Cis−Trans Isomerization of Organic Molecules and Biomolecules: Implications and Applications†

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† C.D. dedicates this review to Professor Andrée Marquet as a mark of his admiration and gratitude.

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I. Introduction

A. Nomenclature

Organic molecules as well as metal complexes may exist as several geometric isomers[1] which display distinct physical properties and chemical reactivities. A molecule containing two atoms (in general, two carbons) attached together by a double bond with substituents W−Z may be found as two isomeric

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Christophe Dugave was born and raised in Paris. After graduating in Chemistry and Biochemistry from the University Pierre and Marie Curie (Paris 6), he prepared his Ph.D. in molecular pharmacology under the guidance of Prof. Andrée Marquet and Prof. Michel Gaudry. Then he moved to the University of Sherbrooke (Quebec, Canada) where he worked under the supervision of Prof. Pierre Deslongchamps. In 1992, he joined the Department of Protein Engineering and Research of the French Atomic Energy Commission at CEA/Saclay near Paris, where he is currently in charge of the labeling of biomolecules. His research interests include the synthesis of nonnatural amino acids, pseudopeptides, peptidomimetics, and radiolabeling of peptides and proteins with tritium and other radioisotopes. He also investigated the mechanism of peptidyl-prolyl cis–trans isomerases, and the design of novel inhibitors of cyclophilins.

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forms as long as W ≠ X and Y ≠ Z (Figure 1). Formally, **A** and **B** are, respectively, the *cis*- and *trans*-isomers, provided W is equal to Y. According to the more recent nomenclature based on the Cahn−Ingold−Prelog system, when W ≠ Y, compound **A** is the *Z*-isomer and **B** is the *E*-isomer. Note that the *Z*-isomer is not necessarily cis. This type of isomerism is also possible with molecules that possess one or two nitrogen atoms. Conjugation of the sp^2 electrons of a heteroatom with a planar π-system may form a pseudo double bond which also exists as both *cis*- and *trans*-isomers (respectively, *E*- and *Z*-isomers). In this case, resonance gives the single bond some double-bond character. Formally, **A**-**B** and **C**-**D** are configurational isomers and **E**-**F** are conformational isomers. Cis−trans isomerism can also exist around a single bond in overcrowded molecules, in particular, in

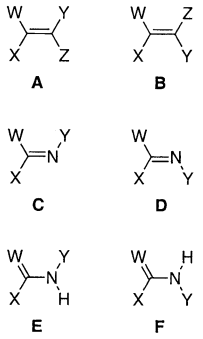


Figure 1. Schematic representation of a π-system containing molecules that display a cis−trans isomerism according to rules of nomenclature established by Cahn−Ingold−Prelog.

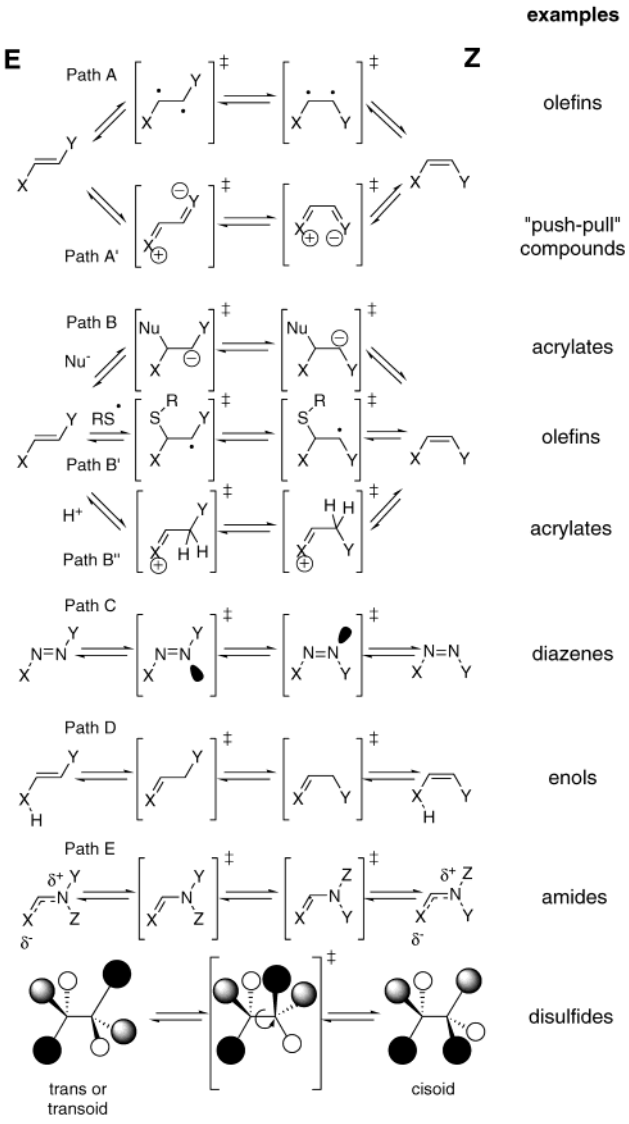
compounds that contain bulky substituents, when rotation around the σ-bond is restrained or when stereoelectronic effects are implicated. In all cases, compounds **A** and **B**, **C** and **D**, and **E** and **F** are diastereomers. This is reflected by differences in physical and chemical properties, and, as anticipated, geometric isomers of biomolecules have distinct biological activities. As long as the energy difference between two isomers is not too large, both may be obtained and they can isomerize, provided the barrier of interconversion is not too high.

B. Possible Mechanisms of Isomerization

Nine different ways of cis−trans isomerization may be proposed. Four of them (path A−B″) imply the formal breaking of the double bond via either a homolytic or a heterolytic process. In some cases, however, the double bond character is decreased by resonance and facilitates the interconversion. A conjugated π-system can isomerize by deconjugation of the pseudo double bond (Scheme 1).

The homolytic breakage of the π-system has been essentially observed in the case of heterogeneous hydrogenation, radical reactions initiated by radical generators (i.e., thermal decomposition of azo compounds), direct photoisomerization or isomerization catalyzed by paramagnetic molecules (oxygen, atomic bromine and iodide, nitrogen oxide, nitroxide radicals...), and thermal isomerization (path A). In some cases, radical addition/elimination processes also cause cis−trans isomerization (path B′). Except for chemical reactions that involve a reactive nucleophilic (path B) or electrophilic (path B″) counterpart, the heterolytic rupture of a double bond is greatly facilitated in a captodative ethylene system where there are electron-withdrawing groups (X) attached to one carbon and electron-donating substituents (Y) on the other. In some molecules, in particular, highly conjugated systems such as dyes, the conjugation may be increased by resonance, which leads to a dramatic fall in the energetic barrier to rotation. Some π-systems including a nitrogen atom with a free electron doublet can also undergo cis−trans isomerization via doublet inversion (path C), and this mechanism often competes with the simple rotation, reflecting the tautomeric effect (i.e., nitroso/oxime tautomerism, path D). Conversely, single bonds with a partial double bond character (formally called

Scheme 1. Possible Mechanisms of *E*−*Z* and Transoid−Cisoid Isomerizations and Relevant Examples



pseudo double bonds), such as amides and thioamides, are able to interconvert slowly in solvents, whereas isomers may be isolated in the crystalline state (path E). Single bonds connecting heteroatoms (i.e., disulfides) or overcrowded systems are also subject to cis−trans isomerism due to stereoelectronic effects, though this usually dramatically favors one of the two isomers. In this review, cis−trans and Z−E isomerizations and related interconversions will be called cis−trans isomerization for conciseness and clarity, except in specific cases where nomenclature should be respected.

C. This Review

A search in the ChemAbstracts and Medline databases using "cis−trans isomerization", "*Z*-*E* isomerization", and "geometric isomerization" as keywords gives access to about 20 000 references that contain these concepts. This amazing number of research articles related to the geometric isomerizations reflects the importance of such a phenomenon in organic and inorganic chemistry as well as in biology. The purpose of the present review is not to list all isomerization processes described in the literature but to provide a comprehensive overview of the implications of cis−trans isomerization of organic molecules, especially biologically active compounds, and the utilization and applications of this phenomenon in biochemistry, pharmacology, and supramolecular chemistry. Instead of giving an exhaustive catalog of all reactions of isomerization and associated mechanisms, we would like to illustrate this review with recent and relevant examples, and hence we will refer to more specific review articles as often as possible. For this reason, all significant works in this area cannot be considered, and therefore, we apologize in advance for omitting some references. Cis−trans isomerisms of cyclic compounds and metal complexes will not be considered.

First, we will examine the general cis−trans isomerism of olefins and their substituted derivatives. In particular, the cis−trans isomerization of unsaturated lipids and carotenoids will be expounded, and we will devote some time to photoisomerization in visual processes and the utilization of this concept for the design of molecular switches and machines. Second, the isomerization of diarylazo compounds will be illustrated by promising applications of cis−trans isomerism in biology and supramolecular chemistry. Third, the cis−trans interconversion of pseudo double bonds will be considered, and we shall most particularly focus our interest on the biological implications and biochemical and medical applications of the peptidy-prolyl cis−trans isomerization. Finally, the existence of restraint rotation about σ-bonds will be mentioned.

II. Carbon–Carbon Double Bonds

A. Olefins: From Stilbene to Retinal

In general, *Z*- and *E*-olefins interconvert neither spontaneously at room temperature nor in daylight in the absence of radical generators. Radical isomerization of olefins (mainly photoisomerization) proceeds either via the π,π^* singlet (S_1) or triplet (T_1) excited states that are driven by the properties of the molecule and the experimental conditions (Figure 2). However, intersystem crossing ($T_1 \rightarrow S_0$ + heat), photosensitization by singlet−singlet or triplet−triplet transfer can also lead to isomerization. Both the S_1-p state and the T_1-p state have a perpendicular instead of a planar geometry, and therefore both isomers may be formed when the molecule drops back to the S_0 state. This explains why the most stable isomer can transform into the less stable isomer upon photoisomerization.[2] As a matter of fact, the cis−trans interconversion of nonconjugated C=C is strongly limited by a high energy barrier that involves $\pi-\pi^*$ (singlet and triplet) and π,σ (Rydberg) states. As an example, in the case of photoisomerization of ethylene, the two lowest transitions are very close and around 170 kcal mol^{-1}. In substituted alkenes, however, the Rydberg π,3s transition, which leads to carbene rearrangements, tends to be at lower energies ($\Delta G^{\ddagger}$ = 141 kcal mol^{-1}) than the singlet $\pi-\pi^*$ state ($\Delta G^{\ddagger}$ = 164 kcal mol^{-1}), whereas the

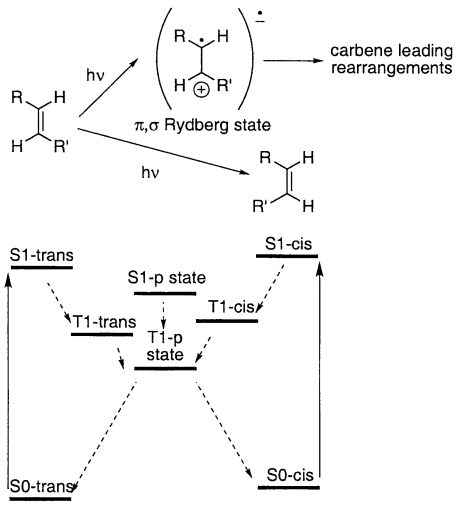
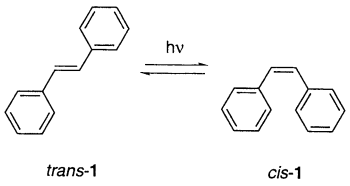


Figure 2. Schematic representation of $E-Z$ photoisomerization pathways of an olefin; corresponding singlet and triplet states.

lowest triplet state has a $\pi-\pi^*$ character and $\Delta G^{\ddagger}$ around 97 kcal mol^{-1}. Free enthalpy of activation is significantly lowered by conjugation of the double bond with π-systems, in particular polyenes. In general, the triplet state has a longer lifetime, sometimes beyond 1 ms, but most cis−trans photoisomerization processes take place in about 10 ns, and some of them occur in less than 100 fs. In any case, photoisomerization must be more rapid than thermal relaxation which produces the reverse reaction.[3,4]

Stilbene **1** is one of the most popular models for studying cis−trans photoisomerization of polyenes, and has been the subject of numerous investigations (Scheme 2). Solvent, pressure, and temperature as

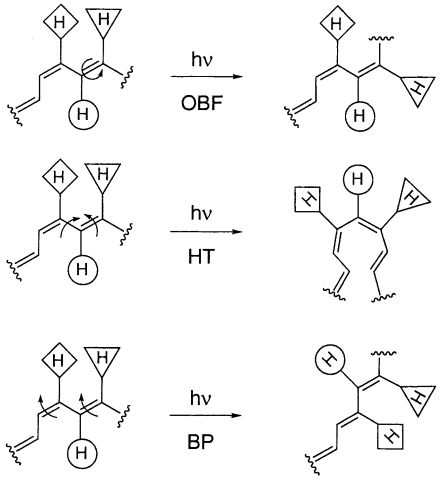
Scheme 2. Cis−Trans Photoisomerization of Stilbene 1



well as the nature and position of substituents strongly influence both the *Z*:*E* ratio as well the isomerization kinetics.[2] Stilbene dendrimers with molecular weights over 6000 undergo a photochemical isomerization in less than 10 ns that corresponds to the lifetime of the excited singlet state, whereas photoisomerization of stilbene usually lasts from 60 to 400 fs.[5]

Although cis−trans isomerization of ethylene derivatives has been studied for a long time, recent ab initio calculations and experimental results have led to the proposal of novel mechanisms.[6,7] As an example, the formation of a complex between stilbene and atomic iodine has been suspected to be an intermediary step of the photocatalyzed isomerization of stilbene by iodine.[8] More general theories such as non-equilibration of excited isomers (NEER), that accounts for predominant chemical reactions in the triplet state of simple dienes, have been experimentally confirmed.[9] Another major advance in the understanding of polyenes cis−trans isomerization was the volume-conserving mechanism, postulated 15 years ago. Geometric isomerization is an important mode of reaction of polyenes in their excited singlet state and the prevailing theory was the one-bond-flip (OBF), which involves the torsional relaxation of the perpendicular excited state via an adiabatic mechanism (Scheme 3). However, such a

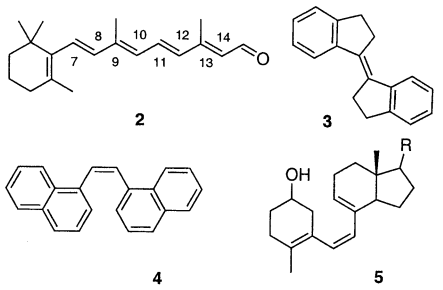
Scheme 3. Three Possible Ways of Cis−Trans Photoisomerization of a Polyene: OBF (One-Bond Flip), HT (Hula Twist), and BP (Bicyclic Pedal) Mechanisms



phenomenon was not consistent with large polyenes such as retinyl chromophores, which are confined inside a protein structure. Therefore, the Hula-Twist (HT) mechanism depicted in Scheme 3 was proposed and has taken over in popularity.[10] The HT mechanism postulates that a C−H unit can undergo a 180° translocation via a diabatic mechanism that is in apparent violation of NEER (Scheme 3).

However, the HT mechanism has been primarily validated with carotenoids in particular in the vitamin D series and later with diene triplets and simple trienes. In the case of carotenoids and other long-chain polyenes, the isomerization implies a high-volume demanding reorganization. This is consistent neither with the position of the chromophoric group that is deeply inserted inside the protein nor with the time-scale of photoisomerization of retinyl chromophores (<1 ps), which is too short for protein reorganization. The bicyclic pedal (BP) mechanism, which predicts a one photon/two-bond isomerization process, was proposed as a volume-conserving process (Scheme 3).[6] However, this mechanism is not consistent with the one-bond isomerized photoproducts observed with retinoids such as retinal **2** (Chart 1).[11] On the other hand, a BP-10,12 process has been proposed for the thermal isomerization of the 10-s-*cis*-*all*-*trans* retinyl chromophore of bathorhodopsin.[12] Although the HT mechanism accounts for most isomerization processes observed, it cannot be considered as a general mechanism for all photoisomerization reactions, in particular, in the case of stilbene analogue **3** that does not allow the HT process.[13] The HT and OBF processes may, however, coexist, and

Chart 1. Retinal 2, Stilbene Analogue 3, *cis*-1,2-bis-α-Naphthylethylene 4 and Previtamin D 5

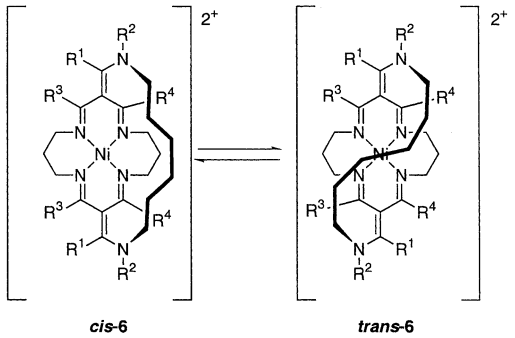


this explains the formation of an unequilibrated mixture of trans products by photoisomerization of *cis*-1,2-bis-α-naphthylethylene **4**. In turn, low-temperature spectroscopic study of previtamin D **5** is in agreement with the HT mechanism.[14]

B. Functionalized Ethylene Derivatives

Conjugation of the carbon−carbon double bond with an heteroatomic π-system dramatically lowers the barrier to isomerization and hence facilitates the rotation via different mechanisms including radical processes, addition/elimination reactions, and isomerization by tautomeric effect. In particular, X=Y−ZH systems are considered as potential 1,3-dipoles.[15] The well-known enamine/imine tautomeric equilibrium accounts for most cases of enamine isomerization,[16] in particular, with push−pull imines.[17] It is favored by bases,[18] acids,[19] Lewis acids,[20] coordinating metals,[21,22] and polar solvents.[23] N-substitution[24,25] and electronic and steric effects[17,26] all strongly influence the imine/enamine tautomerism. Dynamic enamine cis−trans isomerism has been investigated in the cyclidene family of dioxygen carriers **6**, and the formation of the unusual *trans*-linked bridge (Scheme 4) has been proposed as the principal cause of

Scheme 4. Cis−Trans Isomerization of the Enamine Groups of Cyclidene Dioxygen Carrier 6

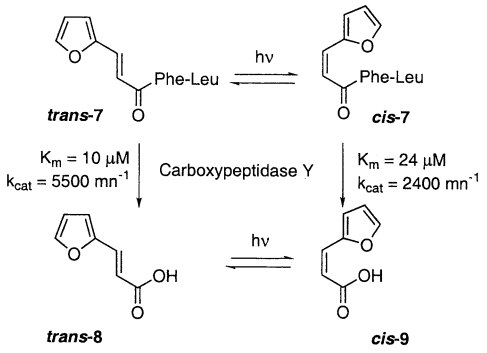


decreased dioxygen affinity in unsubstituted cyclidene.[27] Two cis geometries are possible, although R^2 tends to be nearest R^4. When $R^3 = R^4 = H$, an additional *trans*-isomer coexists with the preferential *cis*-isomer. Variable-temperature ^1H and ^{13}C experiments suggest that both isomers readily interconvert at room temperature.[28]

Keto−enolic tautomerism has been documented for a long time.[29−32] In contrast to enamines, enolates isomerize readily upon light excitation,[33] but in most cases, photoisomerization is not the predominant process due to radical double-bond shift,[34] intermolecular coupling, and inter/intramolecular cyclizations.[35]

Acrylates can undergo cis−trans isomerization via radical processes,[36−38] Lewis acids and metal complexes,[39] protic acids,[40] as well as nucleophiles.[41,42] Photochemical isomerization of furylacryloylpeptides used in kinetic mesurements of proteolytic enzymes has shown that the acrylate moiety quickly isomerizes in plain day light. Even though the proteolysis takes place at a remote site, acrylate isomerization strongly influences the enzymatic transformation and has to be taken into account when using furylacryloyl substrates, as demonstrated in the case of furylacryloyl peptide **7** (Scheme 5).[43] Very recently, it has been

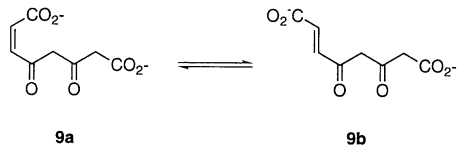
Scheme 5. Trans to Cis Photoisomerization of the Furylacryloyl Moiety of a Carboxypeptidase Y Substrate 7 Decreases Both Affinity and Rates of Hydrolysis



demonstrated that *Z*- and *E*-himbacine have distinct binding properties toward M1−M4 muscarine receptor subtype.[44]

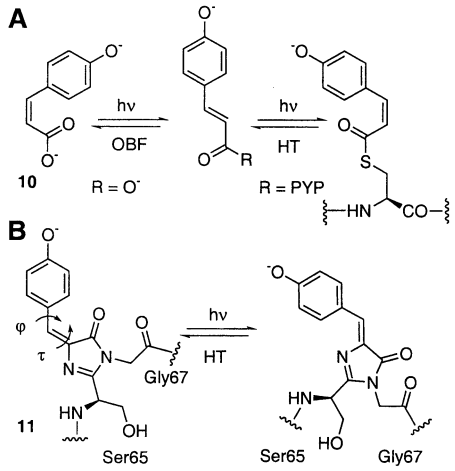
The cis−trans isomerization of maleylacetoacetate **9a**, a product of the degradation of phenylalanine and tyrosine, to fumarylacetoacetate **9b** by the cis−trans isomerase-glutathione system has been investigated: the isotopic effect strongly suggests that the reaction involves a nucleophilic attack at C2 rather than a conjugated addition and the subsequent rotation of the single bond (Scheme 6).[45]

Scheme 6. Cis−Trans Isomerization of Maleyl Acetoacetate 9a to Fumarylacetoacetate 9b by the Cis−Trans Isomerase Gluthatione System



Coumaric acid **10**, the chromophoric species from the photoactive yellow protein (PYP) readily photoisomerizes via a OBF process at the free state,[46,47] but a volume-conserving HT mechanism has been proposed when coumaric acid is bound to the protein[48] (Scheme 7A). The green fluorescent protein (GFP) chromophore **11** (Scheme 7B) is likely to photoisomerize via a HT mechanism which involves the concerted rotation of both τ and φ dihedral angles due to strains on the chromophore that are responsible for the twisted excited state relative to the

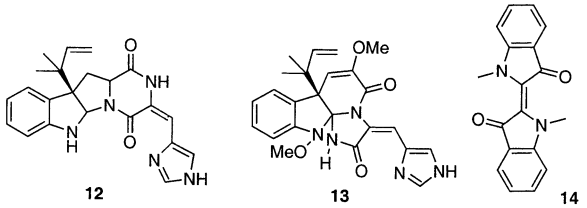
Scheme 7. Distinct Photoisomerization Mechanism Proposed for the Photoisomerization of the Coumaryl Chromophore of PYP (10) Either at the Free State (OBF process) or at the Enzyme-Bound State (HT process) (A); HT Mechanism of Photoisomerization of the GFP Chromophore (11; B).



planar conjugated ground-state. Therefore, recovery of the Z-ground-state (dark form) causes the fluorescence emission.[49] Both three- and four-state models have been very recently discussed in the review article by Zimmer.[50] Photochromic properties of GFP can be tuned by mutations that affect both the steric and electrostatic environment of the chromophore and cause variations of the excitation and emission maxima, molar extinction coefficients, and quantum yield.

Dehydroamino acids found in metabolites from bacteria, fungi, plants, and sponges display interesting structural properties and reactivities.[51] They usually combine the reactivities of both enamines and acrylates and are able to isomerize via photochemical processes[52] to the minor *E*-isomer. Although both isomers may be obtained by synthesis, thermal isomerization usually occurs from the *E*-isomer to the more stable *Z*-isomer.[53,54] The relative configuration of the $\alpha-\beta$ double bond strongly influences the conformation of dehydropeptides as well as their binding properties.[55] Not surprisingly, most dehydroamino acids have a *Z*-configuration,[56] although the neurotoxins roquefortin **12** (Chart 2) and oxalin

Chart 2. Neurotoxins Roquefortin 12 and Oxalin 13; Indigo 14



13 contain an *E*-ΔHis residue.[57] Both *E*- and *Z*-ΔPhe[52] and -ΔTrp[54] residues have been shown to be photointerconvertible either in peptides or as simple protected derivatives. Other 2-aminoacrylic motifs such as indigo **14** interconvert photochemically in solution[58] via an OBF process since constraints do not allow the HT mechanism.[6]

C. Isomerization of Fatty Acids and Phospholipids

Unsaturated fatty acids are important constituents of phospholipids present in the cell membrane and are essential for the control of physical properties of the lipid bilayer. The energy difference between esterified *E*- and *Z*-fatty acids is low (about −1 kcal mol^{-1}), and all-*Z*-fatty acids readily isomerize upon γ-irradiation or heat.[59] As a consequence, they play an essential role in adaptation responses,[60] and the number and geometry of unsaturations are correlated with thermotolerance in plants[61] as well as salt stress resistance[62] and tolerance to toxic substances[63−65] (i.e., organic solvents) in bacteria. *Trans*-phospholipids have also been detected in tissues harvested from breast cancer patients.[66] In normal conditions, most of unsaturations are in a *cis*-conformation which does not introduce perturbation in the relative alkyl chain arrangement inside the lipid bilayer. Modification of lipid composition affects the physicochemical properties of the membrane and hence allows a fine-tuning of membrane microviscosity and molecular mobility.[67] In mammals, the enzymatic pathways for cis−trans isomerization are still unknown, and most of *trans*-fatty acids are likely to be supplied by food intake or bacterial transformations occurring in the stomach of ruminants. In bacteria, however, a linoleic acid isomerase activity is induced in *Pseudomonas putida* by incubation with 0.03−0.04% octanol.[68] Such a phenomenon has been observed for oleic acid and vaccenic acid as well.[69] To our knowledge, the mechanism of action of the isomerase is still unknown despite the cloning and sequencing of the cti gene from *P. putida*. However, a cytochrome C-type heme-binding motif has been localized at the N-terminus.[70] The de novo synthesis of *trans*-fatty acids has been ruled out.[71] The implication of cytochrome is also assumed as a catalyst of cis−trans isomerization of 4-hydroxytamoxifen during metabolization of the therapeutic agent tamoxifen.[72]

Trans-fatty acids are also produced by aborted catalytic hydrogenation, heat, and free radicals,[73,74] in particular, thyil radicals[59,75,76] that act as catalysts both in homogeneous solution and lipid vesicles, and provoke permanent modifications[77] (Scheme 8). Retinoids and carotenoids have been found to inhibit this process and to protect the double bond geometry according to their well-known anti-oxidative activity. Nitrogen dioxide, a toxic free radical produced by spontaneous oxidation of nitrogen oxide as well as enzymatic oxidation of nitrite, is able to induce the cis−trans isomerization of arachidonic acid **15** within cellular phospholipids leading to the formation of a mixture of *trans*-isomers (Chart 3).[73]

The intriguing interconversion of geranial **16a** to neral **16b** (Scheme 9) which is catalyzed by amino acids in alkaline aqueous solution is an example of a noncovalent assisted isomerization process. Zwitterionic forms of asparagine or glutamic acid are able to accelerate substantially the rate of cis−trans isomerization of citral presumably by stabilizing the

Scheme 8. Thyil Radical-Catalyzed Cis−Trans Isomerization of Unsaturated Fatty Acids

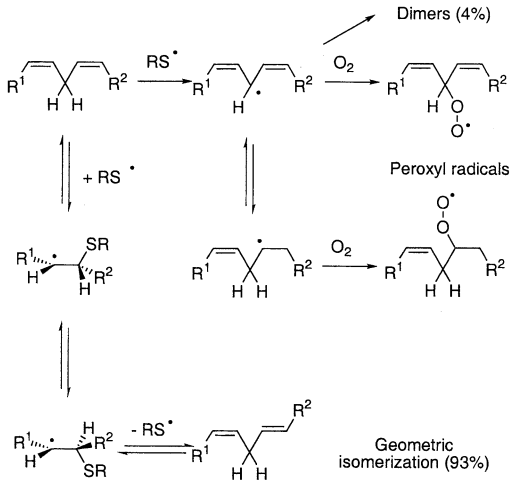
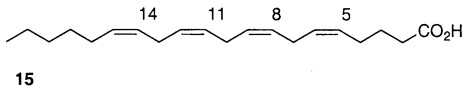


Chart 3. Arachidonic Acid 15: Cis−Trans Isomerization May Occur at C^5=C,^{6} C^8=C,^{9} C^{11}=C,^{12} C^{14}=C.^{15}



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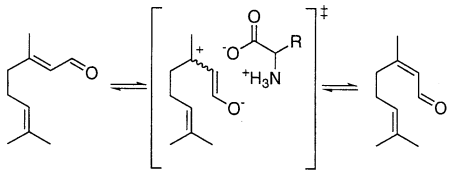
Scheme 9. Proposed Mechanism of the Amino Acid-Catalyzed Isomerization of Geranial 16a to Neral 16b



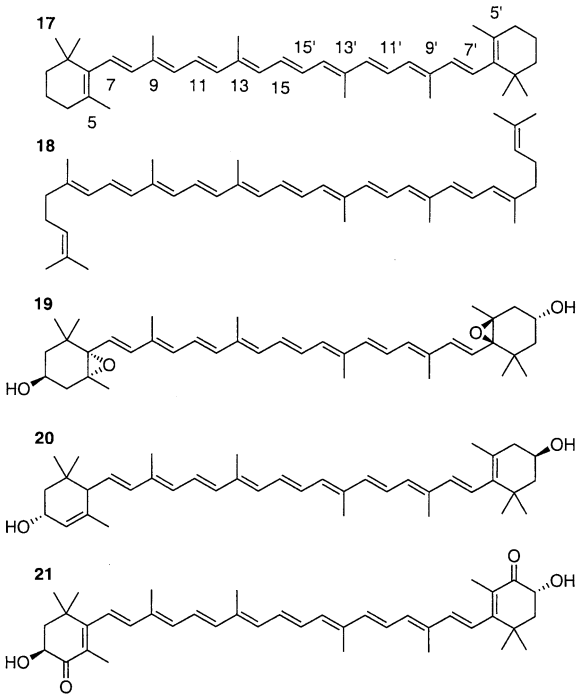
16a 16b

cation/enolate intermediate. It is noteworthy that simple amino acids catalyzed the reaction more efficiently than bovine serum albumin.[78]

D. Isomerization of Carotenoids

There are more than 600 naturally occurring carotenoids, and some are of nutritional importance (Chart 4). In particular, β-carotene **17** is the precursor of β-retinal **2** which plays a central role in vision.[79] Found both in the free state and bound to protein complexes,[80] carotenoids, such as β-carotene and lycopene **18**, are essential photoprotective and antioxidant pigments found in plants, animals, and bacteria, while violaxanthin **19**, lutein **20**, and zeaxanthin **21** are mainly associated with the antenna protein assembly of photosystem II. They play a role as light-harvesting and photoprotective pigments by acting as quenchers of the excited state of chlorophyll and reactive oxygen species both in vivo and in vitro.[81] The most significant change upon illumination is either a trans-to-cis or cis-to-trans isomerization at specific positions of the carotenoid (i.e., C-13 for violaxanthin, C-15 for β-carotene). Carotenoids are present in tissues and pigment proteins in their more stable all-trans form, but specific configurations may have been selected for particular functions.

