptide and protein folding, the effect of water needs to be subsequently simulated.

The reason ab initio methods are being suggested, as the method of investigation, is twofold:

1. the result would be reliable for a considerable length of time;
2. the results would provide a primary standard for future force-field parameterization.

In spite of these good intentions, one may wonder if it is prudent to set our standards so high. Software and hardware development during the past half-century suggest that our computational development in the next half-century will supercede all imagination. Consequently, it seems not only desirable but also practical as well to aim for the more reliable method, even if it is computationally intensive.

Fig. 1 shows the century of development, from 1950 to 2050. In 1950, Boys suggested the use of Gaussian-type orbitals and Roothaan published his SCF procedure in 1957. However, in the 1950s and early 1960s, all digital computers were vacuum-tube based. The first GAUSSIAN calculation on an organic molecule (HCOF), which in fact is isoelectronic to formamide ($HCONH_2$), was carried out in 1963 on an IBM 709 computer that was still using vacuum tubes. The leading software was POLYATOM at that time. The first transistorized mainframe computer (such as the IBM 7090 and 7094) arrived a bit later on the scene.

The vertical bars in Fig. 1 show the hardware development, after transistorization, in units of FLOPS (Floating point Operations Per Second). Clearly there was a dramatic change from mega (10^6) FLOPS through giga (10^9) FLOPS, all the way to the current tera (10^{12}) FLOPS. However, it has been suggested that future computers can solve problems in 30 s—what today's 10^{12}-FLOPS supercomputers would take 10 billion years to solve. The ratio (10^{10} years: 30 s) is of the order of 10^{16}. Thus, we
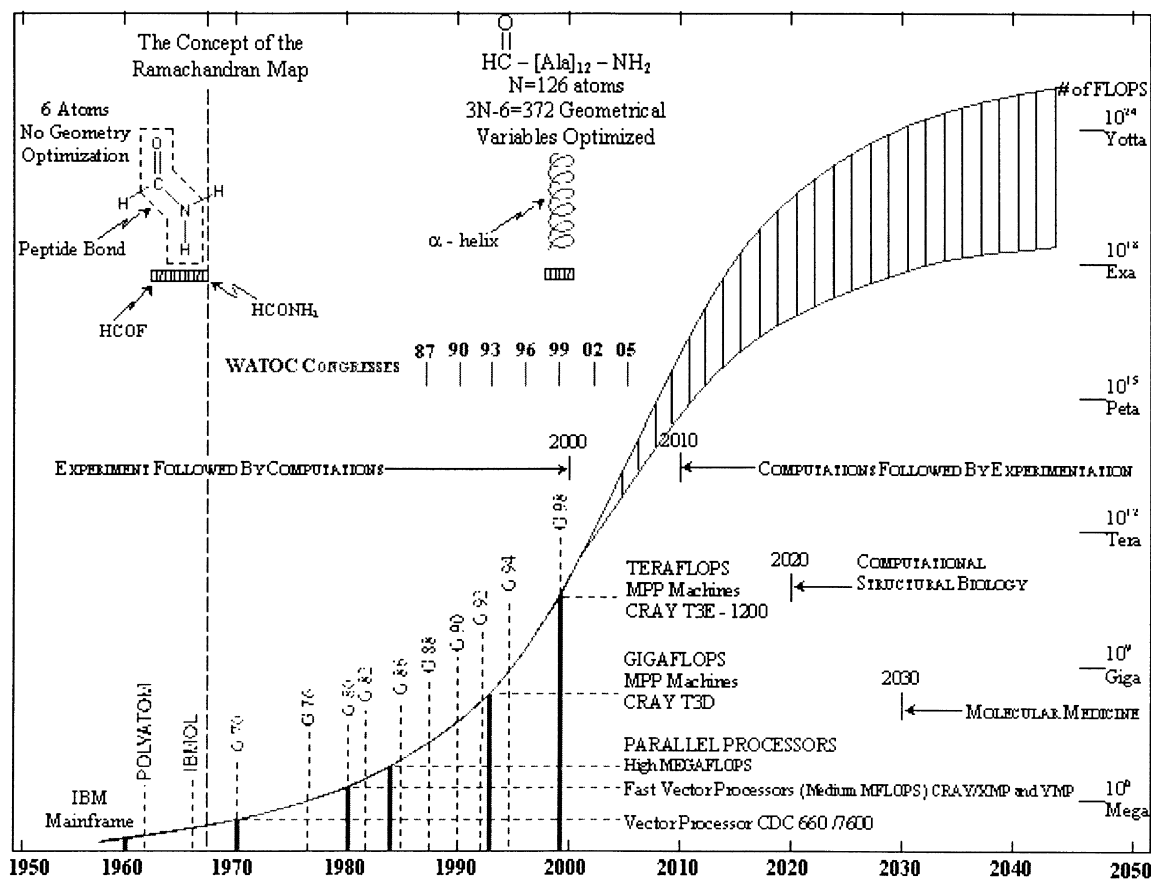
Fig. 1. Development of computer hardware and software as the basis of biomolecular computations. Note that after this figure has been completed (July 1999), IBM announced the creation of the "Blue Gene" ultra supercomputer with one petaflops capability, to be ready by 2005. This makes the optimistic (upper) growth curve a conservative estimate of the future development.

may consider the limit of the growth-curve to be $10^{12} \times 10^{16} = 10^{28}$ FLOPS. Fig. 1 shows two curves, the lower of which is the pessimistic prediction and the upper of which is the optimistic one. Note that even the optimistic curve levels off at about 10^{25} FLOPS, which is about 1000 times more conservative than the prediction currently lingering around (10^{28} FLOPS).

This dramatic hardware development is paralleled by a heroic effort in software development. Such development is not quantified by values of benchmark computations; they are simply presented at their time of appearance. The most durable package is GAUSSIAN [14], which has had several editions from 1970 to 1998. However, other softwares (e.g.

SPARTAN, JAGUAR, etc.), not shown in Fig. 1, are now challenging the GAUSSIAN's dominance. The dates of the WATOC congresses (from 1987 to 2005) where molecular computational chemists and biologists are reporting their progress are also indicated. Finally, the dates of the first and therefore the smallest [HCONH$_2$] [12] and last and therefore largest [HCO–(Ala)$_{12}$–NH$_2$] [13] ab initio peptide calculations of the 20th century are also marked.

3. Peptide conformational background

A polypeptide chain on its own, or as part of a protein molecule, consists of a string of amino acid

residues as shown below in **I**:

I
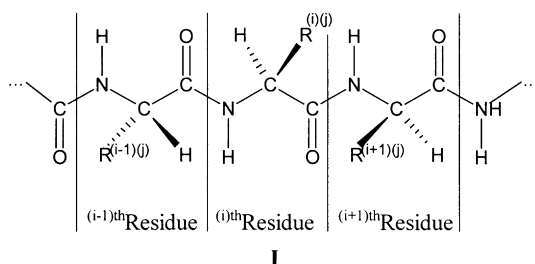
where $R^{(i)}$ specifies the side chain of the ith amino acid residue.

The second index, j, specifies that one of the 20 different side-chains of the 20 naturally occurring amino acid residues[2] [15] may be involved ($1 \leq j \leq 20$). Mono-peptides (**II**), dipeptides (**III**) and tripeptides (**IV**) contain 1, 2 and 3 amino acid residues, respectively. In such models, the chain may be terminated by methyl groups or by hydrogens, as shown below for the above three cases ($P = Q = CH_3$ or H).
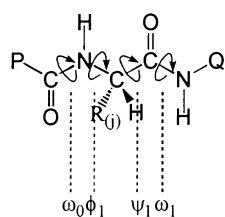
II   **III**
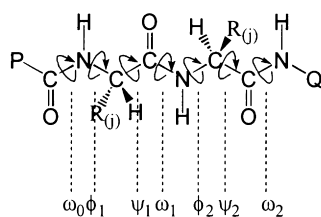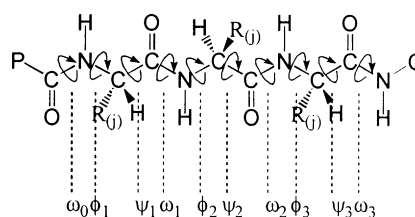
Again, $R^{(j)}$ represents the side-chain of one of the 20 naturally occurring amino acids ($1 \leq j \leq 20$) given in Table 1.

The torsional angles (ω_0, ϕ_1, ψ_1, ω_1,...) are responsible for folding. The energy of folding is a multi-variable function where the torsional angles are the independent variables. The PEHS for the mono(1°)-, di(2°)- and tri(3°)-peptides, given below, are functions containing 4, 7 and 10 independent variables, respectively.

$$E(1°) = E[\omega_0, \omega_1, \phi_1, \psi_1] \tag{1}$$

$$E(2°) = E[\omega_0, \omega_1, \omega_2, \phi_1, \psi_1, \phi_2, \psi_2] \tag{2}$$

[2] The 20 naturally occurring amino acids have both DNA and RNA codons. Selenocysteine nowadays are called the 21st amino acid because it has an RNA codon (UCA stop codon) [15].

$$E(3°) = E[\omega_0, \omega_1, \omega_2, \omega_3, \phi_1, \psi_1, \phi_2, \psi_2, \phi_3, \psi_3] \tag{3}$$

Clearly, for a degree of polymerization of n amino acids, there are n torsional angle pairs of ϕ_i and ψ_i ($1 \leq i \leq n$), two terminal peptide functionalities (ω_0 and ω_n) and ($n - 1$) mid-chain peptide bonds [ω_i for $1 \leq i \leq (n - 1)$]. Thus, the total number of folding variables for a polypeptide containing n amino acids is

$$N = [(n - 1) + 2]\omega + n\phi + n\psi = 3n + 1 \tag{4}$$

The *trans*-peptide bond is more stable than the *cis*-peptide bond. Consequently, it has been traditional to set $\omega_i = 180°$ for all i. This limitation reduces the dimensionality of the problem substantially

$$E(1°) = E_{\text{trans}}[\phi_1, \psi_1] \tag{5}$$

$$E(2°) = E_{\text{trans}}[\phi_1, \psi_1, \phi_2, \psi_2] \tag{6}$$

$$E(3°) = E_{\text{trans}}[\phi_1, \psi_1, \phi_2, \psi_2, \phi_3, \psi_3] \tag{7}$$

IV

The total number of folding variables, after the reduction of the dimensionality, becomes

$$N = n\phi + n\psi = 2n \tag{8}$$

This reduction in dimensionality does not represent a denial of importance of the *cis*-configuration of any given peptide bond; it only means that we are partitioning the problem. First, we study the backbone conformations for *trans*-peptide bonds as this represents the primary problem and subsequently we may study the same problem for any peptide bond being in the *cis*-conformation.

Most of the study carried out so far, has been centered on the generation and analysis of the $E(1°)$ potential energy surface (PES). The contour diagram of this type of PES is frequently referred to as the "Ramachandran Map", in honour of the Indian

Table 1
Structures of amino acid residues. Note that in addition to the 20 naturally occurring amino acids, which have both DNA and RNA codons, the 21st amino acid, Selenocysteine (Sec), which has only an RNA codon, is also included
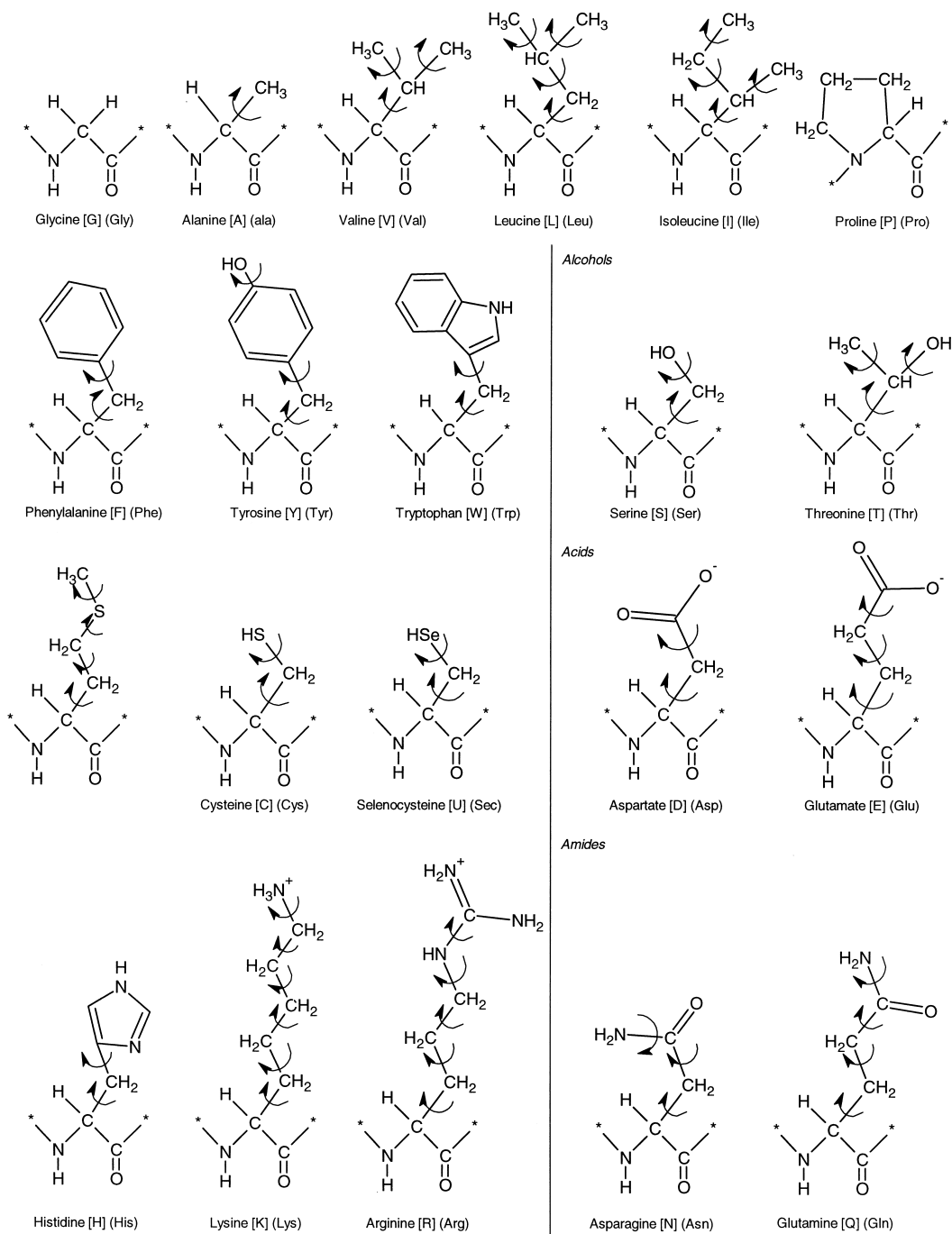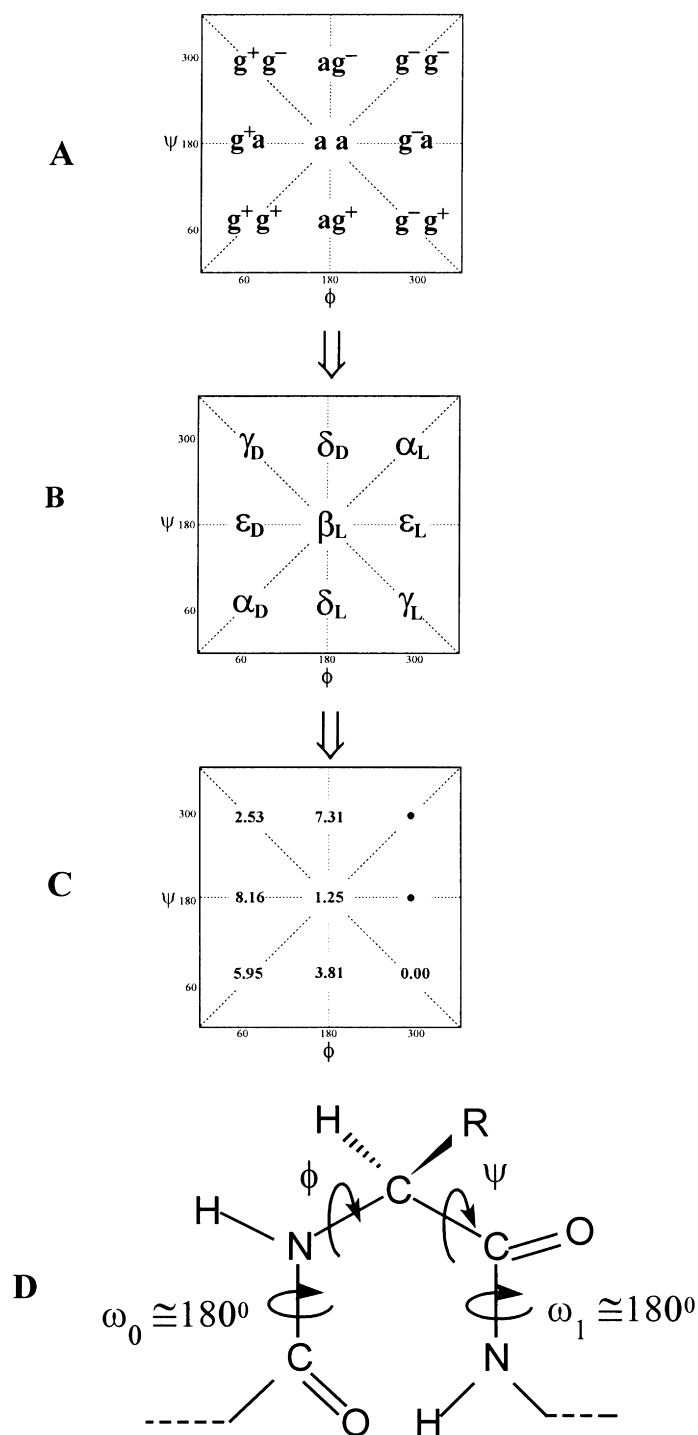
Fig. 2. Topology of a 2D-Ramachandran map. (A) conformational assignments (B) names of conformers (C) relative energies (kcal/mol) of *N*-formyl-L-alanineamide computed at HF/3-21G level of theory (D) structure of a general amino acid residue with the relevant torsional angles.
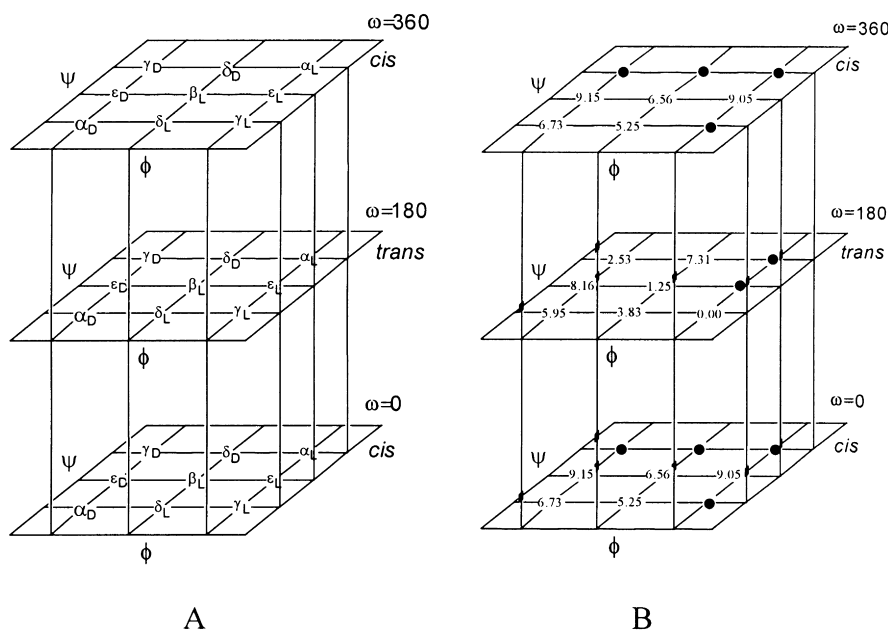
Fig. 3. Topology of a 3D-Ramachandran PEHS. (A) name of conformers (B) relative energies of $HCO-(\omega)-NH-(\phi)-CHMe-(\psi)-CONH_2$.

chemist, Professor Ramachandran, who first called attention to the importance of such a PES.

A topological representation of the Ramachandran map for $R^j = CH_3$ (i.e. for the alanine residue) is shown in Fig. 2. The various minima of the PES, representing the stable conformers, are marked by subscripted Greek letters. Although not all 20 amino acids were subjected to ab initio computational conformational analysis, several amino acids were investigated, and it was recognized that side-chain and backbone conformation can be studied independently, at least in the first approximation.

The relative energies of *N*-formyl alaninamide with *trans*-peptide bond are given in Fig. 2C in the form of a PES topology. The corresponding conformational energy hyper-surface (PEHS) topology, involving both the *trans*- as well as the *cis*-isomers is depicted in Fig. 3.

As yet, these residues have been considered in the absence of conformationally variable side-chains. However, side-chains make contributions to the total energy and they are also involved in backbone/side-chain as well as side-chain/side-chain interactions, thus they ultimately help to determine protein folding. To include these further degrees of freedom in the analytic evaluations requires the extension of the above equations. Labeling of the torsion angle variables in the side-chain is accomplished using χ_1, χ_2, χ_3,... and so on, beginning at the C_α. As the side-chain is the only differentiating structural element between amino acids, each analytic equation also becomes unique. The equations then become:

$$E(1^\circ) = E_{\text{trans}}[\phi_1, \psi_1, (\chi_1^1, \chi_2^1, ..., \chi_k^1)] \quad (9)$$

$$E(2^\circ) = E_{\text{trans}}[\phi_1, \psi_1, (\chi_1^1, \chi_2^1, ..., \chi_k^1), \phi_2, \psi_2,$$
$$(\chi_1^2, \chi_2^2, ..., \chi_k^2)] \quad (10)$$

$$E(3^\circ) = E_{\text{trans}}[\phi_1, \psi_1, (\chi_1^1, \chi_2^1, ..., \chi_k^1), \phi_2, \psi_2,$$
$$(\chi_1^2, \chi_2^2, ..., \chi_k^2), \phi_3, \psi_3(\chi_1^3, \chi_2^3, ..., \chi_k^3)] \quad (11)$$

where $(\chi_1, \chi_2, ..., \chi_k)$ is specific to each amino acid and its side-chain, with the superscript denoting which residue that side-chain belongs to in the poly-peptide chain.