Chart 4. β-Carotene 17, Lycopene 18, Violaxanthin 19, Lutein 20, and Zeaxanthin 21



Different mono- or di-*cis*-geometrical isomers at positions 7, 9, 13, 15, 13′, and 9′ have been identified, and protein environments strongly influence the reactivity of carotenoids.[82,83] For example, carotenoids in the 15-*cis*-configuration are ubiquitously bound to protein complexes that contain quinone-type or iron−sulfur type reaction centers that specifically interconvert them to the all-trans isomer.[84] β-Carotene found in thylakoid membranes of photosynthetic plants is 80% all-trans and 20% cis at various positions.[85] Although their extremely fast isomerization (<200 fs) has been shown to proceed via a triplet excited state in most cases,[84,86] some results suggest that cation radical/dication formation also induces trans-to-cis interconversion of β-carotene and canthaxanthin **19**.[85] Thermal cis−trans isomerization of various carotenoids occurs in vitro[87] and in vivo.[88]

The selective binding of geometric isomers of carotenoids implies the existence of specific carotenoid isomerases (CRTISO), in particular, in dark-grown tissues. Such enzymes have been very recently identified in tangerine[89] and *Arabidopsis*,[90] yet little is known about their structure and mechanism, which seems to be closely associated with desaturases which catalyze oxidation of the hydrocarbon chain during carotenoid biosynthesis. Moreover, CRTISO from tangerine is a redox-type enzyme structurally related to bacterial desaturases.[89]

Nonprotein-bound retinoids also isomerize upon light irradiation, and all 16 possible isomers have been identified and fully characterized. However, photoproducts corresponding to distinct isomerization at C7=C8, C9=C10, C11=C12, and C13=C14 are the most commonly found.[91] Retinal with its n,π^* state lying close to the lowest π,π^* state is not considered as being a valuable model for studying the cis−trans isomerization because it exhibits a high intersystem

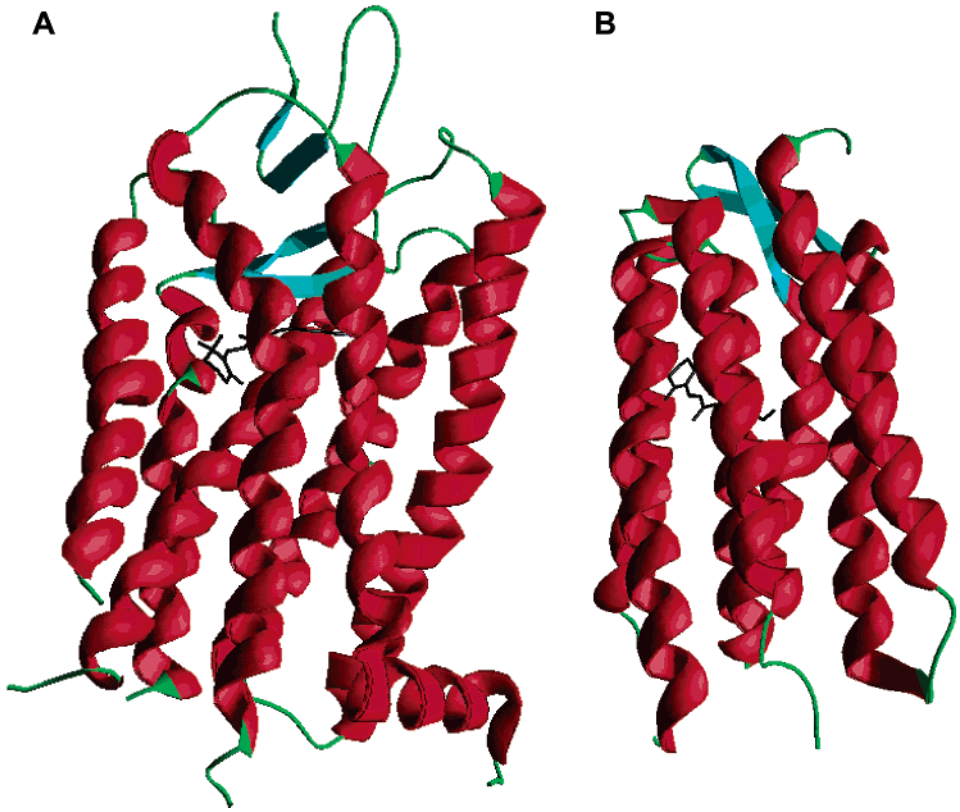


Figure 3. Crystal structures of bovine rhodopsin (1F88) at 2.80 Å resolution (A) from ref 96 and bacteriorhodopsin from *Halobacterium salinarum* (1MOL) at 1.47 Å resolution (B) from ref 151. (Represented using the PDB Viewer Software 3.7b2, Glaxo-Wellcome.) The proteins are illustrated using the ribbons representation, and the chromophores are shown as black backbones.

crossing efficiency. Retinal photochemistry is thus complicated by competing triplet processes. On the other hand, its protein-bound chromophore and vitamin A exhibit exclusive singlet-state processes. Moreover, the cis−trans isomerization reactions of free retinylidene **2** is 2 orders of magnitude slower.[92]

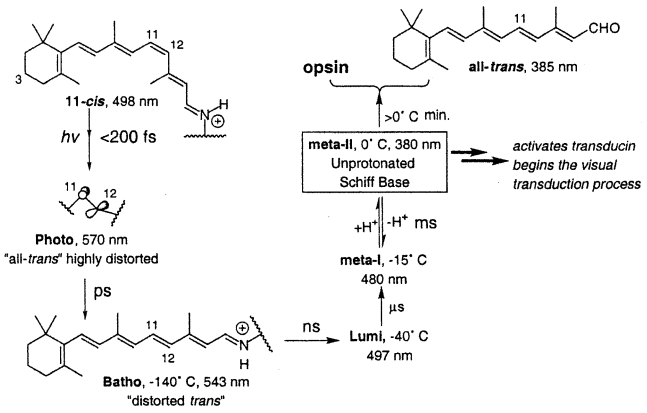
E. Rhodopsin and the Photon Signal Transduction

Rhodopsin is the archetype of visual pigments in animals[93] and algae[94] and remains the subject of intensive research after more than a century of scientific investigation.[95] As a consequence, more than 10 000 scientific reports deal with rhodopsin and its photosensitive prosthetic group retinal. Moreover, retinal has served as a paradigm for theoretical study of the cis−trans isomerization of polyenes and has played a considerable role in the emergence of new concepts in this field.[6] Recently, several crystal structures of rhodopsin,[96] bacteriorhodopsin,[97,98] and halorhodopsin[99] have been published and provide detailed information about the structural features of these proteins. Many other techniques have also been employed to study the dynamics of the conformational changes and chromophore−protein interactions upon light activation:[100] high-resolution solid-state NMR methods,[101,102] realtime spectroscopy,[103,104] resonance Raman spectrometry,[105,106] photothermal methods,[107] fluorescence,[108,109] FTIR,[110] EPR spectroscopy,[111] photoaffinity labeling,[112] the synthesis and analysis of various retinal analogues,[113−116] and mutagenesis of rhodopsin.[117,118] Ab initio methods[119−122] have been employed to compute the ground-state S0 and the two first singlet excited-states S1 and S2.

Rhodopsin (Figure 3A) is an integral membrane G-protein-coupled receptor[123] composed of 348 amino acids which are organized in seven transmembrane α-helices.[124] The chromophore is the *all-trans*-retinal, covalently bound to a lysine residue (Lys296 in bovine rhodopsin) via a Schiff base. Covalent linkage of the retinylidene moiety to the protein is not essential for its function. Upon irradiation, 11-*cis*-retinal specifically isomerizes to *all-trans*-retinal. In contrast to unbound retinal, only isomerization around C^{11}-C^{12} in the counterclockwise direction is permitted in protein-bound retinal[125] due to intramolecular strains inside the protein[119,126] that twist the chromphore backbone conformation[127] and to the presence of internal water molecules.[128] Moreover, the formation of a protonated Schiff base causes a high polarizability of the π-electron system of the unsaturated side-chain.[129] Consequently, photoreaction gives rise to several well-characterized intermediates that have been trapped at low temperature (Scheme 10, Table 3).

The extremely fast primary reaction (λmax = 498 nm) occurs in less than 200 fs to give an *all-trans* highly distorted intermediate (photorhodopsin) which transforms on the picosecond time-scale into a distorted *trans*-retinal (bathorhodopsin). The stored light energy[130,131] is used for the net translocation of a proton accross the membrane.[132] The lumirhodopsin

Scheme 10. Rhodopsin Photocycle and Photointermediates[a]



[a] "Freezing temperatures", maximum absorption wavelength, and typical lifetimes of the photointermediates are indicated (Reprinted with permission from Bohran, B.; Souto, M. L.; Imai, H.; Shichida, Y.; Nakanishi, K. *Science* **2000**, *288*, 2209). Copyright 2000 American Association for the Advancement of Science (http://www.aaas.org).

state, reached in about 30 ns by thermal relaxation of bathorhodopsin, leads to metarhodopsin in approximately 1 μs. There are two distinct protonated meta-I and deprotonated meta-II states which interconvert in 1 ms. Only the metarhodopsin-II is able to activate transducin, a protein that initiates the visual transduction process.[133] An associated proton transport occurs on the millisecond time-scale by transfer from Asp96 to Asp85 of rhodopsin.[97] In a further step, *all-trans*-retinal is hydrolyzed and released from the opsin protein.[112,134] The emergence of X-ray data with sufficient resolution has begun to facilitate understanding of the chromophore−protein interactions at the atomic level as well as chromophore movement inside its binding pocket.[101,112,135] As stated in part A, a simple bond-flip isomerization implies a high-volume demanding reorganization that is not compatible with the protein structure. Simultaneous single chain rotation by the HT mechanism may proceed by a lower energy of activation and less relative movement of the helices in the protein.[100,101,136] The qualitative depiction of the conformational movements of opsin triggered by the cis → trans isomerization of retinal has been suggested to occur by a remarkable flip of the ionone ring of retinal close to Trp265 (helix F) to a position where it is close to Ala169 (helix D; Scheme 11).[112] Furthermore, dramatic changes in the hydrogen-bonding network of the binding pocket involving Glu113, the counterion for the protonated Schiff base, occur.[137] The extramembrane loops connecting helices C/D and E/F have also been implicated in the activation of transducin.[138,139] The influence of the phospholipid acyl chain composition has also been considered important for the modulation of receptor signaling.[140] In addition, internal water molecules in the retinal pocket have been found to play a functional role as revealed by X-ray crystallography.[141]

The reisomerization of retinal after hydrolysis from opsin continues to be investigated. Tritium release experiments and affinity labeling have both shown that two enzymes play important roles in the trans-to-cis interconversion, which is an endothermic process ($\Delta G° = +4$ kcal mol^{-1}). *All-trans*-retinal is first reduced to *all-trans*-retinol, which is transesterified by lecithin retinol acyl transferase to give a high-energy intermediate. Isomerization, catalyzed by an isomerohydrolase, might proceed via an addition/elimination mechanism involving an active site nucleophile that causes the release of the acyl moiety (Scheme 12). This reaction may provide energy to drive the biosynthesis of 11-*cis*-retinol ($\Delta G° = -5$ kcal mol^{-1}). Addition of water has been proposed to lead to a concerted inversion of configuration.[142] Very recently, a novel signal-transduction pathway has been proposed since *all-trans*-retinal might act as a direct regulator of the cyclic nucleotide-gated ion channel.[143]

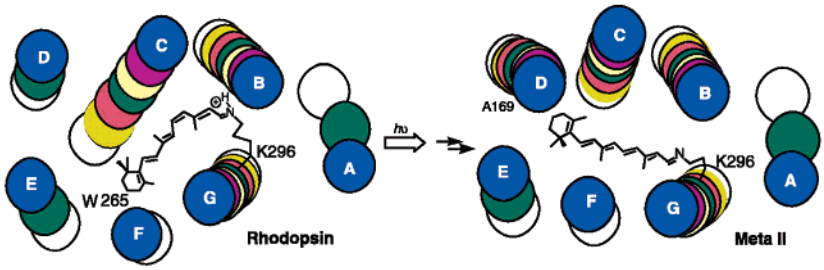
F. Other Retinal-Binding Proteins

1. Retinal Cone Photoreceptors and Pinopsin

Cone photoreceptors contain a 11-*cis*-retinal that photoisomerizes to *all-trans*-retinal and activates the opsin protein, leading to G-protein signal transduction in a way very similar to that of rhodopsin. In contrast, the cellular retinoid-binding protein (CRBP) induces a specific isomerization[144] at C^{13}=C^{14}.

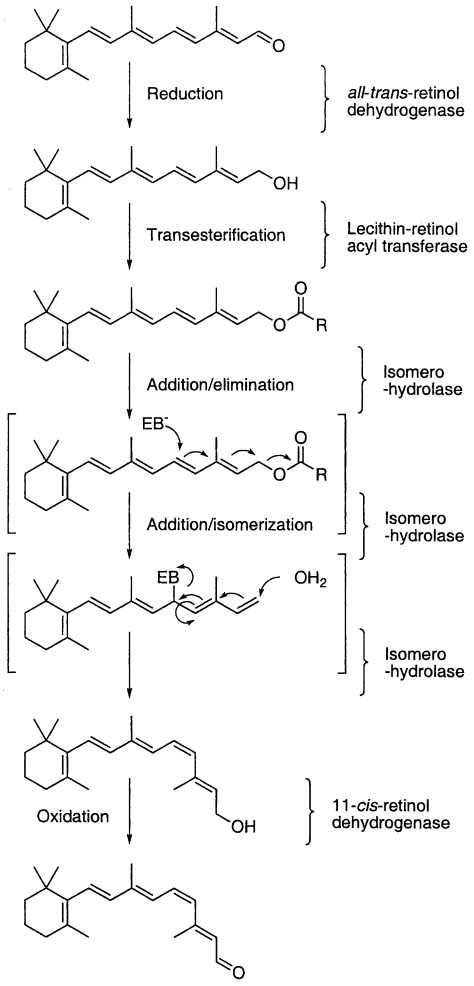
Pinopsin is a blue-sensitive pineal pigment isolated from chicken, which has been classified as a novel subtype of the vertebrate rhodopsin family. Using a combination of low-temperature spectroscopy and transducin activation assays, pinopsin has been shown to be a chimera between rod and cone visual pigments. Cis−trans isomerization of the retinylidene chromophore at C^{11} is fully photoreversible at −196°C, just like retinal visual pigments.[145]

Scheme 11. Schematic Representation of the Relative Motion of Transmembrane Helices of Rhodopsin Due to Retinylidene 11-cis to all-trans Photoisomerization[a]



[a] Only rhodopsin initial and Meta-II states are represented (reprinted with permission from Bohran, B.; Souto, M. L.; Imai, H.; Shichida, Y.; Nakanishi, K. *Science* **2000**, *288*, 2209). Copyright 2000 American Association for the Advancement of Science (http://www.aaas.org).

Scheme 12. Isomerization of 11-*trans*-Retinol to 11-*cis*-Retinol Catalyzed by the Isomero-hydrolase



2. Halobacteria Rhodopsins

Halobacteria contain four distinct retinal-binding proteins that have been proposed as archaeal rhodopsins: bacteriorhodopsin (bR),[146] halorhodopsin (hR),[147] sensory rhodopsin (sR),[148] and phoborhodopsin (pR).[149] Both bR and hR are light-driven ion pumps that act, respectively, as an outward proton pump and an inward chloride pump. The sR and pR act, respectively, as phototactic attractant and repellent responses. All of them are seven-helix integral proteins containing a retinal chromophore bound to a lysine residue.

Bacteriorhodopsin (Figure 3B), the most studied bacterial rhodopsin,[150,151] is a 26-kDa transmembrane protein with a retinylidene moiety bound to Lys216, which undergoes an all-trans to 13-cis photoisomerization upon light irradiation. In the dark, bR relaxes into a 6:4 mixture of B-state (all-trans) and D-state (13-cis). Absorption of a photon and subsequent isomerization initiates a photocycle with several spectroscopic intermediates (Table 1). Although there are many similarities with rhodopsin, bR is characterized by a branched photocycle that drives a vectorial proton flux related to the Schiff base protonation state. Only the $C^{13}=C^{14}$ trans-to-cis isomerization occurs under physiological conditions;[152] however, an all-trans to 9-cis interconversion has been observed in the so-called blue membranes formed under conditions of low pH and deionization.[153] Isomerization at C13 induces a conformational change in the bR core, mainly related to the motion of the Schiff base[154] and rotation of a threonine side-chain. This conformational shift is associated with proton release on the extracellular side and proton uptake from the cytoplasm.[155]

G. Nonretinal Chromophoric Proteins

1. Photoactive Yellow Proteins

Photoactive yellow proteins (PYPs, Figure 4) are members of the xanthopsin family and constitute a new class of eubacterial blue-light receptors with a rhodopsin-like photochemistry.[156−158] The chromophore is a *p*-coumaric acid covalently bound to a cysteine residue (Cys69 in the purple bacterium *Ectothiorhodospira halophila*) via a thioester bond.[159,160] The *p*-coumaryl moiety reversibly isomerizes from trans to cis[161] on a nanosecond scale[162] (Scheme 7A) and activates a photocycle involving a short-lived red-shifted intermediate (pR), followed by a long-lived blue-shifted intermediate (pB) that transforms into the original receptor state (pG). The hydroxy group of the chromophore is very close to the carboxyl moiety of a buried Glu46 residue, which is hydrogen-bonded in pR, deprotonated in pB, and protonated in pG (Figure 5).[163,164]

The structure of PYP has been resolved in the crystal state[165,166] and in solution.[167] The existence of several distinct crystalline form, as well as significant differences between solution and solid state structures, all support the existence of several conformationally distinct intermediates.[168−170] Molecular dynamics have also been used to model the femtosecond modification in PYP.[171] Cryo-trapping of intermediates and time-resolved crystallography[172] have

Table 1. Comparison of Rhodopsin and Bacteriorhodopsin Photointermediates[a]

state (rhodo/bacteriorhodopsin)	λmax (nm)	retinal configuration	Schiff base conformation	Schiff base ionization
rhodopsin/B	498/570	11-cis/all-trans	anti/anti	H^+/H^+
Batho/K	540/586	all-trans/13-cis	anti/anti	H^+/H^+
Lumi/L	497/544	all-trans/13-cis	anti/anti	H^+/H^+
$Meta^I/M^I$	478/409	all-trans/13-cis	anti/anti	$H^+/0$
$Meta^{II}/M^{II}$	380/409	all-trans/13-cis	anti/anti	0/0
$-/M^{III}$	465/−			
−/N	−/562		−/anti	
−/O	−/629	−/all-trans	−/anti	
−/D	−/550	−/13-cis	−/syn	
−/P	−/485	−/9-cis		
−/Q	−/390	−/9-cis		

[a] All characteritics are presented consecutively for rhodopsin/bacteriorhodopsin.

Figure 4. Crystal structure of the photoactive yellow protein (PYP) from *Ectothiorhodospira halophila* in the dark state (2PHY) at 1.40 Å resolution (represented using the PDB Viewer Software 3.7b2, Glaxo-Wellcome). The protein is illustrated using the ribbons representation and the chromophore is shown as a black backbone.

shown that the isomerization is accompanied by a flipping of the thioester linkage[173] avoiding collisions that would result from the large-scale movement of the aromatic ring.[174] The isomerized bond is distorted into a transition state-like conformation, and the resultant stored energy is used to drive the PYP light cycle.[175] The trans → cis isomerization also results in the opening of an arginine gateway that allows exposure of the chromophore to the solvent and causes changes in both local[176,177] and global conformation[178] (Figure 5). Such changes are accompanied by modifications in hydrogen bonding[179] and electrostatic potential at the protein surface[180] as well as by an introduction of water into the interior of the protein.[168,181] Light seems to provoke dramatic conformational modifications that cause the unfolding of PYP.[182−185] The photon signaling pathway of PYP appears to be triggered via important conformational changes rather than a direct proton movement through a hydrogen-bonding network.[186−188] Thus, PYP exemplifies the way cis−trans isomerization of a small prosthetic group can trigger large conformational transitions inside a flexible protein scaffold.[189]

2. Green, Yellow, and Red Fluorescent Proteins

Green fluorescent protein (GFP) has been isolated from *Aequorea victoria*, and there are currently 19 X-ray structures of GFP analogues listed in the Protein Data Bank, including citrine (YFP),[190] the improved yellow variant of GFP,[191] and two X-ray structures of the red fluorescent protein DsRed from *Discosoma* species.[192,193] In the very recent (2002) review devoted to GFP,[50] Zimmer claims that GFP might be considered as "light in a can" because its 11-sheet β-barrel completely isolates the centrally located chromophore from the solvent (Figure 6),[194] aside from protein-bound water molecules, some of which interact with the chromophore.[195]

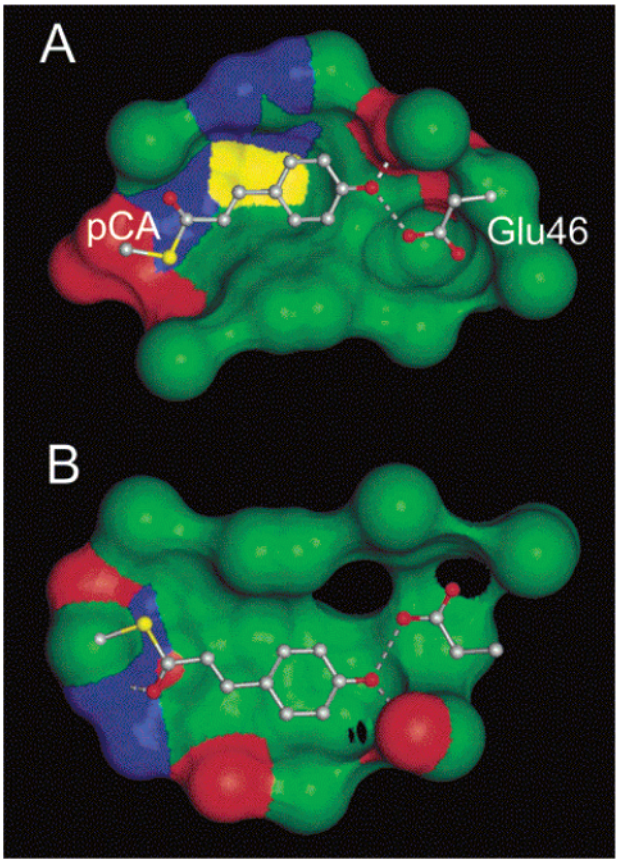


Figure 5. Electrostatic properties of the binding pocket for the coumaryl chromophore of the photoactive yellow protein (PYP) and local pocket reorganization. The green areas (carbon) represent a low-dielectric medium, whereas red (oxygen), blue (nitrogen), and yellow (sulfur) areas denote a high-dielectric environment. The side chain group of Glu46 and the chromophore are illustrated in balls and sticks. Three hydrogen bonds are represented as dashed lines. Reprinted with permission from ref 178. Copyright 2001 American Chemical Society.

The 4-(*p*-hydroxybenzylidene)-imidazolid-5-one chromophore **11** (Scheme 7), which is probably formed by the autocatalytic cyclization of the Ser−Tyr−Gly [65−67] moiety and subsequent oxidation, sits inside a relatively large cavity whose shape is not complementary to the planar chromophore (Scheme 7B).[196] The planar shape of the chromophore is essentially due to π-electron delocalization and protein-induced strain. Studies performed using a chromophore inserted inside a model peptide suggest that fluorescence results from the inhibition of the isomerization of the methylene double bond.[197] GFP has two absorption peaks at 398 nm (major) and 475 nm (minor), which correspond to the neutral and ionized forms of the chromophore. It fluoresces at 503 and 508 nm upon excitation at, respectively, 475 and 308 nm. Many GFP mutants have been described. In YFP, the chromophore phenolate anion is stacked next to a π-system. In DsRed, the phenolate form of the chromophore is conjugated with an additional unsaturation. In the four-state photoisomerization model, GFP allows the rotation of the 4-(*p*-hydroxybenzylidene) moiety via the rotation of either both the φ and τ dihedral angles simultaneously (HT mechanism in the anionic form) or via rotation of φ

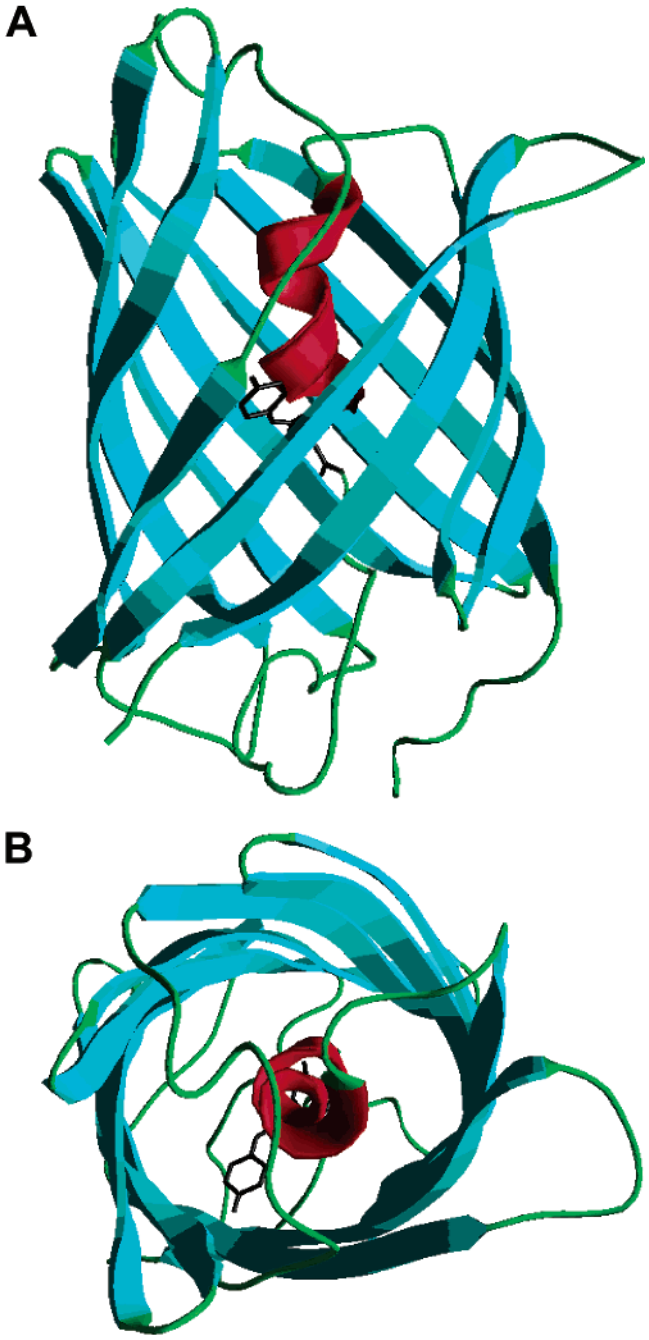


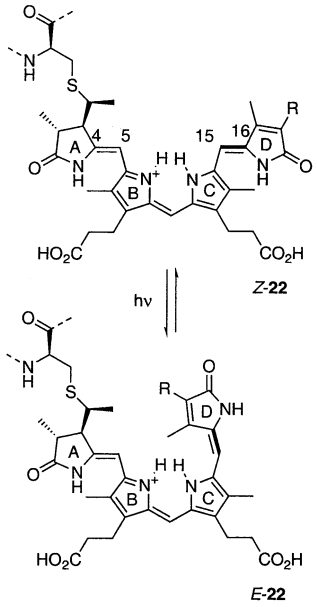
Figure 6. Crystal structure of the green fluorescent protein (GFP) variant YFP−H148Q at 2.10 Å resolution (represented using the PDB Viewer Software 3.7b2, Glaxo-Wellcome). (A) Front-view; (B) top-view. The protein is illustrated using the ribbons representation, and the chromophore is shown as a black backbone. From ref 194.

(zwitterionic form) or τ (neutral form) alone[49] (Scheme 7B). Computational studies have shown that the *E*-isomer is less well coordinated by surrounding protein residues.[49] This model, which proposes the radiationless nonadiabatic crossing from the excited to the ground state (*Z*−*E* isomerization), has not been fully confirmed by experiment. In particular, the cationic and zwitterionic forms were not observed by Raman spectrometry in the wild-type GFP.[198] Electronic excitation S0 → S1 was found to alter the conformation of the chromophore, and a recent study has suggested that the *Z*−*E* isomerization may be a nonradiative pathway for excited-state relaxation of YFP.[199]

3. Phytochromes and Bilirubins

Phytochromes are biliprotein photoreceptors in plants and cyanobacteria,[200] which play a role in light perception and adaptation to environmental modifications.[201,202] These proteins contain bilin chromophores covalently linked to a cysteine residue via a monosulfide bond (compound **22**, Scheme 13). The

Scheme 13. *Z* to *E* Photoisomerization of the Phytochrome-Bound Pigment Bilirubin 22[a]



[a] The isomerizing double bond is represented in bold.

phytochromophores exist as two distinct forms which absorb light in the red (P_r form: λmax = 665 nm) and far red (P_{fr}: λmax = 730 nm) corresponding, respectively, to the *Z*- and *E*-isomer at C^{15}=C^{16} (Scheme 13). Both isomers can interconvert reversibly and incident light controls the P_r:P_{fr} ratio.[203,204] As a consequence, photoisomerization affects signal transduction processes which cause a modification of metabolic response. The elucidation of these molecular[205] and cellular[201] mechanisms is still a subject of active research. However, pico- and femtosecond spectroscopic techniques have provided some novel insight into the first steps of the phototransformation. The first step following light absorption involves a viscosity-dependent *Z*−*E* isomerization of the chromophore requiring intramolecular motion of the chromophore around the D-ring[206] and the proximal amino acid side chains.[207] Resonance Raman spectroscopy has indicated that the isomerization reaction involves simultaneous rotation at the C^{15}-methine bond coupled to C^{15}-H wagging and D-ring rotation.[204] The *Z*−*E* and *E*−*Z* isomerizations are not accompanied by rate-limiting proton transfer as suggested by a lack of effect of H−D exchange,[207,208] a mechanism that was proposed for the light-driven isomerization of the free chromophore.[209,210] The P_r → P_{fr} transformation is a fast process that involves two excited electronic states with lifetimes of 3 and 32 ps,[211] while

the $P_{fr} \rightarrow P_r$ back reaction appears to proceed via two states with 700 and 4.5 ps time constants, respectively.[212]

The $P_r \rightarrow P_{fr}$ transition is associated with a dramatic change in pigment solubility from the P_r form, which is soluble in the cytoplasm to the P_{fr} form which exists as water-insoluble pellets inside the membrane. This process, which may be considered as a major regulation pathway, can be reversed upon irradiation at 730 nm and leads to a redissolution of the phytochrome molecules.[213] Pigment isomerization and local motion are connected with the rearrangement of the C-terminal part of the protein and signal transduction including activation of a G-protein, which results in changes in the Ca^{2+} and cGMP concentrations.[214]

Three years ago, a phytochrome-like protein called P_{pr} was discovered in the purple photosynthetic bacterium *Rhodospirillum centenum*. P_{pr} is formed by the assembly of a PYP N-terminal domain, a central domain similar to phytochrome and a C-terminal histidine kinase domain. The chromophore is the blue light-absorbing *p*-coumaric acid that is spectrally equivalent but kinetically slower than that of PYP.[215]

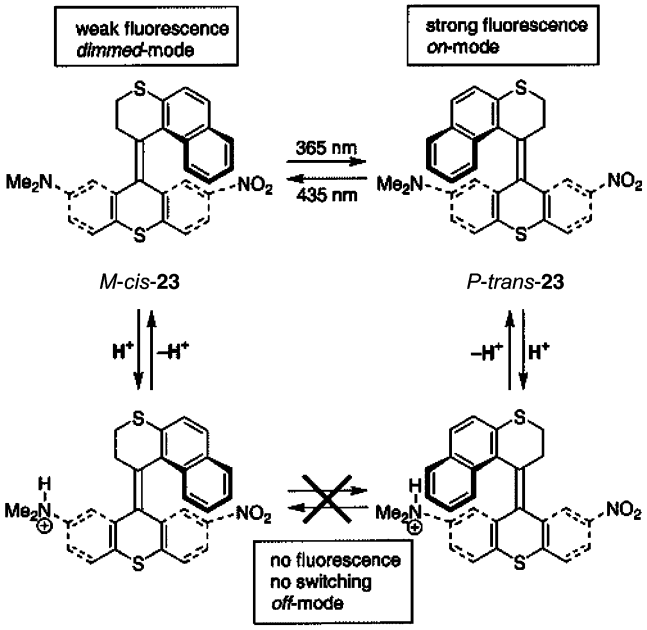
Bilirubin, the chromophoric group of phytochrome, can be isolated in the free-state. At normal concentrations, it mainly binds to a very high-affinity site of human-serum albumin (HSA) in a 1:1 ratio[216] and is also transported by other proteins.[217] Bilirubin is also the hydrophobic and cytotoxic pigment of jaundice, produced by oxidative degradation of heme in mammals and excreted into the bile after modification as mono- and diconjugated derivatives. Accumulation of both conjugated and unconjugated derivatives is responsible for various metabolic[218] and neurologic disorders.[219] However, recent investigations suggest novel roles as antioxidant[220] and neuroprotective agent against stroke and Alzheimer's disease[221] as well as protective agents against coronary artery disease.[222] In the free state, bilirubin exists as a *Z*,*Z*-isomer which readily dimerizes into interconvertible enantiomeric dimers due to H-bond complementarity of the dipyrrinone and carboxylic acid moieties.[223] Both free and HSA-bound bilirubin are able to photoisomerize reversibly from (4*Z*,15*Z*)- to (4*Z*,15*E*)-bilirubin (and to a lesser extent to the (4*E*,15*Z*)-isomer) upon irradiation in the 390−530 region.[224] In the HSA-bound state of bilirubin and bilirubin analogues, isomerization causes very small volume changes. The energy differences between the two isomers has been estimated as less than 1 kcal mol^{-1}.[225] Photoirradiation of bilirubin may produce other reactions such as cyclization,[226] photoautomerization,[227] and photooxidation,[228] and is strongly influenced by the environment.[229−231]

H. Applications of C=C Isomerizable Systems

1. Diarylethylene Switches

Diarylethylene photochromic films containing stilbene or cinnamate moieties grafted on a silica surface exhibit interesting properties as phototunable liquid crystals. The completely reversible cis−trans isomerization of the chromophore induces an alteration of the photoalignment that has been employed for the development of erasable optical recording supports, image processors, laser-addressable devices, etc.[232] Overcrowded chiroptical molecular switches based on *Z*−*E* isomerization of a carbon−carbon double bond are interesting systems for the development of both write−read−erase optical memories and switchable molecular motors.[233−235] In general, *Z*−*E* photochemical isomerization of chiral overcrowded alkenes should lead to racemization; however, polarized light allows a stereoselective interconversion that is driven by the sign of polarization.[236]

Scheme 14. Dual Mode Photoswitching Process Based on the Regulation of Photoisomerization by Reversible Protonation[a]

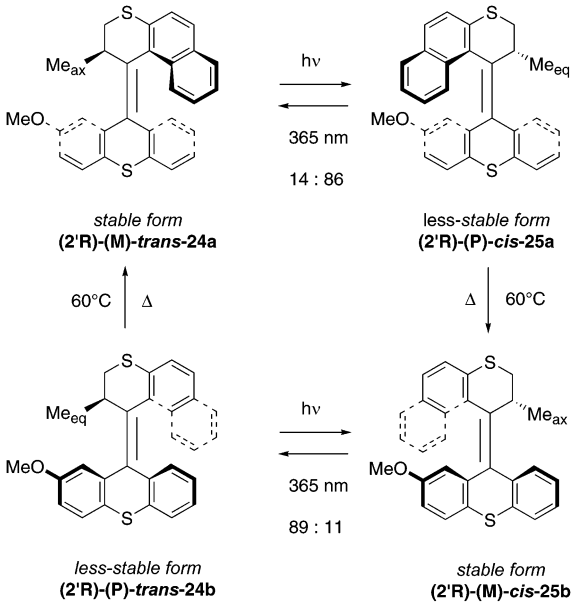


[a] The cis−trans isomerization of the overcrowded alkene **23** is blocked by addition of trifluoroacetic acid. The photochromic system exhibits a gated response behavior that involves three distinct states (on, dimmed, and off-modes) usable to lock stored information (Adapted from Feringa, B. L. *Acc. Chem. Res.* **2001**, *34*, 504).

A dual mode photoswitching process, based on the regulation of the isomerization by reversible protonation, was employed for the design of molecular memory in which information is stored in the chiroptical switch **23** (Scheme 14).

Photoisomerization has been employed for the control of motion in molecular motors.[237] Second-generation light-driven molecular motors employ two cis−trans isomerizations upon irradiation followed by a thermal helix inversion which results in a 360° rotation around the central double bond (Scheme 15). Photochemical conversion proceeds with an almost perfect photoequilibrium of 1:99 (Figure 7). The direction of rotation of compound **24a** is controlled by the single stereogenic center. The speed of complete rotation is governed by the two thermal steps.[238] Some macroscopic effects of the photoisomerization process have been observed such as color change of liquid crystal phases doped with a molecular motor, upon varying irradiation time.[239]

Scheme 15. Four-Step Discrete Isomerization of Chiral Helical Alkene 24a Which Results in an Unidirectional 360° Rotation[a]



[a] Each cycle is initiated by UV irradiation and temperature changes. (Adapted from Koumura, N.; Geertsema, E. M.; van Gelder, M. B.; Meetsma, A.; Feringa, B. L. *J. Am. Chem. Soc.* **2002**, *124*, 5037).

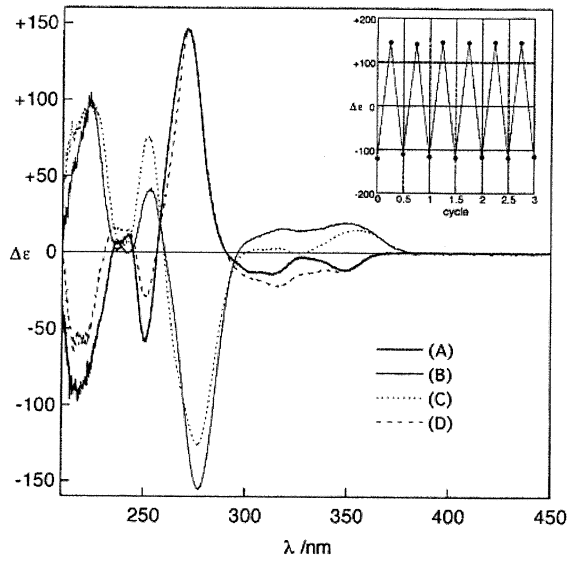
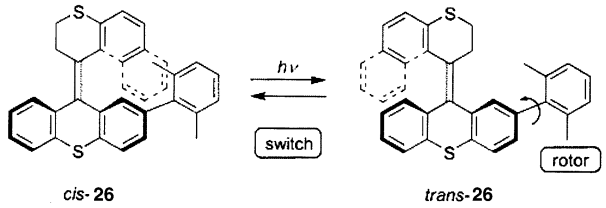


Figure 7. Circular dichroism spectra in each of the four stages of switching of alkene **24a**: (2′R)-(*M*)-*trans*-**24a** (A); (2′R)-(*P*)-*cis*-**25a** (B); (2′R)-(*M*)-*cis*-**25b** (C); (2′R)-(*P*)-*trans*-**24b** (D). Inset: change in CD signal $\Delta\epsilon$ during full rotation of **24a** monitored at 272 nm. Reprinted with permission from ref 238. Copyright 2002 American Chemical Society.

A light-controlled bistable molecular motor based on a dihydronaphthiopyranylidene scaffold bearing a free rotating 1,6-dimethylphenyl group (compound **26**) acts as a molecular brake (Scheme 16) which has barriers to rotation of 19.0 and 19.7 kcal mol^{-1}, respectively, as shown by 2D NMR exchange spectroscopy in DMSO-d_6 at 303 K. This suggests that cis−trans isomerization controls the rate of rotation of the biaryl rotor.[240]

In 2002, the design, synthesis, and study of a photoinduced molecular shuttle **27** composed of a

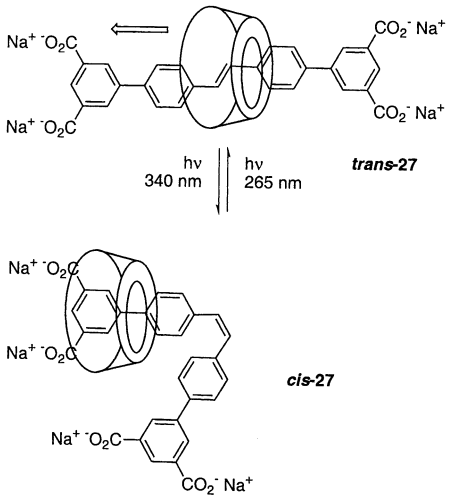
Scheme 16. Photoregulation of the Xylyl Rotor by Photoisomerization of Compound 26[a]



[a] *cis*-**26** acts as a molecular brake whereas rotation is permitted in *trans*-**26**. Adapted from Feringa, B. L. *Acc. Chem. Res* **2001**, *34*, 504.

symmetric stilbene dumbbell and an α-cyclodextrin unit (Scheme 17) was reported. Photoisomerization

Scheme 17. Representation of the Photoinduced Rotaxane 27 with an Axial Stilbene Unit[a]



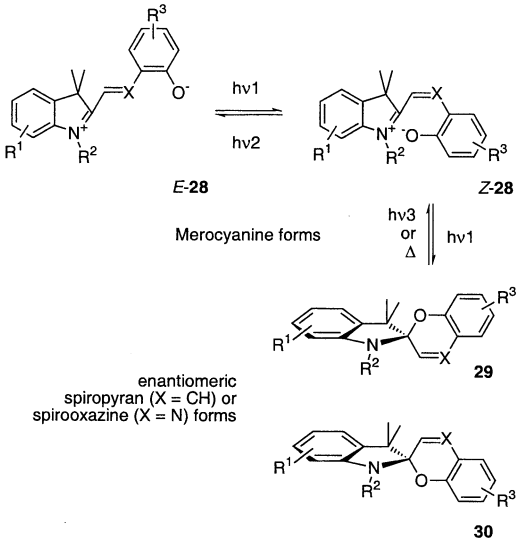
[a] The α-cyclodextrine acts as a unidirectional shuttle which slides to the bis-anionic extremity upon trans to cis photoisomerization of the stilbene moiety. The asymmetry of the α-cyclodextrine unit causes a monodirectional shuttling. (Stanier, C. A.; Alderman, S. J.; Claridge, T. D. W.; Anderson, H. L. *Angew. Chem. Intl. Ed.* **2002**, *41*, 1769.)

of the diarylethylene core at 361 nm results in the unidirectional movement of the unsymmetrical cyclodextrin because sliding in the opposite direction is not allowed due to steric interactions.[241]

2. Spiropyrans, Spirooxazines and Fulgides

Light-induced cis−trans isomerization of merocyanine derivatives **28** not only results in a simple *E* to *Z* transition but is also accompanied by the intramolecular attack of the phenolate at the Schiff base producing the corresponding spiropyrans **29** (X = CH) or spirooxazine **30** (X = N; Scheme 18). This transformation causes large modifications of charge (shift from a zwitterionic to a neutral species), geometry (highly conjugated planar to nonplanar twisted then to tetrahedral), and stereochemistry, with creation of a new stereogenic center. Isomerization upon visible light irradiation is fully reversible in the dark. Exposure to sunlight for a few minutes produces the full conversion of the merocyanine to the spiro form; the back-reaction is much slower and requires 150−250 min to be completed. Opening of the spiro form

Scheme 18. *E* to *Z* Photoisomerization of Merocyanine Derivatives 28 and Subsequent Isomerization of the *Z*-Isomer into Enantiomeric Spiropyran or Spirooxazine Forms

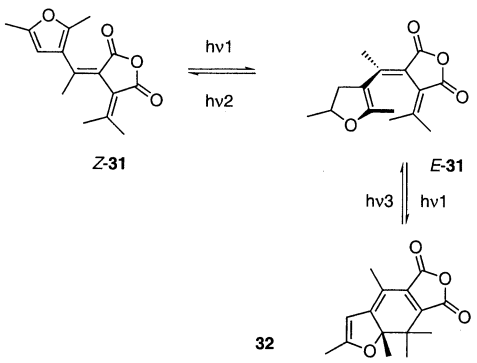


can be induced by UV-light irradiation at 254 nm. Analysis of the rise and decay dynamics has shown that the transformation occurs within a picosecond time range.[242] The two forms have very different physical properties, such as distinct absorption maxima and absorbances that can be modulated by the nature of substituents and matrixes (i.e., solution, molecular assembly, polymer network, and solid surface).[213,242]

The merocyanine to spiropyran or to spirooxazine transformations have potential applications in the development of light filters, optical recording media, photoresponsive monolayers[243,244] and polypeptides,[213] photoswitchable ligands, and photoactivatable modified enzymes.[245] Cis−trans isomerization can thus be harnessed to trigger large modifications of physical properties and chemical reactivity in materials and biomolecules.

Although, the *Z*- and *E*-isomers are both thermally stable, the *E* to *Z* photoisomerization of fulgides **31** occurs within a nanosecond and competes with the ultrafast (<6 ps) electrocyclization reaction of the *E*-isomer (Scheme 19). Because promising applications are based on the electrocyclization reaction, this cis−trans interconversion has been considered as an undesired side reaction.[243−246]

Scheme 19. *Z* to *E* Photoisomerization of 2-[1-(2,5-dimethyl-3-furyl)Ethylidene]-3-isopropylidenesuccinic Anhydride 31 and Subsequent Photocyclization[a]

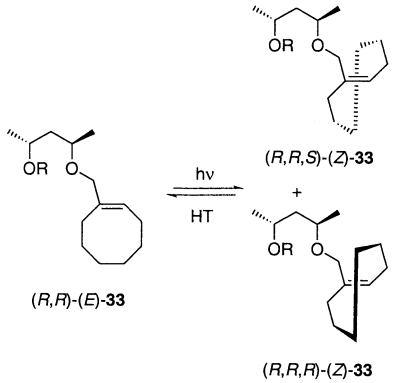


[a] Tamai, N.; Miyasaka, H. *Chem. Rev.* **2000**, 100, 1875.

3. Other Ethylene Switches

Simple olefins can be employed as chiroptical switches. For example, tethered *Z*-cyclooctene **33** (Scheme 20) can be photoisomerized with diastereoselectivity up to 44%.[241] Functionalized 5α-androstane and 5β-androstane skeletons have been employed as molecular photonic wires in which a primarily activated "antenna" chromophore converts singlet energy to triplet energy which is transmitted via a through-bond interaction to a distal olefin that isomerizes from *Z* to *E*. The triplet−triplet energy transfer is relayed inside the steroid skeleton which acts as a molecular photonic wire.[248]

Scheme 20. Diastereoselective Photoisomerization of the Tethered Cyclooctene 33



Cis−trans isomerism in synthetic porphyrins, in particular, [3.0.1.0] and [4.0.0.0] isomers (respectively **34** and **35**) (Figure 8), has recently been studied[249]

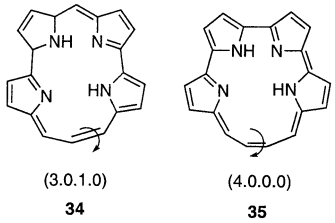


Figure 8. Cis−trans isomerism in synthetic porphyrins **34** and **35** (position of isomerization is indicated by a rotating arrow).

and may provide better understanding of their intriguing metal-coordinating and optical properties.[250] Both atropisomers can be efficiently separated by HPLC.[251] Isomerization can control the size of the coordinated metal ion as well as the stability of the organometallic complex.[252]

4. Bacteriorhodopsin

Halobacterial cell membranes contain a two-dimensional crystalline array constituted by unidirectionally oriented bacteriorhodopsin (BR) molecules. These functional systems associate with lipids to form the purple membrane and are implicated in phototaxis and photosynthesis. BR displays many similarities

with rhodopsin, and, due to the multistate photoreactivity of BR and its high sensitivity, many applications have been suggested for BR, such as devices for converting sunlight into energy, artificial retinas, biosensor applications, and information storage and processing. Most current applications have been recently reviewed,[153] and therefore, we will focus our attention on those in which cis−trans isomerization is directly involved. There are several ways to control the photochromic properties of BR: (i) electric, magnetic fields and pH all may reversibly modify the environment of the molecules; (ii) chemical or biological mutation may modify the protein; and (iii) free and attached chromophores and chemical additives may severely alter the photoresponse of BR.

Long-term 2D data storage on photochromic BR films may allow optical information to be recorded with an unprecedented resolution of about 10^5 lines mm^{-1} compared to conventional optics which do not exceed 2×10^3 lines mm^{-1}. Data storage is based on the *all-trans*-retinal Schiff base (B state) interconversion into the 11-*cis*-isomer (D state). The particular branched photocycle of BR would enable recording and storage of information via a two-step process: apart from the B (*all-trans*) to K (11-*cis*) transition, wild-type BR may be converted to the O-state (*all-trans*) which isomerizes into the P-state (9-*cis*).

The two-photon excitation of bacteriorhodopsin was instrumental in the design of 3D data storage involving repetitive write−read−erase cycles as well as holographic storage. BR can be photoexcited by two perpendicular photons λ_1 and λ_2 provided they coincide and fulfill the condition ($1/\lambda_{excitation} = 1/\lambda_1 + 1/\lambda_2$) (Scheme 21). Reading of information is possible by using two single wavelength perpendicular laser beams to generate a photovoltaic effect.[153] High-resolution long-term storage with BR is presently restricted by several limitations, such as low temperature, usable light wavelength, a difficult read-out procedure, and low diffraction efficiency.

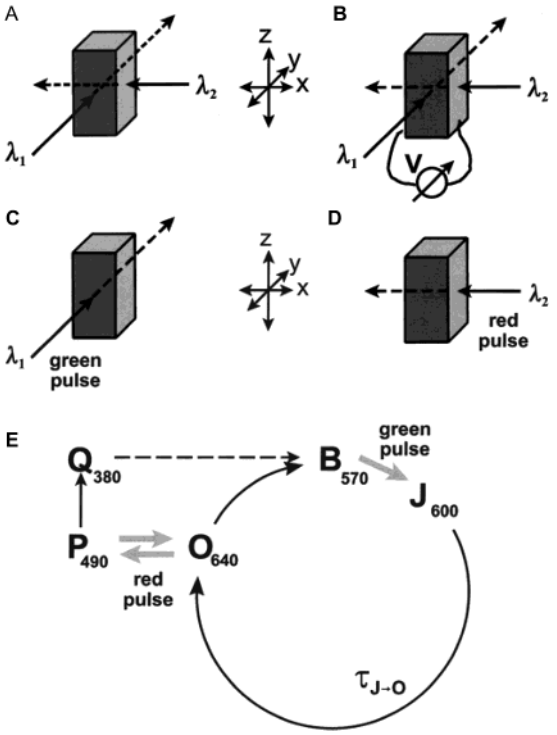
5. GFPs

GFP has been used for many applications in biology, such as a reporter gene and fusion tag. It has served as a tool in studying protein−protein interactions by fluorescence resonance energy transfer (FRET)[50] and photobleaching[253,254] and may be used as a marker for tumor cells. Wild-type and mutant GFP are pH-sensitive and display a strong affinity for Cu(II) and lower affinity for Ni(II). Many yellow and cyan variants of GFP have been designed as pH indicators and metal biosensors.[50]

6. Bilirubins

Bilirubin selectively binds a number of proteins, in particular, human serum albumin (HSA) to form a 1:1 complex, which remains photosensitive. Its *Z*−*E* photoisomerization causes significant CD changes. Only bilirubin-IIIα is usable for information storage due to the existence of a competitive photocyclization reaction of bilirubin-IXα.[255]

Scheme 21[a]



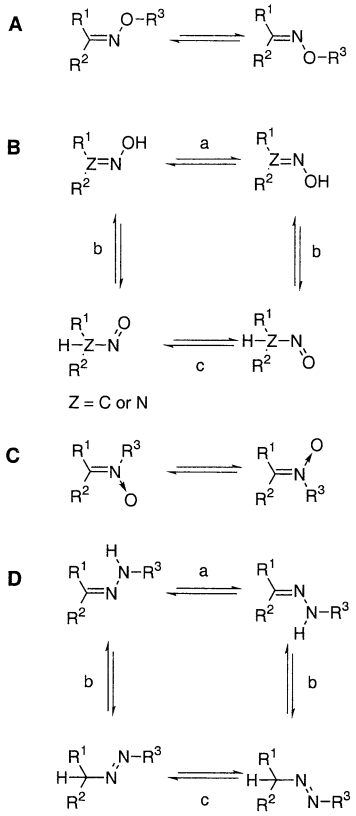
[a] (A, B) Principle of 3-D data storage in bacteriorhodopsin (BR) using two-photon absorption. (A) writing with $\lambda_1 = \lambda_2 = 1140$ nm (B- to M-state transition) and $\lambda_1 = \lambda_2 = 820$ nm (M- to B-state transition). (B) Reading is performed by applying a write pulse which switches BR in the B-state to M-state. This transition is accompanied by a transient voltage that may be detected by external electrodes. (C,D) Principle of 3-D data storage in BR using two one-photon photochemical transitions. (C) A green laser pulse drives the BR from the initial B-state to the J-state from where it relaxes thermally to the O-state. (D) Then a red laser pulse can convert the BR to the P-state which relaxes slowly to the Q-state. If no second laser pulse is applied, the BR returns to the initial state. (E) Simplified representation of the branched BR photocycle (Adapted from Hampp, N. *Chem. Rev.* **2000**, *100*, 1755).

III. Carbon–Nitrogen and Nitrogen–Nitrogen Double Bonds

A. Cis–Trans Isomerization of Nitrogen-Containing π-Systems

In contrast to acrylates, enamines and imines, carbon−nitrogen−oxygen/nitrogen and nitrogen−nitrogen π-systems are not usually found in natural products, with the exception of oximes that have been identified in liver as metabolites of amines such as the adrenergic agent tyramine.[256] Oxime,[257] azo,[258] hydrazone,[259] and triazene[260] motifs have been used in enzyme inhibitors. Nitrones have also served as spin traps that display antioxidative properties.[261] Numerous photoswitchable azo-containing moieties have been developed for the control of the conformation of peptides and other biomolecules. These may play a role in the growing fields of nanotechnology and bioelectronics (for reviews, see *Chem. Rev.* **2000**, *100* and *Acc. Chem. Res.* **2001**, *34*). Several mechanisms have been proposed for the cis−trans isomerization of C=N and N=N systems: (i) inversion of the nitrogen doublet, (ii) deconjugation of the corresponding cation, (iii) tautomeric isomerization, and

Scheme 22. *Z*−*E* Isomerization of oximes (A), nitroso compounds (B), nitrones (C), and Diazenes (D)[a]



[a] Isomerization pathways: doublet inversion (a), prototropy (b), free rotation of the prototropic form (c).

(iv) homolytic cleavage of the double bond (Scheme 22).

Oximes and oxime ethers usually exist as a mixture of *cis*- and the more stable *trans*-isomer that interconvert spontaneously (Scheme 22A),[262] photochemically via relaxation of the perpendicular lowest energy triplet state,[263,264] and by catalyzed rotation involving nitronium ions in both acidic and basic media[265] (see Scheme 1). The mechanism of interconversion (inversion or rotation) depends on the substituents[266] and is strongly limited by steric hindrance.[267]

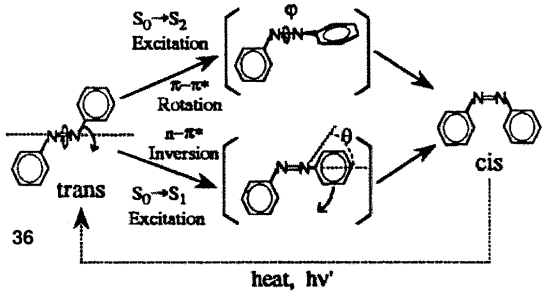
C- and N-nitroso compounds, which are often represented as C−N═O and N−N═O, respectively, are expected to rotate readily around the C−N and N−N single bonds. However, delocalization of the π-electrons entails the existence of an *E*−*Z* equilibrium. The *E*:*Z* ratio as well as the kinetics of interconversion can both be affected by the prototropic effect (Scheme 22B), doublet inversion, as well as dimerization of C-nitroso compounds. The energy barrier for *Z*−*E* isomerization of *N*-nitroso compounds is typically about 12−27 kcal mol^{-1}.[268] Although both isomers are often observed in solution, the *E*-isomer is the most stable in most cases. Acyl nitroso compounds are interesting amide surrogates which display a preferential *E*-conformation due to oxygen−oxygen electronic repulsion.[269]

Nitrones (Scheme 22C) can be reversibly isomerized either by photosensitization via a triplet state or thermally by iodine catalysis.[270] The cis−trans photochemical interconversion may compete with oxazirane formation.[271] Carbonyl-conjugated nitrones can readily isomerize in solvent[272] and by Lewis acid catalysis.[273] Theoretical calculations predict a large dipole moment difference between *Z*- and *E*-isomers.[274]

Hydrazones display properties that are similar to those of imines,[275,276] in particular, cis−trans isomerism. The presence of an additional nitrogen atom decreases the double-bond character of the π-system and facilitates isomerization. They may also be linked to azo compounds due to hydrazino-azo isomerism[277,278] (Scheme 22D). Hydrazone can undergo both photo[279] and thermal[277] isomerization, which is usually facilitated in polar solvents, by acid/base catalysis,[278−280] and by electron-donating substituents.[281] In most cases, the lowest energy configuration is E. However, intramolecular H-bonding[282] and particular strains[283] may offset the equilibrium.