Extending this concept to a generalized form, we obtain the following multi-variable function:

$$E(n^\circ) = E_{\text{trans}}[\phi_1, \psi_1, (\chi_1^1, \chi_2^1, ..., \chi_k^1), ..., \phi_n, \psi_n,$$
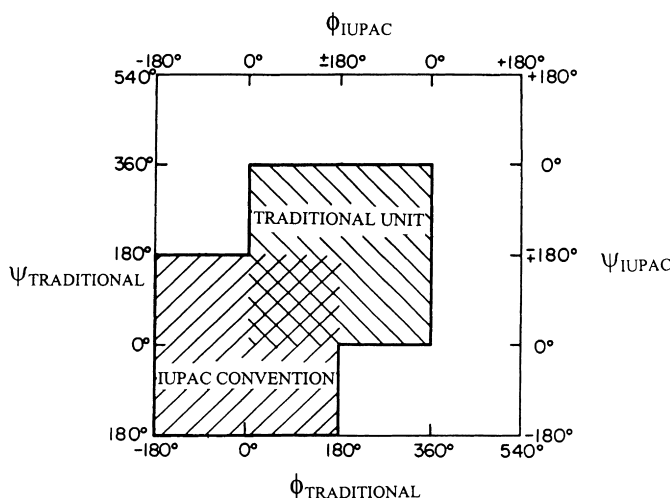$$(\chi_1^n, \chi_2^n, ..., \chi_k^n)] \quad (12)$$

Fig. 4. Two kinds of partition of the PES. The central square corresponds to the traditional cut ($0° \to 180° \to 360°$), while the lower left hand square represents the IUPAC conventional cut ($-180° \to 0° \to +180°$).

4. Conformational study of single amino acid peptide models

There are two ways to represent the rotation about a single bond. Traditionally, the $0° \to 180° \to 360°$ range is used but recently, IUPAC recommended the convention of $-180° \to 0 \to +180°$. This convention has the advantage of designating the $0° \to +180°$ segment as a clockwise rotation and the $0° \to -180°$ segment as a counter clockwise rotation. However, it has the disadvantage that certain minima fall on the edges or the corners of the Ramachandran map. The two representations are presented in Fig. 4.

Peptide models, such as $CH_3CONH-CHR-CONHCH_3$ or simply $HCONH-CHR-CONH_2$ can mimic the ith amino acid residue in a protein chain. The ϕ and ψ torsional angles are defined in Fig. 2D. The conformational assignments (g^+g^+, g^+a, ...etc.) are shown in Fig. 2A.

The names of the minima (Fig. 2B) are subscripted Greek letters. The Greek letters originate from earlier nomenclature (involving α, β and γ) while the L and D subscripts originate from the observation that L-amino acids favor L conformations while D-amino acids favor D conformations (c.f. lower part of Fig. 5). The names also suggest the combination of the chirality of a constitutional structure (R or S configuration) and the chirality of the conformational twist or folding. This is summarized in Fig. 6.

The top of Fig. 7 shows the symbolic representation of a conformational PES for two full cycles of rotation ($-360° \to 0° \to +360°$) of both ϕ and ψ. The PES can be partitioned into four quadrants, in the traditional way, or it can be partitioned according to IUPAC convention, as shown by the broken lines.
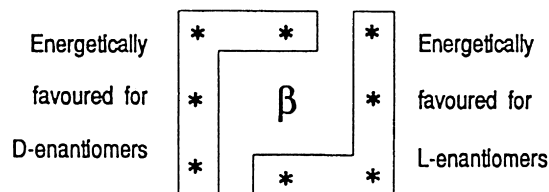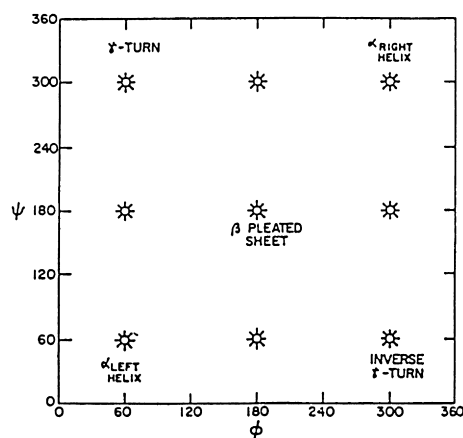

Fig. 5. Underlying principles for choosing subscripted Greek letter (e.g. α_L, α_D, β_L...etc.) as names for the peptide conformations.
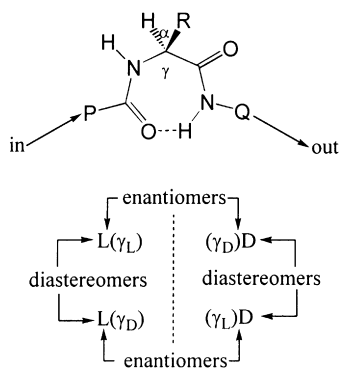
Fig. 6. Stereochemical relationships of γ-turns. Note, that not only the α carbon has chirality, but there is also chirality in the twisting of the backbone. The combination of these two types of chiralities leads to enantiomeric and diastereoisomeric structures. D and L denote the chirality of the C$^\alpha$ configuration, while γ_L and γ_D denote the chirality of the conformation.

An energy contour diagram of the conformational PES of a peptide (PCONH–CHR–CONHQ), presenting two full cycles of rotation ($-360° \rightarrow 0° \rightarrow 360°$) of both ϕ and ψ, is shown at the bottom of Fig. 7. The central square is the IUPAC conventional cut, while the four quadrants are the traditional cuts. One of these traditional cuts (e.g. the upper right hand quadrant) is shown in pseudo-3D-representation in Fig. 8.

There are 20 naturally occurring amino acids. A total of 18 of them have the same type of backbone folding as shown in Fig. 2 (i.e. nine discrete conformations). The two other amino acids are exceptions.

One exception is proline, which is built into proteins like any other amino acid, but its N is locked in a five-membered ring. For proline residue V, ϕ can only be in the vicinity of $-60°$ (i.e. $+300°$) and, therefore, only three backbone conformations are possible: α_L, ϵ_L and γ_L. The other unique amino acid is glycine **VI**, which is achiral.

In the case of the glycine residue, double degeneracy occurs in its conformational PES as shown in Fig. 9.
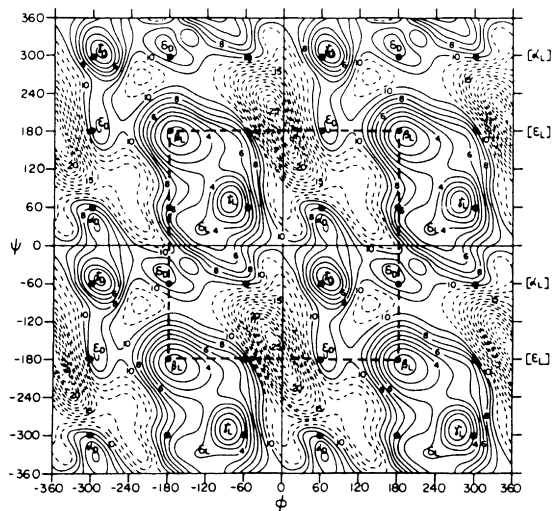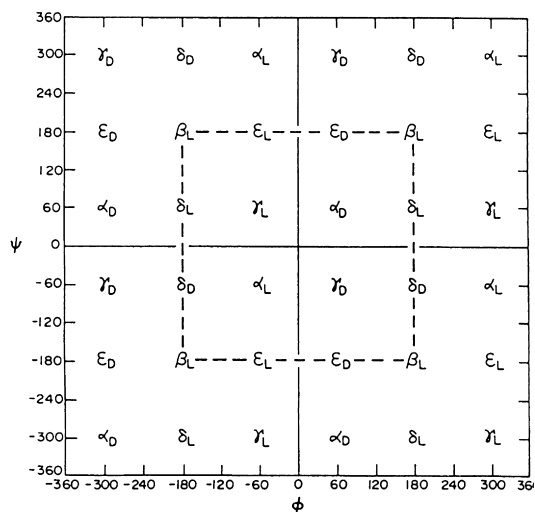

Fig. 7. (Top) A schematic representation of the conformational PES of a peptide (PCONH–CHR–CONHQ). Subscripted Greek letters symbolize the approximate locations of the conformations. (Bottom) Contour diagram of the 2D Ramachandran potential energy surface of HCONH–CHCH$_3$–CONH$_2$, presented in the $-360° \leq \phi \leq 360°$ and $-360° \leq \psi \leq 360°$ range of independent variables. The central square (broken lines) is the IUPAC conventional cut, while the four quadrants are the traditional cuts.

Finally, it should be mentioned that for certain molecular residues molecular computations have established the actual location of the nine minima, as shown above. The values of ϕ and ψ deviate somewhat from the ideal values, although not too significantly. Table 2 lists these numerical values for
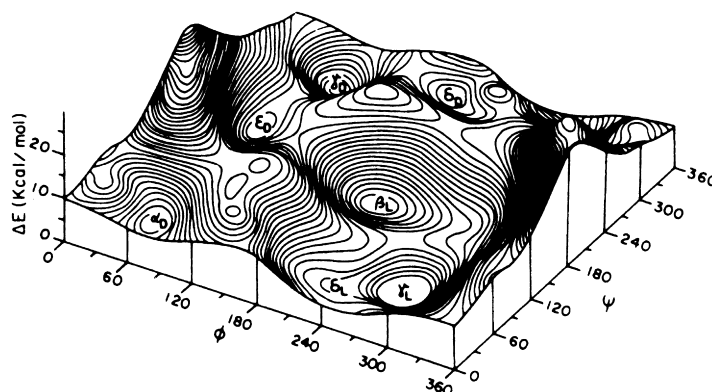
Fig. 8. Pseudo-three-dimensional Ramachandran potential energy surface of $HCONH–CHCH_3–CONH_2$, presented in the $0° \leq \phi \leq 360°$ and $0° \leq \psi \leq 360°$ range of independent variables. This represents one of the four equivalent quadrants in Fig. 7.

alaninediamide. This information is also presented graphically in Fig. 10.

4.1. Previously published single amino acid peptide models

The topological representation of the conformational PES (Ramachandran map) of For-Ala-NH_2 is shown in Fig. 2A, while that of the conformational PEHS is shown in Figs. 7 and 8. Work has been completed for the following N- and C-protected amino acids containing a *trans*-peptide bond: Gly [16,17], Ala [16,17], Val [18], Phe [19,20] and Ser [21–23]. Preliminary studies have been completed on Pro [24], Asp [25], Asn [26], Cys [27], and Sec (selenocysteine) [15].

The following protected amino acid residues, again with *trans*-peptide bonds, are currently under investigation: Arg, Lys, His, Tyr, Leu, Thr and Trp.
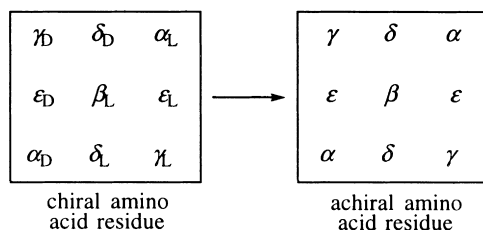

Fig. 9. The development of double degeneracy of the PES when a chiral amino acid residue is changed to an achiral amino acid residue.

Proline is fundamentally different from all the other 18 chiral amino acids in more than one respect:

(i) the R group forms a five-member ring with the backbone;
(ii) there is no peptidic N–H group in the residue to be involved in hydrogen bonding, and
(iii) since there are two carbon atoms connected to the nitrogen, there is a greater chance of *cis/trans* isomerization in the peptide bond.

The potential energy cross-sections of the type $E = E(\psi)$, for the Ramachandran map of $HCO–Pro–NH_2$ containing *cis*- and *trans*- peptide bonds [28] are

Table 2
Optimized ϕ, ψ torsional angle pairs for alanine diamide ($HCONH–CHMe–CONH_2$). The idealized torsional angle pairs, together with their conformational classification, are also shown for the sake of comparison

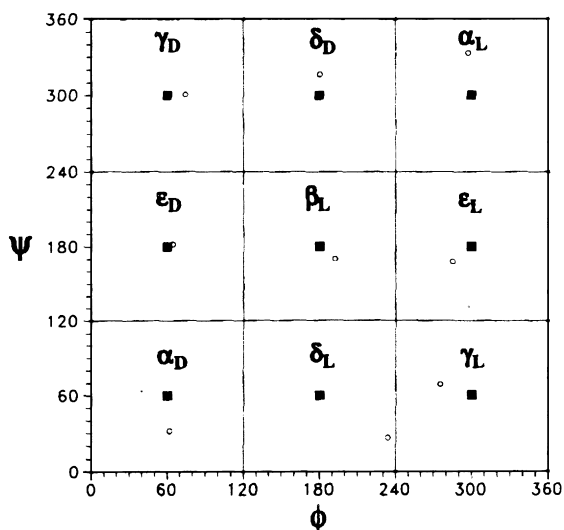| Conformer	Optimized values		Idealized values		Conformational classification
	ϕ	ψ	ϕ	ψ	
α_L	−66.6	−17.5	−60	−60	g^-g^-
α_D	+61.8	+31.9	+60	+60	g^+g^+
β_L	−167.6	+169.9	−180	+180	aa
γ_L	−84.5	−68.7	−60	+60	g^-g^+
γ_D	+74.3	−59.5	+60	−60	g^+g^-
δ_L	−126.2	+26.5	−180	+60	ag^+
δ_D	−179.6	−43.7	−60	−60	ag^-
ϵ_L	−74.7	+167.8	+60	+180	g^-a
ϵ_D	+64.7	−178.6	−180	−180	g^+a

Fig. 10. A schematic illustration of the PES of an average amino acid residue, obtained from the calculations carried out so far on mono-, di- and tri-peptides. The idealized positions are marked by shaded squares and the computationally determined positions are shown as open circles. The names of the conformers are given as subscripted Greek letters. Note that a single amino acid residue might not be able to take on all of the shown conformations.

Fig. 11. Conformational Potential Energy Curves $E = E(\psi)$ for *cis* and *trans*-N formyl-L-prolinamide.

shown in Fig. 11. Preliminary investigation on the *cis*-peptide bond has been completed and the *cis*-Ramachandran map is currently under construction [29].

4.2. Single amino acid peptide models in progress

4.2.1. Aspartic acid and asparagine

Aspartic acid (Asp) and its side-chain deprotonated form, i.e. the aspartate [25] ion as well as its side-chain amide asparagine (Asn) [26] have been studied in a preliminary fashion. In both cases, the *N*-formyl-L-aspartic acidamide, *N*-formyl-L-aspartamide and *N*-formyl-L-asparaginamide were investigated in their γ_L backbone conformation. The side-chain conformational PES for the three compounds are shown in the following illustrations: Figs. 12–14.

4.2.2. Arginine (Arg)

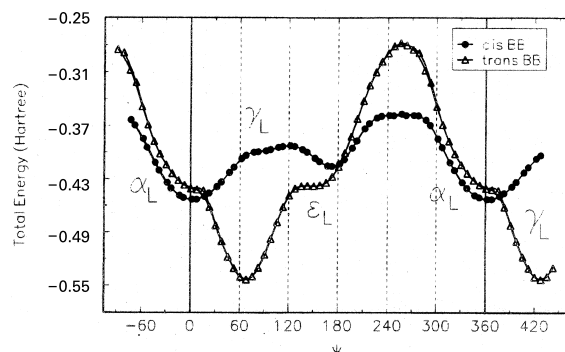Arginine is an amino acid with a positive charge at neutral pH due to its polar side-chain, the guanidium group. The typical pK_a value of arginine is 12.0, the highest pK_a of all amino acids. It is, therefore, important in salt bridge coupling, which is part of certain docking processes.

Arginine is the source of nitric oxide (NO) in biological systems. NO is a free radical which serves as an intracellular second messenger and an intercellular messenger that regulates neighbouring, and possibly distant cells. NO takes part in many biological processes, such as vasodilation for example. NO is also involved in the central nervous system (CNS), such as the modification of pain perception. One of the two equivalent nitrogens of the terminal guanidine in L-arginine undergoes five-electron oxidation in the presence of the enzyme NO synthase (NOS), yielding NO and L-citrulline. *N*-methyl guanidine has been used as a model compound for arginine to study the mechanism of NO release using ab initio molecular computations [30].

Ethylguanidine or ethylguanidium ion is the terminal portion of the side chain (R-group) of the arginine residue, as exemplified by *N*-formyl-argininamide and its side chain terminal *N*-protonated form respectively. Arginine can be divided into two portions: the backbone, which is analogous to glycine (or alanine) and the side chain, which is an alkyl guanidine.