Diarylazo compounds such as compound **36** have been widely employed as dyes and inserted inside biological probes, photoresponsive polymers, and novel materials used in nanotechnology and supramolecular chemistry. As a consequence, azo compounds have been widely investigated.[284] The diarylazo moiety exclusively exists as the *trans*-isomer in the dark and readily isomerizes to the less stable cis-isomer upon excitation at 320−350 nm. The reaction is fully reversible either by irradiation around 400−450 nm or by heating. The maximum excitation wavelength depends on the nature of the substituents on the aryl groups. Push−pull diazenes have red-shifted excitation wavelengths. Azo groups that possess lone pair electrons on both nitrogen atoms can isomerize via two distinct mechanisms because the presence of doublets allows the $n-\pi^*$ electronic transition with inversion of the nitrogen as well as the $\pi-\pi^*$ transition (rotation mechanism) involving a singlet pathway similar to that of stilbene (Scheme 23).[242]

Scheme 23. Proposed Reaction Mechanism of Wavelength-Dependent Photoisomerization of *trans*-Azobenzene 36[a]

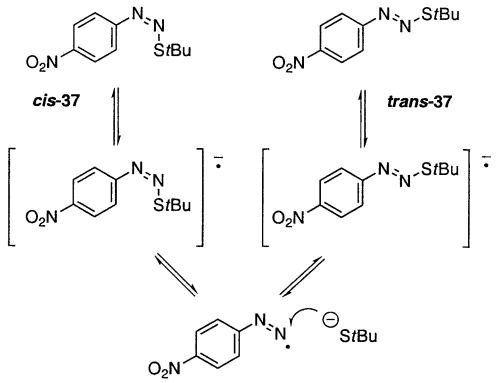


[a] Reprinted with permission from *Chem. Rev.* **2000**, *100*, 1875−1890. Copyright 2000 American Chemical Society.

The nature of the electronic transition likely depends on the excitation wavelength. In the inversion mechanism, a semilinear transition state has been proposed because a linear transition state is expected to have a higher energy. The inversion mechanism has been particularly useful in explaining the isomerization of restricted azobenzene compounds because the rotation mechanism is not permitted due to the

steric hindrance.[242] Time-resolved fluorescence experiments have shown that trans → cis and cis → trans isomerizations are extremely fast processes that reach the limits of detection of the method and have been estimated to be below the picosecond timescale. Isomerizations via $n-\pi^*$ and $\pi-\pi^*$ electronic transitions take place in 200−400 fs. *Z*-4-nitrophenyl *tert*-butylazosulfide **37** isomerizes about 1000 times faster than azobenzene. This remarkable acceleration has been explained by a novel cleavage/recombination mechanism (reversible cleavage of the radical anion, Scheme 24).[285] Factors, such as solvent viscosity and

Scheme 24. Extremely Fast Cleavage-recombination Mechanism of *Z*-4-Nitrophenyl *tert*-butylazosulfide 37



polarity, and hydrogen bonding[286−288] can influence cis−trans isomerization, especially in the case of push−pull azo compounds.

The influence of protonation in the thermal cis−trans isomerization of diaryl azo compound **38** has been experimentally demonstrated[277,289] and rationalized.[290] In this case, the acid−base-catalyzed tautomerization of *cis*-ammonium ions to azonium ions produces a decrease in the double-bond character which enables the rotation around the −N=N− bond (Scheme 25).

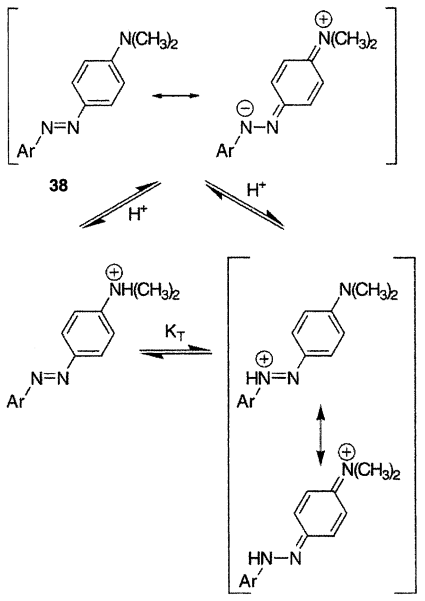
Triazene compounds display properties that are very similar to those of azo compounds,[291] except that a polar rotation mechanism has been proposed for the rotation about the N−N=N double bond.[292] Moreover, triazenium salts shares some features with the corresponding triazenes, suggesting that triazene could isomerize in the protonated state.[293,294]

B. Applications of Azo Compounds in Biology and Supramolecular Chemistry

1. Light-Switchable Ligands and Photomodulation of Peptide Conformation

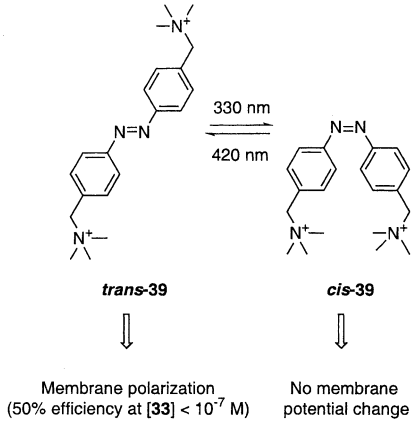
Controlling the conformation and hence the activity of biomolecules in a reversible way without adding any reagent is an attractive strategy which was first applied to functional and structural studies of the nicotinic acetylcholine receptor (AChR) in the late 60s.[295] The 3,3′-bis[α-(trimethylammonium)methyl]-azobenzene **39** readily isomerizes to the major *cis*-isomer upon irradiation at about 330 nm, whereas the reaction may be reversed at 420 nm (Scheme 26).[296,297] Specific interaction of both isomers with

Scheme 25. Acid−Base Catalyzed Cis−Trans Isomerization of Methyl Orange 38[a]



[a] Reprinted from Sanchez, A.; Barra, M.; De Rossi, R. H. *J. Org. Chem.* **1999**, *64*, 1604. Copyright 1999 American Chemical Society.

Scheme 26. 3,3′-bis[A-(trimethylammonium)-methyl]azobenzene 39 as a Photoswitchable Ligand of the Acetylcholine Receptor



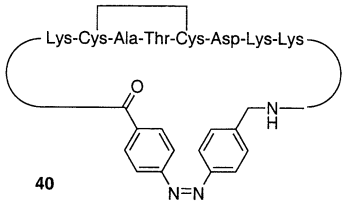
AChR results in a modulation of potential across the excitable membrane and is the first model illustrating that it is possible to photoregulate the membrane potential.[298]

Since then, the chemistry of azobenzyl compounds has opened the way to numerous applications including the photomodulation of the conformation of linear[299−302] and cyclic[303−308] peptides and DNA[309] by introducing photoswitches both inside the peptide backbone and at side chains. Subpicosecond light-driven cis−trans interconversion can induce structural changes on the subnanosecond time-scale,[310] such as random coil/α-helix transitions and helix-sense reversals as well as solubility modifications. These photoinduced structural changes usually occur as cooperative transitions enabling the transduction and amplification of the primary photochemical event.[213,311]

For example, Moroder and co-workers recently reported the design of a soluble photoswitchable

cyclic peptide containing the thioredoxin-derived Cys−Ala−Thr−Cys sequence and investigated the folding efficiency of RNAse A in the presence of both *cis*- and *trans*-isomers (Chart 5). Light irradiation at

Chart 5. Photoswitchable Cyclic Peptide 40 Containing a Thioredoxin-Like Sequence



40

360 nm induced a significant modification of the peptide backbone conformation of compound **40** which resulted in a dramatic rise in redox potential (trans: $E_0' = -200$ mV) to a more oxidizing value (cis: $E_0' = -146$ mV). This redox potential makes this isomer very similar to the native enzyme PDI ($E_0' = -147/-159$ mV) and correlates very well with the NMR conformational analysis (Table 2). Photoregulation of

Table 2. Apparent Redox Potentials of Linear and Cyclic Peptides Containing the Thioredoxin-Derived Cys−Ala−Thr−Cys Sequence at pH 7.0[a]

compound	K_{ox} (M)	E_0' (mV)
thioredoxin		−270
thioredoxin reductase		−250
glutathione		−240
thioredoxin reductase [134−141]	0.123	−210
trans-**40**	0.050	−200
native PDI enzymes		−147/−159
cis-**40**	8×10^{-4}	−146
DsbA		−122

[a] Cattani-Scholz, A.; Renner, C.; Cabrele, C.; Behrendt, R.; Oesterhelt, D.; Moroder, L. *Angew. Chem. Intl. Ed.* **2002**, *41*, 289.

the protein folding by photoswitchable thioredoxin derivative **40** suggests that the large difference between redox potentials observed in the class of disulfide oxidoreductases (−270 to −122 mV), which possess a common thioredoxin fold,[305,312] might be related to local constraints inside the fold.

The photocontrol of the solubility of azo-modified polypeptides has also been investigated.[213] Intermolecular stacking of *trans*-diarylazo moieties causes dimerization of grafted α-helices of poly(L-glutamic acid), poly(L-ornithine), and poly(L-α,β-diaminopropanoic acid) bearing the photoswitchable moiety. The resulting formation of aggregates in hexafluoro-2-propanol/water is photoinduced by irradiation at 450 nm, and precipitation is fully reversed by light-irradiation at 350 nm. Several aggregation−disaggregation cycles were photoinduced.[213] This is typically a cooperative process which is very similar to the P_r-P_{fr} transitions of phytochrome pigment.

Gene expression can be regulated by tethering an azobenzene moiety to a DNA promoter. Transcription by T7 RNA polymerase was impeded by the presence of a *trans*-azobenzyl group which presumably intercalates between the base-pairs. UV-driven trans-to-cis isomerization flipped the chromophore out of the helix and then switched on the transcription.[313]

The azo motif was also employed for the modulation of the size,[314] self-assembly,[315] and optical[316] and encapsulation[317] properties of dendrimers.[318,319] In contrast with most other diarylazo-grafted polymers, no cooperativity was exhibited by azodendrimers, and all possible discrete states of isomerization were observed because $Z-E$ interconversion of a single unit did not influence other azo moieties.[314,320] Because photoisomerization results in a strong modification of polarity, fine-tuning of molecular structure and physical and biological properties may be possible with such dendrimers. Most of these molecules are not water-soluble, but photoresponsive dendritic azobenzene peptide **41** may be covalently attached to other biomolecules to photomodulate their biological properties (Chart 6).[321]

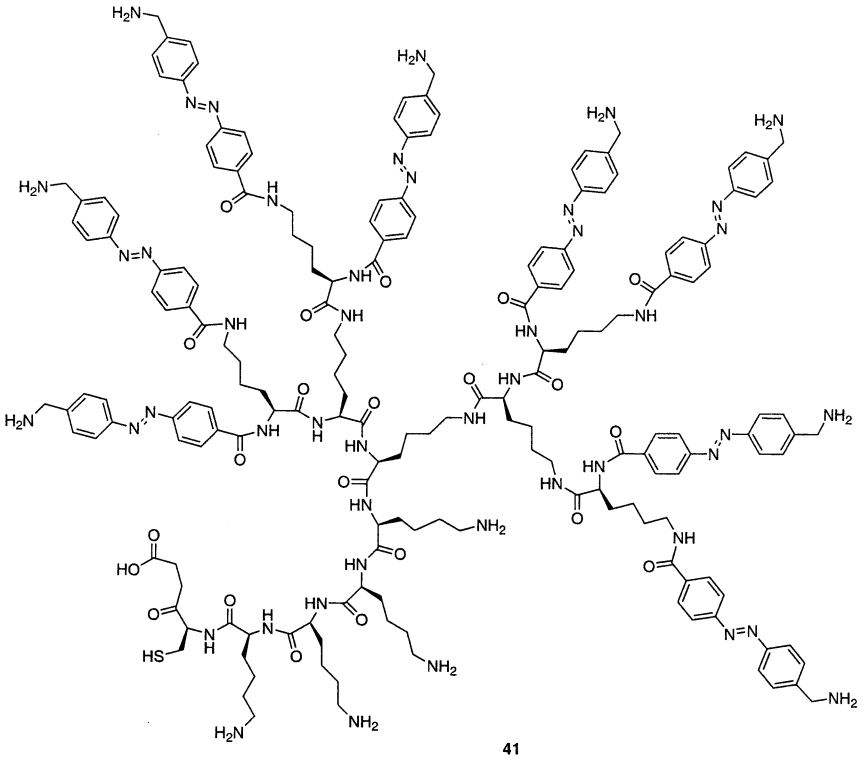
2. Light-Switchable Receptors and Sensors

Light-controlled molecular recognition is a fascinating challenge that has found many applications in biology. Photoresponsive motifs can be used to tune the properties of artificial receptors, sensors, and enzymes. The phototuning of ion specific aza-crowns **42**−**45**,[284] aza-calixarenes **46**,[322] and aza-calixcrowns **47**[323] has been reported in several papers (Chart 7). In particular, azobenzene crown *p*-*tert*-butylcalix[4]-arenes **46** are photoswitchable receptors with a marked preference for Na^+ in the *cis*-conformation and an exclusive affinity for K^+ in the *trans*-isomer.[322] Cation selectivity is controlled by ring size and conformation as well as significant cation−π interactions with the two aromatic rings of the diarylazo moiety.[323,324] Azo/hydrazono tautomerism can play an important role in the selective binding of cations such as Ag^+.[325] Cation complexation may be accompanied by color[326] and absorbance[327] changes, and can be monitored electrochemically using Langmuir−Blodgett monolayers.[328]

Different geometric macrocyclic supramolecules can be prepared by assembling dipyridylazo bridging ligands on one hand, and Pd(II) and Re(I) complexes on the other hand. The trans → cis isomerization process of the azo moiety initiated by irradiation at 366 or 313 nm generates large strains on the square structure of complex **48** and cause breaking of metal−nitrogen bonds and the tetranuclear species thus transforms into a dinuclear complex **49** (Scheme 27). The reaction was reverted by heating, suggesting that it is therefore possible to photomodulate the size of self-assembly of macrocylclic compounds containing metal complexes.[329] A similar behavior was also observed with stilbene-containing complexes.[329,330]

Cyclodextrins have also been converted to photoswitchable receptors (compound **50**) to regulate the formation of an inclusion complex (Scheme 28).[331] Because β-cyclodextrin specifically recognizes the *E*-diarylazo moiety of a bipyridinium-azobenzene diad **51**, formation of the inclusion complex can be photochemically initiated in a completely reversible manner. Such a process may be used for microgravimetric transduction of optical signals recorded by the bipyridinium-azobenzene diad via a β-cyclodextrin

Chart 6. Photoresponsive Dendritic Azobenzene Peptide 41



41

monolayer prepared on an Au electrode (Scheme 29).[332−334] Cis−trans isomerization of diarylazo compounds can also be used to probe the microenvironment in gels using spectroscopic methods.[335]

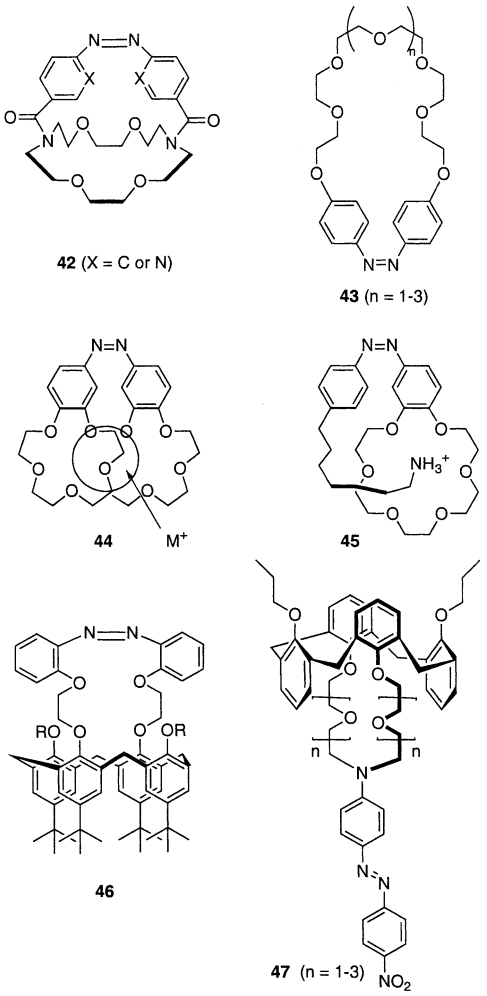
A diarylazo moiety tethering two cyclic octapeptides with the sequence [(L-Phe-D^{Me}N-Ala)$_3$-L-Cys-D-^{Me}N-Ala] **52**, was employed to modulate the stacking of each peptide unit.[336] The flat, ring-shaped peptide system tends to form very stable layers at the air−water surface in both *E*- and *Z*-configurations and switches photochemically in a reversible way with a remarkable 70 Å^2 variation of surface per molecule (Scheme 30). In addition, this process takes place in thin films on solid support and enables the control of supramolecular surfaces.[337]

Photomodulation of enzymes has been investigated for a long time.[338] In a recent report, the design of a photosensitive azoaldolase demonstrated photomodulation of an enzyme without affecting its catalytic activity or its Michaelian kinetics. Moreover, this photoresponsive azoaldolase can be considered as a model for a possible mechanism of light regulation in biological systems, based on variations in the molecular recognition of the substrate by the active protein.[339] Functional photoswitchable horseradish peroxidase mutants containing a *p*-phenylazophenylalanine residue at varying positions have also been described.[340]

3. Diazene-Containing Polymers and Light-Addressable Memories

Many azo dyes such as Congo red, methyl orange, or orange II have been commonly employed in industry. They have also been applied as protein ligands and enzyme inhibitors.[341] Very promising advances, recently reviewed by Delaire[342] and Ichimura,[232] have been obtained in the field of light-sensitive

Chart 7. Phototunable Ion-specific Aza-Crowns and Calixarene Compounds



42 (X = C or N)

43 (n = 1-3)

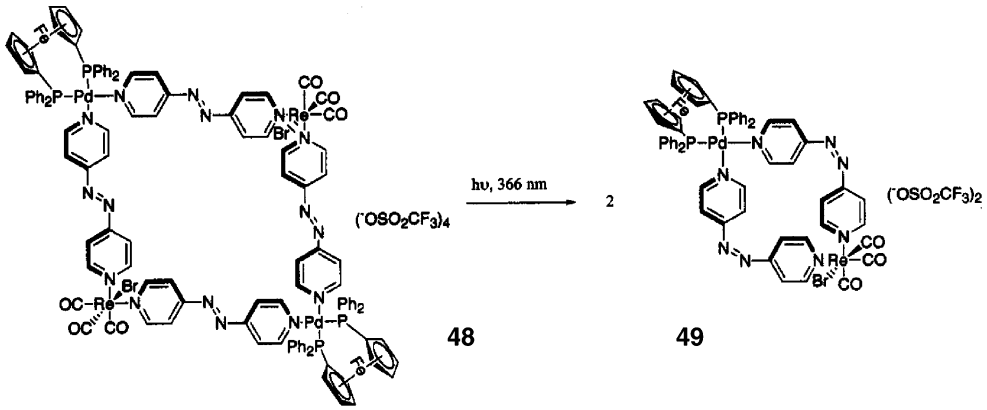
44 M^+

45

46

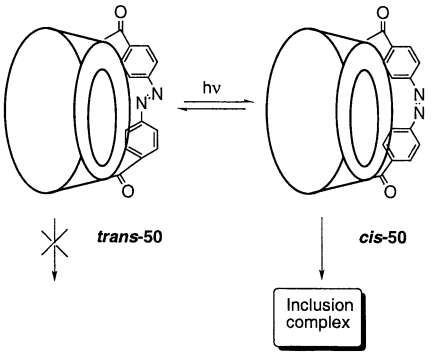
47 (n = 1-3)

Scheme 27. Light-Induced Trans to Cis Isomerization of a Photoswitchable Terameric Re_2/Pd_2 Complex 48[a]

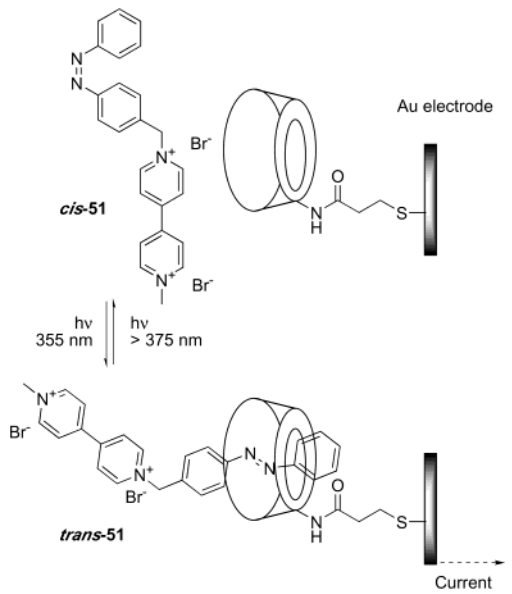


[a] Adapted from *J. Am. Chem. Soc.* **2000**, *122*, *37*, 8956.

Scheme 28. Photoregulation of the Formation of Inclusion Complex Using Azobenzene-Capped β-Cyclodextrine 50



Scheme 29. Schematic Representation of a Sensor Constituted by a β-Cyclodextrin Monolayer on a Gold Electrode[a]



[a] *Z* to *E* photoisomerization of the ligand **51** and subsequent interaction with the β-cyclodextrin monolayer results in an electrochemical and microgravimetric transduction of the signal.

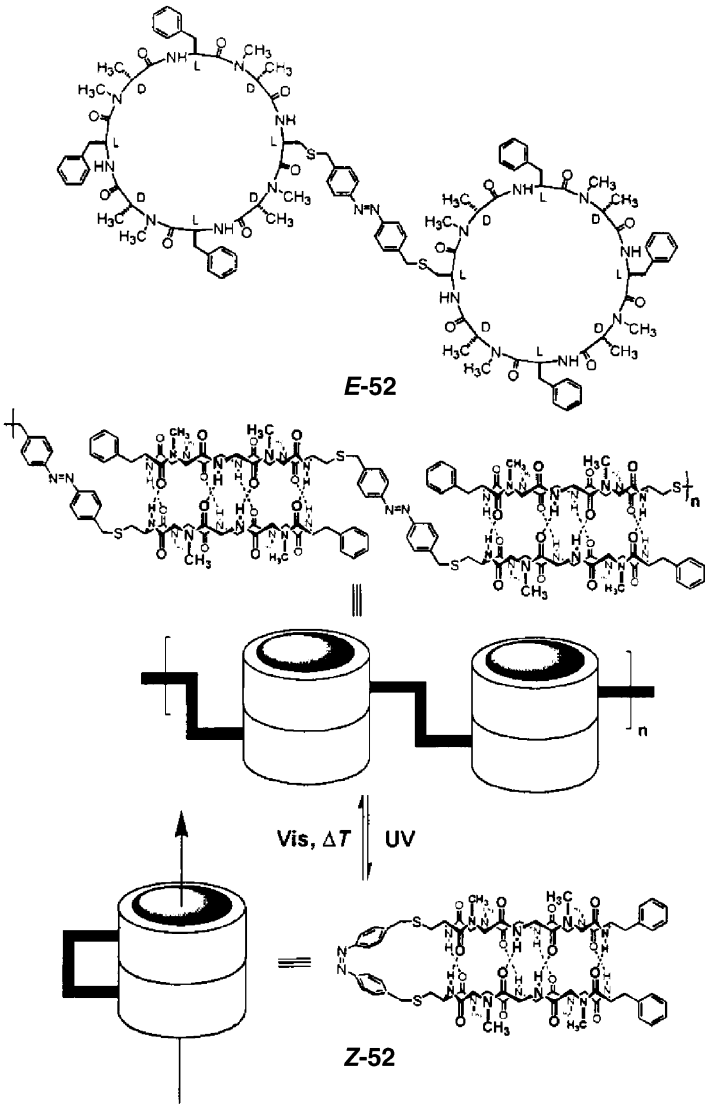
polymers. Among other processes such as electrocyclic reactions and photodimerization, *E*−*Z* isomerization is a widespread reaction employed for liquid crystal photoaligment (Chart 8).[232]

An impressive number of liquid crystalline polymers featuring diarylazo groups have been investigated. They display tunable optical and photochromic properties, contingent on the nature of the polymer matrix, the grafting nature, material density, and aryl substituents. Fine-tuning of $n-\pi^*$ and $\pi-\pi^*$ transitions can be achieved by regulating the $Z \rightarrow E$ and $E \rightarrow Z$ energy barriers.[342] Photoinduced anisotropy of photochromic polymers by linearly polarized light does not result from a simple $Z \rightarrow E$ process but rather comes from a rotation of the molecules during a $Z \rightarrow E \rightarrow Z$ photoisomerization cycle (Scheme 31). This leads to a permanent reorientation of molecules that accumulate in a direction of the smallest probability of pumping and therefore secure the perpetuation of the anisotropy. On the other hand, molecules that are perpendicular to the polarization direction undergo a number of random reorientations through reversible cis−trans isomerization cycles.[243] Other chromophoric groups such as α-hydrazono-β-ketoesters (Chart 8D) and stilbene (Chart 8B) share with diazenes a common propensity to out-of-plane photoalignment upon light irradiation.[232]

Helical stacked azobenzene-substituted peptide oligomers are promising materials for erasable holographic information storage because the peptide backbone can favor stacking of chromophores and limit the number of possible orientations. As an example, photoisomerization of azobenzene-grafted poly-aspartate **53** at wavelengths below 400 nm leads to the amplification of the signal: upon irradiation, the exclusive left-handed helix in the all-trans form switches to a 70% preference for the right-handed helix with only 9.7% azo groups in the *cis*-conformation. The process, which is fully reversible upon light irradiation at $\lambda > 400$ nm, can be monitored by CD spectroscopy in the chromophore region (Figure 9).[243,343] Although photochromic materials may have low thermal stability of their *cis*-azobenzene isomer, azobenzenesulfonyl-grafted polylysine polymers **54** display a highly stable *cis*-form at room temperature for several weeks and exhibit an interesting wavelength-dependent coil-α-helix transition (Figure 10).[344]

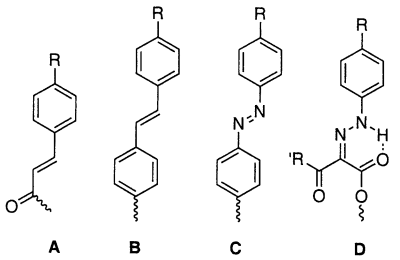
Another approach was developed with isocyanate polymers bearing an azobenzene moiety and a stereocenter. Polyisocyanates form helices that can either twist locally (fragment twist, compound **55**)[345]

Scheme 30. Layer Formation and Variation of Surface of Photoisomerizable Molecule 52 Constituted by a Diaryazomoiety Tethering Two Cyclic Octapeptides[a]



[a] Adapted from Steinem, C.; Janshoff, A.; Vollmer, M. S.; Ghadiri, M. R. *Langmuir* **1999**, *15*, 3956.

Chart 8. Photoisomerizable Moieties Usually Grafted on Polymers[a]

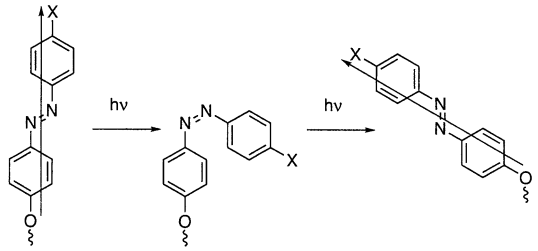


[a] Cinamate (A); stilbene (B); diarylazo (C); α-hydrazono-β-ketoesters (each isomeric form is stabilized via an intramolecular H-bond (D). Adapted from *J. Am. Chem. Soc.* **1999**, *121*, 4738−4743.

without change of helix sense, or inverse the helix twist sense (compound **56**, Scheme 32).[346] In all cases, photoirradiation induces a strong cooperative response associated with an impressive amplification of chirality.

Besides temperature sensitivity, a major drawback in the current use of azobenzene-based materials for

Scheme 31. Permanent Reorientation of Diarylazo Moieties of Photochromic Polymers via a Two-Consecutive-Isomerizations Process Results in a Photoinduced Anisotropy



holographic information storage is the long exposure time (typically 200 s) required for efficient response of the polymer, both for recording and erasing. Recently, a proline-based azobenzene peptide **57** (Chart 9) was described as having an extraordinarily short irradiation time (<1 s) and high diffraction efficiency (up to 80%).[347]

The reversible photochemical control of gel formation associated with a modulation of optical trans-

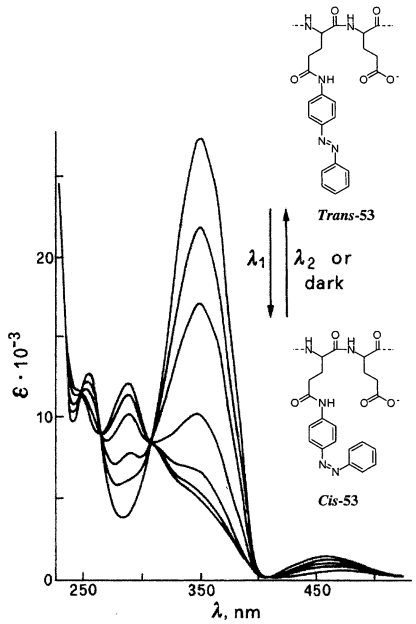


Figure 9. Reversible light-induced variations of azo-modified polyglutamic acid **53**. Adapted from Figure 1, ref 213..