When a planar moiety is twisted about a single bond with respect to a tetrahedral moiety, in principle, up to six minima may appear. In the case of ethylguanidine [**VII**] and ethylguanidium ion [**VIII**], there are two torsional angles χ_3 and χ_4. These are two types of bonds, C–C and C–N, with respect to
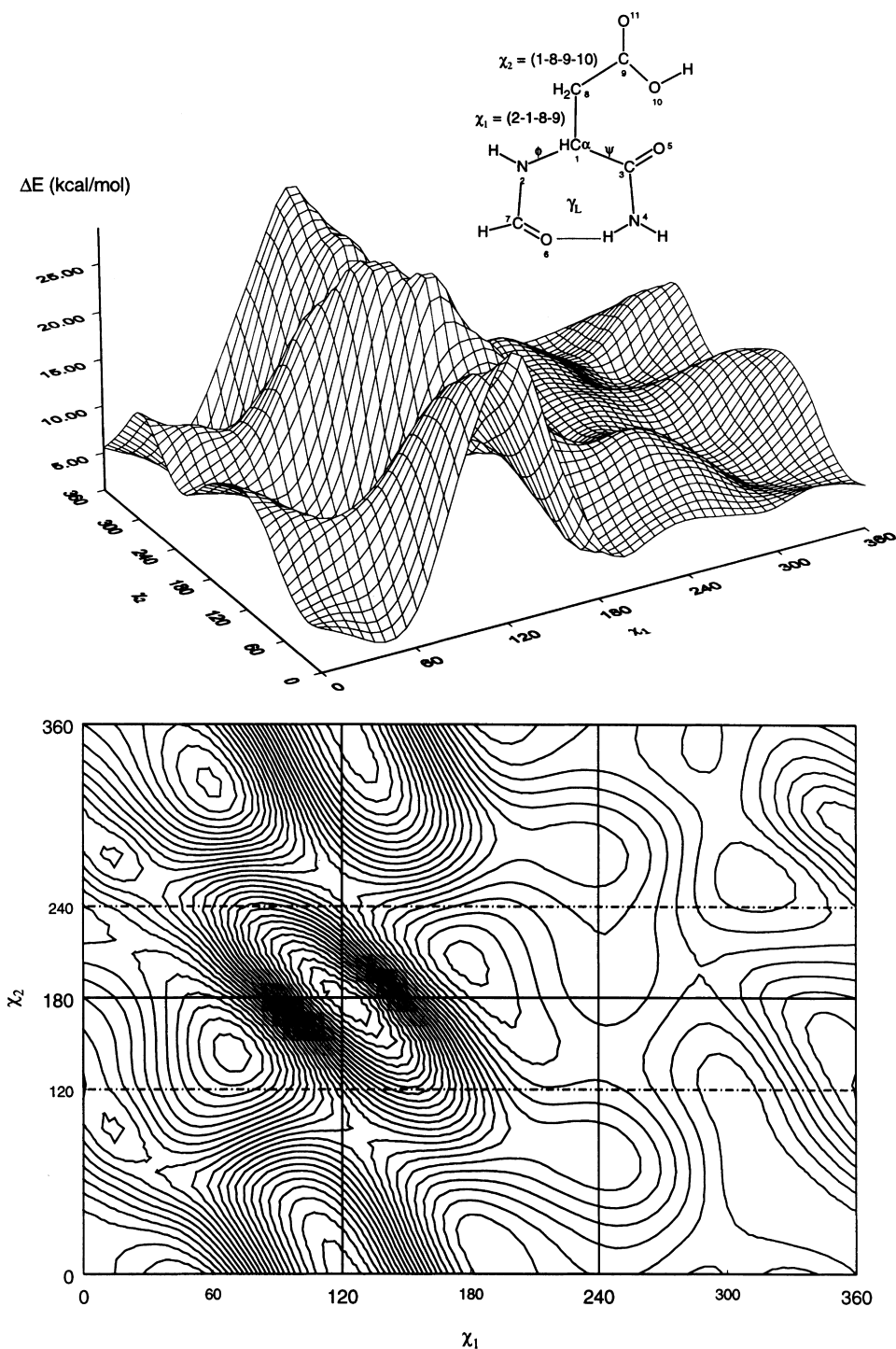
Fig. 12. Side-chain conformational PEHS of *N*-formyl-L-asparticacidemide. (Top) Landscape representation. (Bottom) Contour diagram representation.
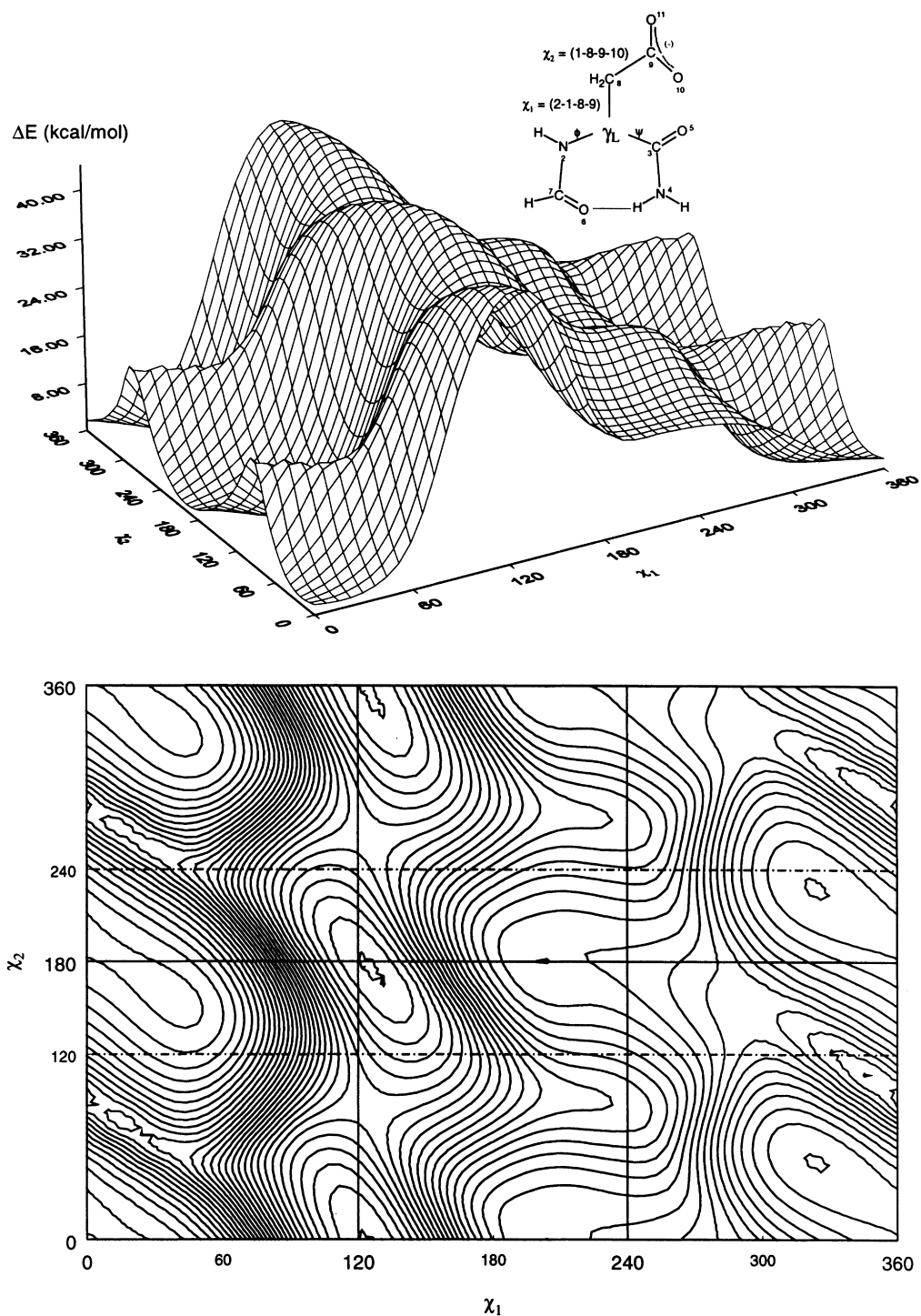
Fig. 13. Side chain conformational PES of *N*-formyl-L-aspartatamide. (Top) Landscape representation. (Bottom) Contour diagram representation.
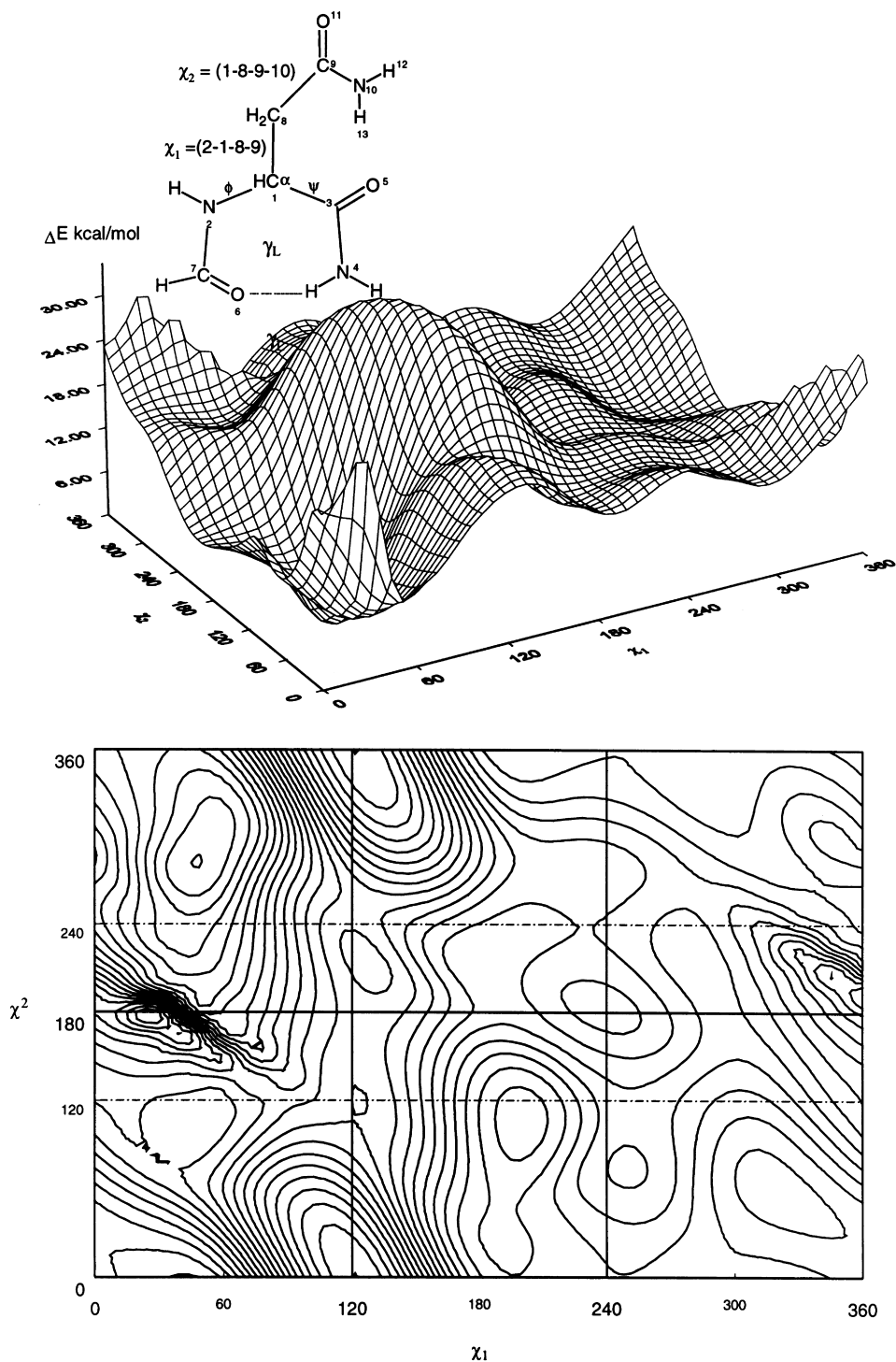
Fig. 14. Side-chain conformational PES of N-Formyl-L-asparaginamide. (Top) Landscape representation. (Bottom) Contour diagram representation.
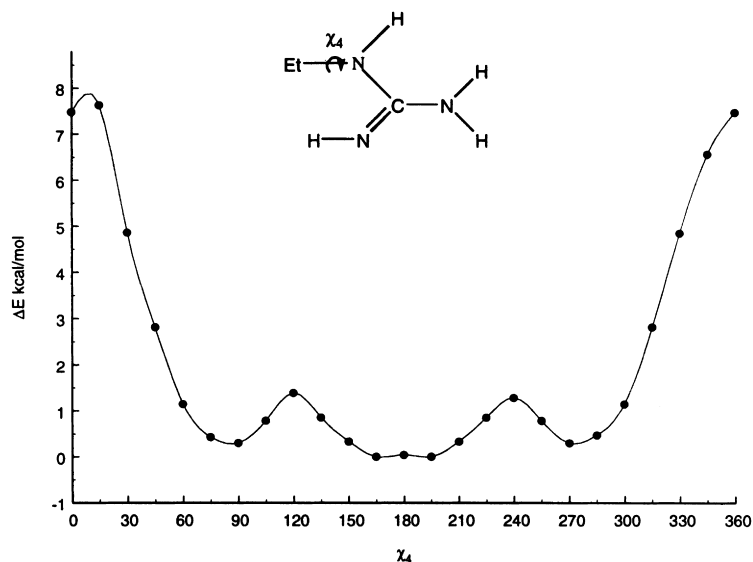
Fig. 15. Conformational PEC of ethyl guanidine, which may model the rotation (χ_4) about the C–N bond in neutral arginine.
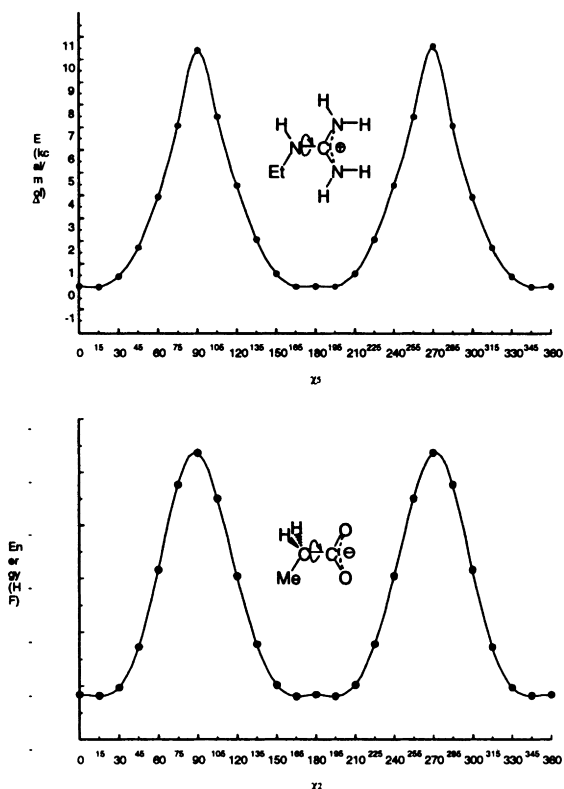

Fig. 16. Rotational potential energy curves of ethylguadinium ion (top) and propionate ion (bottom). Note that each molecule has only one discrete conformation.

which the guanidine group could be eclipsed or be perpendicular to it.

Fig. 15 shows the torsional potential, as a function of χ_4 for ethylguanidine in its *s-cis*/*exo* [**VII(a)**] structure calculated using two basis sets. For each basis set, the global minimum occurs at $180°$ (the anti form). The energy values of the 1D-scans were computed at the HF/6-31G level of theory. For all four structures [**Ia,Ib,Ic** and **Id**] g^+, a, g^- were shown to have energy minima. The upper portion of Fig. 16 shows the potential energy curve $E = E(\chi_5)$ of structure **VIII** calculated at HF/6-31G [31].

The protonated form of the ethyl guanidine [**VII**] is the ethyl guanidium structural ion [**VIII**]. Due to the internal symmetry of the N–C(NH$_2$)$_2^+$ moiety, this species has only one arrangement.

Two vertical proton affinity values may be identified using these PECs. The two vertical ones involve $g \to g$ and $a \to a$.

The full side-chain orientation of N- and C-protected arginine and its conjugate acid depends on χ_1, χ_2, χ_3, and χ_4 as illustrated by molecular structures **IX** and **XI**.

Consider the diamides of the two amino acids arginine **IX** and aspartic acid **X**. Under physiological conditions the amino acid side chains occur in the protonated **XI** and deprotonated **XII** forms

**VII (a)**   **VII (b)**   **VII (c)**

VII (d)   **VIII**
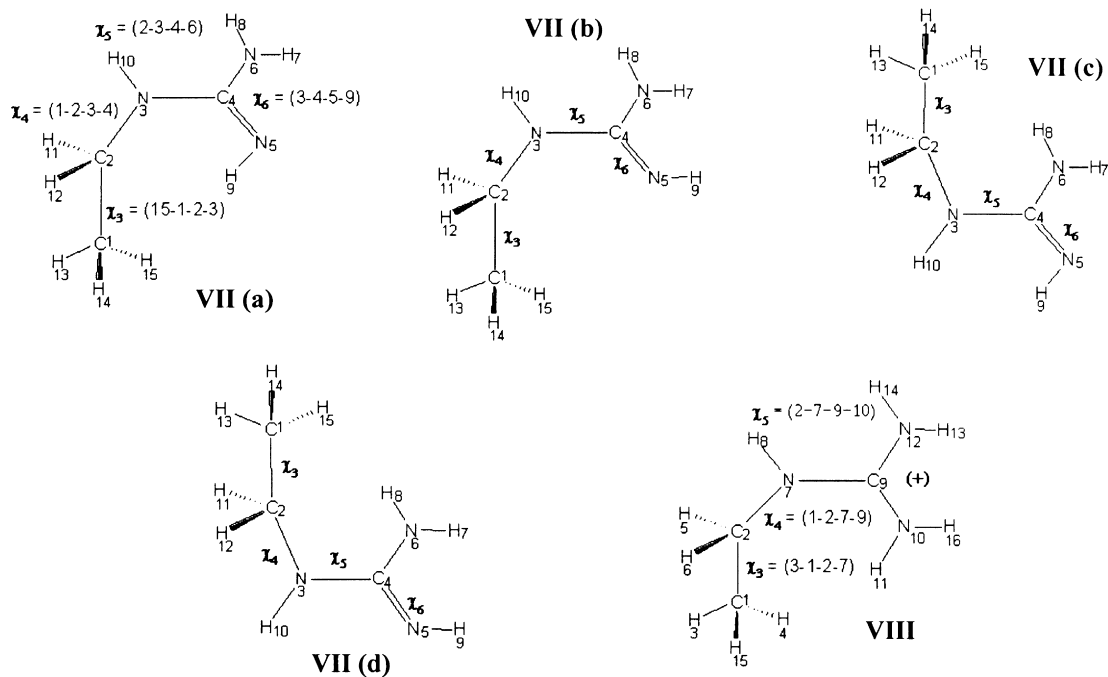
respectively. The side chain conformations could be mimicked for **XI** by **XIII** and to some extent **XIV** and for **XII** by **XV**. The potential energy curve, $E = E(\chi_5)$, for **XIV** and that for **XV**, $E = E(\chi_2)$, is shown in Fig. 16. The side chain PES of **XII** has also been reported and is shown in Fig. 13. It is clear that torsional angles within the amino acid side chains do increase the dimensionality of the conformational space. Nevertheless, they are very important, because the side chain makes each

amino acid unique. Such distinction can be made because, to a good degree of approximation, the side chain conformational pattern is independent of the backbone conformational behavior. For all practical purposes, the backbone of all amino acids shows the same conformational pattern (c.f. Figs. 2, 7, 8 and 10).

4.2.3. Cysteine (Cys) and selenocysteine (Sec)
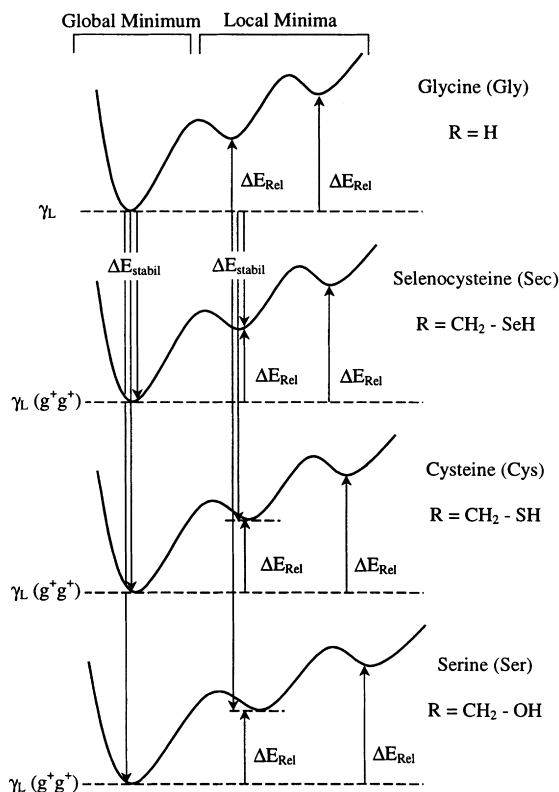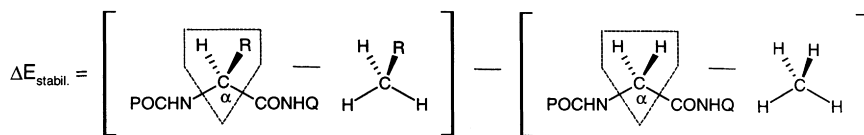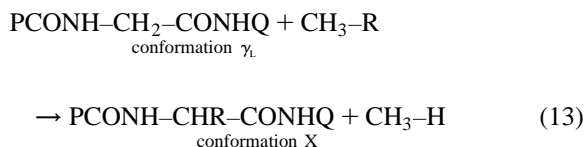
Each of serine [21–23,32], cysteine [27] and

XI   **IX**   **X**   **XII**

XIII   **XIV**   **XV**
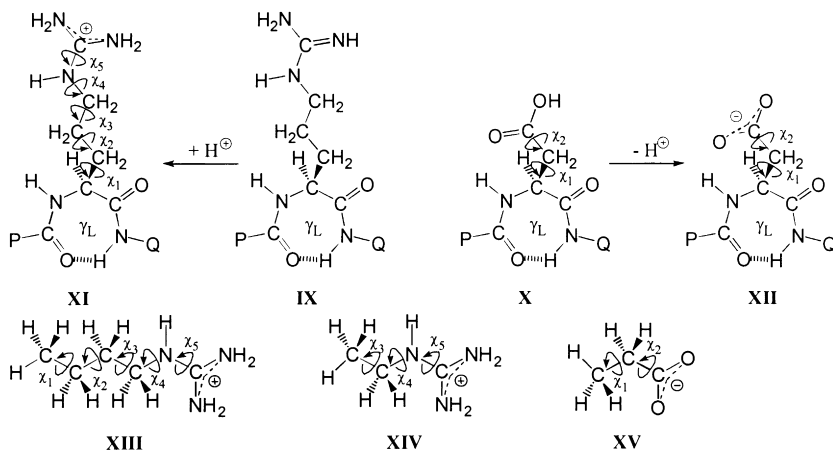
Fig. 17. Calculation of stabilization energies ($\Delta E_{\text{stabil.}}$) with various substituents (R may be CH_2-OH, CH_2-SH and CH_2-SeH) relative to the γ_L conformation of Glycine ($R = H$).

selenocysteine [15] is expected to have $9 \times 9 = 81$ conformations [$3 \times 3 = 9$ backbone: $\psi(g^+, a, g^-) \times \phi(g^+, a, g^-)$ and $3 \times 3 = 9$ side-chain: $\chi_1(g^+, a, g^-) \times \chi_2(g^+, a, g^-)$]. To investigate the effects of side-chain/backbone conformational interactions, all torsional modes of the side-chain (χ_1: rotation about the C^α–C^β and χ_2: rotation about the C^β–X bonds) were studied in the relaxed γ_L backbone [$(\phi, \psi) = (g^-, g^+)$] conformation. Six out of the nine expected minima for serine and seven out of the nine expected minima for cysteine and selenocysteine were found at the RHF/3-21G level of theory.

The stabilization energy exerted by the –CH_2–SeH

side-chain has been compared to that of –CH_2–SH and –CH_2–OH. The stabilization energies were calculated with respect to the γ_L backbone conformation of N- and C-protected glycine [33,34] using the following isodesmic (same number of bonds) reaction, where P and Q may be H or CH_3 and R = CH_2–XH(X = O,S,Se):

$$\text{PCONH–CH}_2\text{–CONHQ} + \text{CH}_3\text{–R}$$
$$\text{conformation } \gamma_L$$

$$\rightarrow \text{PCONH–CHR–CONHQ} + \text{CH}_3\text{–H} \qquad (13)$$
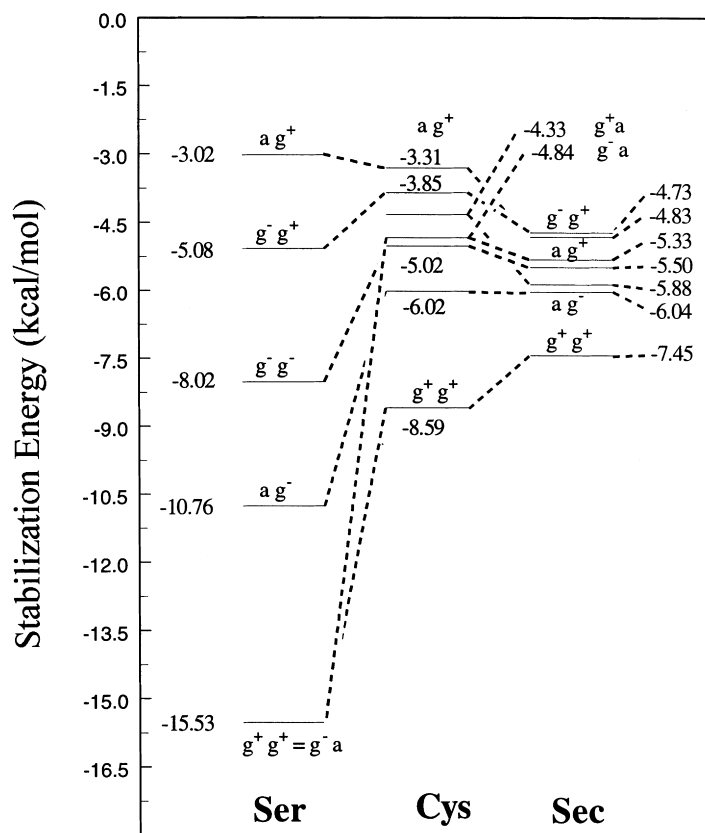$$\text{conformation X}$$

Fig. 18. Spectrum of conformational dependence of the side-chain stabilization energy on the γ_L backbone conformation of formyl Ser-, Cys- and Sec-amide.

The stabilization energy may be calculated as follows:

$$\Delta E_{stabilization} = \{E[PCONH-CHR-CONHQ]_x$$

$$+ E[CH_3-H]\}$$

$$- \{E[PCONH-CH_2-CONHQ]_{\gamma L}$$

$$+ CH_3 - R]\} \qquad (14)$$

These equations are also illustrated in Fig. 17.

From Fig. 18, it can be observed that the effect of side-chain orientation with respect to the peptide backbone, going from Serine to Selenocysteine, is gradually decreasing. This is due to the increase in the size of the atoms $O \rightarrow S \rightarrow Se$ and the resulting gradual lengthening of $C-O \rightarrow C-S \rightarrow C-Se$ bond lengths as well as the reduced hydrogen bonding ability in going from OH to SH and all the way to SeH.

Topological analysis of the electron density has been performed for cysteine and selenocysteine, using Bader's Atoms in Molecule (AIM) approach at the B3LYP/6-31G(d,p) level of theory. The hydrogen bonds were verified by the existence of bond critical points. Three conformations: $(g^+ g^+)$, $(g^+ a)$ and $(a\ g^-)$ exhibited such interactions as illustrated graphically in Fig. 19 for For-Sec-NH$_2$.

4.2.4. Leucine (Leu)

Leucine is one of the naturally occurring essential amino acids. It is an important amino acid, having many implications in medicine, one of them being its role in Maple Syrup Urine disease (MSUD). Maple syrup disease occurs when plasma leucine, isoleucine, alloisoleucine and valine are elevated to very high levels. These amino acids will also be present in the urine and hence the urine releases
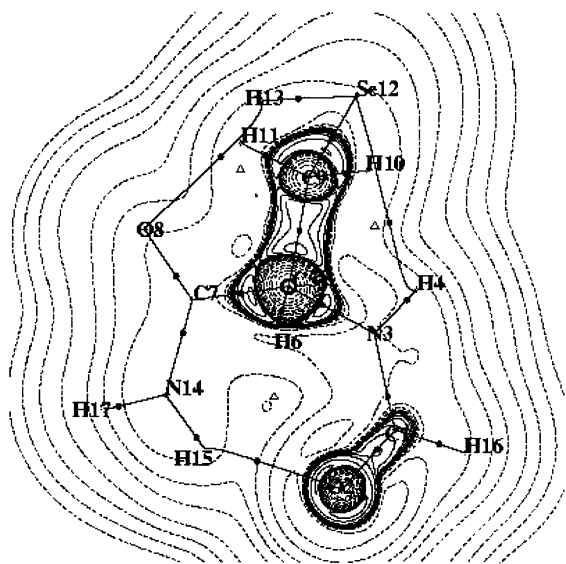
Fig. 19. The contour map of the Laplacian of the electron density for the (g^+g^+) conformation of For-L-Sec-NH$_2$ calculated at the B3LYP/6-31+G(d,p)//RHF/3-21G level of theory. Bond paths are denoted by lines, bond critical points (BCPs) are denoted by black dots, ring critical points (RCPs) are denoted by open triangles and the nuclei are denoted by crosses.