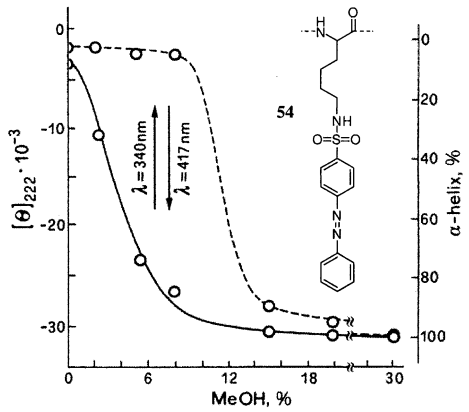


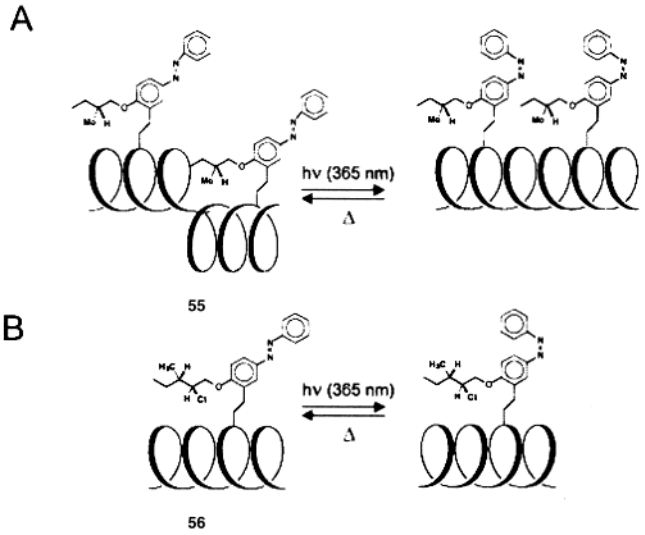
Figure 10. Intensity of the CD band at 222 nm and α-helix percentage of a poly(azobenzenesulfonyl-L-lysine **54** in a mixture of hexafluoro-2-propanol/methanol as a function of methanol concentration for samples irradiated at 417 nm (continuous line) and 340 nm (dashed line). (Pieroni, O.; Hoben, J. L.; Fissi, A.; Constantino, P.; Ciardelli, P. *J. Am. Chem. Soc.* **1980**, *102*, 5913). Adapted from *Acc. Chem. Res.* **2001**, *34*, 9−17, Figure 8.

mittance has also been reported for 4-methoxyazobenzoyl cholesterol. *E* to *Z* photoisomerization was performed in the 330−380 nm range. Back isomerization occurred under thermal conditions (slow) and light irradiation at $\lambda > 460$ nm (fast). The photoreaction, associated with a sol−gel phase transition, resulted in a modification of optical properties ($\Delta_{transmitance} = 2.2\%$).[243]

4. Azo-Based Molecular Machines

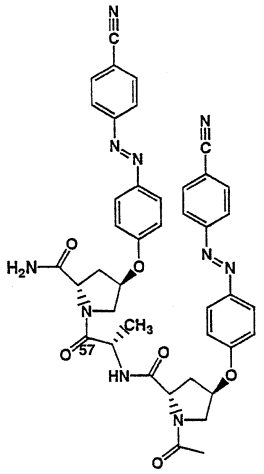
Another interesting application of the arylazo/cyclodextrin-based supramolecular chemistry is the development of light-switchable molecular shuttles: A hydrophobic aliphatic chain, terminated by two charged bipyridinium locks, was demonstrated to act as an "electric trap" for cyclodextrin[348,349] or azobiphenoxy units.[350] The central or off center position

Scheme 32. Controlled Fragment Twist (A) or Change of Helicity (B) by Irradiation of a Polyisocyanate Helices 55 and 56 Grafted with Chiral Diarylazo Groups (Müller 1994, Maxein 1995)[a]



[a] Adapted from *Chem. Rev.* **2000**, *100*, 1789−1816.

Chart 9. Proline-Based Azobenzene Peptide 57 Used for Holographic Storage



of the cyclodextrin ring was regulated by light, once again in a completely reversible way.[351] Such rotaxane molecular shuttles are very similar to their stilbene equivalents (see Scheme 17).[241]

IV. Pseudo-Double Bonds

A. Restricted Rotation in Conjugated π-Systems

1. Thermodynamic Aspects

Ester and amide groups are of fundamental importance in biological systems and the existence of similar energy *Z*- and *E*-isomers has aroused considerable interest in understanding the molecular mechanism for their isomerization in chemistry and biology. Other groups such as carbamates and ureas, which are mainly found in materials and therapeutics, display similar properties. These motifs are characterized by a potential deconjugation of the heteroatom−(σ-bond)−carbon−(π-bond)−heteroatom sys-

tem which restricts the free rotation about the formal single σ-bond, and therefore, two isomers may coexist.[352] The nomenclature of ester, amide, carbamate, and urea isomers is depicted in Figure 11.

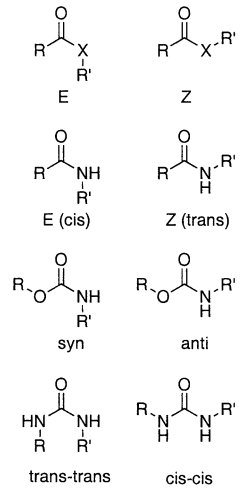


Figure 11. Nomenclature used for calling most stable rotamers of esters, amides, carbamates, and ureas.

The general preferences of esters for a *Z*-conformation (*trans*-conformation by analogy with olefins) with an energy difference of about 3 kcal mol^{-1} may be explained by possible steric interactions when R and R′ are large (Figure 11). Dipole−dipole interactions have been held to account for this conformational preference. Although *Z*-formate esters are favored, the free energy difference decreases for bulky R′. Moreover, "aromaticity" and an additional secondary electronic effect (e.g., $n_0 \rightarrow p^*$) in the *Z*- compared to the *E*-ester form have been suggested to stabilize the *Z*-isomer.[353] For example, *E*-methyl formate is only observed ($\leq$ 0.3%) in favorable solvents at low temperature (−83 °C, Table 3).[354] In *tert*-butyl formate,

Table 3. Comparative R′ Substituent Effect on the Free Energy Differences of Esters, Thiol Esters, and Amides (R = H) in kcal mol^{-1}

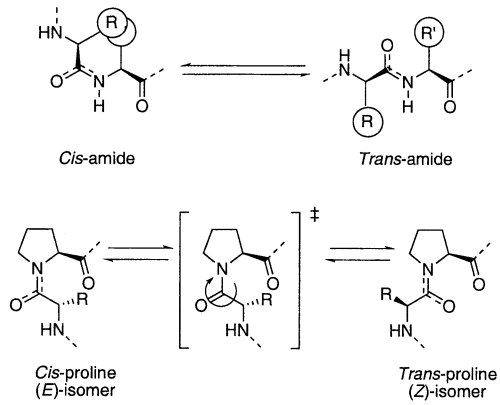
R′	RCOOR′	RCOSR′	RCONHR′
methyl	2.15	1.29	1.22
ethyl			0.93
vinyl	0.95		0.42
propyl	0.43		0.93
cyclopropyl		0.31	0.35
iso-propyl		0.93	0.83
cyclobutyl			0.65
cyclopentyl		0.90	0.82
tert-butyl	0.57	0.51	0.17
phenyl		0.13	0.14

there must be a strong electronic repulsion between the carbonyl oxygen and the *tert*-butyl group in the *Z*-isomer. However, this form still predominates (90%).[355,356]

In the case of thioesters HCOSR, determination of *Z*:*E* ratio and free energy differences by dynamic NMR spectroscopy showed that both parameters are strongly influenced by the solvent and the nature of R′. Methyl thionoformate was shown to have 3.0 and 10.6% of the *E*-conformation, respectively, in acetone ($\Delta G°$ = 1.29 kcal mol^{-1}) and dichloromethane ($\Delta G°$ = 1.57 kcal mol^{-1}). Cyclopropylthionoformate has 29.3% of *E*-isomer in acetone ($\Delta G°$ = 0.31 kcal mol^{-1}) at low temperature (−81 to −96 °C).[357]

Amides in solution show a similar, though less pronounced, preference for the *trans*-isomer (*Z*-isomer). The enthalpic difference between the two conformers is also relatively low and has been shown to lie anywhere between 0.5 and 2.5 kcal mol^{-1} for secondary and tertiary amides.[358−360] The marked pseudo-double bond character of the amide enables the cis−trans interconversion with a moderate free energy barrier ($\Delta G^{\ddagger}$ = 16−22 kcal mol^{-1}, Scheme 33).

Scheme 33. Local Variations of Steric Interaction Related to the Amide Cis−Trans Isomerization Process in Peptides and Proteins



The $\Delta S^{\ddagger}$ for rotation of amides and similar compounds is usually close to zero. It is noteworthy that isomerization is easier in the amide series relative to the corresponding thioamides.[361,362] Free enthalpies of activation are correlated linearly by the formula $\Delta G^{\ddagger}_{\text{thioamide}} = 1.13 + 1.11\ \Delta G^{\ddagger}_{\text{amide}}$.[363] Photoisomerization of arylthioamide has been reported to proceed via both the singlet and triplet excited states.[364,365]

Secondary and tertiary amides, the connecting motifs in peptides and proteins, are among the most widespread functions in biomolecules. Amide cis−trans isomerism thus plays a central role in the conformations and activities of peptides and proteins.[366] The consequences of peptide backbone reorientation from cis−trans isomerism include distinct hydrogen bonding patterns, hydrophobic interactions, and solvations.[367] *Cis*-tertiary amides, in particular proline, tend to form 10-(β-turn), 7-(Pro γ-turn), and 13-membered intramolecular H-bonds.[368,369] *Cis*- and *trans*-isomers have different hydration shells, and therefore water molecules interacting with the them play a crucial role in determining the relative cis and trans populations.[370] Therefore, in vacuo simulations that ignore interactions with water molecules may be subject to criticism, and any reliable molecular dynamics calculations must consider the first-shell water molecule.[371] The solvation/desolvation process also accounts for permeation of hydrophobic cyclic peptides such as cyclosporin A accross the membrane.[372]

Although oxalamide and hydrazide moieties are not usually found in biomolecules, they have been employed as pseudopeptide templates in bioorganic and medicinal chemistry. Due to the existence of multiple ω dihedral angles, oxalamide and hydrazide groups

display more than two isomers and molecular modeling and gas-phase simulations have demonstrated that *N*,*N*′-dimethyloxalamide **58** and *N*,*N*′-diacetylhydrazine **59** exist, respectively, as three (trans−trans−trans, cis−trans−trans, and cis−syn−cis, noted respectively ttt, ctt and csc) and four (trans−gauche−trans, trans−trans−trans, cis−gauche−trans, and cis−syn−cis noted, respectively, tgt, ttt, cgt, and csc) possible isomers. Free energy differences computed in water suggest that *ttt*-**58** and *cgt*-**59** are the most stable conformations (Figure 12). Oxalamide **58** has

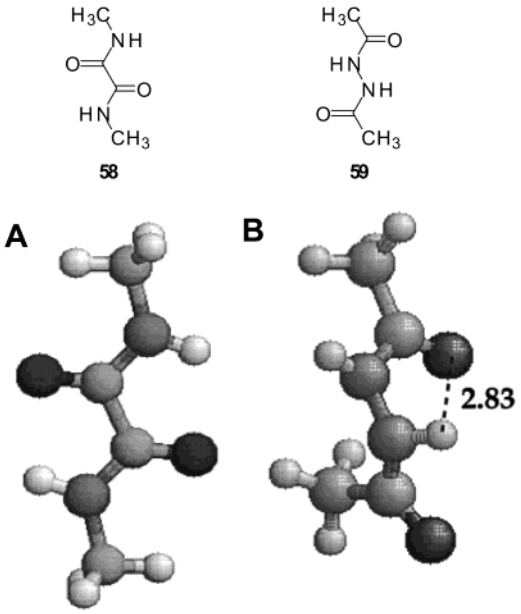


Figure 12. Lower energy conformation of *N*,*N*′-dimethyloxalamide **58** (ttt) and *N*,*N*′-diacetylhydrazide **59** (cgt) in solution (data obtained at the MP2/6-31G level). Adapted from Figures 2a and 3b, ref 373.

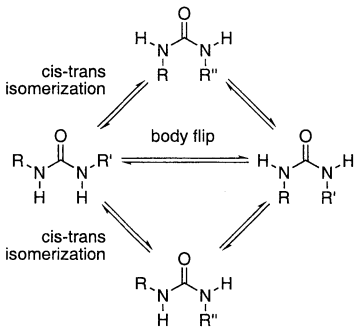
some similarity with the corresponding monoamide, and its conformational preferences are quite different from those of hydrazide **59** irrespective from the environment. Conversely, hydrazide **59** is significantly different because the pyramidal nitrogen atoms exhibit a reduced amide resonance.[373] Therefore, the N−N bond behaves as a single bond.

Hydrazino peptides have recently been employed as β-peptides analogues with an enhanced ability to adopt various conformations. Increasing the number of dihedral angles and potential H-bonding sites gives access to a large variety of stable secondary structures.[374−376]

Carbamates are close structural relatives to amides, but they display a less marked preference for the *anti*-isomer (Figure 11) because the free enthalpy difference lies between 1.0 and 1.5 kcal mol^{-1} for steric and electrostatic reasons.[377] Hydrogen bonding has been shown to alter this equilibrium.[378,379] The barrier to rotation is fairly high (Table 3), and, in many cases, a Boc group may appear as two singlets at a greatly upfield position in the ^{1}H NMR spectrum (singlets at 1.23 and 1.28 ppm vs a lone singlet at 1.45 ppm) at room temperature.[380]

Ureas have barriers to rotation lower than the corresponding amides as a result of competitive conjugation. They can isomerize (Scheme 34) via

Scheme 34. Sequential Cis−Trans Isomerization an Whole Body Flip of Ureas



either a classical cis−trans isomerization ($\Delta G^{\ddagger} = 18.5 \pm 1.6$ kcal mol^{-1}) or a whole-body motion (urea flip, $\Delta G^{\ddagger} = 15.5 \pm 1.2$ kcal mol^{-1}).[381] As a general rule, *N*,*N*′-disubstituted ureas exhibit a *trans*−*trans*-*tt*-conformation, so that both N−H are oriented in a position opposed to that of the carbonyl.[382] However, the urea-containing model pseudopeptide **60** has been recently described in an exclusive *cis*−*trans*- *ct*-conformation in solution, due to the setting of an intramolecular H-bond that stabilizes an eight-membered pseudocycle[383] (Figure 13).

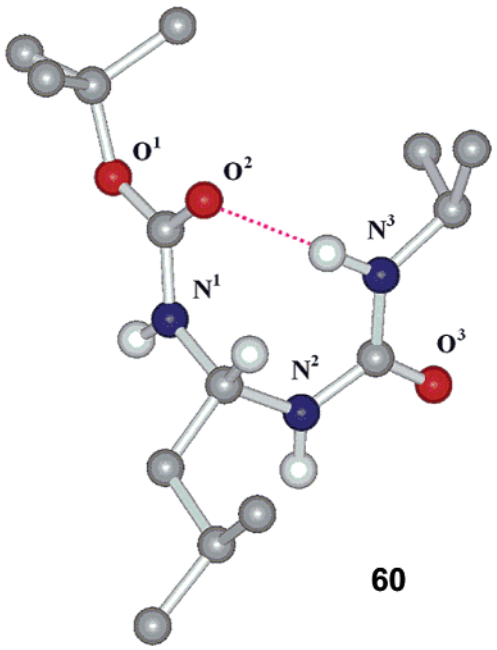


Figure 13. Stabilization in an exclusive cis−trans conformation of a urea-containing pseudopeptide **60** by an intramolecular H-bond. Adapted from Figure 4, ref 383.

Some other amide-derived groups, such as N-aminated and N-hydroxylated amides on one hand and aminimide on the other hand, have been reported in the literature (Figure 14). N-amination and N-hydroxylation of peptide bond nitrogen have little influence on the local conformation, but both affect the hydrogen-bonding network since N-amino group is a weak proton donor, whereas the N-hydroxy group is a strong proton donor. For example, the N-hydroxy-containing pseudopeptide Piv-Proψ[CO−N(OH)]Gly-NH-^{i}Pr **61** exists as a mixture of γ-like and βII-turns presenting, respectively, an 8- and 11-membered

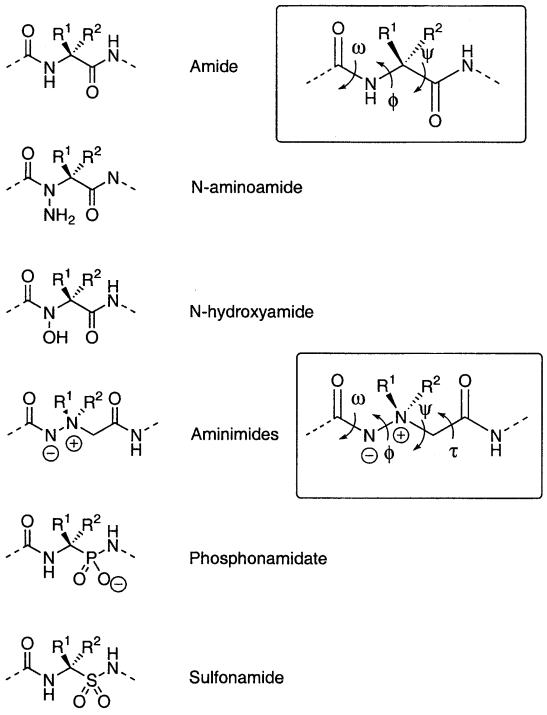


Figure 14. Dihedral angles in peptides and pseudopeptides.

cycle in dichloromethane instead of the classical 10-membered cycle found in the corresponding peptide Piv-Proψ[CO−NH]Gly-NH-^{i}Pr **62**.[384] Dynamics simulation in water has suggested that the tetrapeptide Ac−Ala−Proψ[CO−N(OH)]Ala−Ala−NH−Me **63** tends to nucleate in a reverse-turn structure.[385]

The amide surrogate aminimide displays high conformational flexibility due to additional backbone dihedral angles (ω, ϕ, τ, and ψ):[386] energy minima are found at ϕ = 60, 180, and 300° (Figure 14).

Although P−N and S−N bonds are often considered as single σ-bonds, the free electron doublets can be involved in a deconjugation with a π-system (2p(N) → 3d (P)) in a way that restricts the rotation.[387] In contrast to amides, sulfonamide- and phosphonamide-containing compounds (Figure 14) do not display *cis*- and *trans*-confomers,[388] but they may exist as mixtures of rotational isomers with enantiomeric and diastereomeric intermediary conformations. Computer simulations[389] as well as biochemical assays have demonstrated that phosphonamides and sulfonamides may be considered as mimics of the tetrahedral transition state involved in amide hydrolysis[390−393] (Figure 15).

2. Influence of the Substituents

The influence of the R substituent on free-energy difference of the *cis*- and *trans*-isomers in acetonitrile has been illustrated in the case of methyl formate (R = H) and acetate (R = CH_3), which are, respectively, 1.66 and 5.24 kcal mol^{-1} (Table 4).[394] Steric hindrance at R′ tends to increase the *E*-population (*cis*-isomer) in esters, thiol esters, and amides as indicated by the free-energy differences of formate derivatives (Table 3). Steric effects do not account for

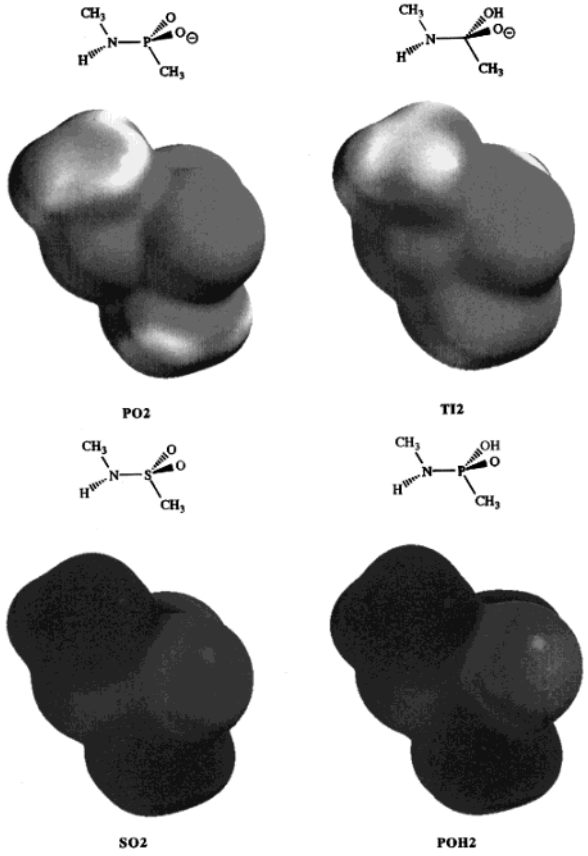


Figure 15. RHF/6-31+G* electrostatic potentials of minimum energy conformations of phosphonamidate PO2, the tetrahedral intermediate TI2, sulfonamide SO_2, and phosphonamide POH2 graphed over the range of −100 to −0 kcal/mol. Reprinted with permission from *J. Org. Chem.* **1998**, *63*, 1419−1428. Copyright American Chemical Society.

all modifications observed and electronegativity of R′ must be considered because it may affect the conformation in several ways including changes in the dipole moment and bond angles. For example, the *E*-population is doubled when R′ is changed from an ethyl group ([E] = 16.8%) to a vinyl group ([E] = 32.7%).[357] Intra-[395−398] and intermolecular[378,379,399] hydrogen bonding may also perturb the cis−trans equilibrium of amides and the syn−anti isomer equilibrium of carbamates. As an example, pentafluorophenyl-lone pair interaction is sufficiently strong to shift the cis:trans ratio of secondary amides: the *cis*-isomer is entropically favored, suggesting that the pentafluorophenyl group acts as a Lewis acid.[399]

3. Influence of Solvent, Salts, and pH

Solvent and salts strongly influence the free energy difference of the *E*- and *Z*-isomers of esters and thioesters,[357] amides and carbamates,[377,379] as well as ureas.[400] Interactions with solvent such as trifluoroethanol (TFE)[401] or tetrahydrofuranne (THF),[402] and Lewis acids such as Li^+, control the isomer ratio. For example, addition of 0.47 M LiCl to solutions of peptide **74a** in THF and TFE shifts the conformational equilibrium to the *cis*-isomer (Table 5). Conversely, sudden addition of a LiCl/TFE solution of

Table 4. Comparative *Z*:*E* (trans:cis) Ratio (K), Free Energy Differences ($\Delta G°$), and Free Energy Barriers ($\Delta G^{\ddagger}$) in kcal mol^{-1} of a Set of Esters 64–65, Thioacid Ester 66, Amides 67–70, Hydrazide 71, Carbamate 72, and Urea 73

no.	product	*T* (°C)	solvent	K	$\Delta G°$ (kcal mol^{-1})	$\Delta G^{\ddagger}_{Z\rightarrow E}$ (kcal mol^{-1})
64	$HCO-OCH_3$	−83	acetonitrile	332	1.66	8.0
65	$CH_3CO-OCH_3$	calc	acetonitrile		5.24	
66	$HCS-OCH_3$	−85	acetone	32	1.29	10.6
67	$HCO-NHCH_3$	rt	water	8	1.22	
68	$CH_3CO-NHCH_3$	rt	water	70		21.7
69	$CH_3CO-N(CH_3)_2$	rt	water			21.3
70	$CH_3NH-CO-CO-NHCH_3$	cal	water		6.2	
71	$CH_3CO-NH-NH-COCH_3$		water		1.4	
72	$CH_3OCO-NHCH_3$	rt	acetonitrile		1.1	15.3
73	$CH_3NH-CO-NHCH_3$	rt		Z only		15.5/18.5

Table 5. Effect of Solvent and LiCl on the Cis Content of a Model Tetrapeptide Suc–Ala–Ala–Pro–Phe–*p*NA 74a and Cyclosporin A 75[a]

peptide	solvent	LiCl	% cis
Suc–Ala–Ala–Pro–Phe–*p*NA **74a**	H_2O	−	10
Suc–Ala–Ala–Pro–Phe–*p*NA **74a**	THF	−	10
Suc–Ala–Ala–Pro–Phe–*p*NA **74a**	THF	+	40
Suc–Ala–Ala–Pro–Phe–*p*NA **74a**	TFE	−	10
Suc–Ala–Ala–Pro–Phe–*p*NA **74a**	TFE	+	70
cyclosporin A **75**	THF	−	100
cyclosporin A **75**	THF	+	0

[a] Without LiCl (−) or with 0.47 M LiCl (+).

peptide to a buffer (solvent jump) shifts the cis:trans ratio from 70:30 to 10:90.[402] Although the mode of action of TFE is poorly understood,[403] LiCl/TFE seems to favor the formation of unusual conformations in peptides and a *cis*-conformation of the amino acyl–prolyl peptide bond by both breaking intramolecular H-bonds and complexing of lone pairs from the backbone to the Lewis acidic Li^+ cation.[309,404] LiCl/TFE also affects the conformation of cyclosporin A (CsA) **75**, a cyclic undecapeptide (Figure 16), which

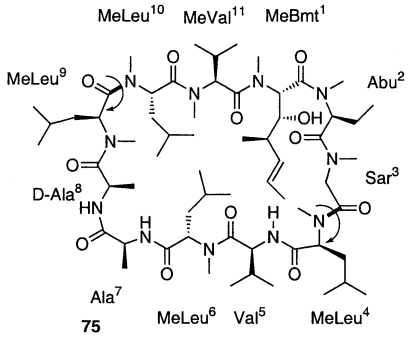


Figure 16. Structure of cyclosporine A **75**; position of cis–trans isomerization upon solvent and salt jumps are indicated by rotating arrows.

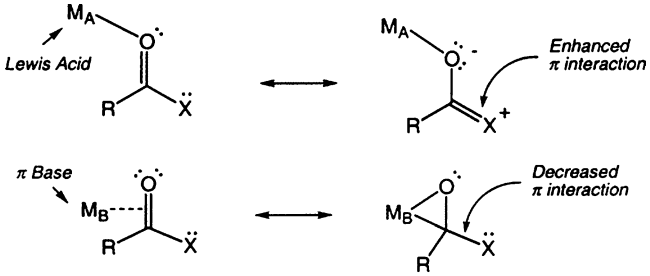
was shown to exist as multiple conformers with an exclusive *trans*-conformation between the MeLeu[9] and MeLeu[10] residues (which was clearly different from the *trans*-conformer observed in apolar solvents).[405,406] In $CDCl_3$, two isomers possessing a *cis*-conformation at MeLeu[9]–MeLeu[10] and Sar[3]–MeLeu[4] (6%) were characterized.[407] In DMSO, slowly interconverting conformers were observed by NMR spectroscopy.[408]

Sudden dilution of concentrated solutions of peptides in LiCl/TFE (LiCl-mediated solvent jumps) has been shown to pertub the cis–trans isomerism of secondary amides in model dipeptides in a manner reminiscent of the tertiary amides, although less is known about secondary amide isomerization in peptides due to the lack of suitable probes.[409]

Computational studies of the *N*-acetyl-*N*′-methylprolylamide cis–trans isomerization, performed in a set of solvents, have rationalized the influence of solvent on both cis and trans populations as well as on the energy barrier for interconversion.[410] The effect of Li^+ may not be general to all amides. For example, LiCl does not influence the isomerization of simple amides and is limited to certain amino acids preceding proline.[366] In cyclic peptides that contain both secondary and tertiary amides, CO–NH bonds may isomerize preferentially. This was observed in the cyclic tetrapeptide tentoxin c(MeAla–Leu–MePhe[(*Z*)Δ]–Gly) **76**, which exists as a mixture of four isomers that interconvert in aqueous solution by rotating about the MeAla–Leu and MePhe[(*Z*)Δ]–Gly peptide bonds.[411]

Asymmetric metal complexes such as rhenium π-bases are able to interact with the carbonyl group in a π-fashion that removes the π-interaction between the bound carbonyl and the nitrogen and thus facilitates the cis–trans isomerization. In this regard, the rhenium complex has the opposite effect compared to η^1 complexes with Lewis acids (Scheme 35).[412]

Scheme 35. Proposed Mechanism for the Rhenium-Catalyzed Isomerization of Amides.



[a] Reprinted from *J. Am. Chem. Soc.* **2002**, *124*, 13506. Copyright 2002 American Chemical Society.

Solvent pH may also alter the cis/trans isomer ratio (Figure 17) as well as the rate constant $k_{cis\rightarrow trans}$ (Figure 18) as observed by spectrophotometric[409] and NMR techniques.[413] Ionization states strongly influence the electronic distribution in the NH–C=O moiety and may, therefore, perturb the electronic transition during cis–trans isomerization.

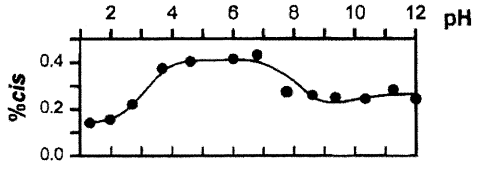


Figure 17. pH dependence of the Ala−Tyr cis content in percent. The solid line represents the calculated curves according to a modified Henderson−Hasselbalch equation using the three pK_a values 3.08 (COOH), 8.05 (NH_3^+), and 10.00 (Tyr-OH) for the trans isomer and 2.89, 8.32, and 9.99 for the cis isomer, respectively. (Schiene-Fischer, C.; Fischer, G. *J. Am. Chem. Soc.* **2001**, *123*, 6227). Reprinted with permission from ref 409. Copyright 2001 American Chemical Society

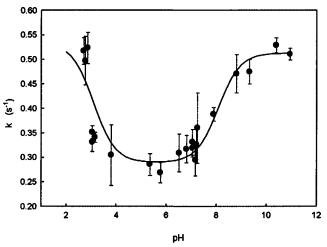


Figure 18. pH dependence of rate constants $k_{cis\rightarrow trans}$ for the cis/trans isomerization of Gly−Gly. The solid line represents the calculated curve according to a modified Henderson−Hasselbalch equation using the pK_a values 3.1 (COOH) and 8.08 (NH_3^+). (Scherer, G.; Kramer, M. L.; Schutkowski, M.; Reimer, U.; Fischer, G. *J. Am. Chem. Soc.* **1998**, *120*, 5568). Reprinted with permission from ref 413. Copyright 1998 American Chemical Society.

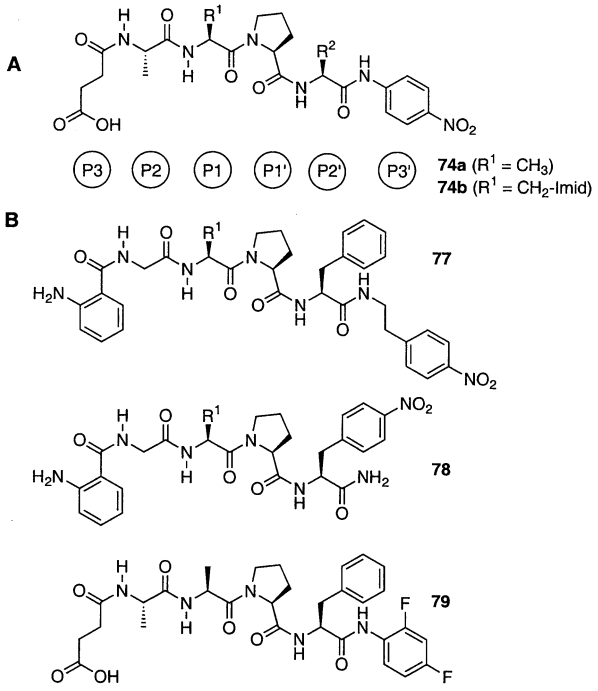
B. Monitoring the Cis–Trans Isomerization of Amides

As stated above, *cis*- and *trans*-amide isomers display steric and electronic differences which may modify intra- and intermolecular interactions as well as solvation (see Scheme 33). This implies that the two isomers may be differentiated using physical, chemical, and biochemical techniques. Several spectrophotometric and fluorimetric assays have been used to monitor the cis−trans isomerization of peptides. Most of them are based on the isomer-specific hydrolysis[414] of model tetrapeptides Suc−Ala−Xa1−Pro−Xa2−*p*NA (where *p*NA is the *p*-nitroaniline chromophoric group, Chart 10A) at 283 K with α-chymotrypsin (Xa2 = Phe),[402,415] trypsin (Xa2 = Arg),[361] dipeptidyl peptidase IV,[416,417] pepsin,[418] and subtilisin (Xa2 = Tyr).[401] In a typical experiment, sudden dilution of a concentrated solution of the model peptide to the appropriate buffer containing a nonlimiting concentration of protease leads to a very fast hydrolysis of the preexisting *trans*-isomer which drives a slow cis → trans isomerization of the nonhydrolyzed *cis*-isomer. Monitoring of the *p*NA release at 390 nm gives access to kinetic data for isomerization, while interpolation of the two-slope curve reflects the initial cis:trans ratios (Figure 19). The sensitivity of the test was greatly improved by operating at low temperature (4−10 °C) and by dissolving the model peptide in a 0.47 M LiCl/TFE solution which is known to favor the *cis*-isomer.[406]

Some protease-free assays have been also reported. In particular, isomerization of a chimeric peptide **77** (Chart 10B) containing both an *o*-aminobenzoyl fluorophore at the N-terminus and a *p*-nitrobenzoyl quencher at the C-terminus can be followed by monitoring the increase of fluorescence at 410 nm upon excitation at 337 nm.[420] Two spectrophotometric protease-free assays employing either the *o*-nitrotyrosine residue (compound **78**)[421] or the 2,4-difluoroanilide chromophore (compound **79**)[404] have been used in biochemical studies,[423,424] the later method having low sensitivity. A routine semiautomated microtiter plate assay has also been developed.[425] Very recently, a general approach for following the time-course of cis−trans isomerization of nonproline-containing dipeptides has been described and employs dramatic and rapid changes of pH and solvent jump experiments. Although *cis*- and *trans*-isomers present some significant spectral differences, their difference in relative absorbance does not exceed 0.8% at 220 nm.[409] Spectrophotometric assays supply important thermodynamic information such as the cis/trans ratio (K) and kinetic information such as $k_{cis\rightarrow trans}$ (k_{obs}). As a consequence, $k_{trans\rightarrow cis}$ may be deduced from the previous data (K = [cis]/[trans] = $k_{cis\rightarrow trans}/k_{trans\rightarrow cis}$). However, the value may be inaccurate due to additive experimental errors. Proteins may be used as probes for the isomerization process by monitoring the fluorescence of phenylalanine, tyrosine, or tryptophan residues which may vary, depending on the environment of the fluorophore. As an example, the folding of RNAse T1 was followed by monitoring the fluorescence increase at 320 nm upon excitation at 262 nm.[426−432]

NMR spectroscopy has been widely employed to determine the cis−trans ratios of the Xaa−Pro moiety, since in many cases, cis−trans interconver-

Chart 10. Chromophoric Peptides Employed for the Monitoring of the Peptidyl-Proline Cis−Trans Isomerization[a]



[a] (A) *p*NA-terminated peptides **74a** and **74b** employed in the protease-coupled assay; (B) peptides **77**−**79** used in the protease-free assays.

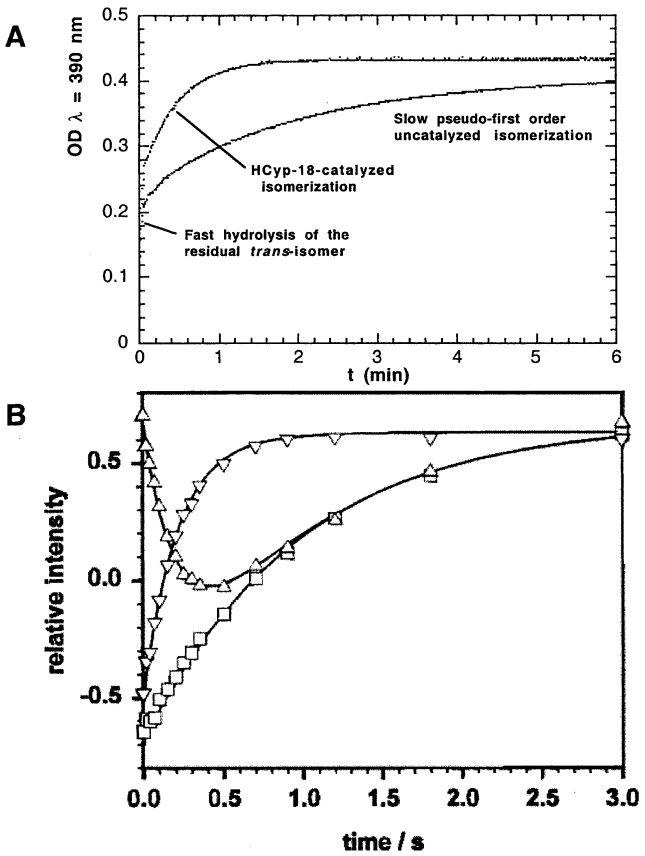


Figure 19. Examples of monitoring of the peptidyl-prolyl cis−trans isomerization (A) spectrophotometric protease coupled assay: *trans*-Suc−Ala−Ala−Pro−Phe−*p*NA is specifically hydrolyzed by a nonlimiting concentration of α-chymotrypsin in a 35 mM Hepes buffer pH 7.6 at 10 °C and release of *p*NA is monitored at 390 nm; (B) Relative intensities of the time-dependent magnetization of cis CH_3-alanine for Ala−Tyr in 9:1 H_2O/D_2O, pH 5.9 in a T1 experiment (□) and the magnetization-transfer experiments with the cis signal parallel (△) and antiparallel to the stationary magnetic field (▽) at 316 K. Solid lines represent the correspondingly fitted biexponential decays with T1 = 0.984 s, $k_{cis \rightarrow trans} = 3.7\ s^{-1}$, and a cis content of 0.61% as parameters. Reprinted with permission from *J. Am. Chem. Soc.* **1998**, *120*, 5568−5574. Copyright American Chemical Society.

sion is sufficiently slow on the NMR time-scale. Prolyl amide isomers are often distinguishable by their particular chemical shifts (^1H, ^{13}C, ^{15}N) and some differences may be observed on remote positions of peptides.[433] The *cis*- and *trans*-conformations are usually unambiguously assigned by two-dimensional NOE experiments.[434] The kinetics of amino acyl-proline isomerization may be analyzed by dynamic NMR spectroscopy including relaxation experiments,[435] magnetization transfer experiments,[436−438] and line shape analysis,[439] as well as homo-[440] and heteronuclear single quantum coherence experiments.[441] These techniques may also be adapted for monitoring Xaa−Pro cis−trans isomerization in proteins[442,443] and large and cyclic peptides[444] and for the study of small peptides[439,445] and non-peptide models.[395−398,446]

Infrared spectrophotometry[397] and circular dichroism spectroscopy[447] can also be used for the detection of *cis*- and *trans*-conformations; however, in most cases, they only provide additional experimental evidence which corroborates other results.[448,449] Fragmentation pathways in mass spectrometry of peptides such as Gly−Gly−Gly are also isomer-dependent due to different protonation of each conformational state.[450] Mutagenesis of proline residues also provide important information about folding kinetics that might implicate particular proline residues; however, the results must be confirmed by complementary techniques.[451]

In most cases, cis−trans isomerism is significantly faster than methodologies employed for the purification of molecules (i.e., HPLC) and does not enable the separation of isomers. However, there are some examples of peak splitting (i.e., erythropoietin,[452] enalapril)[453] and peak separation either at low[434] or room temperature.[454−456] Dynamic capillary electrophoresis coupled to computer simulations can give access to kinetic and thermodynamic parameters of amino acyl-proline dipeptides.[457]

C. Amides in Peptides and Proteins: The Particular Behavior of Proline

1. Secondary and Tertiary Amides in Peptides and Proteins

Secondary and tertiary amides play a major role in the control of the conformation of peptides and proteins by reducing the possible rotation around the ω dihedral angle (Figure 14). Usually, ω is about 0° (cis) and 180° (trans), but examples have been reported of nonplanar amides.[458−460] Secondary amides are known to occur predominantly in a *trans*-conformation in peptides **80**−**84** and *cis*-content tends to decrease in larger peptides **85** and **86** (Table 6).

In proteins, this tendency is confirmed since the frequency is about 0.28% in a set of 571 nonredundant protein structures deposited in the Brookhaven Protein Data Base.[461,462] This percentage might increase in the years to come since occurrence of the *cis*-peptide bond seems to be related in part to resolution (Table 7). *Cis*-amides are most currently found in turns and inside loops. Most frequent cis-amide bonds are found in Cys−Thr (3.0%), Ser−Gln (2.2%), and Arg−Asp (2.0%) sequences.[359] The particular disulfide-linked dipeptide Cys−Cys has a propensity for the *cis*-conformation due to cyclic constraints,[463,464] but general rules cannot be drawn for all proteins.[465]

The overall frequency of *cis*-Xaa−Pro listed in the PDB is about 5−6%, a value that compares well with the typical percentage observed in model peptides.[462,466] It is noteworthy that an increasing number of identical or nearly identical proteins have been found to differ in their isomeric state by a single amino acyl-proline peptide bond. Therefore, as proposed by Fischer in 1994, isomers coexisting in solution might have distinct biochemical properties toward potential reaction partners.[366] A remarkable example has been provided very recently by the nonreceptor protein kinase interleukin-2 tyrosine kinase (itk). NMR spectroscopic data suggest that cis−trans isomerism about the Asn286−Pro287 amide bond is responsible for the coexistence of two different itk conformations that display distinct biological activities.[467]

Table 6. Thermodynamic Constants for the Cis−Trans Isomerization of Secondary Peptide Bonds Determined by ^{1}H NMR Spectroscopy[a]

compound	bond	$\Delta G^{\circ b}$ (kcal mol^{-1})	% cis	$\Delta G^{\ddagger}_{c\rightarrow t}$[c] (kcal mol^{-1})	$\Delta G^{\ddagger}_{t\rightarrow c}$ (kcal mol^{-1})	$k_{c\rightarrow t}$ (10^{-3} s^{-1})	$k_{t\rightarrow c}$ (s^{-1})	pH
Ala−Phe **80**	Ala−Phe	14.0 ± 0.6	0.36	18.1 ± 0.4	21.7 ± 0.5	650	2.3	5.9
Phe−Ala **81**	Phe−Ala	13.5 ± 0.2	0.43	18.5 ± 0.6	21.6 ± 0.5	340	1.5	5.7
Tyr−Ala **82**	Tyr−Ala	13.2 ± 1.6	0.48	19.1 ± 0.6	22.4 ± 0.7	240	1.2	5.5
Ala−Tyr **83**	Ala−Tyr	13.6 ± 1.1	0.41	18.3 ± 0.4	21.5 ± 0.5	570	2.4	5.9
Ala−Ala−Tyr **84**	Ala−Tyr	15.3 ± 2.0	0.21	19.6 ± 0.7	23.7 ± 1.2	290	0.6	4.4
Ala−Ala−Tyr−Ala **85**	Ala−Tyr	16.0 ± 1.5	0.16	15.6 ± 1.4	19.4 ± 1.4	161	2.6	6.3
	Tyr−Ala	15.5 ± 2.0	0.19	17.8 ± 1.4	22.1 ± 1.4	440	0.8	6.3
Ala−Ala−Tyr−Ala−Ala **86**	Ala−Tyr	16.3 ± 1.8	0.14	15.5 ± 0.4	17.6 ± 0.5	238	3.3	4.1
	Tyr−Ala	16.8 ± 2.0	0.11	18.5 ± 0.7	18.5 ± 1.4	177	2.0	4.1

[a] Ref 413. [b] From the Arrhenius equation. [c] From the Van't Hoff equation for 298 K.

Table 7. Frequency of *cis*-Peptide Bond in Proteins Depending on the Sequence (25% Protein Data Base)[a]

peptide bond	cis (%)
Xaa-aliphatic[b]	0.015
Xaa-polar[c]	0.030
Xaa-aromatic[d]	0.074
all	0.029
aliphatic-non-Pro	0.029
polar-non-Pro	0.023
aromatic-non-Pro	0.060
all	0.029
aliphatic-Pro	5.31
polar-Pro	4.38
aromatic	7.96
all	5.21

[a] Ref 461. [b] Aliphatic: Ala, Gly, Leu, Ile, Met, Val. [c] Polar: Arg, Asn, Asp, Cys, Gln, Glu, Lys, Ser, Thr. [d] Aromatic: His, Phe, Tyr, Trp.

2. Influence of Length and Sequence

cis-Xaa−Pro motifs are more frequent for Tyr−Pro (25.0%), Ser−Pro (11.0%), and Phe−Pro (9.6%).[359] In the case of Tyr and Phe residues, a particular interaction between the aromatic side chain and the pyrrolidine ring of proline might stabilize the *cis*-isomer. This was not observed in proteins with tryptophan (*cis*-Trp−Pro: 0%), yet may be due to the very low occurrence of this sequence in proteins. For example, the relative population of *cis*-Trp−Pro is as high as that of the *cis*-Tyr−Pro moiety in the model peptide Ac−Ala−Xaa−Pro−Ala−Lys−NH_2 (Figure 20). Rates of spontaneous cis to trans isomerization of Xaa−Pro amide in these peptides in aqueous solution pH 5.0 at 4 °C are very low for Trp and Tyr (in both the neutral and anionic state), moderate for Phe and maximum for Ala, Gly, Ser, Thr, and Asp (Figure 18).[366] Electrostatic interactions have been suggested to account for this behavior in the case of the contryphan family of cyclic octapeptides.[468] Capping of the N- and C-termini usually does not significantly affect the cis/trans ratio in water. In the case of the *o*-aminobenzoyl moiety (compound **88**), however, the population of the *cis*-isomer and the isomerization rates for cis to trans are decreased and trans to cis is increased relative to unacylated peptide **87** (Table 8).[419] The activation barrier for cis−trans isomerization is almost entirely enthalpic for proline and is 1−2 kcal mol^{-1} lower than that of acyclic tertiary amides.[469]

The influence of chirality on both the cis:trans ratio and the kinetic constants of spontaneous isomerization was also investigated using derivatives of the canonical model peptides R−Ala−Ala−Pro−Phe−*p*NA **89**−**93** (Table 8). A D-amino acid scanning showed that except for D-Ala at P2 (compound **90**), inversion of the stereochemistry strongly affects the percentage of *cis*-isomer as well as $k_{trans\rightarrow cis}$, whereas $k_{cis\rightarrow trans}$ remains relatively constant.[419] This was recently confirmed with shorter proline derivatives.[470]

O-Glycosylation of the Ser−Pro sequence of peptides **94** and **95**[471] as well as *O*-phosphorylation[472] of the Ser/Thr/Tyr−Pro sequences (peptides **96**−**101**) both decrease the percentage of *cis*-isomer. *cis*-Amide content was not decreased as a consequence of phosphorylation of Ser/Thr−Pro moieties in τ peptides **102**−**107**[473] (Table 9). Phosphorylation also affects the kinetics of the cis−trans isomerization of Xaa−Pro amide bond. The rates of interconversion were up to 7-fold slower for the Thr(PO_3H_2)−Pro-containing model peptide versus the alcohol counterpart Thr−Pro. Conversely, phosphorylation of the Ser/Tyr−Pro sequences only slowed the cis−trans isomerization by 2-fold. Determination of thermodynamic constants as well as pH-dependence studies (Figure 21) strongly suggested that phosphorylations of Ser−Pro and Thr−Pro have distinct effects on cis−trans isomerism[472] and may therefore be distinct signaling regulatory mechanisms.[474]

3. Environmental Effects

Both the amide isomer ratio and the amide isomerization rate constants are insensitive to pH unless ionizable groups are present close to the peptide bond.[409,413] Secondary isotope effects have suggested that the cis−trans isomerization of the amino acyl-proline amide bond does not involve a solvent-assisted mechanism with a complete disruption of the π-character of the acyl C═O.[475] Among the few observed solvent effects, protic solvents restrict the isomerization of Ac−Gly−Pro−OMe and other amides by forming a hydrogen bond with the carbonyl oxygen. Hydrophobic environments tend to accelerate cis−trans interconversion.[464] This suggests that desolvation of the amide is a prerequisite to efficient isomerization. Twenty years ago, Drakenberg showed that isomerization of *N*,*N*′-dimethylacetamide was favored in cyclohexane which might stabilize a transition state of low polarity.[476] The effect of pH on the cis/trans ratio of the cysteine-4-hydroxyproline peptide bond of contryphan-R suggests a paradigm for "locally determined but globally selected" folding for cyclic peptides and constrained protein loops.[468]

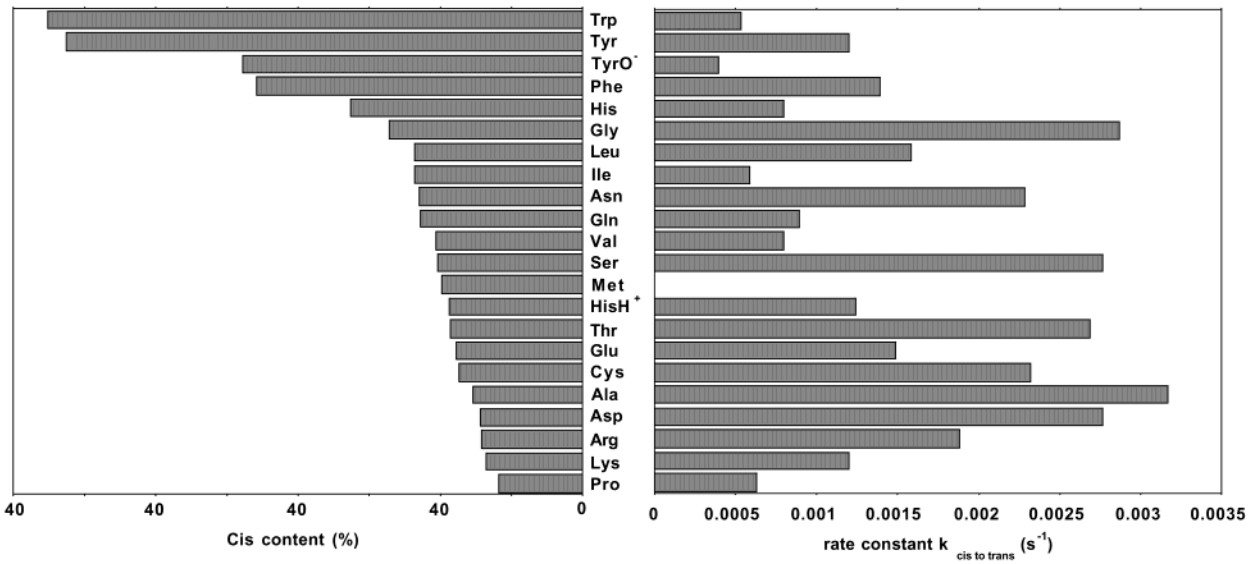


Figure 20. *Cis*-isomer content and rate constant for the cis-to-trans isomerization of Ac−Ala−Xaa−Pro−Ala−Lys−NH_2 in aqueous solution pH 5.0 at 4 °C.

Table 8. Influences of the C-Terminus and Ala Scanning in Tetrapeptides on Both the cis Content and Uncatalyzed Rates of Isomerization in Water[a]

position D-Xaa	tetrapeptide	cis content in water (%)	$k_{c\rightarrow t}$ (10^{-3} s^{-1})	$k_{t\rightarrow c}$ (10^{-3} s^{-1})
	Ala−Ala−Pro−Phe−*p*NA **87**	10.7	6.8 ± 0.5	0.81
	Suc−Ala−Ala−Pro−Phe−*p*NA **74a**	10.0	8.6	0.86
	Abz−Ala−Ala−Pro−Phe−*p*NA **88**	20.1	4.7 ± 0.4	1.18
P2	D-Ala−Ala−Pro−Phe−*p*NA **89**	11.1	4.4 ± 0.3	0.55
P1	Ala−D-Ala−Pro−Phe−*p*NA **90**	22.5	5.7 ± 0.1	1.65
P1′	Ala−Ala−D-Pro−Phe−*p*NA **91**	24.5	4.5 ± 0.3	1.46
P2′	Ala−Ala−Pro−D-Phe−*p*NA **92**	6.5	ND	ND
P2′	Abz−Ala−Ala−Pro−D-Phe−*p*NA **93**	16.0	6.6	1.26

[a] Schiene, C.; Reimer, U.; Schutkowski, M.; Fischer, G. *FEBS Lett.* **1998**, *432*, 202.

Table 9. Influences of the *O*-Glycosylation and *O*-Phosphorylation on Both the Cis Content and Uncatalyzed Rates of Isomerization in Water or TFE

no.	peptide derivative	cis content (%)	$k_{cis\rightarrow trans}$ (10^{-3} s^{-1})
94	Phe−Ala−Pro−Gly−Asn−Ser(α-GlcNac)−Pro−Ala−Leu	0	
95	Phe−Ala−Pro−Gly−Asn−Ser(*β*-GlcNac)−Pro−Ala−Leu	0	
96	Ac−Ala−Ser−Pro−Tyr−*p*NA	13.4 ± 0.2	7.8 ± 0.5
97	Ac−Ala−Ser(PO_3H_2)−Pro−Tyr−*p*NA	18.7 ± 0.3	4.0 ± 0.3
98	Ac−Ala−Thr−Pro−Tyr−*p*NA	13.2 ± 0.4	12.9 ± 0.5
99	Ac−Ala−Thr(PO_3H_2)−Pro−Tyr−*p*NA	11.1 ± 0.3	1.8 ± 0.2
100	Ac−Ala−Tyr−Pro−Tyr−*p*NA	30.9 ± 0.6	5.1 ± 0.3
101	Ac−Ala−Tyr(PO_3H_2)−Pro−Tyr−*p*NA	28.6 ± 0.8	3.9 ± 0.3
102	...-**Thr**−**Pro**−Pro−Lys−Ser−Pro-... ($\tau^{224-240}$)	4 (H_2O)/0 (TFE)	
102	...-Thr−Pro−Pro−Lys−**Ser**−**Pro**-... ($\tau^{224-240}$)	9 (H_2O)/0 (TFE)	
103	...-**Thr(PO_3H_2)**−**Pro**−Pro−Lys−Ser(PO_3H_2)−Pro-...	3 (H_2O)/0 (TFE)	
103	...-**Thr(PO_3H_2)**−**Pro**−Pro−Lys−Ser(PO_3H_2)−Pro-...	9 (H_2O)/ 0 (TFE)	
104	...-**Tyr**−**Pro**−Pro−Lys−Ser−Pro-...	ND (H_2O)/20 (TFE)	
105	...-**Tyr(PO_3H_2)**−Pro−Pro−Lys−Ser−Pro-...	ND (H_2O)/10 (TFE)	
106	...-Thr−Pro−Pro−Lys−**Tyr**−**Pro**-...	ND (H_2O)/25 (TFE)	
107	...-Thr−Pro−Pro−Lys−**Tyr(PO_3H_2)**−Pro-...	ND (H_2O)/10 (TFE)	

Surprisingly, surfactants such as phosphatidyl choline or *N*-dodecyl-*N*,*N*-dimethylammonium-3-propanesulfonate were found to increase the trans content and dramatically affect the kinetics of cis−trans isomerization of proline-containing peptides. For example, interconversion of the model tetrapeptide Suc−Ala−Ala−Pro−Phe−*p*NA is accelerated about 20-fold in micelles.[477] The effects of micelles and membranes may be unpredictable: bombolitin, a 17-mer peptide, displayed a *cis*-Ile1−Lys2 amide in aqueous dodecyl sulfate.[478] Although their mechanism of action is not clearly understood, surfactants are anticipated to induce conformational changes in the peptide (putatively related to desolvation) which may favor intramolecular-assisted rotation of the amide.[469]

D. The Mechanism of Amino Acyl-Proline Cis–Trans Isomerization

1. The Different Families of Peptidyl-Prolyl Cis–Trans Isomerases and Their Putative Catalytic Mechanisms

Very early, the isomerization of amides was proposed one of the limiting steps of protein folding and, considering the extremely slow folding of RNAse T1, the need for ubiquitous biocatalysts seemed criti-

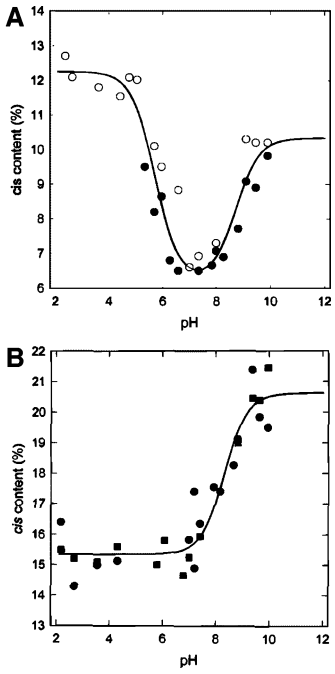


Figure 21. pH-dependence of the content of *cis*-isomer of the phosphorylated Thr−Pro and Ser−Pro bonds (A) in Ala−Ala−Thr(PO_3H_2)−Pro−Phe−pNA; (B) in Ala−Ala−Ser(PO_3H_2)−Pro−Phe−pNA; by ^1H (○ and ■) and ^{31}P (●) NMR spectroscopy. Adapted from *Biochemistry* **1998**, *37*, 5566−5575, (A) Figure 5, (B) Figure 6.

cal.[479] Because amide isomerization may be rate-limiting,[461,480] amino acyl-proline cis−trans interconversion plays a major role in the folding of proteins. The peptidyl-prolyl cis−trans isomerases (PPIases) are enzymes that significantly accelerate the reaction in model peptides as well the folding of denatured proteins. They were first described by Fischer and co-workers in 1984,[481] and, a few years later, PPIase was found to be identical to the cyclosporin (CsA)-binding protein cyclophilin A (CypA).[432,481−484] Another PPIase, the FK506-binding protein (FKBP) was identified as the specific receptor for the immunosuppressive agent FK506.[484] Recently, the human nuclear PPIase Pin-1 has been shown to be selectively inhibited by juglone. The three proteins display distinct properties and selectivities: in particular, there was no cross-inhibition by CsA, FK506, and juglone.[485] This strongly suggested that CypA, FKBP, and Pin1 belong to different families with distinct catalytic mechanisms,[486−488] and further studies have confirmed this proposal.[489−491] Two other PPIases, the trigger factor and the parvulins from *E. coli* (the bacterial homologue of Pin1), were discovered respectively in 1988 and 1994.[492] The main features of the above-mentioned enzymes are summarized in Table 10 and X-ray structures of either the substrate-bound state or the inhibitor-state (there is no structure of hFKBP-12 binding a substrate) are illustrated in Figure 22. Very recently, Fischer's group demonstrated that the hsp70 chaperone DnaK is a secondary amide peptide bond cis−trans isomerase (APIase), a novel class of enzymes that might play an important role in protein folding;[493] however, little is known about this enzyme and further study will undoubtely highlight its importance in protein folding.

Cyclophilin is the smallest member of the immunophilin family, and other members are characterized by additional sequences that confer the proteins particular activities, specificities, and localizations. The same pattern is observed in the FKBP family, and some members contain several partial or complete FK506 domains. *E. coli* parvulin, which contains only 92 amino acid residues, is the smallest PPIase.[492] The three families of PPIases have been isolated from human: cyclophilin hCyp-18, hFKBP-12, and parvulin Pin1 are the archetypal members of each family. Their biological implications have inspired extensive efforts to elucidate their catalytic mechanisms.