Fig. 20. Relative energies of the N- and C-protected leucine with varying side chain conformations for the β_L backbone conformation retaining *trans* peptide bonds calculated at HF/3-21G level of theory.

an odor similar to maple syrup. This disease has severe neurological and gastrointestinal effects. The current treatment for it involves intense control of the intake of leucine, isoleucine and valine. This careful restriction of leucine also includes monitoring of the branched chain amino acids in plasma.

Leucine is also found in some small proteoglycans. This group of small, interstitial proteoglycans has a high degree of homology in their protein core sequence. Each has between 10 and 12 highly conserved leucine rich tandem repeats which makes up the central portion of the core protein. The current members of the group are fibromodulin and lumican, which are keratan sulphate substituted, as well as decorin and biglycan, which are both chondroitin sulphate substituted. Other proteoglycans which have had functions ascribed to them are the small leucine rich interstitial proteoglycans decorin and fibromodulin which have been shown to modulate collagen fibrillogenesis.

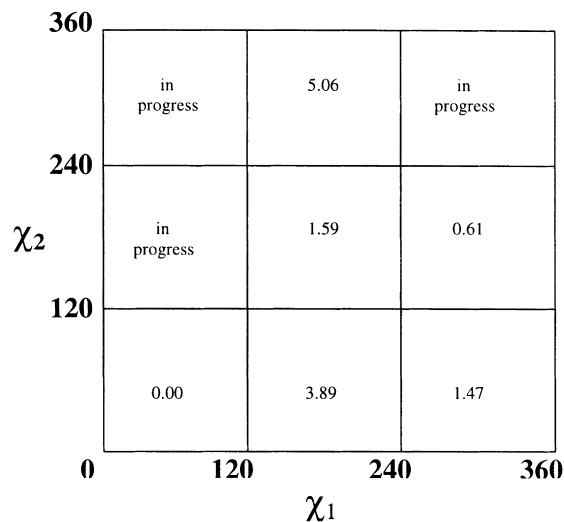The knowledge of the conformational potential energy surface for leucine would allow a better under-

standing of diseases such as MSUD and the function of leucine in biological processes such as collagen fibrillogenesis. Each of the nine possible backbone conformations is associated with nine possible side chain conformations. In addition, there are four chain-end conformations associated with the *cis* and *trans* peptide bond that also must be explored. Thus the total number of conformations to be considered is $9 \times 9 \times 4 = 324$. The results of some calculations that have been carried out for the β_L backbone conformation are shown in Fig. 20.

4.2.5. Tryptophan (Trp)

Tryptophan is one of the essential amino acids the body cannot manufacture itself. It is the least abundant in proteins and is destroyed easily by the liver. This amino acid is necessary for the production of the vitamin B niacin, which is essential for the brain to manufacture the key neurotransmitter serotonin. Low levels of serotonin have been linked with insomnia, anxiety and depression.

Tryptophan is believed to be effective for insomnia and jet lag. It is also believed to have anti-anxiety effects and control aggressive behavior in some individuals. Other studies have shown that its anti-depressant effect lasts longer than that of the popular
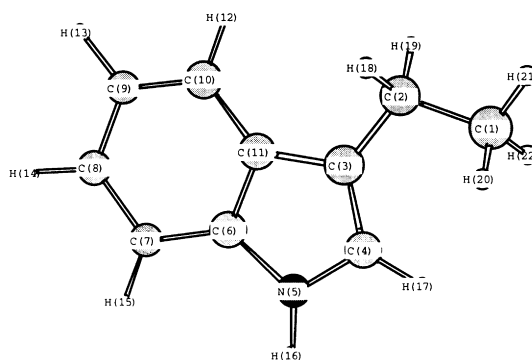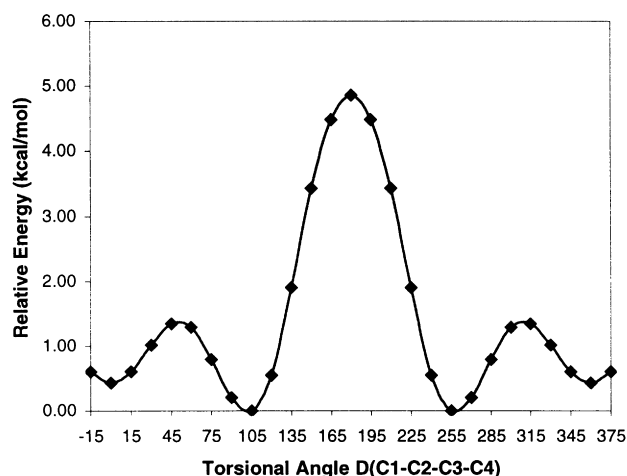
	RHF Regular Optimization			RHF Tight Optimization
	STO-3G	3-21G	6-31G	3-21G
Planar (0 degrees)	-434.1966671 (hartree)	-437.0948503 (hartree)	-439.3773570 (hartree)	-437.0948513 (hartree)
Perpendicular	-	-437.0955447 [a] (hartree)	-439.3775512 [b] (hartree)	-437.0955634 [c] (hartree)

[a]Torsional angle was found to be 105.00 degrees.
[b]Torsional angle was found to be 104.46 degrees.
[c]Torsional angle was found to be 102.34 degrees.

Fig. 21. Potential energy curve of D(C1–C2–C3–C4) of ethyl indole.

anti-depressant drug, Imipramine. In addition, some preliminary studies of combined Vitamin B-6 and tryptophan show that they may be effective in reducing the severity of hyperventilation as well as the panic attacks produced by it.

Although it is safe to use, tryptophan is no longer available in supplement form, because of potential adverse reactions. Tryptophan supplements are not recommended in cases of pregnancy, asthmatics, or auto-immune disorders like Lupus or Scleroderma. As a preliminary investigation, a scan at HF/3-21G level of theory was performed on ethyl indole (Fig. 21).

Minima were found to exist at torsional angles (*D*) of 0 and in the vicinity of 105°.

4.2.6. Lysine (Lys)

Similar to arginine, lysine **XVI** is a basic hydrophilic amino acid. At neutral pH, it is positively charged, with a typical pK_a of 10.0. One can imagine that the two amino acids would be able to assume similar roles in biological systems. They may increase the solubility of proteins, furthermore, they can provide binding sites at the surface of proteins. However, there is a main stereochemical difference between the two molecules. Lysine has a hydrocarbon chain with a terminal tetrahedral $NH_3(+)$, whereas the terminal guanydilic ion of arginine is planar. This difference may be important in the fine-tuning of molecular interactions, for example, between substrate and the active site.


Structure of lysine **XVI**
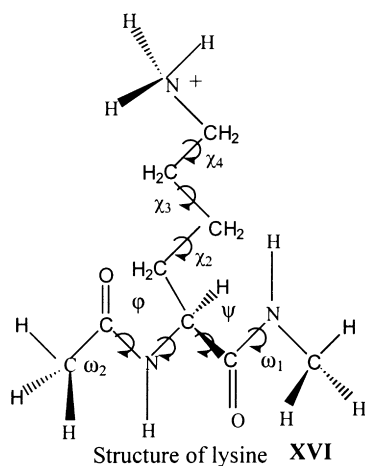
Both, the side chain **XVII** and the lysine residue **XVI** with the protected backbone, are currently being studied.

5. Dipeptide, tripeptide, and tetrapeptide

Most of the oligopeptides studied so far at the ab initio level of theory involved alanine. All backbone conformers of dialanine diamides have been studied [35–38]. Analogously trialanine diamide (tripeptide) [39] and tetraalanine diamide (tetrapeptide) [40]

have also been investigated at the ab initio level but only a selected few conformers were studied. In this paper, we wish to illustrate in the case of tripeptide Arg-Gly-Asp (or RGD for short) and in the case of tetrapeptide Pro-Pro-Thr-Pro (or PPTP for short) the medical importance of such small oligopeptide fragments.

Single amino acid diamides or "monopeptides" for short, have nine unique conformers. For diamino-acid diamides, or "dipeptides" for short, each amino acid residue has nine conformers. This leads to $9 \times 9 = 81$ conformers for dipeptides. In the case of tripeptides, for each of the 81 dipeptides conformations there are 9 conformers for the third amino acid. This results in $9 \times 9 \times 9 = 729$ conformers. The name of each of the 729 conformers, given in subscripted Greek letters, is summarized in Fig. 22. These 729 conformers are derived within the law of MDCA, and in that respect

Side chain of lysine **XVII**
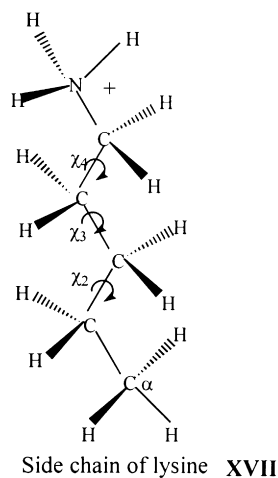
they may be regarded as "legitimate" conformers. Not all 729 conformers are expected to exist. In certain folding patterns, the terminal groups may come too close and such minima may be annihilated due to steric repulsion. The pattern shown in Table 2 has been previously recognized for $HCON-(Ala)_n-NH_2$ via ab initio computations (Table 3).

Mono-peptides, dipeptides and tripeptides contain 1, 2 and 3 amino acid residues, respectively. In such models, the chain may be terminated by methyl groups or by hydrogens, (P = Q = CH_3 or H) as shown for the above three cases by

Fig. 22. Names, in terms of subscripted Greek letters, of the 729 legitimate backbone conformers of tripeptides.

Table 3
Legitimate and existing conformers of small peptides

n	Legitimate conformers	Conformers found
1	9	7
2	81	49[a]
3	729	Not investigated

[a] These 49 conformers were found by optimizing the 81 "legitimate" conformers. Subsequently, Prof. Lothar Schafer and coworkers [Can. J. Chem. 76 (1998) 566] have scanned the conformational space computing 11 664 grid points at the HF/4-31G level of theory and located one more conformer. Now the number of conformers stands at 50.

II, **III** and **IV**. The symbol $R^{(j)}$ represents the side-chain of one of the 20 naturally occurring amino acids $(1 \leq j \leq 20)$.
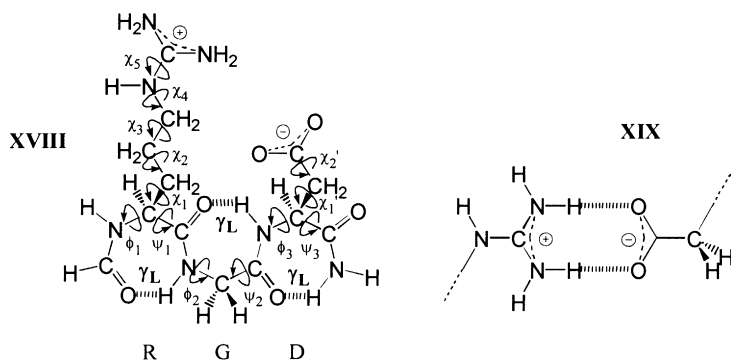
5.1. Tripeptide sequence, RGD

The tripeptide Arg-Gly-Asp (RGD) is an adhesive molecule which has a positively and a negatively charged side chain as shown in **XVIII** in accordance with **XI** and **XII**. The positively and negatively charged side chains may be engaged in salt bridge **XIX** formation either via an intra- or an intermolecular connection.

The structure shown in **XVIII** is the amide of the *N*-formyl derivative of the RGD-motif and it is given in its $\gamma_L \gamma_L \gamma_L$ backbone and extended (fully *anti*) side chain conformations. Once again, assuming that all peptide bonds are of *trans* configuration, the PES can be represented analytically by the following function **XX**.

The backbone may be represented by a 6D-conformation subspace $(\phi_1, \psi_1, \phi_2, \psi_2, \phi_3, \psi_3)$. Since we may

expect only one distinct conformer along χ_5 and χ_2', the side chain may be represented by a 5D-conformational chain subspace $(\chi_1, \chi_2, \chi_3, \chi_4, \chi_1')$. Consequently, we may expect $3^5 = 243$ side-chain conformations. The total number of distinct RGD conformers may, therefore, be $729 \times 243 = 177147$. In view of the enormity of the problem only a selected few conformations were subjected to molecular computations.

Table 4 summarizes the geometrical parameters (e.g. torsional angles), conformational assignments and relative stabilities of the RGD structures optimized at HF/3-21G level of theory.

The last two structures of the table are depicted in Fig. 23. Note that one of them is without the involvement of Ca^{2+}, and therefore, it exhibits intramolecular salt bridge. The other structure is with Ca^{2+}, implying that the previously negatively charged side chain $(-COO^-)$ now contains a mono-positive $(-COO^- \cdots Ca^{++})$ ending. Consequently, there is no chance for intramolecular salt-bridge formation. This charge difference in side-chain has implication for the docking mechanism. Fig. 24 shows a possible docking mechanism without the involvement of Ca^{2+}.

Fig. 25 indicates that in the presence of Ca^{2+}, the receptor would need two negatively charged side chains (i.e. two carboxylate ions).

It is interesting to recall the result of the physiological study [41], involving GRGDS(β-OH) (c.f. Fig. 26) and GRGDS(β-O–SO_3^-). The final results are summarized in Table 5.

It seems that when Ca^{2+} ion is not present the mechanism given in Fig. 24 is operative and thus an extra negative charge at the C-terminal makes no

Table 4
Geometrical parameters, conformational assignment, total energies and relative stabilities of collected RGD conformers without (A–E) and with (F) the inclusion of Ca^{2+}, optimized at the HF/3-21G level of theory

Residue		A		B		C		D		E		F[a]	
		Geometry	Confor-mation	Geometry	Confor-mation	Geometry	Confor-mation	Geometry	Confor-mation	Geometry	Confor-mation	Geometry	Confor-mation
Arginine (R)	ω^0	177.5804	–	172.5255	–	−175.5717	–	−177.5620	–	−171.2285	–	−176.4709	–
	ϕ^1	−153.0601	δ_L	−155.4259	δ_L	46.2751	α_D	−81.9119	γ_L	−82.2705	γ_L	−153.6854	β_L
	ψ^1	47.7288		53.3474		41.4783		66.0202		66.0650		165.4315	
	ω^1	−179.4318	–	173.4531	–	−178.5048	–	−179.8780	–	−179.5433	–	175.9534	–
	χ^1	44.2825	g^+	58.1487	g^+	57.9267	g^-	57.1272	g^-	−61.4889	g^-	−60.7727	g^-
	χ^2	75.7941	g^-	123.3340	a	96.1472	g^+	142.7702	a	−64.8448	g^-	−66.1861	g^-
	χ^3	−92.5497	g^-	−166.2056	a	−144.5144	a	−64.6406	g^-	−57.8581	g^-	−175.4661	a
	χ^4	155.5904	a	79.6676	g^-	96.1651	g^+	169.7310	a	142.8879	a	−96.2058	g^-
Glycine (G)	ϕ^2	120.6121	β_L	96.0964	δ_L	123.2492	δ_D	−87.4209	γ_L	−65.5310	α_L	173.2842	β_L
	ψ^2	−131.5270		−103.2997		−34.2922		58.5616		−26.2768		179.6252	
	ω^2	−175.4871	–	171.9142	–	171.0358	–	−159.1781	–	−169.9989	–	−174.1726	–
Aspartate (D)	ϕ^3	−66.4906	ϵ_L	−84.2133	ϵ_L	−161.4273	δ_D	−93.1384	γ_L	−81.2064	γ_L	−83.6510	γ_L
	ψ^3	171.5693		−141.3300		−119.3301		53.6705		69.7315		52.2155	
	ω^3	−177.6658	–	−155.8824	–	−158.9678	–	−171.2286	–	173.9757	–	−172.5103	–
	χ^1	59.2826	g^+	64.5079	g^+	48.5048	g^+	37.1034	g^+	53.9209	g^-	168.2582	a
	χ^2	89.7330	g^+	106.2422	g^+	89.8874	g^+	29.3742	g^+	155.2624	a	37.4610	g^-
Energy (hartree)		−1328.6774261		−1328.7079568		−1328.6899608		−1328.6819226		−1328.69329353		−2001.8202808	
ΔE (kcal/mol)		19.16		0.00		11.29		16.34		9.20		N/A	
Backbone conformation		$\delta_L\delta_L\epsilon_L$		$\delta_L\delta_L\epsilon_L$		$\alpha_D\delta_D\delta_D$		$\gamma_L\gamma_L\gamma_L$		$\gamma_L\alpha_L\gamma_L$		$\beta_L\beta_L\gamma_L$	

[a] Includes a Ca^{2+} in the vicinity of the asparate side chaincarboxylate moiety.
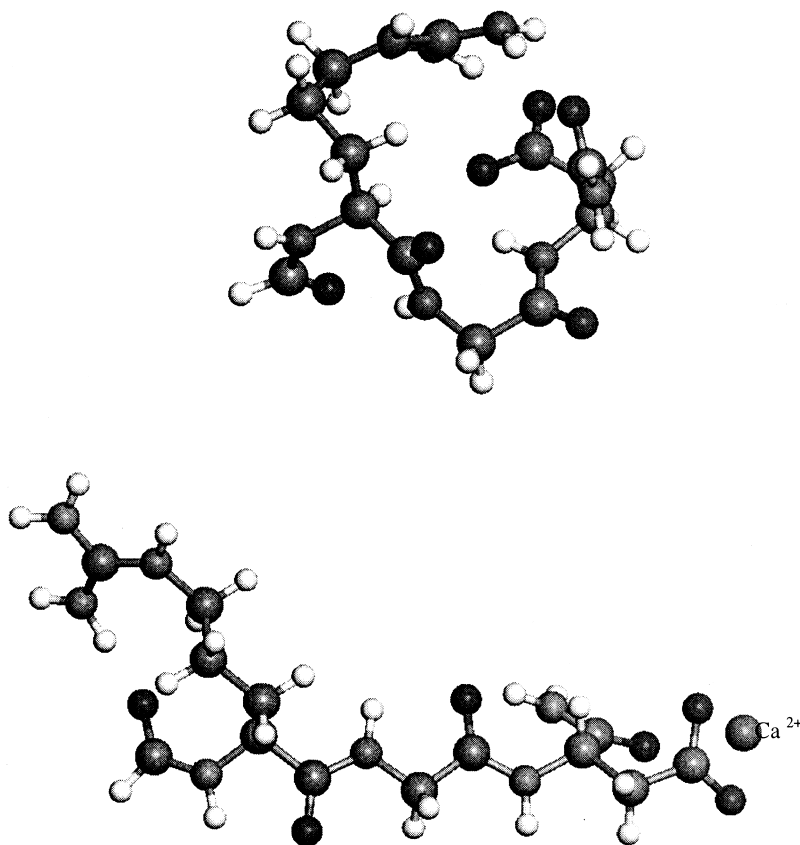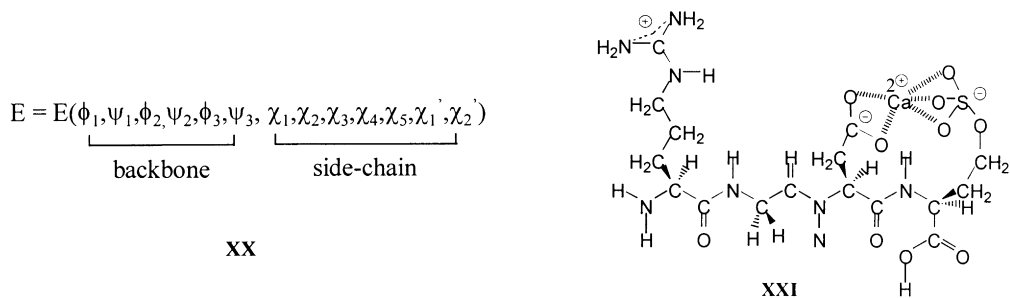
Fig. 23. Two conformers of RGD without (top) and with Ca^{2+} (bottom) optimized at the HF/3-21G level of theory corresponding to structures E and F respectively in Table 3.

significant change. However, when Ca^{2+} ion is present, GRGDS(β-OH) becomes very active, favoring the complex formation according to Fig. 25. However, the Ca-salt of GRGDS(β-OSO_3^-) has only one charge, a positive charge at the Arg side chain, but no charge at the DS(β-OSO_3^-) moiety since the two

$(+)$ and the two $(-)$ will lead to a neutralization as illustrated in **XXI**. It is not surprising therefore that under these conditions the activity is reduced greatly. One structure using Mg ion rather than Ca ion is optimized at the HF/3-21G level of theory and is shown in Fig. 26.
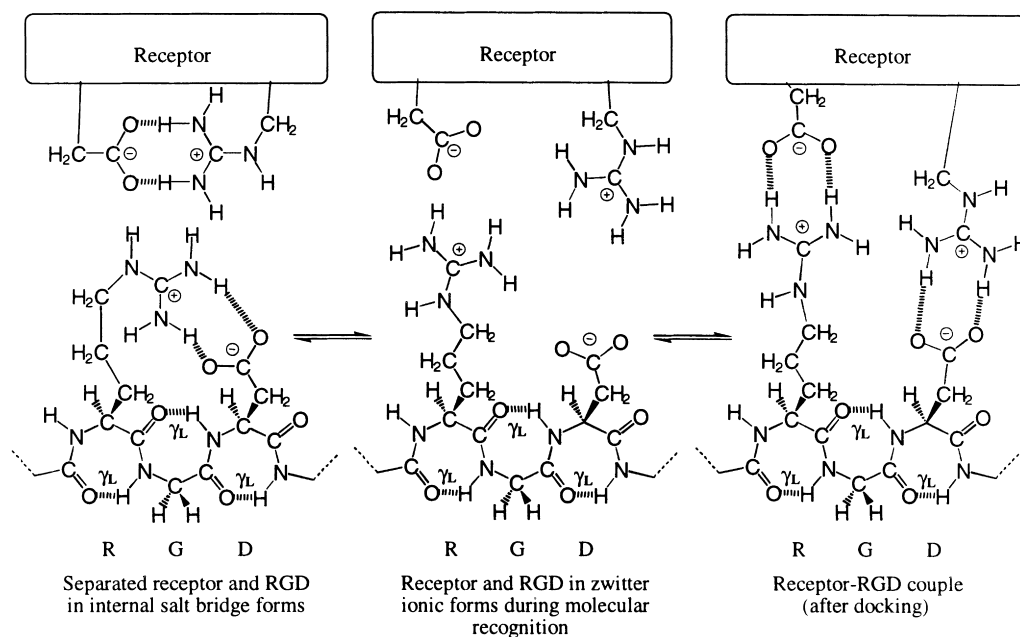
$$E = E(\phi_1, \psi_1, \phi_2, \psi_2, \phi_3, \psi_3, \ \chi_1, \chi_2, \chi_3, \chi_4, \chi_5, \chi_1^{'}, \chi_2^{'})$$

$\underbrace{}_{\text{backbone}} \underbrace{\phantom{\chi_1, \chi_2, \chi_3, \chi_4, \chi_5, \chi_1^{'}, \chi_2^{'})}}_{\text{side-chain}}$

XX

XXI

Fig. 24. A possible docking mechanism of RGD operative in the absence of Ca^{2+} ion. Note that the backbone is shown arbitrarily in its $\gamma_L\gamma_L\gamma_L$ conformation.