The mechanism of uncatalyzed isomerization of amides remained ambiguous until the end of the century. Particular points were unclear, due to the heterogeneity of the Xaa−Pro amide bond. Several distinct mechanisms for amide isomerization (Scheme 36) were considered and confirmed using simple models. Resolution of several PPIase/substrate complexes or PPIase/inhibitor complexes have provided important additional information about the possible catalytic mechanisms.[494]

All PPIases are characterized by a hydrophobic pocket that is specific to the proline pyrrolidine ring. Both X-ray data and mutagenesis experiments have shown that there are different patterns for interaction inside the catalytic sites (Figure 23). The ability of PPIases to accommodate nonproline amino acids also varies. Cyclophilin can only catalyze the cis−trans isomerization of Xaa−Pro peptide bonds,[495] although it binds to peptides that do not contain proline,[496−498] as shown in the case of the yeast cyclophilin[499] and the *E. coli* trigger factor.[497] Isomerization of pseudoproline-containing peptides[498] and fluoroproline-containing peptides[500] is also accelerated by hCyp-18. Amides of the four- and six-membered ring equivalents of proline, respectively, azetidine carboxyl and pipecolinyl moieties, can also be isomerized. However, modification of ring size dramatically lowers the catalytic efficiency. Potent hFKBP inhibitors contain the pipecolinate motif.[486]

All cyclophilins that display a significant PPIase activity contain two key residues Arg and Asn (respectively, at positions 55 and 102 in hCyp-18) which interact with the substrate and are able to stabilize the putative transition state (Figure 24).[489] Furthermore, Trp121 has been shown to be a critical residue for interaction with CsA,[501,502] model peptides and small hydrophobic molecules.[496] Structure-based molecular dynamics simulations of the transition state and both *cis*- and *trans*-ground states inside the hCyp-18 active site suggest that Asn102 interacts more favorably with the C=O at P1′ in the transition state: amide rotation involves a pyramidalization of the nitrogen which is assumed to interact via an H-bond with the Arg55 guanidinium group (d_{N-N} = 3.42 Å), an interaction which is not permitted in both ground-states (d_{N-N} = 4.13 Å). Moreover, the Asn102 backbone nitrogen is closer to the rotating carbonyl in the transition state (d_{N-O} = 2.98 Å) than in the *cis*-ground state (d_{N-O} = 3.18 Å) (Figure 24).[489,503] Mutagenesis experiments have confirmed the impor-

Table 10. Main Characteristics of Cyclophilin hCyp-18, hFKBP-12, and the Human Parvulin Pin1

characteristics	hCyp-18	hFKBP-12	Pin1
molecular mass (kDa)	18	12	18
number of residues	165	108	163
structure	central large PPIase domain	single PPIase domain	WW N-terminus, PPIase C-terminus
localization	cytosol	cytosol	nuclear
main function	protein folding and trafficking, immunosuppression, cell surface binding, HIV-1 virus assembly, chaperone	protein folding and trafficking, immunosuppression, chaperone	regulation of mitosis (effects on replication and transcription)
related pathologies	AIDS	neurodegenerative diseases	neurodegenerative diseases including BSE
substrate preference (P1′−P1)	Ala−Pro, Val−Pro	Leu−Pro	Ser^{P}−Pro, Thr^{P}−Pro, Glu−Pro
inhibitors	cyclosporin A	FK506, rapamycin, ascomycin	juglone
catalytic residues	Arg55, Asn102	(hydrophobic surrounding residues)	Cys 113 (His 59, Ser154, His157)
probable catalytic mechanism	nucleophilic, non covalent	amide distortion/ intramolecular H-bond	nucleophilic covalent/ general acid−base

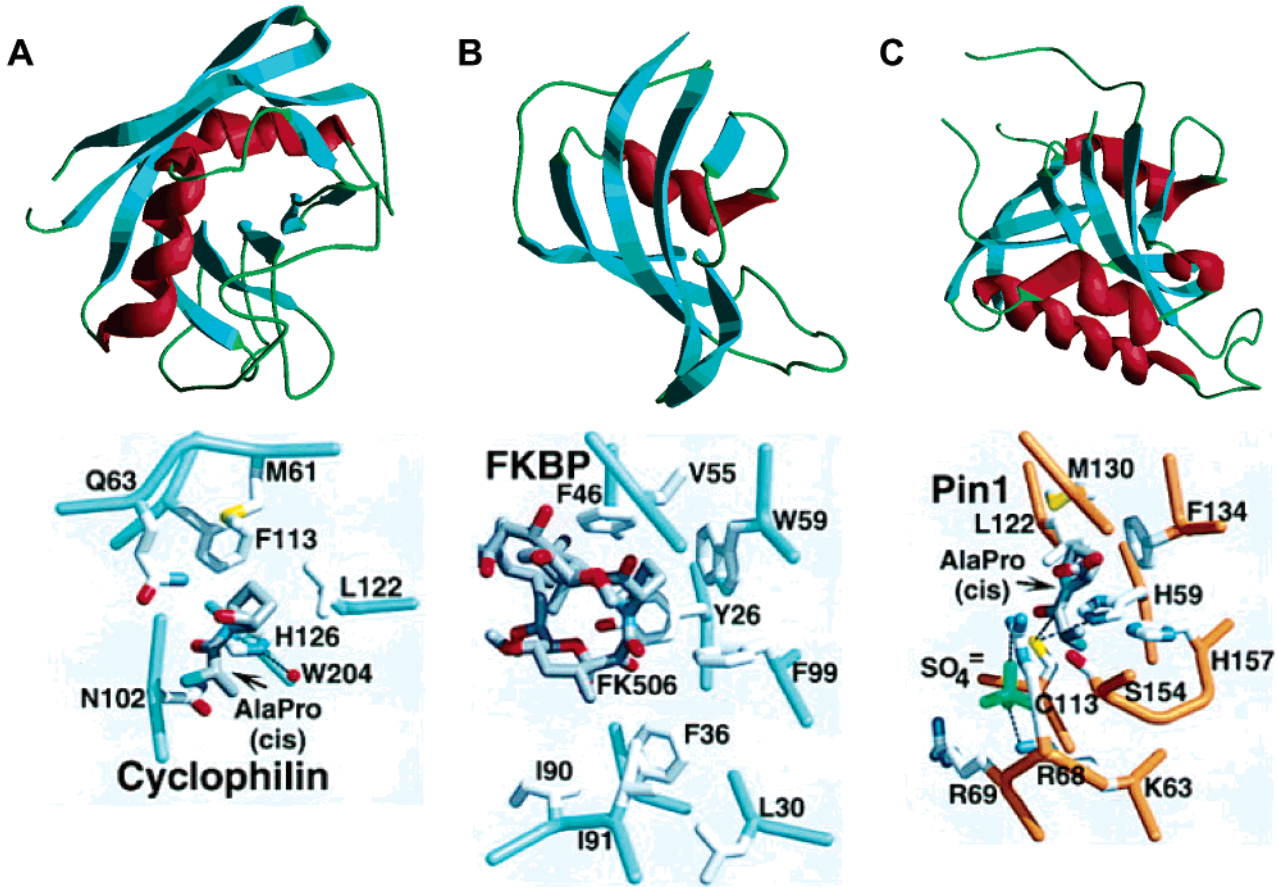


Figure 22. Ribbons representation of cyclophilin hCyp18 (A), hFKBP-12 (B) and the human parvulin Pin1. Active site residues (colored frames) are in cylindrical bond representation and labeled. Reprinted with permission from ref 490. Copyright 1997 Cell Press.

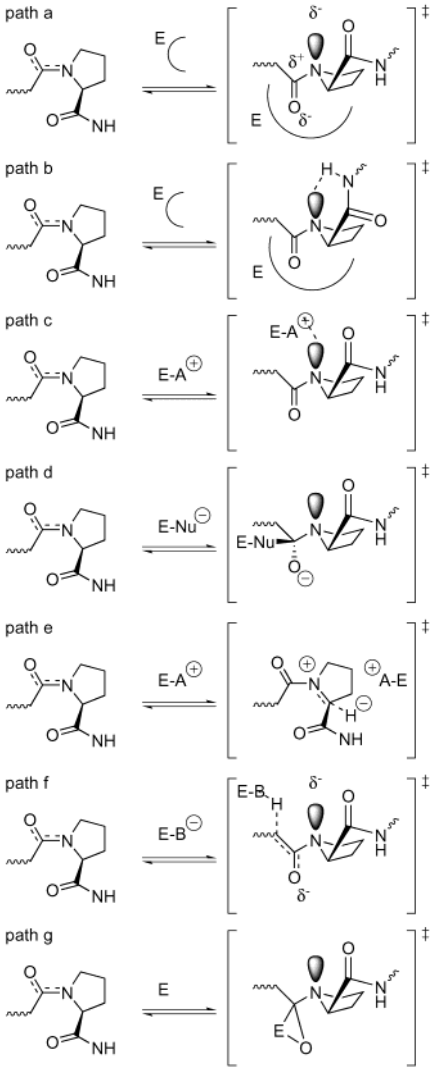
tance of Arg55 in the catalytic process.[504] Finally, the importance of Arg55 for the catalysis was confirmed by ^{15}N NMR spin relaxation experiments which correlated motion of the hCyp-18 backbone with the catalytic activity.[505]

A solvant-assisted mechanism was proposed in the case of dipeptide substrates,[506] but the involvement of water molecules closely associated with the active site residues seems to be strictly limited to this single example which is probably not representative as a relevant mechanism of action for the cyclophilins.[507]

The generation of catalytic antibodies (abzymes) that display a significant PPIase activity was accomplished using haptens **108**[508] and **109**[509] that contain a twisted amide surrogate ketoamide.[510] This motif is found in many FKBP inhibitors and some hCyp-18 inhibitors[509,511] (Chart 11, Scheme 37).

The only reliable structures of hFKBP-12 in the bound-state have been obtained from enzyme/inhibitor complexes, in particular, hFKBP-12/FK506.[512] Resolution of the interaction pattern has highlighted the intriguing catalytic mechanism. Ab initio detailed

Scheme 36. Possible Mechanisms for the Cis−Trans Isomerization of the Amino Acyl-proline Peptide Bond[a]



[a] Deconjugation in the enzyme hydrophobic environement (path a); induction of an intramolecular assistance to isomerization (path b); acid-catalyzed isomerization (path c); nucleophilic catalysis (path d) and π-base-catalyzed Isomerization (path g). Paths e and f will not be discussed herein.

mechanism analysis suggests that FKBPs bind substrate in a type VIa β-turn and cyclophilins bind substrates in a type VIb β-turn.[491] In contrast to hCyp-18, hFKBP-12 does not possess an H-bond donor able to interact with the isomerizing O=C−N moiety which is isolated deeply inside the catalytic pocket surrounded by aromatic side-chains. Therefore, it may be suggested that hFKBP-12 induces a substrate conformation which enables a substrate-assisted mechanism of the reaction (see Figure 23B).[513]

X-ray data of Pin1, complexed with a dipeptide substrate, has revealed the spatial proximity of nucleophilic side-chains from His59, Cys113, Ser154, and His157 symmetrically distributed around the P1′ carbonyl of the rotating amide bond. In particular, Cys113 is well-positioned for a nucleophilic attack of the amide carbonyl which is favored by S−H deprotonation by His59. The newly formed negatively charged tetrahedral intermediate may then be stabilized through electrostatic interactions with His157. This high-energy intermediate may then relax back to either the *cis*- or *trans*-conformer. This putative mechanism was supported by mutations of Cys113 to Ser and Ala, which resulted, respectively, in 20- and 123-fold decreases in catalytic efficiency (see Figure 23C).[490] In contrast to Pin1, individual mutations of Cys at positions 52, 62, 115, 161 in cyclophilin hCyp-18 had no effect on catalysis.[514] These results suggest that Pin1 exerts its catalysis through a unique covalent mechanism that is an exception in the PPIase class of enzymes.

2. Uncatalyzed Isomerizations: Models for the Enzyme-Catalyzed Reaction

In the mid-1990s, spontaneous isomerization was considered as the result of the free rotation of a deconjugated (hyperpolarized) amide. The transition state was represented as a twisted keto-amine intermediate with an increased polarization of the carbonyl[515] and a pyramidal nitrogen.[469,486] This mechanism explained why ketoamides **108** and **109**, which are mimics of a rotating-amide transition state with the ketone perpendicular to the amide, were inhibitors of PPIase activity[509] (see Scheme 37). As a consequence, four transition state configurations are possible (Figure 25),[489] and their relative stabilities depend on the environment.[516] This was confirmed by the secondary isotopic effect[517] as well as the positive influence of hydrophobic solvents on the rates of isomerization.

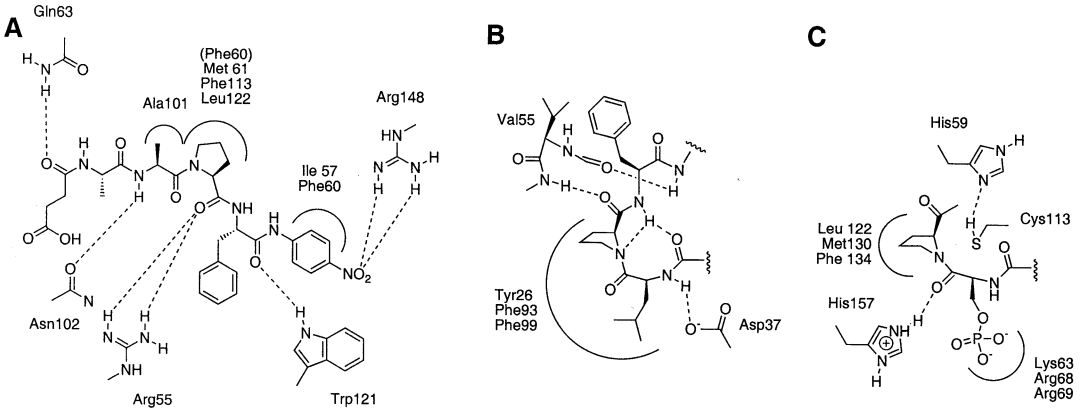


Figure 23. Detailed representation of the interaction of hCyp-18 (A), hFKBP-12 (B), Pin-1 (C) with their substrates and schematic representation of the interaction pattern inside the catalytic sites.

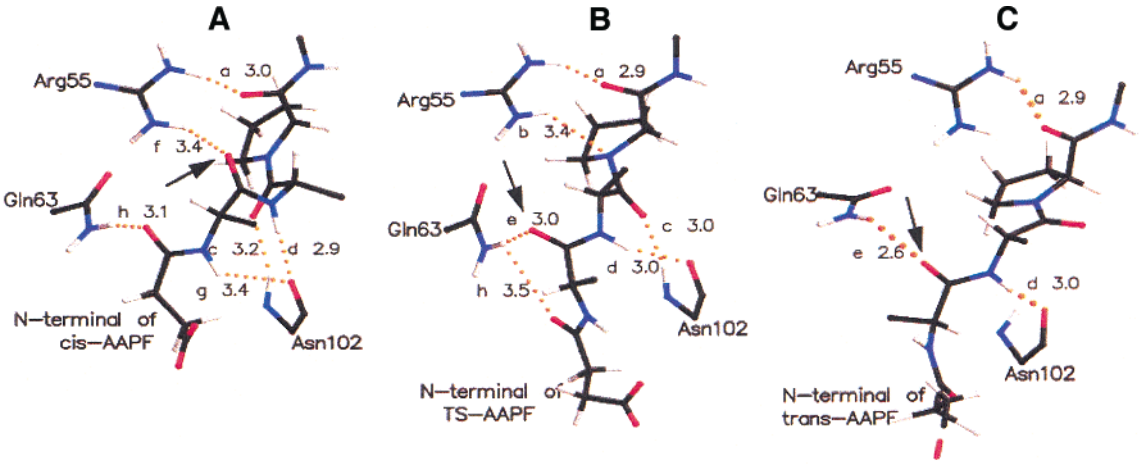
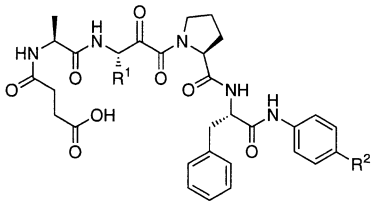


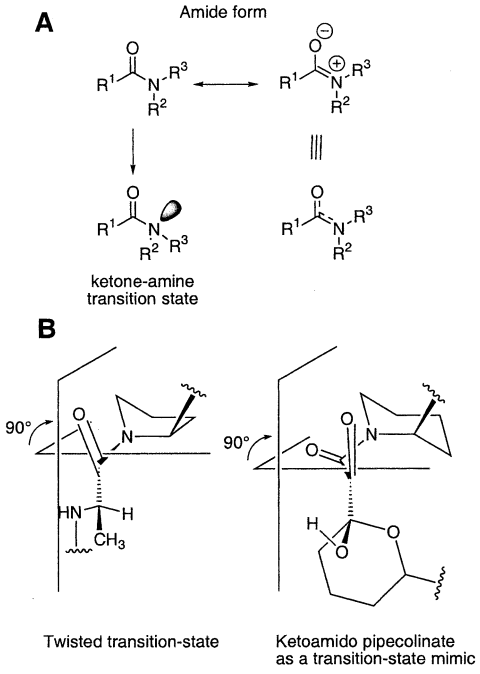
Figure 24. Proposed mechanism for the cyclophilin-catalyzed isomerization of tetrapeptide Ala–Ala–Pro–Phe (AAPF) obtained by molecular dynamics computed simulations: (A) *cis*-isomer; (B) transition-state (TS); (C) *trans*-isomer. Adapted from Figure 3, ref 489.

Chart 11. Keto-Amide-Containing Transition-State Isostere Inhibitors 108 and 109 of the Cis–Trans Isomerization Catalyzed by hCyp-18



108 $R^1 = CH(CH_3)_2$ $R^2 = CO(CH_2)_3CO_2H$
109 $R^1 = CH_3$ $R^2 = NO_2$

Scheme 37. Hyperpolarization of the Rotating Deconjugated Amino Acyl-Prolyl Amide Bond (A) and Mimicking of the Perpendicular Transition-State by a Ketoamido Pipecolinate (B)



Although the isomerization kinetics are dramatically increased by protonation of the amide oxygen,[518,519] the C=O is not the preferential site of protonation.[520] Because this general acidic catalysis takes place only at very low pH (i.e., pH 1.8 for dimethylacetamide),[518] cis–trans isomerization does not usually depend on pH at values between 5 and 9. An exception to this trend was the 2–4-fold increase in the rate of isomerization of Suc–Ala–His–Pro–Phe–pNA **74b** and angiotensin III when the pH was lowered from 7 to 5.5, and may be attributed to the protonation of the proximal histidine imidazole which facilitates the deconjugation of the amide via an intramolecular H-bond (Figure 26).[445] Other protonated chemical groups such as guanidinium are also able to facilitate the pyramidalization of the nitrogen and hence assist the amino acyl-proline cis–trans isomerization as observed in dehydrofolate reductase.[443] This effect was not observed with model peptides Suc–Ala–Xaa–Pro–Phe–pNA **74** when Xaa was Arg or Lys,[445] but this may be the result of particular strains in the peptide backbone that do not allow the guanidinium or ammonium groups to adopt a proper position for the setting of an H-bond with the pyrrolidine nitrogen.

In naphthalene derivative **110**, the addition of acid substantially lowered the energy barrier to isomerization, demonstrating that hydrogen bonding to the amide nitrogen facilitates cis–trans isomerization (Scheme 38).[396]

The positive effect of hydrophobic solvents on the cis–trans isomerization was suggested to result from

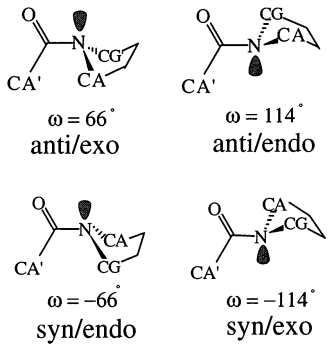


Figure 25. Four possible transition-state proposed for the acyl-proline cis–trans isomerization. Reprinted with permission from *J. Am. Chem. Soc.* **2002**, *124*, 7303–7313. Copyright 2002 American Chemical Society.

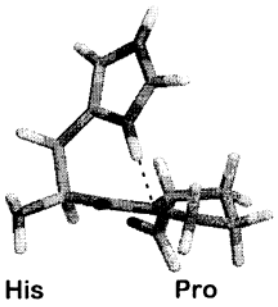
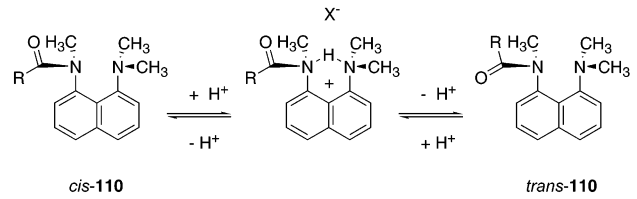


Figure 26. Schematic drawing of the His−Pro moiety: the proposed H-bond between an imidazole nitrogen-linked proton and the pyrrolidine nitrogen might explain the acceleration factor obtained by dividing the rate constants in acid and basic conditions. Reprinted with permission from ref 645. Copyright 1997 Portland Press.

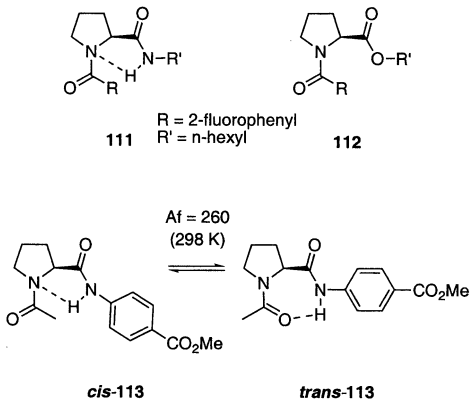
Scheme 38. Acid-Catalyzed Cis−Trans Isomerization of Amide 110[a]



[a] Adapted from *Org. Lett.* **1999**, *1*, 749−752.

conformational modifications as well as the hyperpolarization of the amide. The influence of solvent on the isomerization barrier ($\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{\text{water/acetone}} - \Delta G^{\ddagger}_{\text{chloroform}}$) was much higher for amide **111** ($\Delta\Delta G^{\ddagger}_{\text{trans}\rightarrow\text{cis}} = 2.0 \pm 0.3$ kcal mol^{-1}) than for the corresponding ester **112** ($\Delta\Delta G^{\ddagger}_{\text{trans}\rightarrow\text{cis}} = 0.7 \pm 0.1$ kcal mol^{-1}) for both cis → trans and trans → cis reactions. The amide N−H has been implicated in assisting rotation by stabilizing the transition state (Scheme 39).[398,521] Solvent-induced conformational

Scheme 39. Intramolecular Assistance to the Acyl-prolyl cis−trans Isomerization of Compounds 111−113



modification of the proline derivative **113** was suggested to facilitate intramolecular H-bonding between the amide N−H at P2 and the pyrrolidine nitrogen, which resulted in the 260-fold rate enhancement of amide isomerization ($\Delta\Delta G^{\ddagger}_{\text{cis}\rightarrow\text{trans}} = 3.3$ kcal mol^{-1}) in the case of compound **113**.[397]

Dramatic increase in rates of isomerization was also observed with analogues containing oxazolidine **114**, thiazolidines **115** and **116**,[498,522] and (*2S*,*4S*)-4-fluoroproline **117**.[500] The puckering of pseudoprolines[523,524] and a gauche effect of fluorine,[525,526] as well as the electroattractive effects of ring-heteroatom substituents and fluorine on the proline amide bond may also participate in the lowering of the energy barriers for isomerization.[527] Ab initio and functional density computational studies suggest that the *cis*-isomers of compounds **114** and **115** have a conformation very similar to that of the anticipated transition state (Figure 27).[523,524] A proline peptidomimetic with

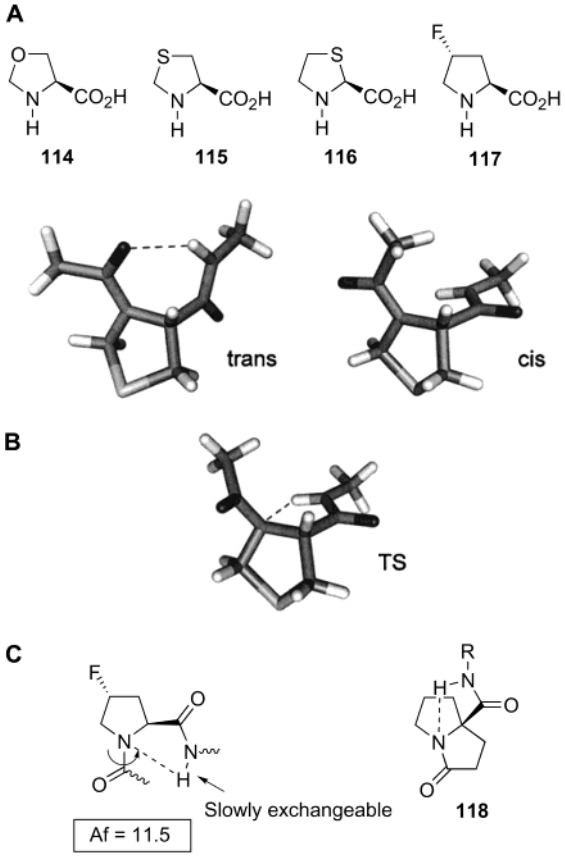


Figure 27. Structures of compounds **114**−**118**; optimized trans, cis, and transition-state (TS) structures of Ac-**115**−NHMe (hydrogen bonds are represented by doted lines). Adapted from Figure 3, ref 523..

a locked *cis*-amide bond (compound **118**) was used to characterized the putative intramolecular H-bond whithout interference with the *trans*-conformation.[397,398] This was also proposed in the case of **122** which isomerizes 11.5-fold faster than the corresponding Pro-containing peptide (Figure 27C and Table 11).[500] The high percentage of *cis*-conformer, the low D/H exchange of the P2 amide, as well as the reduced sensitivity to solvent changes, all suggest the existence of an H-bond between the pyrrolidine nitrogen at P1 and the H−N at P2 and may account for the observed acceleration of cis−trans isomerization. A similar autocatalytic process initiated by an amide N−H has been also proposed in the case of a cyclic disulfide-containing peptide **126** (Table 11) and validated by Monte Carlo mechanics simulations.[444] All these data suggest that a particular peptide backbone conformation is able to facilitate the aminoacyl-proline cis−trans isomerization either by an

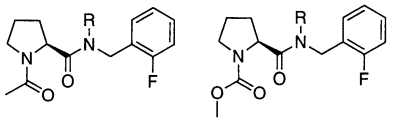
Table 11. Trans:Cis Ratio (K = %trans/%cis) and Acceleration Factor (A_f $k_{proline\ surrogate}/k_{proline}$) of a Set of Peptides Containing Either a Proline Residue or Proline Surrogates

no.	compounds	K	A_f (T °C)
74a	Suc−Ala−Ala−Pro−Phe−*p*NA	8.1	
	113	65.1	260 (25)
119	Ala−Gly−**114**−Pro−Phe−*p*NA	1.6	92 (27)
120	Ala−Gly−**115**−Pro−Phe−*p*NA	2.6	111 (27)
121	Ala−Gly−**116**−Pro−Phe−*p*NA	11.5	167 (27)
122	Suc−Ala−Ala-**117**−Phe−*p*NA	8.1	11.5 (10)
123	Suc−Ala−His−Pro−Phe−*p*NA		2.0 (10)
124	Ac−Ala−His−Pro−Ala−Arg−NH_2		6.3 (4) (pH < 5.5)
125	Arg−Val−Tyr−Ile−His−Pro−Phe		9.6 (48.1)
126	Ac−*c*(Cys−Pro−Phe−Cys)−NH_2	9.8	2 (25)

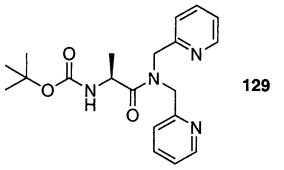
inter- or intramolecular process. Therefore, FKBP may act as an inducer of a type VIa β-turn (intramolecular-assisted isomerization) and cyclophilin binds its substrate in a type VIb β-turn (enzyme-catalyzed isomerization).[491] FKBP may be considered as a receptor which might favor an autocatalytic conformation rather than an enzyme that interacts covalently with its substrate.[513] However, the gain in free enthalpy of activation ($\Delta G^{\ddagger}_{uncat}$ = 19 kcal mol^{-1}; $\Delta G^{\ddagger}_{cat}$ = 6 kcal mol^{-1}) is considerably higher than what would be expected in a simple autocatalysis, and may reflect contributions from desolvation of the amide carbonyl, ground-state destabilization, and preferential transition state binding.[491]

Copper-catalyzed amide isomerization has been demonstrated with proline derivatives **127a** and **127b** and carbamates **128a** and **128b** (Chart 12).[447]

Chart 12. Structures of Compounds 127−129 Used for the Study of the Isomerization Catalyzed by Cu^{2+} and Cd^{2+}



127a R = H **127b** R = Bn **128a** R = H **128b** R = Bn

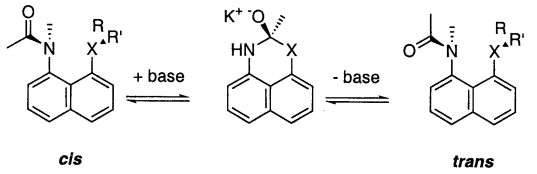


129

N-coordination of Cd^{2+} by Boc-Ala-*N*,*N*-bis(picolyl)-amide **129** gave rise similarly to a significant reduction of the cis−trans isomerization barriers.[528] Moreover, the *cis*,*cis*-conformation of the tetrapeptide Tyr−Pro−Phe−Pro was stabilized in the presence of Cu^{2+} at 293K, tt:tc:ct:cc = 28:34:29:9 in the free state and 13:36:20:31 in the metal-bound state.[529]

The nucleophilic solvent-assisted catalysis of isomerization was demonstrated 30 years ago with dimethylacetamide at pH 11.8. Under these conditions, entropy differences contributed to the free energy of activation, suggesting that hydroxonium directly participated in the catalysis. Conjugation of the amide was disrupted by the formation of a tetrahedral intermediate.[518] Intramolecular nucleophilic catalysis was also observed on addition of potassium hexamethyldisilazane to amide **130** and the tetrahedral intermediate was unambiguously identified.[395] A similar observation was made with compound **131** (Scheme 40) for which a significant decrease of $\Delta G^{\ddagger}$

Scheme 40. Nucleophilic Intramolecular Catalysis of Amides 130 and 131



cis *trans*

130 X = N R = CH_3, R' = H base = KHMDS

131 X = S R, R' = H base = KIm

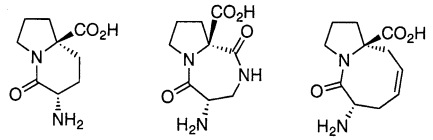
(up to 4.5 kcal mol^{-1}) and a dramatic modification of the cis:trans ratio were seen upon addition of the potassium salt of imidazole, and this was suggested to result from an intramolecular process.[395] These studies showed that cis−trans isomerization of amides can be catalyzed by nitrogen, oxygen, and sulfur nucleophiles by formation of tetrahedral intermediates.