Fig. 25. Receptor-RGD-complex after docking, in the presence of Ca^{2+} ion.

5.2. Tetrapeptide sequence, PPTP

For a discussion of a tetrapeptide example, let us briefly review immunoglobulin A (IgA). Antibodies or immunoglobulins (Ig) represent the great defense force in the army of the immune system. Ig molecules are produced during a humoral immune response to bind and neutralize invading antigens. Subsequently, the antigen is labeled for removal by phagocytosis. The fight, however, is not one-sided. To help neutralize the role of Ig in antigen recognition, bacteria have developed effective deactivation mechanisms. One of them involves a site-specific cleavage of an Ig molecule at the hinge region with the aid of an extracellular protease produced by the bacteria.

Although there are differences in their catalytic mechanisms, all reported IgAses fall into one of three types: serine-, cysteine-, or metallo-proteases [42]. The IgAse from *N. gonorrhea* has been classified as a serine protease [43]. A comparison between the gene and amino acid sequences of *N. gonorrhea* and *H. influenzae* [44,45] showed that they are 50% identical and contain a fingerprint region for the chymotrypsin/trypsin family of serine proteases [42]. The most conserved sequence,
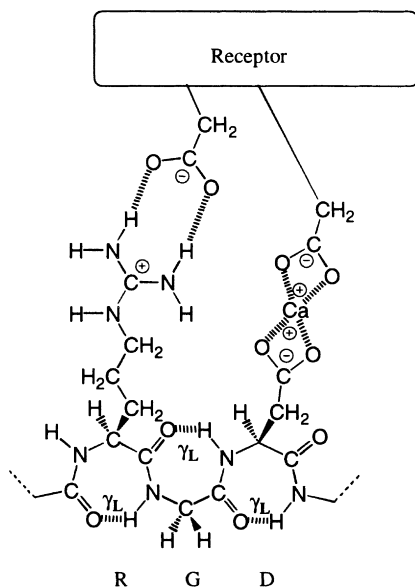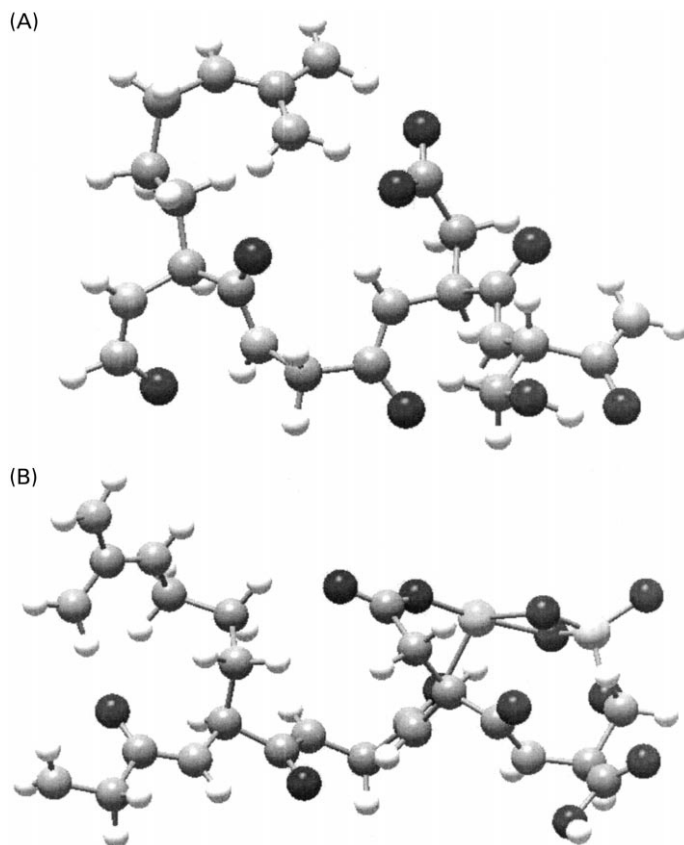
Fig. 26. (A) An internal salt-bridge forming conformer of RGDS(β-OH). Conformer optimized at the HF/3-21G level of theory. (B) An Mg^{2+} complex of RGD (β-OSO3-). The conformer shown was optimized at the HF/3-21G level of theory.

GDSGGPL (S = active serine site) is found in the vicinity of Ser 250 in *N. gonorrhea* IgAse.

There is an equal amount of anionic forming (Glu, Asp, Tyr) and cationic forming (Arg, Lys, His) amino acid side chains present [44]. Based on the nature of the inhibitors, there would be reason to believe that these oppositely charged moieties are not uniformly distributed in the tertiary structure. Instead, it is likely that this enzyme contains concentrated areas of positive charge that would enable the IgAse to seek out substrates that are negatively charged **XXII**. The fact that serine proteases contain histidine at the active site suggests that the active site of the enzyme will also be positively charged.
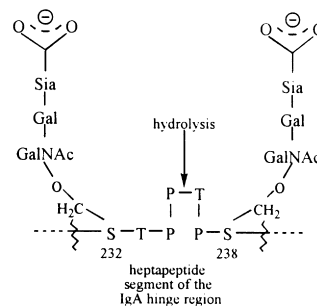
Table 5
Summary of physiological effects of GRGDS(β-OH) and GRGDS(β-OSO$_3^-$) in the absence and in the presence of Ca ion

Biological phenomena	[Ca^{2+}]	Peptide sequence	
		GRGDS(β-OH)	GRGDS(β-OSO$_3^-$)
Platelet aggregation	Not present	Active	Active
Vasodilator effect	Present	Very active	Not active
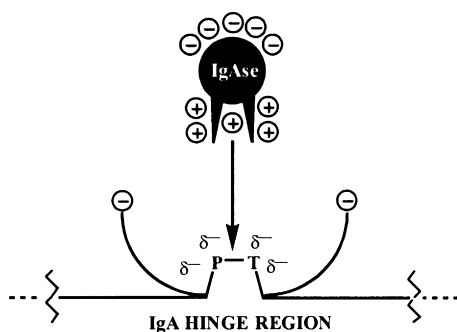
XXII

XXIII

There have also been inhibition studies done on short peptide segments of the hinge region [46], such as Pro-Pro-Thr-Pro, as well as longer ones and these have been found to act as mild inhibitors. They exhibit IC_{50} values in the micro-molar $(10^{-6}$ M$)$ range or higher. Thus, they are weak inhibitors when compared with the ideal nano-molar $(10^{-9}$ M$)$ range values expected for a drug candidate.

Successful drug candidates, therefore, may need to mimic the heptapeptide segment of the hinge region containing the negative charges of the two sialylic acid moieties **XXIII**. The structure must be appropriately folded and should not exceed a certain molecular weight. Because bacteria became resistant to most antibiotics such an inhibitor may become a successful lead compound for the development of several possible antibacterial agents. While these target oriented research projects may be laudable nevertheless it is lamentable whether we have enough information to search for these highly desirable compound. What is needed is an extensive database, which includes peptide, cyclo-peptide, and peptidomimetic conformations.

To date, neither X-ray structure for the IgA hinge region nor for the IgA protease produced by *N. gonorrhea* are available. Until such structures become available, we can carry out some necessary conformational structure analyses. With the aid of the enzyme structure, the drug design process may proceed at an accelerated pace. The most obvious place to start is with the tetrapeptide Pro-Pro-Thr-

Pro. The scission occurs at the Pro-Thr peptide bond and we have included at least the nearest neighbours at each end (XXII).

Clearly, this Pro-Pro-Thr-Pro tetrapeptide is more complicated than Ala-Ala-Ala-Ala [40] because Pro may exhibit *cis*- and *trans*-isomerism in its peptide bonds, as well as *syn*- and *anti*-ring puckering. Thus, $2 \times 4 = 4$ isomeric forms may exist for each backbone conformer. Up to three backbone conformers $(\gamma_L, \epsilon_L, \alpha_L)$ may be expected; thus each proline moiety may have $4 \times 3 = 12$ conformations (Table 6). Consequently, for the dipeptide Me-CO-Pro-Pro-CO-NHMe, we may anticipate $12 \times 12 = 144$ conformations. However, even if one disregards ring puckering and considers only *trans*-peptide bonds, Pro-Pro-Thr-Pro may exhibit $3 \times 3 \times 9 \times 3 = 243$ backbone conformations. Including the $3 \times 3 = 9$ side chain conformations of the Thr side chain moiety, this will lead to a total of 243×9 or 2187 conformers. Thus, the 243 backbone conformations may be best studied on Pro-Pro-Ala-Pro and the Ala side chain may be extended to a Thr side chain subsequently. One of the 2187 conformers has been optimized in our preliminary study and the structure is shown in Fig. 27. The central Pro-Thr structure turned out to be $\gamma_L \gamma_D$, which has been previously identified as a new type of β-turn [36]. It is interesting to note that several of the oxygen atoms occupy a region of the space creating an electron-rich domain. Such a display of high electron density may play a role in the docking of the PPTP moiety within the hinge into the cavity of the IgAse.

Table 6
Side chain dimensionality of selected representative compounds and their corresponding amino acids—excluding methyl-group rotation (proline and glycine have no dimensionality; alanine has methyl rotation dimensionality only)

Model compound		Amino acid residue	
Dimensionality	Structure	Compound	Dimensionality
0	$CH_3-CH-(CH_3)_2$	Valine (Val)	1
1	$CH_3-CH_2-CH-(CH_3)_2$	Leucine (Leu)	2
1	$CH_3-CH(CH_3)-CH_2-CH_3$	Isoleucine (Ile)	2
1	CH_3-CH_2-Ph	Phenylalanine (Phe)	2
1	CH_3-CH_2-COOH	Aspartic Acid (Asp)	2
1	$CH_3-CH_2-CONH_2$	Asparagine (Asn)	2
1	$CH_3-CH_2-C=CH-NH-CH_2=N:$	Histidine (His)	2
1	CH_3-CH_2-she	Selnocysteine (Sec)	2
1	CH_3-CH_2-SH	Cysteine (Cys)	2
1	CH_3-CH_2-OH	Serine (Ser)	2
1	$CH_3-HCOH-CH_3$	Threonine (Thr)	2
1	$CH_3-CH_2-Indole$	Tryptophan (Trp)	2
2	$CH_3-(CH_2)_2-S-CH_3$	Methionine (Met)	3
2	$CH_3-CH_2-Ph-OH$	Tyrosine (Tyr)	3
2	$CH_3-(CH_2)_2-COOH$	Glutamic Acid (Glu)	3
2	$CH_3-(CH_2)_2-CONH_2$	Glutamine (Gln)	3
3	$CH_3-(CH_2)_4-NH_3^{(+)}$	Lysine (Lys)	4
3	$CH_3-(CH_2)_3-NH-C-(NH_2)_2(+)$	Arginine (Arg)	4

6. Cyclopeptide conformations for $C_6H_9N_3O_3$ and $C_8H_{12}N_4O_4$

Cyclopeptide structures **XXIV** have not been studied as extensively as their open chain counterparts. Once the ring size increases, it is more difficult to optimize all stable conformations in order to explore the conformational space of these types of cyclic molecules. Ab initio MO (HF/STO-3G, HF/3-21G, HF/6-31G**) and DFT/6-31G** (using B3LYP functional) computations are to be carried out on various cyclopeptides $(C_2H_3NO)_n$ for $3 \leq n \leq 4$. All-*cis*-CONH and all-*trans*-CONH structures were considered. Typical optimized structures are shown in Figs. 28 and 29.
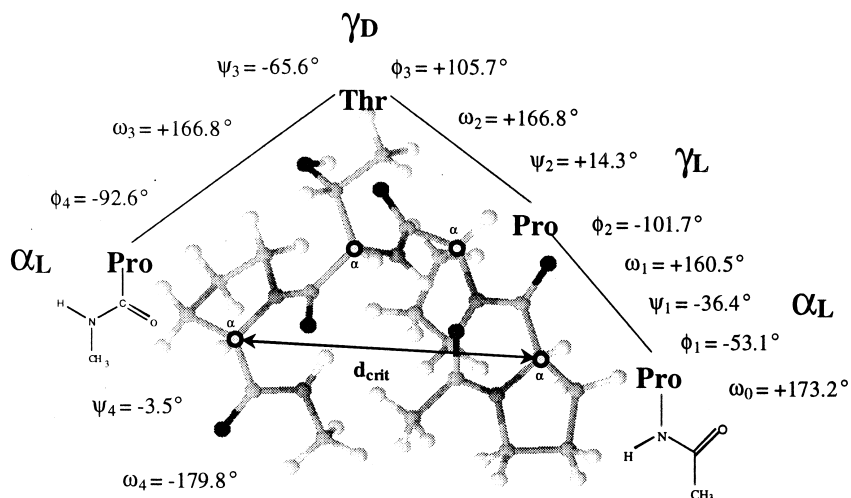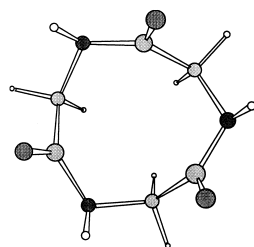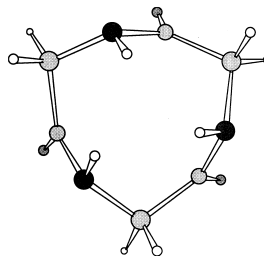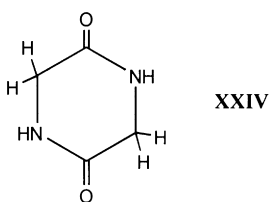

Fig. 27. An optimized structure of the tetrapeptide Pro-Pro-Thr-Pro (PPTP).

Conformer no. 1

Conformer no. 2

Fig. 28. Conformers of all-*cis*-cyclotriglycine and all-*trans*-cyclotriglycine.

XXIV

For small rings, such as diketropyperazine $(C_2H_3NO)_2$, **XXIV**, only *cis*-peptide bonds are allowed. As the ring size increases, the ring will tolerate *trans*-peptide bonds although for cyclic triglycine $(C_2H_3NO)_3$, the ring is very strained. The situation is somewhat better of cyclotetraglycine $(C_2H_3NO)_4$.

7. Conformational analysis of simple peptidomimetics

7.1. Dehydroalanine (Dha or ΔAla)

The α,β-dehydroamino acid residues occur widely

in various classes of natural and synthetic drugs, although they are most commonly found in classes of antibiotics such as "lantibiotics" (lanthionine containing gene-encoded peptides with antibiotic properties such as nisin [47,48], subtilin [47,49] and ancovenin [47]) and the thiopeptide antibiotics [50,51] berninamycin A, B, C and D. They are also increasingly used in the synthesis of enkephalin analogues having different potencies and selectivities from the parent compound [52–54].

The simplest α,β-dehydroamino acid is ΔAla Fig. 30, having no substituents on its beta carbon. Because it is the only unsubstituted α,β-dehydroamino acid, its experimentally observed conformations differ significantly from those of the other dehydro residues [55,56]. In this sense, ΔAla is reminiscent of glycine, which also has very different properties from the rest of the naturally occurring amino acids, due to its lack of a side chain [16,17].

Through ab initio calculations, it has been found that *trans*-For-ΔAla-NH$_2$ can take on seven (β_{DL}, δ_D, δ_L, ϵ_D, ϵ_L, γ_D and γ_L) of the nine theoretical conformations of a

Conformer no. 1

Conformer no. 2

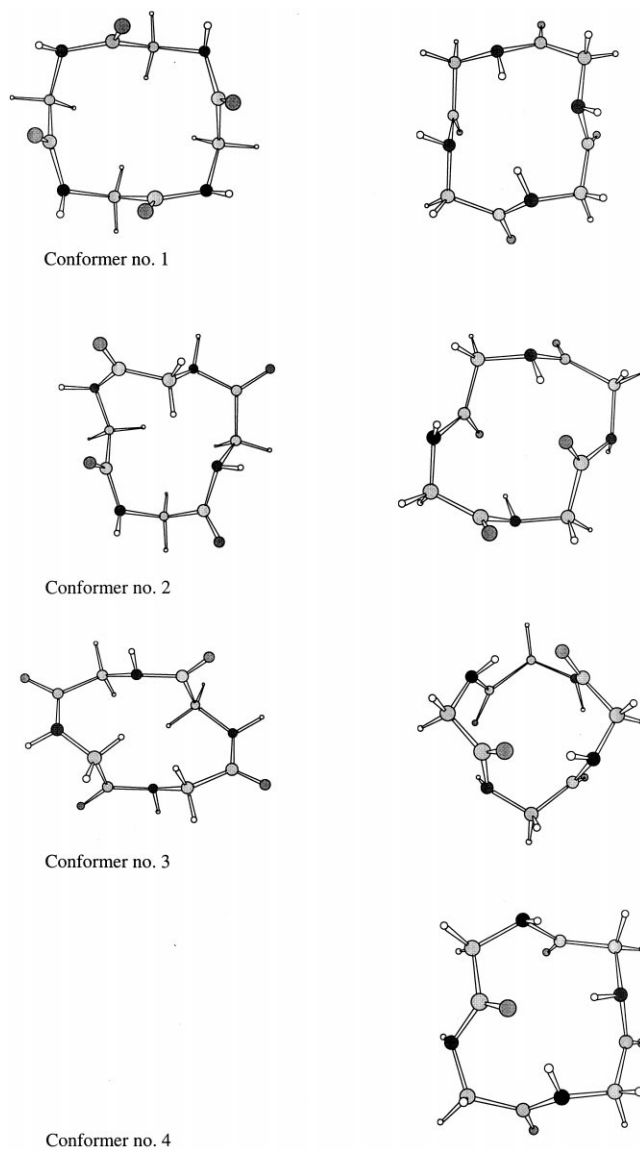Conformer no. 3

Conformer no. 4

Fig. 29. Conformers of all-*cis*-cyclotetraglycine and all-*trans*-cyclotetraglycine.
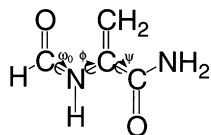
Fig. 30. Torsional angles ω_0, ϕ and ψ of N- and C-protected 2,3-didehydroalanine. ω_0 is approximately 180° for *trans*-2,3-didehydroalanine.

saturated amino acid residue Fig. 31, whereas, even N and C protected glycine, can actually only take on five.

Furthermore, it has also been found, by means of relative and stabilization energies, that although ΔAla is more rigid than glycine Fig. 32, it has a much greater stabilizing effect on the peptide chain as shown in Fig. 33. In fact, ΔAla has a greater stabilizing
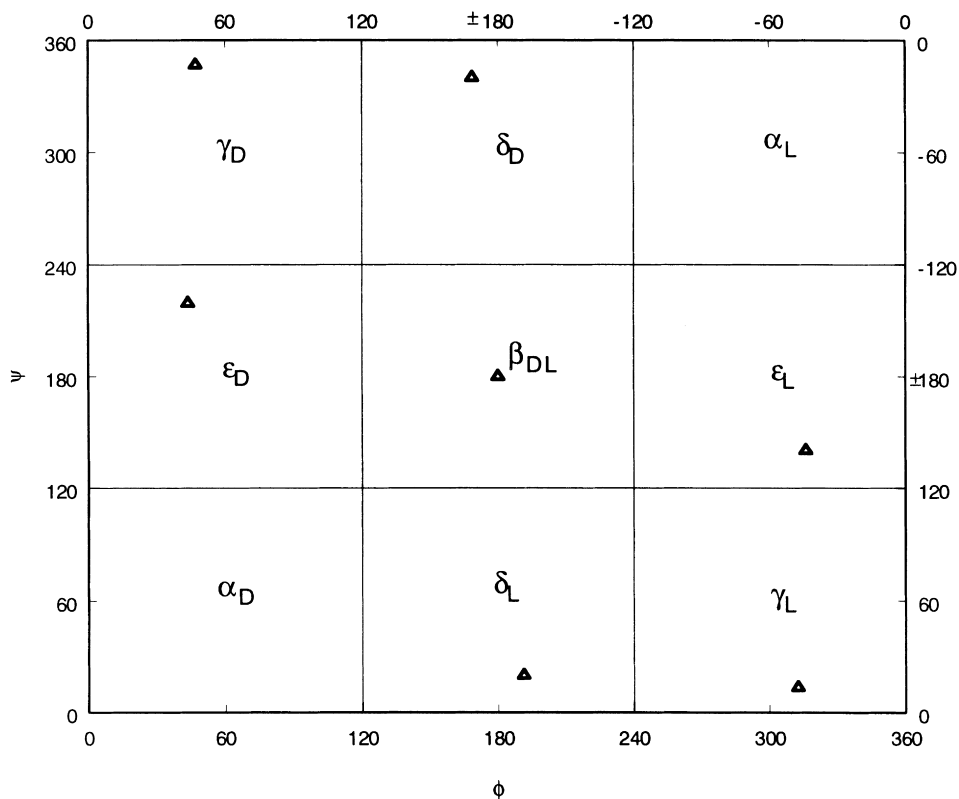
Fig. 31. The seven stable conformations of N- and C-protected *trans*-2,3-didehydroalanine as computed at B3LYP/6-31G*.
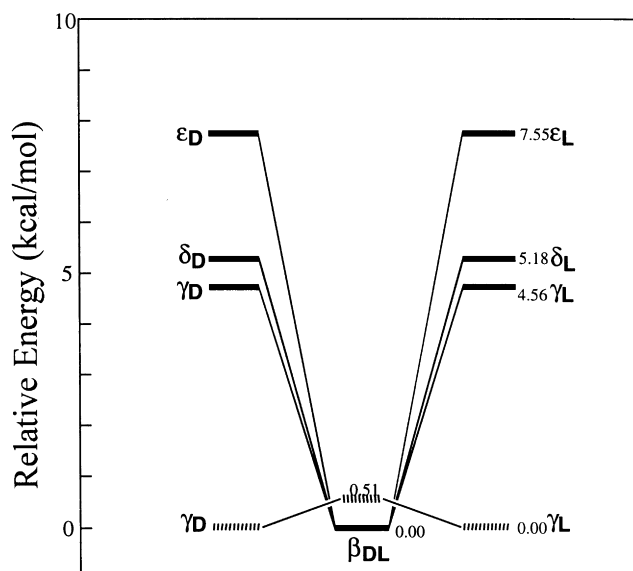

Fig. 32. Relative energies of the various conformations of N- and C-protected *trans*-2,3-didehydroalanine (solid line) and *trans*-glycine (dashed line) computed at B3LYP/6-31G* level of theory.
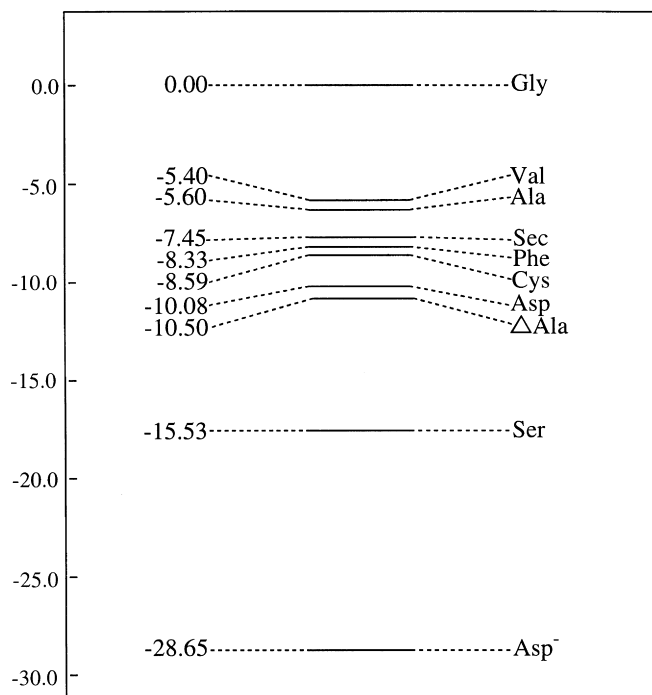
Fig. 33. Relative stabilization energies of various side chains for *trans*-For-CHR-NH$_2$, calculated according to the equation given at the top of Fig. 17. The most stabilizing conformer of *trans*-For-ΔAla-NH$_2$ is the β$_{DL}$ one and hence its stabilization energy, instead of that of the γ$_L$ conformer, is given. This value was calculated according to the following isodesmic equation: HCONH–CH$_2$–CONH$_2$ + H$_2$C=CH$_2$ → HCONH–C(=CH$_2$)–CONH$_2$ + CH$_4$.

effect than most of the saturated residues examined so far, except for serine and deprotonated aspartic acid [57]. These results can have great implications for the future of peptide based drug design.

7.2. Chiral pseudo-peptide and selected derivatives

Due to their newly discovered pharmacological properties, pseudo-peptides are able not only to substitute peptides, but also essentially improve their pharmacological action [58–63]. In order to assist the search for new compounds with desired pharmacological properties, conformational proper-

ties of three model pseudo-peptides were investigated by semi-empirical and ab initio MO methods. The three models include the protonated form of *N*-formyl pseudo-alaninamide HCONH–CHMe–CH$_2$NH$_3^+$, *N*-formyl pseudo-alanine itself HCONH–CHMe–CH$_2$NH$_2$ as well as the formylated *N*-formyl pseudo-alanine (i.e. propylene-di(*N*-formylamine)) HCONH–CHMe–CH$_2$NHCOH [64]: **XXV**, **XXVI**, **XXVII**.

For compound **XXV**, which is an *N*-protonated pseudo-peptide derived from *N*-formylalaninamide, both *trans*- and *cis*-configurations were considered (i.e. ω = 180° and 0°).
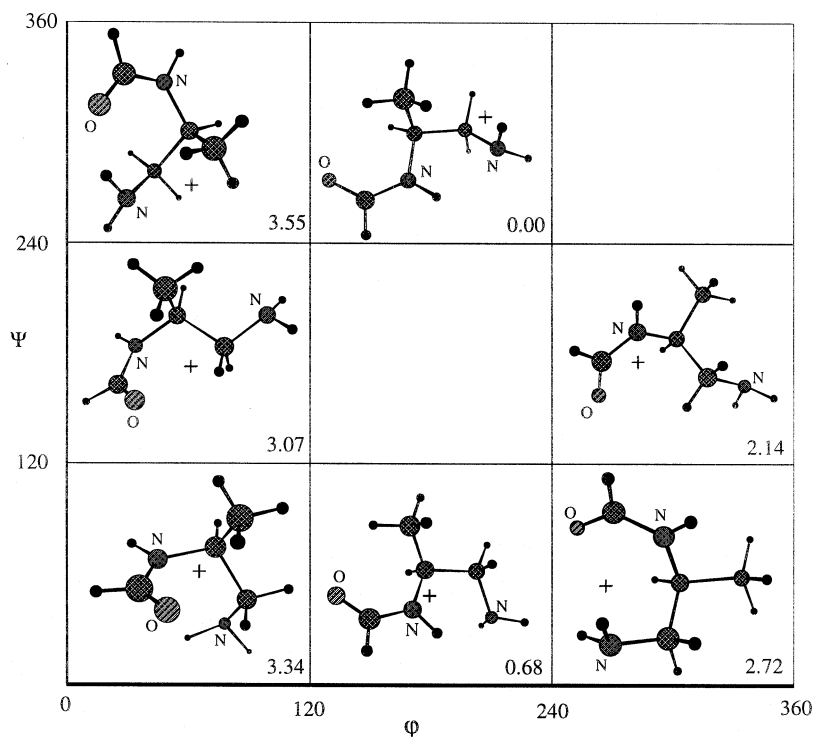

XXV   **XXVI**   **XXVII**
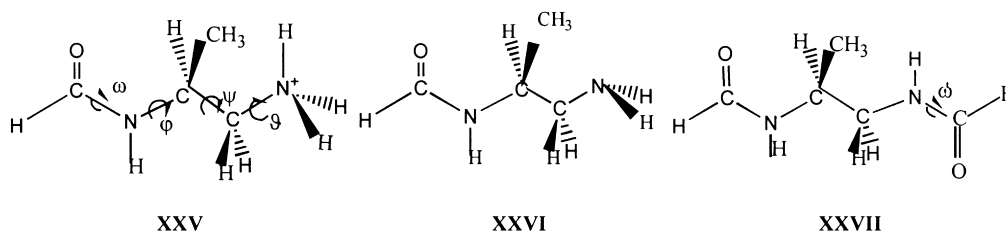
Fig. 34. Topology of a Ramachandran-type PES of the simplest chiral pseudo-peptide derived from *N*-formylalaninamide.

Taking into the account the dominant role of the peptide backbone, it was important to assess how the backbone changes under the transition from peptides to pseudo-peptides. To characterize the backbones for compounds **XXV** and **XXVI**, four dihedral angles were chosen, namely ω, φ, ψ, θ. In addition, compound **XVII** was characterized by a fifth dihedral angle ω. The conformations of the simplest pseudo-peptides were conveniently described by a 2D-Ramachandran map, i.e. a 2D-conformational potential energy surface (PES). This surface was defined in terms of two dihedral angles, φ and ψ, varying between 0° and 360° [16,17,57,64–66]. To describe pseudo-peptide conformations, the 9 expected legitimate minima for model compounds **XXV**, **XXVI** and **XXVII** were carefully optimized.

Both semi-empirical [67] and ab initio [14] RHF SCF calculations were performed on typical backbone conformations. For ab initio calculations, the RHF/3-21G level of theory was used for all compounds under consideration. To check the validity of the results in some cases, calculations were performed on RHF/6-31G(d) level of theory. It was found that the geometries of pseudo-peptides in their global minima are determined by H-bonds.

According to the computational results, pseudo-peptides, in general, can mimic the backbone of the parent peptides, as shown by the experimental data [62,63]. Pseudo-alanine was shown to be more flexible, than the parent alanine. Fig. 34 shows the topology of the E (ϕ, ψ) PES which is analogous to the Ramachandran map.

8. Non-peptidic bioactive molecules:

8.1. Antioxidants

During the metabolism of foodstuffs, carbon is oxidized to CO_2 with the help of the inhaled oxygen. Parallel to that, O_2 is reduced through a multistep process to H_2O.

$$O_2 \rightarrow O_2^- \rightarrow\rightarrow H_2O_2 \rightarrow\rightarrow 2H_2O \qquad (15)$$
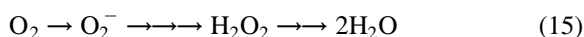
Somewhere along this line, hydroxy radical (HO˙) is released in small amounts. Due to the non-discriminative reactivity of (HO˙), it damages a number of key organic compounds in the human body. Among these are DNA, the damage of which leads to premature aging as well as numerous including cancer. Collectively, these processes are labeled as "oxidative stress" and can be relieved by free radical scavengers, called antioxidants, such as flavones, selenocysteine and lycopene. It has also been recognized recently that certain drugs can act as antioxidants.

8.1.1. Flavone conformations

Flavone is the parent molecule of a broad range of compounds belonging to the family of flavonoids. Flavonoids are polyphenolic compounds, which occur as yellow pigments in plants. They are believed to be effective against HIV, cancer, coronary artery disease and aging [68].

Conformational analyses were performed on flavone and three other related compounds, namely 2-phenyl pyranone, β-phenyl naphthalene and biphenyl. In particular, the phenyl rotation with respect to the rest of the molecule was studied using Hartree–Fock (HF) calculations performed on Gaussian 94. The basis sets employed were STO-3G and 3-21G. It was concluded that the hydrocarbons (biphenyl and β-phenyl naphthalene) are noticeably different from oxygen containing heterocyclic analogues such as 2-phenyl pyranone and flavone, as far as phenyl rotation is concerned. One would also expect a difference in their reactivity towards free radicals. It seems that only the oxygen heterocyclic analogues can be reactive in nucleophilic and radical attack because they contain positively charged atoms, as shown by computed Mulliken charges. Nevertheless, the topology at the potential energy curves (number of minima and transitions structures) is the same for all four compounds.

The potential energy curve of flavone is shown in Fig. 35. The antioxidant mechanism of flavone skeleton is now under investigation.

8.1.2. Selenocysteine as antioxidant

Selenocysteine is involved in a variety of biochemical interactions and has recently been considered as the 21st amino acid used by nature for RNA directed protein synthesis [69,70]. Several selenoproteins containing selenocysteine at their active sites have been identified using neutron activation analysis. At the end of the 20th century, possibly 28 of these proteins are known to exist. At present, however, we know very little about the structure and function of most of these proteins. Those that have been characterized and for which functions are known include the following:

(a) Four species of glutathione peroxidase [71–73] (cellular or classic, extracellular or plasma, gastrointestinal and phospholipid hydroperoxide glutathione peroxidase) have been identified. Glutathione (GSH) peroxidase catalyses (Fig. 36) the reduction of a variety of hydroperoxides including lipid hydroperoxides (which, in the presence of trace metals, can form toxic lipid radicals). This mechanism is believed to protect biomembranes and other essential cellular components against oxidative challenge [74–76], cancer [77] and gastric ulceration [78].

(b) Selenoprotein P is an extra-cellular protein presumably containing 10 Selenocysteine residues that are encoded by the UGA stop codon in the open reading frame of the mRNA. Some indirect evidence suggests that selenoprotein P acts as a free radical scavenger [79].

(c) Selenoprotein W is found in muscles [80].

(d) Iodothyronine deiodinase catalyzes the de-iodination of thyroxine to the biologically more active thyroid hormone triidothyronine [81].

(e) Thiroredoxin reductase catalyzes the NADPH-dependent reduction of the redox protein thioredoxin. Thioredoxin stimulates cell growth and is over-expressed in a number of human cancerous cells [82,83].

(f) Mitochondrial selenoprotein [84] was localized in the mitochondrial membranes of endocrine organs.

(g) Prostatic selenoprotein and testicular selenoprotein were found in spermatid nuclei. Recent findings suggest that it takes part in the process of replacing and condensation of the DNA, which occur in the spermatid nuclei, and thus may have an important function in sperm development [85,86].

In addition to the above, new evidence, based on sequence analysis, suggests that HIV-1 encodes a
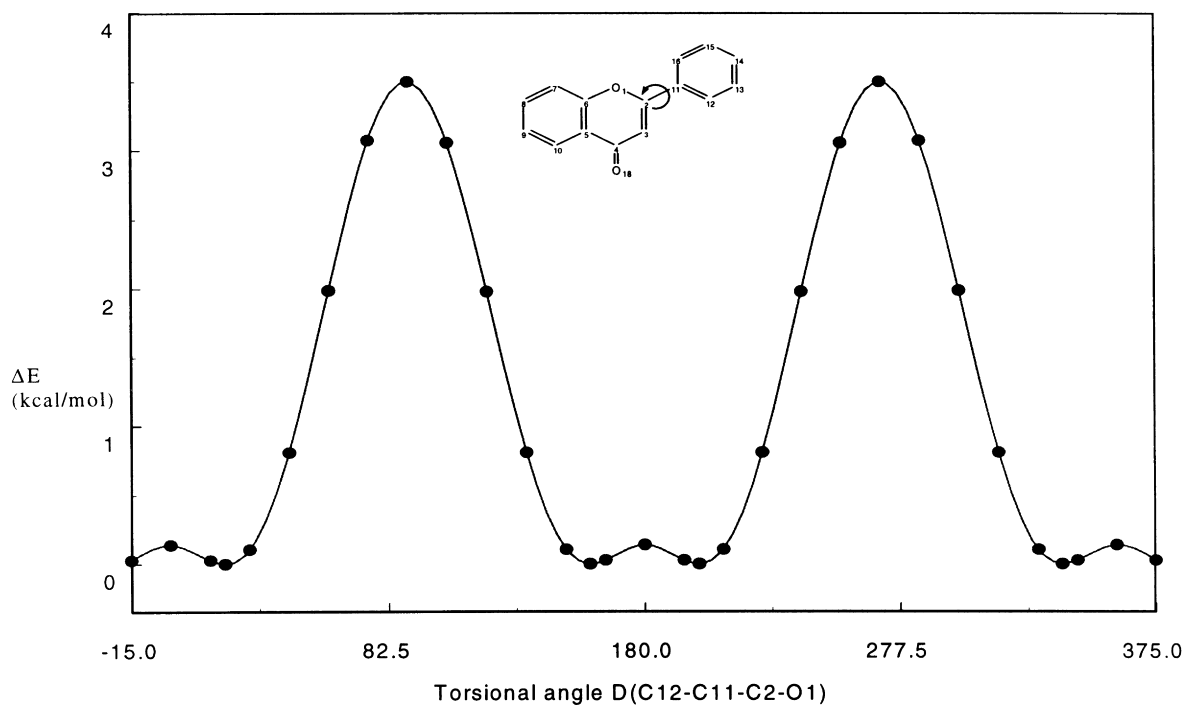
Regular Optimization		
RHF		
	STO-3G	3-21G
Energy (hartree)	-714.5491401	-719.5533998
Deviation from coplanarity (degrees)	20.82	8.84

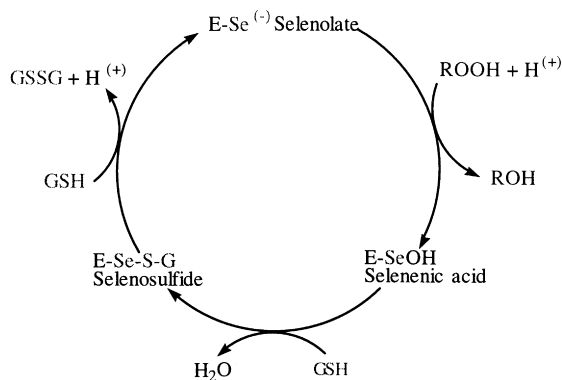Fig. 35. Phenyl torsional potential of the flavone skeleton.

Fig. 36. Putative catalytic mechanism of glutathione peroxidase.

protein containing selenocysteine (Sec) [87–89]. It has been observed previously that plasma selenium and glutathione levels are subnormal in HIV-infected individuals [90] and that specifically, four ^{75}Se-containing proteins are lower in HIV-infected cell populations than in uninfected cell populations.

8.1.3. Lycopene and free radicals

Lycopene is a very important free radical scavenger [91,92]. It is a polyene with 15 conjugated double bonds. There are numerous cis- and trans-isomers of lycopene (c.f. Fig. 37). The all trans isomer is the predominant species in tomatoes and tomato products (≈95%). The 5 cis-, 13 cis- and 9 cis- lycopene are

all-*trans* lycopene

15-*cis* lycopene
13-*cis* lycopene
11-*cis* lycopene
9-*cis* lycopene
7-*cis* lycopene
5-*cis* lycopene
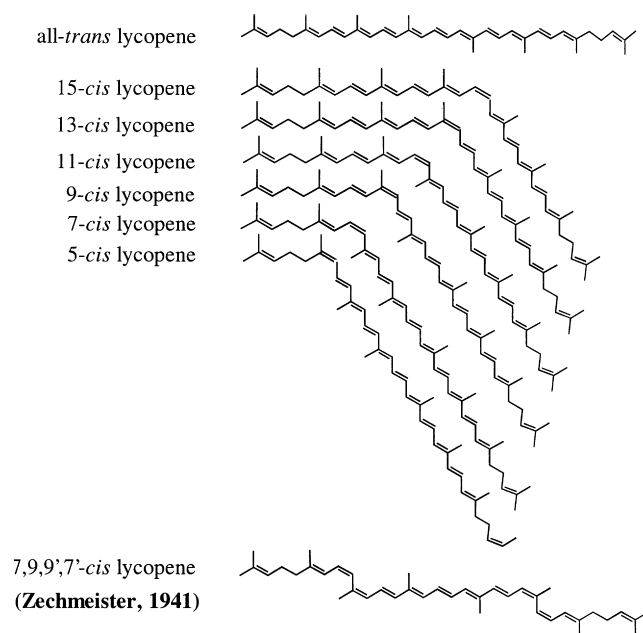
7,9,9',7'-*cis* lycopene
(Zechmeister, 1941)
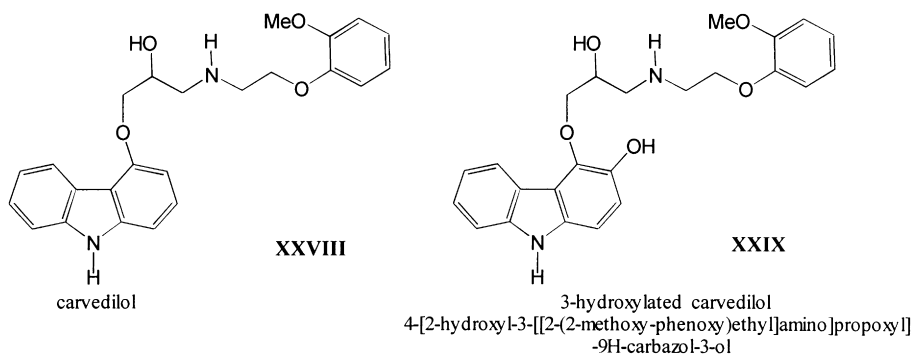
Fig. 37. Geometrical isomers of lycopene.

found in human serum, making up approximately half the total lycopene content. Isomers with multiple *cis*-double bonds are also known. For example 7,9,9$'$,7$'$ *cis*-lycopene is the naturally occurring form of lycopene in fresh Tangerine-type tomatoes is also shown in Fig. 37.

Two isomers of 1,3,5 hexatriene were used to model *cis*- and *trans*-lycopene. The central double bond (Δ [4–6]) of hexatriene was used in the *cis*- and *trans*-forms. For the computational convenience, free radical fluorine atom (˙F) was used instead of the biologically more important hydroxy radical (˙OH).

Through ab initio calculations, using ROHF/6-31++G(d,p) and UHF/6-31G(d,p) methods, it has been found that if C2 or C3 of the 1,3,5 hexatriene is fluorinated, both the *trans* and *cis* isomers have the same relative energies. On the other hand, fluorinating C1 makes a difference of 3.7 kcal/mol between the *trans* and the *cis* isomer, the *trans* isomer being more stable Fig. 38.

8.1.4. Carvedilol as a new antioxidant

The Merck Index recognizes carvedilol (**XXVIII**) as a nonselective β-blocker with vasodilating activity,

XXVIII

carvedilol

XXIX

3-hydroxylated carvedilol
4-[2-hydroxyl-3-[[2-(2-methoxy-phenoxy)ethyl]amino]propoxyl]
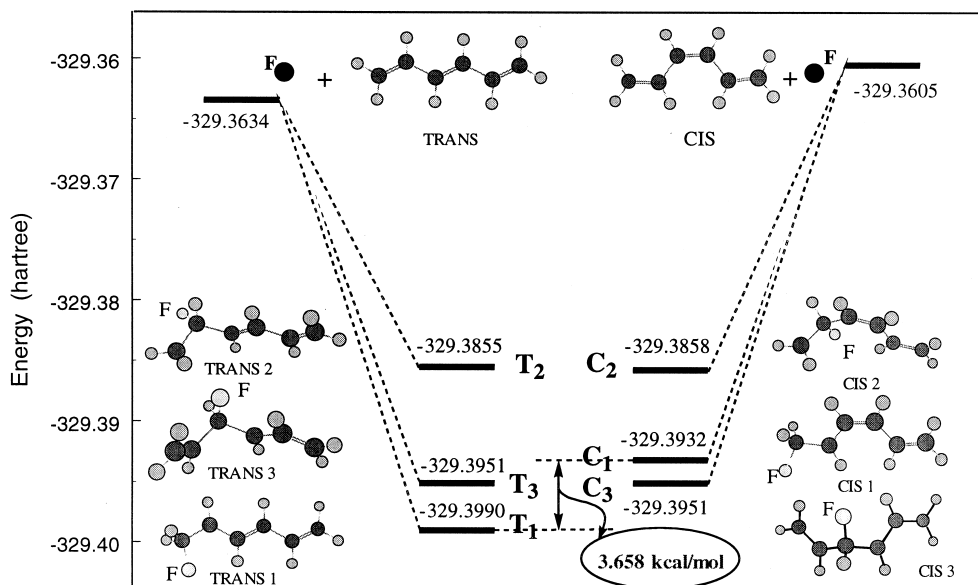-9H-carbazol-3-ol

LYCOPENE MODEL

Fig. 38. Relative energies of radical addition products to lycopene models computed at RHF/3-21G level of theory.

which is mediated by its antagonizing effect on α-adrenoceptors. It was firstly patented in Germany in 1979 and subsequently in the US in 1985.

Carvedilol has been used in the treatment of hypertension [93], angina pectoris and congestive heart failure. In these fields, carvedilol is at least as effective as reference drugs (metoprolol [94], enalapril, verapamil [95]) but its side effect profile is better, and it has more beneficial effects.

According to clinical trials, carvedilol effectively reduced the morbidity and mortality rates in-patients suffering from chronic heart failure to such an extent that the US Data and Safety Monitoring Board stopped, for ethical reasons, the investigation before its completion [96]. This was the first time in the history of drug investigations that a clinical trial was stopped because the drug was too effective. It seemed unethical to deprive the medication from placebo group. Carvedilol also reduces the frequency of reversible myocardial ischaemic events after thrombolysis [97] and prevents cardiac remodeling in-patients suffering from left ventricular dysfunction after acute

myocardial infraction [98]. This latter effect has been attributed to its scavenging action of oxygen free radicals and preventing the lipid peroxidation [97]. These reactive radicals are implicated in the process of programmed cardiac cells death (apoptosis) [99] and the concomitant loss of myocardial cells leading to progressive decrease of left ventricular mass and function [100].

Originated from this antioxidant property, carvedilol may also prevent the development of nitrate tolerance in, patients receiving continuous nitrate therapy [101,102].

Apart from carvedilol, at least one of its 3-hydroxylated metabolites (**XXIX**) has marked antioxidant properties. The metabolite is approximately a thousandfold more effective than vitamin E in certain experimental setups and more effective than carvedilol itself. Although the exact antioxidant mechanism of these compounds is not known, recent structure activity relationships indicate that its activity resides in the carbazol ring. A computational mechanistic study on this exceptional antioxidant capability is now in progress.

8.2. Medicinal chemistry and drugs

Most of today's marketed medications were found by chance or by systematic screening of large collections of compounds (called "libraries") either from natural or man-made sources. Current emphasis on high-throughput screening and combinatorial chemistry suggests that an educated guess is pivotal for the discovery of tomorrow's drugs. Computer assisted molecular modeling (CAMM) is a relatively new and rapidly developing tool in drug design. Computer graphics techniques allow the transformation of complex data sets, obtained e.g. from theoretical chemical calculations or X-ray diffraction patterns, into an image on a computer screen. Chemical structures and their properties may thus be visualized, manipulated, and matched or combined with other relevant molecules. The availability of the three-dimensional architecture of HIV protease, thymidylate synthase, thrombin, carbonic anhydrase, and many more has led to at least an equal number of new chemical entities being evaluated and used now in clinics. In such cases, CAMM has certainly led to a more rational approach toward drug discovery, in particular when the binding domains for inhibitors and substrates are known from cocrystallization experiments. Knowing the "lock" definitely helps in designing the "key", although the interaction between newly synthesized ligands and the target macromolecule may appear sometimes unexpectedly.

Locking into certain stereochemically important conformations is sometimes achieved by heavy substitution, such as o,o' difluorophenyl groups. This principle has been applied in one of the anti-HIV drugs where o,o' difluoride substitutions are used [103].

XXX
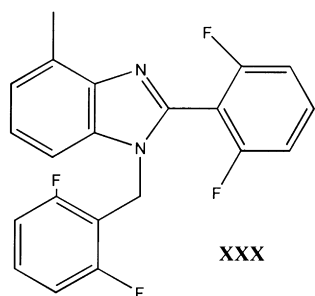
Calculations of the barriers and associated one-dimensional torsional potential are performed for the internal rotation of the nitro-group in nitrobenzene as well as for o-fluoro- and o,o'-difluoro-nitrobenzenes [104]. This structure is related to certain drugs, such as the one shown in **XXX**, that are under investigation. It is shown that the presence of substituents in ortho-positions forces the benzene ring to rotate about the C–N bond, out of the plane of the nitro group. In these conjugated molecules, the inclusion of electron correlation is shown to be necessary for reliable barrier prediction. The potential curves at HF level are shown on Fig. 39. Corresponding MP2 values are given by " + " symbol. It is found that the π-stabilization (resonance) is of relatively small contribution to the structure of the nitroaromatics, whereas the steric repulsion between the ortho-substituents and oxygen atoms of the NO_2 group is rather significant. The interplay between them accounts for the fact that for o,o'-$C_6H_3F_2NO_2$, the barrier height at $0°$ is larger than that at $90°$, when electron correlation is included. The values for the barrier at $0°$ were 2.67 and 3.81 kcal/mol computed without and with electron correlation and the corresponding values at $90°$ were 1.42 and 0.91 kcal/mol, respectively.

A fairly large area of drug design is related to G-protein coupled receptors. A preliminary structural analysis of the β_2 adrenergic G-protein coupled receptor was considered. This receptor is a transmembrane protein, and it usually consists of seven bundles of α-helices. Each of these seven helices consists of approximately 21–24 amino acids, as required to span the cell membrane. In order to determine the type of interaction between the helices, an α-helix made up of seven alanine residues was built using HYPERCHEM. Two other helices were built in the same manner, except one of the amino acids was changed to serine and tryptophan respectively. It was found that amino acids arrange so that many of the polar amino acids can face the cavity and non-polar amino acids can face away from the cavity. This is expected because a hydrophobic lipid membrane surrounds the outer circumference of the bundle of seven helices. Interactions between residues of a helix such as that between tryptophan and serine are practically thermo-neutral. Interaction between the Ala-Ala helices has a stabilization energy of -3.0 kcal/mol, while that between the
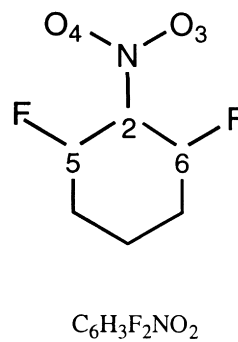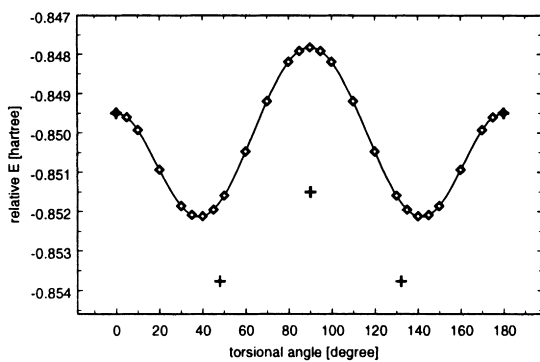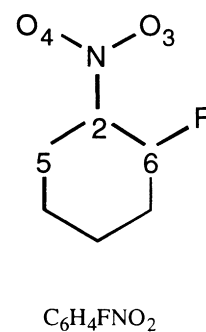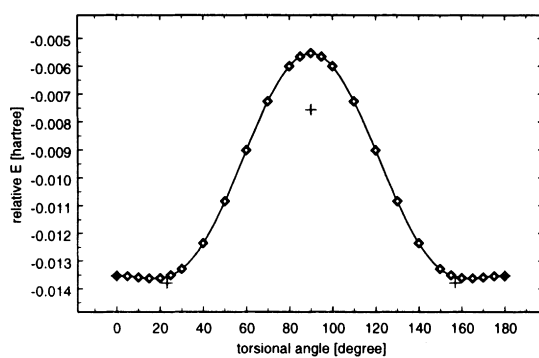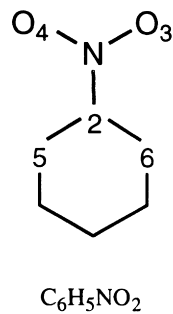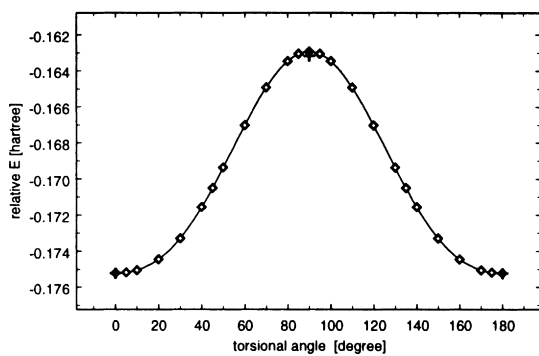
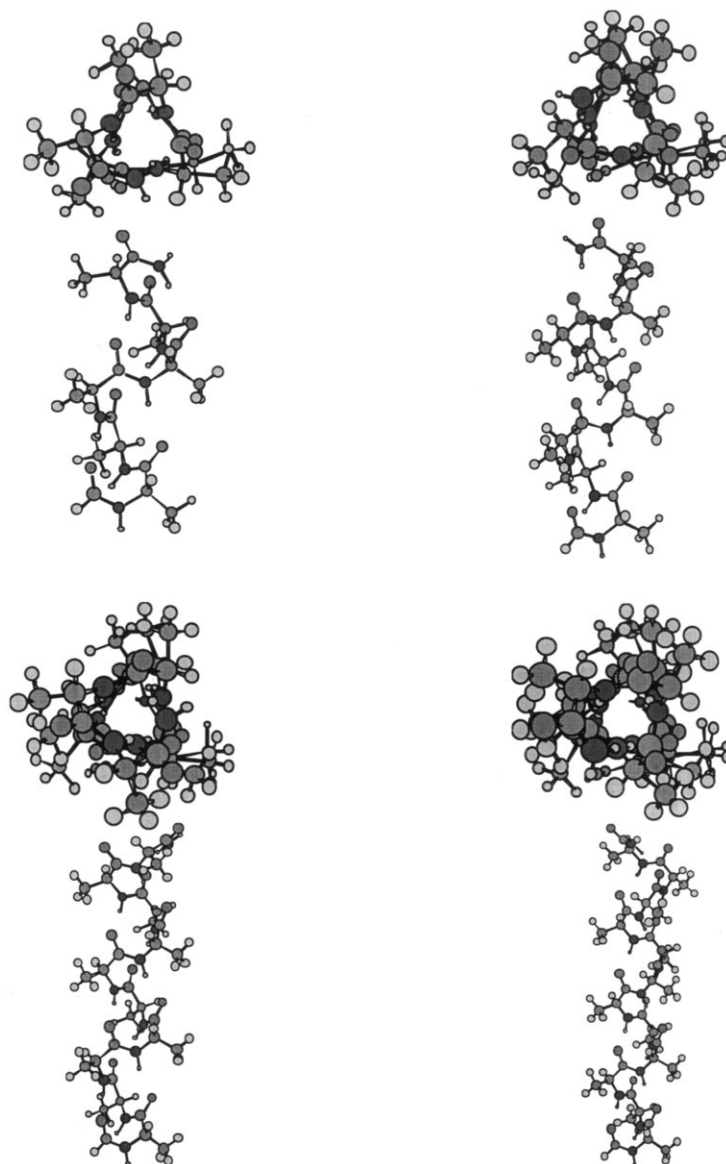Fig. 39. Phenyl rotation in nitrobenzene, o-fluronitrobenzene and o,o′-difluronitrobenzene.

Fig. 40. Helical structure of N- and C-protected polyalanines HCO-(Ala)$_n$-NH$_2$ ($n = 6, 8, 10, 12$).

Ser-Ser helices has a stabilization energy of -8.2 kcal/mol [105]. The interaction between two serines result in a greater stabilization energy than that of the two alanine residues interacting. Thus, replacing the alanine with serine resulted in larger stabilization energy but replacing serine with tryptophan did not significantly change the stabilization energy. An ab initio study on the helical structure of For-(Ala)$_n$–NH$_2$ has been carried out for $n = 6, 8, 10$ and 12 (c.f. Fig. 40).

Cell cycle inhibitors or modulators that halt uncontrollable tumor growth are regarded as highly promising new therapeutic agents against human cancer. Certain sulfonamides [106] inhibit microtubule assembly owing to its reversible binding to the colchicine-binding site on tubulin. They also
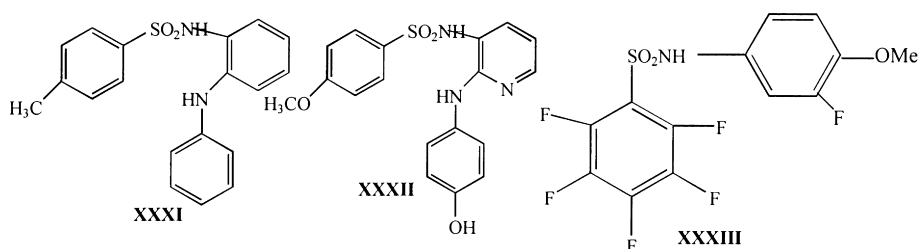
exhibit good in vivo antitumor activity against various rodent tumors and human tumor xenografts. The focus on making a sulfonamide compound library is based upon template **XXXI** ever since compound **XXXII** was found to inhibit cellular growth and mitosis in vitro, but not quite potent in vivo. In the design of this template, the sulfonamide moiety located between two aromatic rings was fixed as a basic motif, and the NH group at the *ortho* position of the sulfonamide was considered a key functionality for substantial antiproliferative activity in cell-based assays [106].

A substantially different aromatic sulfonamide (**XXXIII**) has also been reported [107] to interact with microtubules during cell division. Concerning the recently reported polyfluorodiarylsulfonamide (**XXXIII**), we are now involved in assessing its mechanism of action.

9. Multidimensional conformational analysis of allyl methyl disulfide: a key component of garlic

Garlic has been one of the most popular medically researched plants, with over 1300 research articles in only the last 100 years [108]. Organosulfur compounds in garlic, like allyl methyl disulfide, have been found to be involved in antimutagenic, anticarcinogenic, antithrombotic, and lipid-lowering activities, and it has also been found to act as an antioxidant. Ab initio molecular computations were performed on allyl methyl disulfide (**XXXIV**)
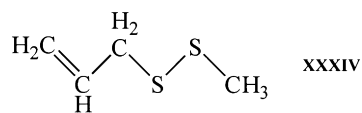

with respect to torsional angles $\tau_1 = \tau(H_3C_2 \cdot CH_2 \cdot S{-}S \cdot CH_3)$, $\tau_2 = \tau(H_3C_2 \cdot CH_2{-}S{-}S \cdot CH_3)$, and $\tau_3 = \tau(H_3C_2{-}CH_2 \cdot S \cdot S \cdot CH_3)$. Potential energy scans,

resulting in potential energy curves (PEC), were performed along **a**, **b** and **c** as shown in Fig. 40, at the HF/3-21G level of theory. All conformations were optimized at the HF/6-31G* level of theory and their energy values are provided in Fig. 41 as well. The potential energy hypersurface surface (PEHS) of **XXXIV**, i.e. $E = E(\tau_1, \tau_2, \tau_3)$, revealed six lower energy pairs of enantiomeric minima (i.e., $[g^+g^+g^+|g^-g^-g^-]$; $[g^+ag^-|g^-ag^+]$; $[g^+g^-g^+|g^-g^+g^-]$; $[g^+g^+g^-|g^-g^-g^+]$; $[g^+ag^-|g^-ag^+]$; and $[g^+g^-g^-|g^-g^+g^+)$ as well as three higher energy minima (i.e. $g^+g^+s|g^-g^-s]$; $g^+as|g^-as]$; and $[g^+g^-s|g^-g^+s]$) were optimized at $\tau_1 = \pm 90°$ using the two HF/6-31G* and B3LYP/6-31G* methods at this level of theory (Fig. 40). In our previous study on the multidimensional conformational analysis of ethyl benzene [109], we found that very often two minima result when a planar moiety is rotated against a tetrahedral moiety, one of them being higher (0° rotation) and the other being lower (90° rotation) in energy.

As illustrated in Fig. 41, it is apparently clear that the center of symmetry of the PEHS of **XXXIV** is at $\tau_1 = \tau_2 = \tau_3 = 180°$, i.e. at the fully *anti–anti–anti* orientation. Therefore, if an imaginary line can be drawn through this fully *anti* center from one energy platform to the other on the PEHS of **XXXIV**, then the structures along such a line are enantiomers. Conversely, if an imaginary line joining two conformers cannot be drawn through fully *anti* center, then the structures are diastereomers. Therefore, although there are no stereocentres in **XXXIII**, there is chirality in the conformational twist with respect to the fully symmetrical *anti-anti-anti* structure [*aaa*] through $\tau_1 = \tau_2 = \tau_3 = 180°$.

In order to denote the enantiomeric relationship of each pair of conformers, a convenient notation has been adopted, with the symbol "|" representing a

A

B
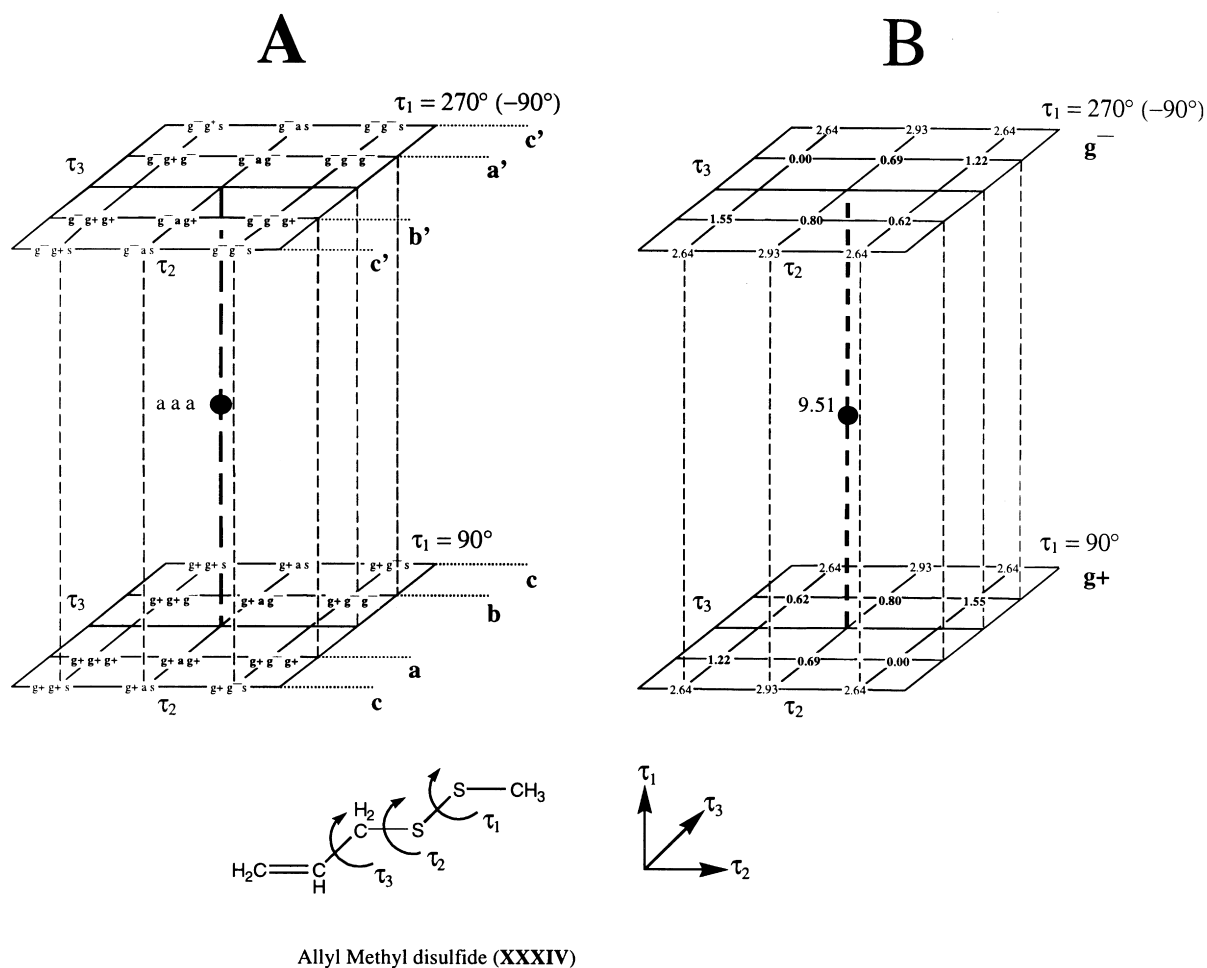
Allyl Methyl disulfide (**XXXIV**)

Fig. 41. Topology of the conformational the PEHS of **XXXIV** (allyl methyl disulfide), where $E = E(\tau_1, \tau_2, \tau_3)$. (A) The notation of the 6 pairs of lower energy conformers (along a, a$'$, b and b$'$), the 3 pairs of higher energy conformers (along c and c$'$) and the fully symmetrical *anti* structure. (B) The relative energies in kcal/mol of the 6 pairs of lower energy conformers, the 3 pairs of higher energy conformers, and the fully symmetrical *anti* structure. (All energy values obtained at the HF/6-31G* level of theory). Note that the centre of symmetry is denoted by a solid dot and corresponds to the $\tau_1 = \tau_2 = \tau_3 = 180°$, fully *anti* conformation (*a a a*) as shown in Fig. 42. [Cross-sections were studied at a,a$'$,b,b$'$,c and c$'$]. All conformations were optimized at the HF/6-31G* level of theory.

mirror between two enantiomers. The global minimum was determined to be the $[g^+ g^- g^+ | g^- g^+ g^-]$ conformer, and the fully symmetrical *anti* [*aaa*] conformer was determined to be a second order saddle point (Fig. 42) [126].

Based on the energies and MO diagrams of **XXXIV**, the HOMO and LUMO + 1 orbitals were determined to be involved in electron donating and accepting activity of **XXXIV**. The putative anti-carcinogenic and cholesterol lowering mechanism of activity of **XXXIV** is presented in Fig. 43 [126].

10. Macromolecular interactions

10.1. Protein–protein interactions

Molecular recognition forms the basis of protein–protein interactions. Proteins that contain covalently bound oligosaccharides, called glycoproteins, are often tailored to be specific recognition sites. They form a diverse group that includes enzymes, hormones structural proteins, and transport proteins.

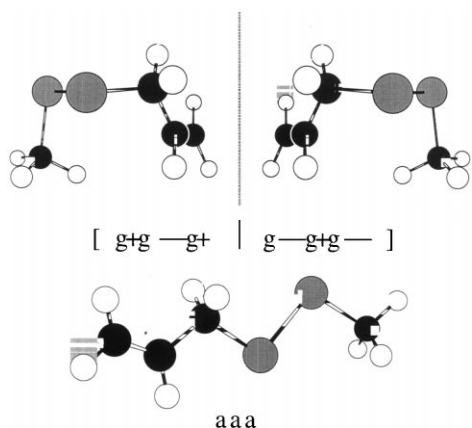The presence of one or more oligosaccharide chains

Fig. 42. Structures of the degenerate $g^+g^-g^+$ and $g^-g^+g^-$ conformers ($\lambda = 0$), and the fully symmetric $a\,a\,a$ conformer ($\lambda = 2$). Note that the global minima conformers, $g^+g^-g^+$ and $g^-g^+g^-$, are enantiomeric and that this relationship is denoted with the symbol "|", indicating a mirror plane.

on a protein can alter its physical properties, such as its size, solubility and stability. These changes can affect biological activities such as immunogenicity. A number of mammalian hormones are dimers of glycoproteins whose oligosaccharide chains assist in the assembly of the dimer. As a result, they confer resistance to proteolysis. Another role of glycoproteins is the recognition of cell by another cell in processes such as cell migration or oocyte fertilization. These events are dependent partly on the surface of one cell binding to the sugar portions of specific glycoproteins on another cell's surface. Studies of a wide range of lectins have shown that oligosaccharide chains of proteins are always located on the outer surface of a cell membrane, and not to the cystolic surface.

10.2. Protein–lipid interactions

The backbone conformation of a polypeptide chain may follow some well-defined pattern, which could be drastically modified by various side chains. These will lead to side-chain side-chain or side-chain backbone interactions in a polypeptide chain, which very often involve hydrogen bonding. Side chains of arginine and lysine could play important roles on many occasions.

The overwhelming presence of arginine and lysine (approximately 30 out of the 170 residues) in myelin basic protein (MBP) is crucial in the protein's role in stabilizing myelin. The positively charged side chains can interact with the hydrophilic head groups of the lipid bilayer. Consequently, MBP may be involved in bridging distant points on surfaces of myelin. MBP exhibits charge microheterogeneity as a result of various processes such as post-translational deamination, phosphorylation and the deamination of arginine to citrulline. Such changes lead to a decrease in the stability of the lipid-MBP complex [109]. Eighteen out of the nineteen arginine residues are citrullinated (MBP Citl8) in the fulminant form of multiple sclerosis (MS), known as Maburg's Disease [110]. The interaction between MBP and the lipid bilayer could be studied experimentally using either NMR or atomic force microscopy (AFM), or a combination of both. It may be expected that the spectroscopic pattern associated with the complex will be different from the sum of the isolated components. The altered observation of the complex could be regarded as diagnostic of the interactions between the two components.

The interaction of the two components could also be modeled at the molecular level. The simplest interaction that one can imagine between the guanidinium ion functionality of the arginine side chain and the carboxylate ion functionality is shown in **XXXV**. The actual interaction is, of course, far more complicated because phospholipids (see structures **XXXVI–XXIX**, where X = head group) have a number of electron acceptor sites. In particular, binding could occur between the two NH moieties of the guanidinium ion and one of the following three pairs of proton acceptor, as illustrated in **XXXV**. Binding occurs in the fashion shown in **XXXV**; the pairs of arrows in **XXXVII–XXXIX** indicate the locations of the binding sites.

10.3. Protein–nucleic acids interactions and protein biosynthesis

Protein–nucleic acid interaction is of special importance. It occurs even at the birth of a protein molecule at its biosynthesis. Translation of the genetic message into proteins implies the precise correspondence between the 64 base triplets and the 20 canonical amino acids. In this process, tRNA plays a central
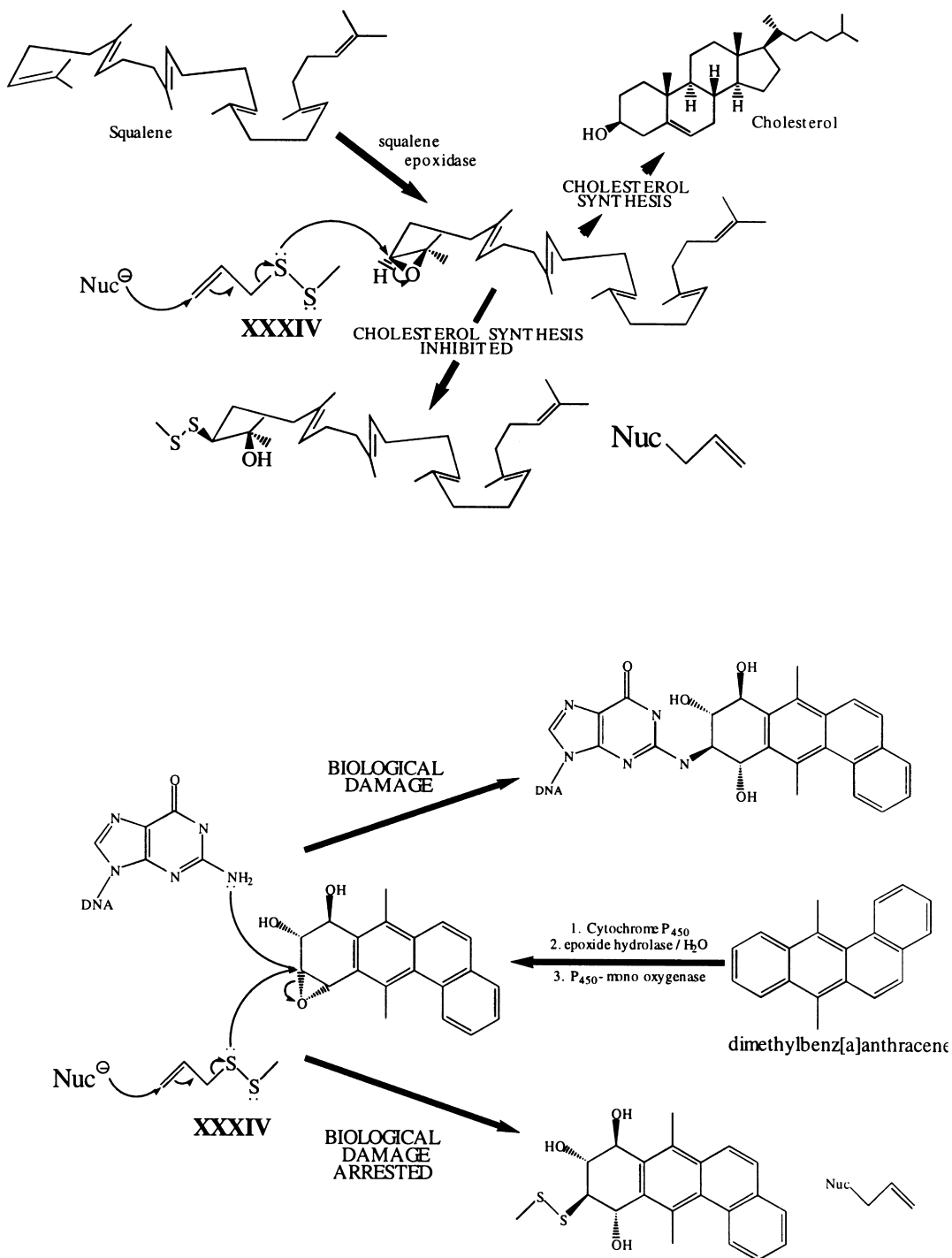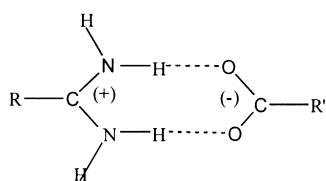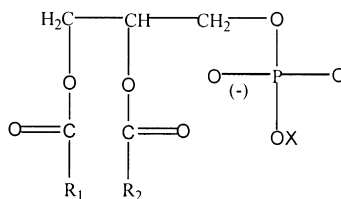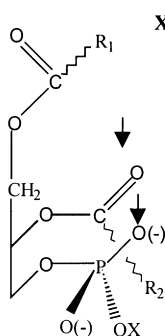
Fig. 43. Putative mechanism of the (A) anticholesterol and the (B) anticarcinogenic activity of **XXXIV** (allyl methyl disulfide).

XXXV
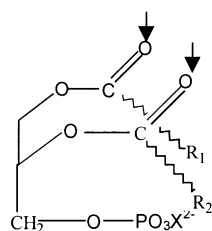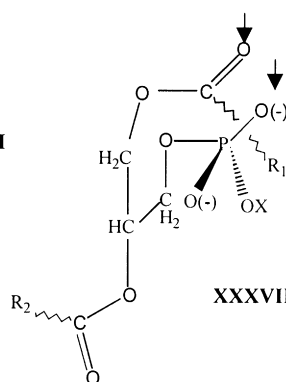

XXXVI
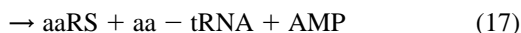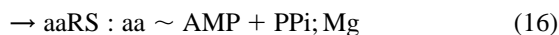

XXXIX

XXXVIII

XXXVII

role by providing the nascent polypeptide with the amino acids to which they are esterified in response to codons on RNA. In this step, the amino acylated tRNAs decipher the base triplet (codon) using Watson–Crick base pairings between mRNA and a complimentary region on the tRNA. The anticodon is independent of the nature of the amino acid esterified to the tRNA. Therefore, the accuracy of the tRNA aminoacylation reaction, ensured by the aminoacylation tRNA synthetases aaRS (a given aminoacyl-tRNA synthetase is abbreviated by its cognate amino acid three-letter code followed by RS; e.g., GlnRS stands for glutaminyl-tRNA synthetase), is of primary importance in all living cells, since it will govern to a large extent the fidelity of the translation process [111].

Aminoacylation of tRNA proceeds through a two step mechanism. The first step involves the formation of a stable aaRS-amino acyladenylate complex

resulting from the specific binding and reaction with ATP:Mg^{2+} of the amino acid; pyrophosphate is subsequently released. In the following step the $3'$ terminal adenosine of enzyme-bound tRNA reacts with the aminoacyladenylate, leading to both the esterification of the tRNA and the release of AMP. Notably, the binding of tRNA occurs in the presence of a polyamine or a divalent cation, usually magnesium [112].

$$aaRS + ATP : Mg^{2+} + aa$$

$$\rightarrow aaRS : aa \sim AMP + PPi; Mg \qquad (16)$$

$$aaRS : aa \sim AMP + tRNA$$

$$\rightarrow aaRS + aa - tRNA + AMP \qquad (17)$$

where aa stands for amino acid; aa $\sim$ AMP for aminoacyladenylate.

Because the two steps involve the specific recognition of both the amino acid and the tRNA they impose specificity throughout the overall reaction. As a general rule, there is one synthetase for each of the 20 canonical amino acids in a given organism or organelle. Two main functions are carried out by an aaRS: the activation of the amino acid and the recognition of the tRNA molecule. In the activation of the amino acid, the carboxylate of the amino acid becomes linked to the AMP moiety of an ATP molecule to form the aminoacyladenylate, which remains firmly bound to the synthetase [113].

The mixed anhydride bond between the carboxylate and the phosphoryl group of AMP is highly susceptible to the attack by a hydroxyl group, thereby facilitating further transfer of the amino acid moiety onto the tRNA. Consequently, the reaction of the activated amino acid with tRNA can be viewed as the result of a productive positioning of the $3'$-terminal adenosine of the nucleic acid within the active center of the synthetase. This allows the attachment of the amino acyl-AMP by either the $2'$- or $3'$ hydroxyl group of the terminal ribose. In this context, the accuracy of the activation reaction is of importance in the further specificity of the tRNA amino acetylation reaction. In all cases the rapid formation of a ternary complex of the enzyme with both ATP:Mg^{2+} and amino acid was evident. The amino acid acyladenylate is produced through a rate-limiting isomerization of this ternary complex PP:-Mg, one of the products, is then released.

The other function of the aaRS is the recognition of the tRNA molecule. Many recent studies have pointed out that the occurrence of discrete nucleotidic recognition elements on a tRNA molecule dictates the specificity of aminoacylation by the corresponding aaRS. Briefly, three major recognition sites have been identified:

(i) the acceptor stem, including the "discriminator base";
(ii) the three bases of the anticodon;
(iii) the D-loop.

In some systems, the three sites are important for correct aminoacylation by the corresponding amino acid, whereas in others, one site is enough. However, at least two sites appear to be required systematically for achievement of maximal aminoacylation rates.

Because a limited number of recognition sites are present on the tRNA molecule, it appears that there may be a limited number of recognition sites (peptides regions) on the cognate aaRS, too. The aaRS–amino acid interaction is based on protein structure rules, but other kinds of rules govern the tRNA–aaRS interaction. An unsolved question in the molecular biology of gene expression is the determination of these rules. Discrimination between tRNAs by the aaRS enzymes is complex because all tRNAs are about 76 nucleotide residues in length and form the typical tRNA cloverleaf secondary structure [114–117].

In the process of investigating protein synthesis in general, an exploratory set of ab initio molecular orbital computations were carried out on the various conformations of ribose and deoxy-ribose model compounds to establish the nature of possible interactions between the $5'$-hydroxyl and the oxygen of the ribose ring. Moreover, the investigation was extended to the hydrogen bonding between the two adjacent hydroxyls, $2'$- and $3'$-, to explore their effect on the stabilization of the whole system. The consistent presence of a rather unusual weak hydrogen bond between the proton of $C_{2'}$–H and the $O_{5'}$ of the hydroxyl methyl group for the g^-g^- conformation of the $-CH_2$-OH functional group has been studied using Bader's atoms in molecules (AIM) method.

Density functional calculations coupled with Bader's Atoms in Molecule (AIM) method are becoming a very important tool in examining intramolecular interactions that are responsible for the system's stabilization. As has been demonstrated [118], an unusual hydrogen bond between $C_{5'}$–$O_{5'}$$\cdots$$H_{2'}$ is present for the specific g^-g^- orientation of the $-CH_2$-OH moiety in the ribose system.

10.4. Carbohydrates and molecular recognition

Carbohydrates are sometime referred to as *information-molecules*. This characterization of carbohydrates is due to the fact that carbohydrates have a very large number of structural variants. First of all there are several hexoses and they can connect to each other to form oligosaccharides in different sequences. The chain can be branched or unbranched and the connection can be via α- or β-glycosidic linkage. Furthermore, the numerous OH groups can assume g^+, a and g^- rotamers. Such tremendous structural

variation can code a great deal of information. Oligo-saccharides are frequently attached to proteins via O- or N-glycosidic linkage. The former one involves either serine or threonine while the latter utilizes almost exclusively asparagine. These carbohydrate antennas are involved in molecular recognition processes. During the molecular recognition process, hydrogen bonds change from intramolecular to inter-molecular type. These processes could be studied in terms of glycopeptides. However, before embarking to the field of glycopeptide research one must under-stood the conformational intricacies of carbohydrate conformations.

We have modeled hydrogen bonding between adjacent OH groups in ethylene glycole [119,120]. We have also investigate internal hydrogen bonding in a 6-dehydro hexose: L-fucose [121,122]. D-glucose [123,124] as well as D-mannose [124] have also been studied in some details. An explanatory study of an oligosaccharide: the Lewis X trisaccharide [125] has just been completed. We are now engaged to study glycopeptides.

11. The mystery of protein folding

Protein synthesis occurs sequentially, one amino acid at a time. Initially, it was tacitly assumed that once the synthesis of the primary sequence is completed, the nascent macromolecule is folded to its bioactive conformation. Originally, it was further assumed that the folded structure is the global mini-mum, and thermodynamics will drive the structure to this uniquely folded structure. Relatively soon, it became obvious that it takes an enormous amount of time "to search the entire conformational space available for a polypeptide chain" [8]. This principle became known as the Levinthal's paradox [7]. Latter, it was envisaged that the bioactive form may not be the global minimum, but in fact it could be one of the local minima. Thus, thermody-namics could not be treated as "Maxwell's demons" to guarantee such a specifically folded structure, even if the necessary time was available. At that time, chaperones were invoked as an explanation of post biosynthesis folding. This view effectively allows the interaction between the chaperone, and the nascent polypeptide while it is being

synthesized. Thus, the folding could in fact occur while the synthesis is in progress.

Nowadays, it is customary to look upon folding in terms of statistical ensembles of states. However, such ensembles of states are nothing more than energeti-cally and conformationally closely spaced minima on a rather complex multivariable potential energy hypersurface (PEHS). Professor Honig greets the "light at the end of the tunnel" with the following acclamation [8]:

> It is likely that a complete solution to all aspects of the protein folding problem will require that the information available from the analysis of large structural and sequence databases be combined with the principles and methods of physical chemistry and computational chemistry.

We concur with his view. However, we wish to add that computational chemistry should go beyond currently used empirical force fields. It must start with ab initio molecular computations, which will eventually lead to an accurate analytic function that encompasses all possible protein conformers as the roots of the gradient of such an analytic function. The search for the secret of folding patterns can really start in the possession of such roots. The mystery associated with protein folding will gradually fade in the light of new understanding.

12. Proposal for a millennial mega-project and conclusion

Once a complete set of data exists for all 21 amino acids and all of their 'found' backbone conformations, a database may be constructed. This large data set may then be used in the construction of di-, tri- and even large arrays of peptides, with the ability of accepting changes at any time. However, as the number of units in a polypeptide chain grows linearly, the number of possible combinations and conforma-tions grows exponentially. Therefore, without ques-tion, an automated procedure is necessary for both the construction of input files as well for the extraction and tabulation of relevant output parameters.

Even with a successful and efficient digital script or program to perform these tasks, the current problem

of insufficient computational power and other hardware resources would still exist. This is the largest and omnipresent limitation in computational chemistry, amicably referred to as the 'limiting reagent' in the synthesis of ab initio solutions to chemical problems. The present solution of continually upgrading to larger and faster hardware will not bring about the elimination of this low yield.

The implication of truly parallelized software and more efficient optimization algorithms is one part of the solution. The other lies in the creation of a distributed network, through which calculations may be sent out to various individuals, institutions and organizations willing to donate free CPU down-time.

As PC's have come to the forefront of the cost to performance ratio, many nodes are now available to run large arrays of computations, both quickly and efficiently. All that is required is a means to manage the incoming and outgoing data, in an organized and error-free manner. The fragmentation of jobs, for parallelization, may be accomplished by sending a copy of the electron-density and Fock matrices to each node in the array. Portions of the Hessian are then constructed and assembled into a complete set afterwards.

This may be accomplished using a standardized numbering method for the molecules to be studied. With this common input parameter definition, polypeptides may be constructed without error and optimized quickly.

A novel and sound proposal has been made to optimize all possible tri-peptide (**IV**) combinations and conformations. The reason for choosing tri-peptides lies in that fact that all *N*-acetyl and *N*-methyl interactions and resultant conformations possible with a central peptide would be evaluated. Hence, once completed, two tri-peptide blocks could then be bound together and optimized more quickly than a single calculation of a hexapeptide. This is due to the fact that the internal structure of each tri-peptide unit would not change dramatically, upon biding to a second one. These 'blocks' could subsequently be used to give insight into the most stable geometry of much larger polypeptide chains, both quickly and efficiently.

For the automation of this input generation and subsequent data extraction from the log files, the numbering of the atomic sequence in the peptides

must be standardized. In this way, scripts and programs may be used to reorganize immediate patterns necessary for one's chemical analysis. As an example, we use Arginine **XL** for this:
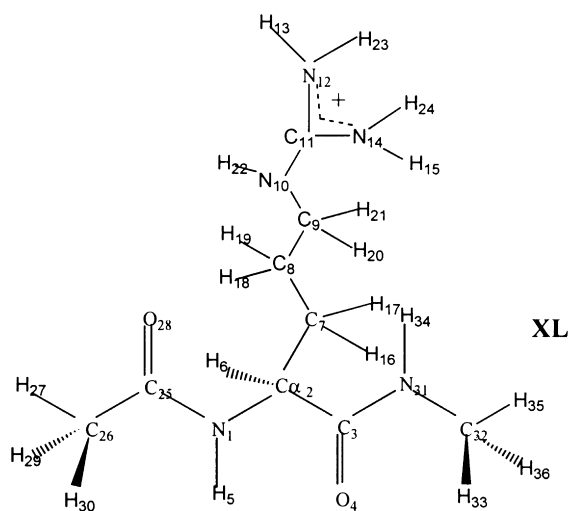
As all amino acids contain the same backbone sequence, the numbering is started from the nitrogen attached to the alpha C, following through to the carbonyl carbon all the way to the carbonyl oxygen. The groups on the nitrogen and subsequently the alpha C are numbered next, with the side chain the hydrogen attached to the alpha C. The *N*-Acetyl or *N*-Methyl groups are defined separately and then added at any time, as their coordinates do not disturb the definitions of the internal parameters specific to the core (i.e. amino acid residue).

In the case of di-peptides or larger polypeptides lengths, the numbering is left 'open', in the form of variables, so that the files may be modified at any time at any of these groups without disturbing the structure of the remaining coordinates, or the molecular environment it defines. As the internal coordinate system relies on fixing subsequent atoms to previously defined ones, the next amino acid would join to the carbonyl carbon of the first. Hence, any of the standardized amino acids in this database, numbered beginning at the N attached to the C_α, would then 'mesh' with the internal coordinates of the first without problem. In fact, any of the groups or atoms attached to the backbone may be denoted by a variable of choice and modified or truncated accordingly.

Although an extensive explanation of the above numbering sequence would be required to show all the inherent benefits of this system, the reader is encouraged to attempt trials on various input files.

In this way, it is possible to make an additive or integrateable database for the 20 amino acids, with the point of attachment in a polypeptide chain being defined as the #1 atom, in the coordinate system used. Other previously optimized structures can then be added to the database, including the *N*-acetyl and *N*-methyl portions, or even long chain fatty acids, provided they use the standardized numbering system. Simply adding the coordinate definitions onto the internal coordinate system of that original structure may then extend an optimized structure. Any polypeptide combination of any possible conformation can be easily created and submitted for optimization efficiently and can be easily automated. With the new millennium, a massive mega-project is proposed to use the above process to explore the large number of potential energy surfaces created by this gigantic number of peptide structures. For example, a tripeptide sequence would contain many millions of possible combinations and conformations to be optimized; this number grows exponentially with the extension of the polypeptide chain.

To create a surface large enough to help in the unraveling of the protein folding problem, one must continue to explore such surfaces with progressively larger sections of the amino acid polymer. This creates a massive demand for both CPU time, hard storage space and data management and extraction; the latter being a much less significant obstacle. The all too common problem of CPU power being the 'bottle neck' in the creation of meaningful ab initio results may be solved via a different avenue. Rather than throwing massive amounts of hardware in tightly coupled networks at the challenge, instead it is proposed to farm-out the CPU time to the millions of stand alone PC's and workstations residing on the loosely coupled network of the internet.

Some computational problems and data analysis ventures have indeed used this approach with much success. SETI@home is one example, whereby radio-telescope data from Arescebo in Puerto Rico are analyzed by millions of individuals in their homes, using their PC's. At last count, over 1.3 million people were contributing computer time toward this effort.

The process is automated to use 'down CPU time', whereby the individual's computer usage is not affected by the running program, nor are they burdened by any direct responsibility of managing their chunk of the data. Once the user needs their computer, the calculating program is stopped and the CPU is entirely free for the user. Another such venture is the DES/RC5 project, whose goal is to show how US export limitations on the strength of cryptographic algorithms is naive. They are undertaking this by having contributors' computers try every possible decryption key in sequence against a cipher. With over 100,000 CPUs working on the job, cracking such encryption is relatively easy. The megaproject could indeed make use of a similar such automated and 'loosely coupled' network, whereby surfaces of the many independent variables may be analyzed. Those who wish to participate may register at the following e-mail address: **protein@velocet.ca.**

It is part of human nature to ask what good will come out of a project that aims to decode the secret of protein folding. Such a discovery may well be regarded as the greatest human achievement in history. Its social impact would be greater than the combined social impact of:

the discovery of fire;
the discovery of writing;
the discovery of the wheel.

Even though this topic is very important for the whole human race, it cannot be done due to lack of funds unless individual contributions come from millions of people.

The deciphering of the law of protein folding would open new dimensions to all life sciences, including medicine. For example, the drug discovery process would be shortened dramatically, by a more cost-effective method for the production of pharmaceutical products. Not only would the cure be found reasonably soon (say by the year of 2025) for cancer, HIV, as well as other diseases, preventive medicine would also reach new heights whereby pathogen infections (bacteria, viruses and prions) could be eliminated.

Anyone, who has lost a loved one to an incurable disease, appreciates the prospects of eliminating all human sufferings. This heartache includes the pain of the ill, as well as the anguish of those who would

mourn after the patient has passed away. Needless to say, a person lost prematurely by a family is also lost by society as a whole, with all of his/her talents and productivity. Global hunger could also be eliminated by improved agricultural and food-processing technologies, in which the knowledge of protein folding also plays a significant role.

The peptide folding project, which one can participate in, at the above e-mail address, does not offer a quick fix, but gradually it will lead to a new understanding of protein folding, which in turn will produce a healthier society enjoyed by generations to come.

Acknowledgements

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