E. Tailoring the Cis–Trans Isomerization Using Proline Derivatives and Pseudoprolines

The conformational influence of amide cis−trans isomerism on the activity of peptides and proteins has inspired extensive research and the design of constrained proline derivatives and proline mimics. It is now possible to mimic either a *cis*- or a *trans*-proline in a rigid defined conformation (Chart 13).[530−537] Proline analogues may be designed to mimic both the geometry as well as the dynamics of the cis−trans equilibrium. We will focus on the latter

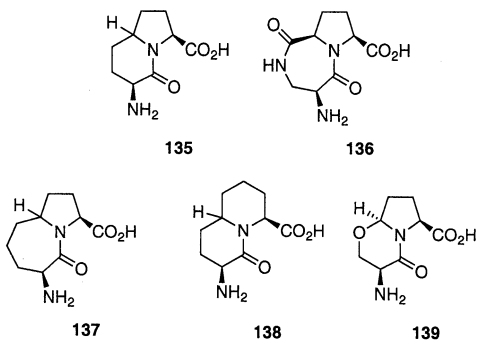
Chart 13. Examples of Locked Proline Mimetics Used for Restraining the Conformation of Peptides

Locked *cis*-proline mimetics



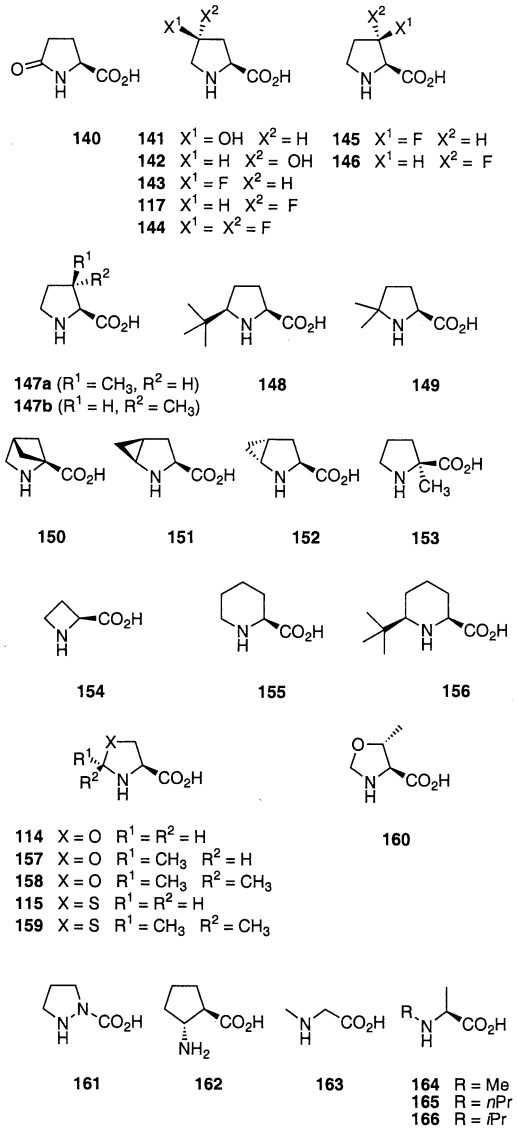
132 **133** **134**

Locked *trans*-proline mimetics



135 **136** **137** **138** **139**

Chart 14. Proline Analogues and Pseudoprolines Used for Studying the Amide Cis−Trans Isomerization



using the structures depicted in Chart 14 and the results summarized in Table 12.

5-Oxoproline **140** exhibits an exclusive *trans*-amide isomer which is stabilized by both steric and electronic effects in part due to the replacement of the tertiary amide bond by an imide structure.[538] Hydroxyprolines **141** and **142** and fluoroprolines **117** and **143**−**146**[500,539,540] exhibit modified cis:trans ratios and energy barriers for amide isomerization relative to proline. The *trans*-isomer content of acetyl-methyl ester derivatives of proline, hydroxyproline **142**, and fluoroproline **146** was increased ($K_{trans:cis}$ values were, respectively, 4.3, 5.8, and 6.2 in water).[527] This tendency, confirmed in the case of 4-difluoroproline **144**,[541] may be explained by a shortening of the proline C4−C5 bond, a consequence of the inductive effect of the substituents. Significant differences in $K_{trans:cis}$ value were also observed depending on the C−F[450,525] and C−OH[525] absolute configuration. Introduction of a fluorine on C3 slightly favored the *trans*-amide isomer of the corresponding acetyl methyl ester derivative. This trend is not confirmed when (3*S*,2*R*)-3-fluoroproline is inserted inside a model peptide (Table 12, entries 9 and 11).[500] The inductive effect also lowered the pK_a value of the proline amine group (respectively, 10.8, 9.7, and 9.2 for proline, hydroxyproline **142**, and fluoroproline **146**)[527] and was anticipated to decrease the energetic barrier to isomerization when inserted inside peptides. Substituents on the C3 and C4 positions lower the $\Delta G^{\ddagger}$ value in Ac−Xaa−OMe when Xaa is a 3- or a 4-fluoroproline[500,541] except when Xaa is (*4R*)-hydroxyproline.[527] These difference may be ascribed to stereoelectronic effects and intramolecular interactions of the fluorine and hydroxy groups with other polar functional groups.[542] The gauche effect of substituents, which alter the puckering of the fluoroproline pyrrolidine ring, strongly affects the pyramidalization of the pyrrolidine nitrogen.[527,542] In particular, *trans*-isomers are stabilized by (*4R*) substituents and *cis*-isomers are favored by (*4S*) electronegative substituents.[542] In peptides and peptide analogues, however, additional intramolecular interactions such as C═O- - -H−N and N- - -H−N hydrogen bonds must be considered.[543] For example, the $K_{trans:cis}$ was 4.6 and 5.7 in Ac−Xaa−OMe and 8.1 and 3.8 when Xaa was, respectively, proline and (*2R,3S*)-3-fluoroproline residue. Intramolecular H-bonds may also affect the kinetics of isomerization by increasing the pyramidalization of the pyrrolidine nitrogen and thus decreasing amide double bond character, in particular, in the case of (2*S*,4*R*)-4-fluoroproline **117**.[500,544]

Hydrocarbon substituents may also orientate the conformation of the preceding amino acyl peptide bond. For example, peptides containing either *syn*- or *anti*-3-methylproline (respectively, **147a** and **147b**) exist as a 25:75 mixture of cis/trans isomers.[545] (2*S*,5*R*)-5-*tert*-butylproline **148** favors predominantly a *cis*-conformation due to steric hindrance in short peptide models that adopt a VIa and VIb β-turn[428] geometry contingent on the stereochemistry of the N-terminal residue. Steric effects have also been used to augment the cis/trans isomer population in larger peptides.[546,547] 5,5-Dimethylproline **149**[548] was shown to exist solely in the *cis*-conformation in the tripeptide Ac−Tyr−5-Me$_2$Pro−Asn by NMR spectroscopy experiments in a mixture of H_2O/D_2O 90:10 at pH 5.3 between 279 and 333 K. Conversely, the cis:trans isomer ratio of Ac−Asn−5-Me$_2$Pro−Tyr varied from 90:10 at 279 K up to 21:79 at 353 K.[549]

Another way to influence the amide isomerization has been to constrain the proline five-membered cycle by adding respectively a methylene group between the 2,4- and 4,5-positions (compounds **150** and **151**/**152**) to influence the preceding amide bond by steric hindrance and "flattening" of the puckered pyrrolidine.[550,551] In 2,4-methanoproline-containing dipeptides Ac−Xaa−2,4-MePro−NHMe (with Xaa is Ala and Tyr), the *trans*-isomer of the peptide bond preceding the methanoproline residue is strongly stabilized over the *cis*-isomer by at least 5.9 kcal mol^{-1}.[552] The kinetics of cis−trans isomerization of N-acyl derivatives of methanoprolines have yet to be reported. No *cis*-isomer was detected for Ac-**153**−NHMe in water and chloroform due to steric interactions between the α-methyl and the acetyl group.[545]

Table 12. Effect of Replacement of Proline by a Proline Surrogate on the Cis Content and Enthalpy of Activation in Peptides and Proteins[a]

entry	Xaa	%cis(Xaa)/%cis(Pro)	$\Delta\Delta G^{\ddagger}$ (kcal mol^{-1})	Cmpd	solvent
1	**141**	1.6	ND	Ac−**141**−OMe	A
2	**142**	0.8	+0.4	Ac−**142**−OMe	A
3	**143**	1.6	−0.1	Ac−**143**−OMe	A
4		1.9	ND	Suc−Ala−Ala−**143**−Phe−*p*NA	B
5	**117**	0.7	−0.5	Ac−**117**−OMe	A
6		1	ND	Suc-−Ala−Ala−**117**−Phe−*p*NA	B
7	**144**	0.7	−0.3	Ac−**144**−OMe	A
8	**145**	0.7	−0.2	Ac−**145**−OMe	A
9		0.9	ND	Suc−Ala−Ala−**145**−Phe−*p*NA	B
10	**146**	0.8	−1.4	Ac−**146**−OMe	A
11		1.9	ND	Suc−Ala−Ala−**146**−Phe−*p*NA	B
12	**147**	0.18	ND	Ac−Ala−**147**−NHMe	C
13	**148**	3.3	−1.6	Ala−**148**−*p*NA	C
14	**149**	2.2	−2.3	Ala−**149**−*p*NA	C
15	**114**	2.5	−2.8	Ala−**114**−*p*NA	C
16	**150**	1.4	ND	Ala−Gly−**150**−Phe−*p*NA	D
17	**115**	1.2	−2.6	Ala−**115**−*p*NA	A
18	**151**	20	−1.2	Ala−**151**−*p*NA	A
19	**116**	0.3	ND	Ala−Gly−**116**−Phe−*p*NA	D
20	**161**	(100% cis)		Boc−Ala−**161**−NH*i*Pr	A
21	**163**	1.3	ND	Ala−Gly−**163**−Phe−*p*NA	D
22	**164**	1.1	ND	Ala−Gly−**164**−Phe−*p*NA	D
23	**165**	1.4	ND	Ala−Gly−**165**−Phe−*p*NA	D
24	**166**	1.6	ND	Ala−Gly−**166**−Phe−*p*NA	D

[a] Cis content of proline surrogate (%cis(Xaa)) was compared with cis content for proline in the same conditions (compound, solvent, temperature); $\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{Xaa} - \Delta G^{\ddagger}_{Pro}$ in the same conditions (compound, solvent, temperature); ND: not determined. Solvents used: (A) water; (B) pH 7.6 35 mM Hepes buffer; (C) D_2O; (D) pH 6.0 10 mM sodium phosphate buffer.

2-Methylproline **153** was shown to stabilize reverse β-turns in peptides.[553]

The replacement of proline with four- and six-membered ring proline analogues, respectively, 2-azetidine carboxylate (Aze) **154** and pipecolinates **155** (Pip), and **156** as well as N-alkyl glycine **163** and N-alkyl alanines **164**−**166**, strongly affect the cis:trans ratio. *Cis*-isomer contents of Ala−Aze−*p*NA, Ala−Pip−*p*NA, and Ala−Pro−*p*NA are, respectively, 20, 13, and 6%. Rates of isomerization are accelerated by, respectively, 14.5- and 49-fold relative to the proline-containing peptide.[498,554]

In contrast to methanoprolines, pseudoprolines **114** (4-Oxa), **115** (4-Thz), **157** (4-MeOxa), **158** (4-Me_2Oxa), and **159** (4-Me_2Thz) have been thoroughly investigated these recent years.[498,522,523,555−557] These compounds are commonly prepared from serine, threonine, or cysteine, respectively. They exhibit an impressive ability to shift the cis:trans ratio to very high values, and this explains why these compounds have gained in popularity. Moreover, this effect is also observed in peptides and proteins,[558] although an exclusive *cis*-conformation is only observed with 5,5-dimethyl compounds **158** and **159**.[556] In a general way, substitution of a pyrrolidine methylene group results in a significant decrease in the rotational energy barrier.[498,522,556,557] Moreover, it is possible to tune the cis:trans ratio in the oxazolidine and thiazolidine series simply by changing the substituent nature and geometry at position 2 (which corresponds to position 5 in proline).[556,559] As recently reported by Kang, the calculated preference for the *cis*-isomer is in the order 4-Oxa > 4-Thz > Pro. The facilitated isomerization for 4-Thz ($\Delta G^{\ddagger}$ in the order Pro > 4-Oxa > 4-Thz) may be explained by the puckering of the proline analogues and a transition state more efficiently stabilized by a pertinent N−H···N hydrogen bond (see Figure 27). This conformational effect has been suggested to be predominant compared to the ring-heteroatom inductive effect.[523]

Therefore, it is possible to tailor the cis−trans isomerization in peptides: type VI β-turns with drecreased energetic barriers to isomerization may be induced in peptides as a result of minor structural modifications of the pyrrolidine ring. Cyclophilin, which is poorly permissive to modifications of the pyrrolidine ring, catalyzes the cis:trans isomerization of peptides containing 4-Thz **115** and 2-Thz **116**, Aze **154**, Pip **155**, but not 4-Oxa **114**.[498] In the same way, grafting of a fluorine at positions C^3 and C^4 resulted in a reduction of catalytic efficiency up to 1 order of magnitude relative to the Pro-containing peptide.[500] AzaPro **161**, the intriguing hydrazido analogue of proline exhibits an exclusive pseudo *cis*-conformation in dipeptides with a marked pyramidalization of the two nitrogen atoms.[560]

Proline mimetics have been successfully employed for tuning the peptidyl-prolyl cis−trans isomerization in peptides and proteins. Chemical introduction of proline analogues is usually performed by standard peptide synthesis, but coupling of C2-alkylated compounds requires special procedures. The pseudoproline thiaproline **115** and the 4-fluoroprolines **117** and **143** were introduced, respectively, in annexin V[558] and barstar[541] in proline-auxotrophic *E. coli* mutants. The successful expression of annexin V and barstar mutants confirmed that thiaproline **115** and 4-fluoroprolines are recognized by their cognate aminoacyl-tRNA synthetases and are incorporated into proteins as efficiently as proline. Thermal denaturation experiments showed that the *per*-thiaproline mutant of annexin V[558] and (*2S*,*4S*)-fluoroproline (**143**) mutant of barstar[541] displayed a higher thermal stability relative to the wild-type proteins. The per-(*2S*,*4R*)-

Table 13. Replacement of Proline with Proline Surrogates in Morphiceptin Analogues: Effects on the Cis Content, Selectivity (Respectively K_i for δ versus μ Opioid Receptors in Displacement Assays and $IC_{50}^{MVD}/IC_{50}^{GPI}$ Bioassays)[a]

no.	compound	pro analog	cis content (%)	K_i^{δ}/K_i^{μ}	$IC_{50}^{MVD}/IC_{50}^{GPI}$
167	H-Tyr−Pro−D-Phe−Pro−NH_2		39	>284	5.45
168	H-Tyr−Cys($\psi^{H,H}$pro)−D-Phe−Pro−NH_2	**115**	40	286	2.55
169	H-Tyr−Cys($\psi^{Me,Me}$pro)−D-Phe−Pro−NH_2	**159**	>98	>265	5.77
170	H-Tyr−Ser($\psi^{H,H}$pro)−D-Phe−Pro−NH_2	**114**	45	>170	2.17
171	H-Tyr−Ser($\psi^{Me,Me}$pro)−D-Phe−Pro−NH_2	**158**	>98	>104	0.99
172	H-Tyr−Pro−Phe−Phe−NH_2			1910	1.98
173	H-Tyr−Cys($\psi^{H,H}$pro)−Phe−Phe−NH_2	**115**	40	309	6.22
174	H-Tyr−Cys($\psi^{Me,Me}$pro)−Phe−Phe−NH_2	**159**	>98	>198	ND
175	H-Tyr−Ser($\psi^{H,H}$pro)−Phe−Phe-NH_2	**114**	45	>509	3.62
176	H-Tyr−Ser($\psi^{Me,Me}$pro)−Phe−Phe−NH_2	**158**	>98	>241	1.99
	[Leu5]enkephalin			0.268	0.05

[a] Keller, M.; Boissard, C.; Patiny, L.; Chung, N. N.; Lemieux, C.; Mutter, M.; Schiller, P. W. *J. Med. Chem.* **2001**, *44*, 3896.

fluoroproline (**117**) mutant of barstar had a lower stability than barstar. These results suggest that 4-fluoroprolines might be used to monitor protein folding and activity by ^{19}F NMR[561] and to tune the properties of proteins.[558]

Several peptides containing a pseudoproline or a fluoroproline residue have been tested as substrates of hCyp-18. Cyclophilin hCyp-18 is highly specific for the proline pyrrolidine ring which is deeply inserted inside a narrow hydrophobic pocket. Replacement of proline in the tetrapeptide Ala−Gly−Pro−Phe−*p*NA with 4-thiazolidine **115**, 2-thiazolidine **116**, azetidine carboxylate **154**, and pipecolinate **155** caused a decrease in catalytic efficiency k_{cat}/K_m of at least 1 order of magnitude, suggesting that cyclophilin catalyzes the cis−trans isomerization of these peptides with a reduced efficiency.[498] Tetrapeptides Suc−Ala−Ala−Xaa−Phe−*p*NA (Xaa is either a 3- or a 4-fluoroproline) are also weaker substrates of hCyp-18 except when Xaa is (*2S*,*4R*)-fluoroproline.[500]

F. Biological Implications of Amide Cis–Trans Isomerism

1. Peptidyl-Prolyl Isomerization and the Regulation of the Activity of Peptides and Proteins

Amino acyl-proline cis−trans isomerization is likely implicated in the control of the bioactivity of Pro-containing peptides;[562] however, in most cases, rates of uncatalyzed interconversion are pretty high and evidence for a preferential bioactive conformation is provided either by structural investigation or structure−activity relationship studies. Local structural changes caused by peptidyl-prolyl isomerization are relatively small. In the majority of cases, the magnitude of Cα atom displacement around the isomeric amino acyl-proline bond is below 1.3 ± 0.6 Å and does not exceed 3 Å even at positions remote to proline; however, the magnitude of intramolecular isomer-specific Cα atom displacement revealed a lever-arm amplification of structural changes in the proteic backbone.[563] In the native state of a number of proteins, both isomers around the Xaa−Pro bond may coexist in particular when Xaa is Ser, Gly, or Glu, and in Pro−Xaa1−Xaa2−Pro sequences.[563]

Constrained proline analogues which adopt a preferential or exclusive conformation and mimic a defined amino acyl-proline isomer were employed for this purpose. Proline residues of the tetrapeptide morphiceptin Tyr−Pro−Phe−Pro−NH_2 were replaced with a (*1S*,*2R*)-aminocyclopentene carboxyl motif **162** which is anticipated to give only *trans*-amide bonds in peptides.[564,565] Biochemical assays and structural studies suggested that the *cis*-conformation about the Tyr−Pro amide linkage is likely required for the biological activity of morphiceptin-related analogues. Recently, the study of morphiceptin analogues containing **114**, **115**, **158**, **159** (Chart 14, Table 13) has confirmed these results.[546] A highly predominant *cis*-conformation around the Tyr−Pro amide bond of endomorphin-2 was also detected with analogues containing proline mimics Ser[$\psi^{Me,Me}$pro] **158** and Cys[$\psi^{Me,Me}$pro] **159**. These dimethylated analogues retained a high biochemical activity, indicating that a *cis*-Tyr−Pro conformation is required for μ-receptor binding and selectivity. Peptides containing a thiaproline **115** have a cis:trans ratio near 1, whereas peptides containing compounds **158** and **159** have a highly predominant *cis*-conformation.[454,557] The substitution of proline with 2,4-methanoproline **150** in [Ile[5]]angiotensin II gave direct evidence that a *trans*-conformation of the amino acyl-proline amide bond is the one recognized by the angiotensin II receptor.[551] A *cis*-conformation between two hydroxyproline residues at positions 7 and 8 was also required for muscle-selective μ-conotoxins GIIIB for blocking voltage-sensitive sodium channels.[566] Pro-to-Ala mutations in interleukin-3, a 133-amino acids cytokine, has shown the important role of the *cis*-conformation at Arg29−Pro30 and Pro30−Pro31 for optimal interaction with the α-subunit of the receptor.[567] The Bowman-Birk inhibitors are a family of serine protease inhibitors which contain a canonical disulfide-linked nine residue-loop. Pro-to-Ala mutation inside the loop of inhibitor fragments led to a 1:1 mixture of slowly exchanging *cis*- and *trans*-amide isomers with a reduced affinity, suggesting that, although Pro is not essential for the interaction with the protease, it stabilizes the peptide in a biologically active *cis*-conformation.[568]

Proline conformation may also regulate the agonist/antagonist activity of peptides. As an example, several oxytocin analogues containing a 5-*tert*-butylproline **148** were prepared and were tested for their receptor-binding affinities and uterogenic activities in vitro. 5-*t*BuPro-containing peptides exhibited higher

cis-isomer content than the corresponding [Pro7]-peptides. Two peptides, [5-*t*BuPro^7]oxytocin and [Mpa^1,5-*t*BuPro^7]oxytocin displayed strongly reduced binding affinities for oxytocin receptor but unchanged pharmacophore characteristics, whereas [D-Pen^1,5-*t*BuPro^7]oxytocin displayed stronger inhibitory potency than the proline equivalent, and no partial agonist activity. This suggested that *cis*-oxytocin might favor antagonism, whereas a *trans*-conformation is required for agonist activity.[446] Therefore, a single molecule might be able to display distinct biological activities depending on amino acyl-proline cis−trans isomerization.

Proline may act as a molecular hinge that modulates recognition by controlling the relative orientation of protein-binding surfaces. As an example, interleukin-2 tyrosine kinase, a T-cell-specific protein required for proper immune response, contains a conformationally heterogeneous proline residue inside its SH2 domain. Cis−trans isomerization mediates conformer-specific recognition of the ligand that might play its role in T-cell signaling.[569]

The amino acyl-proline cis−trans isomerization can also affect the anion-binding properties of cyclic peptides containing 4-hydroxyproline residues by switching from an *all-cis* to an *all-trans* conformation.[570]

2. Amide Cis–Trans Isomerization and Protein Folding

Amide cis−trans isomerization, in particular, amino acyl-proline cis−trans isomerization, is implicated in nascent protein folding and trafficking as well as refolding of denatured proteins.[571] Proline occupies a special place among amino acids:[572] (i) it restricts the conformational space for the peptide chain; (ii) the energy difference between *cis*- and *trans*-isomer is about 2 kcal mol^{-1} and the energy barrier to isomerization is $\Delta G^{\ddagger} = 20 \pm 3$ kcal mol^{-1}. Consequently, the amino acyl-proline cis−trans isomerization may become rate-limiting in protein folding. Classical models for protein folding postulated a unique pathway with folding intermediates that accumulates because of kinetic traps caused by partial misfolding as a result of slow isomerization about prolyl peptide bonds. Recent studies carried out with α-lactalbumine and apomyoglobine argue that these intermediates are rather stable intermediates which are more highly folded than early intermediates. This new view suggests that the folding is hierarchical.[573] Classical view implied that proline cis−trans isomerization occurs before folding was initiated, however, folding kinetics of pectate lyase C reveals that a rate-limiting cis−trans isomerization of a peptidyl−prolyl amide bond occurs in a loop region connecting individual strands of a preformed β-helix.[574] The cis−trans isomerization of several Xaa−Pro amide bonds may be the source of the multiple folding pathways of tryptophan synthase as suggested by mutation of the 18 proline residues which gave rise to kinetically distinct forms.[575] In proteins such as the α-subunit of tryptophan synthase, a single peptidyl-prolyl isomerization has been reported to dominate the folding by driving the trans to cis isomerization of several nonprolyl peptide bonds. This implies that the local stabilization is sufficient to favor *cis*-isomers for secondary amide bonds.[576]

Proline cis−trans isomerization is accelerated by PPIases which thus play a central role in cell life and multiplication by controlling the production of proteins in their active conformation.[366,571,577] Although the nonproline amide bond is likely implicated in protein folding,[480,493,578] the amino acyl-proline peptide bond plays a major role in the complex succession of events that give a protein its biologically active conformation,[479] and many examples illustrate the importance of proline cis−trans isomerization in protein folding.[473,574,579−583] In most cases, the amino acyl-prolyl cis−trans isomerization limits the folding of proteins[576,583] by inducing large magnitude remodeling at positions remote from Pro;[563] however, the two isomers may coexist in active proteins especially when the proline residue is located in a relatively flexible loop.[584] PPIases have been shown to accelerate the rates of folding though they do not generally form a stable complex with their protein counterpart.[583] In some cases, however, PPIases remain bound to their protein substrates[586,587] as in the unactivated steroid receptor[588] and HIV-1 capsid/hCyp-18 complex.[589]

Amino acyl-proline cis−trans isomerism not only plays a role in protein folding and activity but is also an important determinant of protein stability. As an example, it plays a role in collagen nucleation and stability[590,591] and might be directly implicated in misfolding of collagen in connective tissues diseases.[592] Collagen, the most abundant protein in vertebrates, is composed of approximately 300 repetitive units of the sequence Xa1−Xa2−Gly where Xa1 and Xa2 are L-proline and 4-(*R*)-hydroxy-L-proline (Hyp). Consequently, the abundance of Pro and Hyp residues restricts the collagen backbone which tends to form left-handed PII helices exclusively. All Xaa−Pro and Xaa−Hyp bonds are in the *trans*-conformation in the collagen triple helix. Although cis−trans isomerization is the limiting step of collagen folding,[592] many other effects may be adduced to explain its particular conformational stability: local (intraresidue) effects such as the gauche effect,[591] pyrrolidine up and down puckering[593] and intramolecular H-bonding[500,544,593] as well as vicinal and long-range interesidue effects,[527] all play an important role.

3. Direct Assistance to Other Catalytic Processes

Immunophilins assist the catalytic function of other enzymes such as the protein disulfide isomerase (PDI), a protein related to the oxidative folding of reduced proteins. PPIases improve the formation of the correct disulfide bonds by PDI, possibly via the formation of correct prolyl isomers which are better substrates of the disulfide isomerase.[427]

They can also participate in the processing of peptides and proteins by various proteases such as serine- and aspartic-proteases and this specificity has been exploited for the development of PPIase test tube assays as stated above (see part IVB.). In particular, hCyp-18 has been proposed to modulate the processing of the different polyproteins of the virus HIV-1 by the viral protease which specifically hydrolyzes the *trans*-Xaa−Pro sequence (Xaa is an aromatic residue).[584,594−596] PPIases might act as

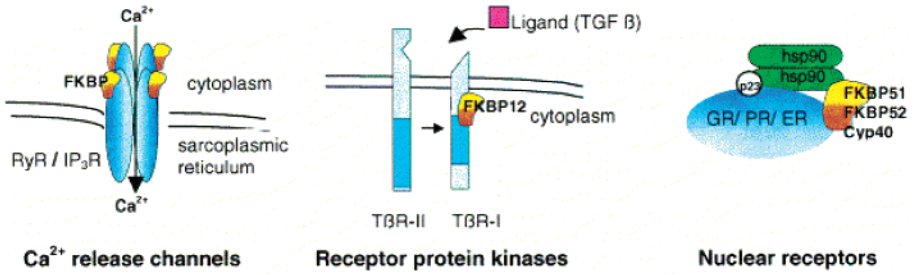


Figure 28. Interaction of FKBP with molecular channels and nuclear receptors. Reprinted with permission from *FEBS Lett.* **2001**, *495*, 1−6. Copyright 2001 Elsevier Science.

helper enzymes in many translational and posttranslational processes which lead to the formation of properly folded proteins. As an example, the Gag polyprotein is the precursor of the four viral capsid protein, respectively the p17 matrix protein (MA), p24 capsid protein (CA), and p7 nucleocapsid protein (NC), and the small p6 protein. It interacts with hCyp-18 via an accessible Gly89−Pro90 moiety located inside an accessible loop of the CA domain. HCyp-18 is likely to catalyze a cis−trans isomerization of the Gly-Pro motif,[594] and hence induces long-range conformational reorganization which enables the polyprotein processing.[589]

PPIases often function in parallel and act on common targets. Consequently, cyclophilins and FKBPs are individually dispensable for cell viability due to functional overlap between the different enzymes.[597] However, Pin1 (and its yeast analogue Ess1), which plays a critical role in cell multiplication and specifically recognizes phosphorylated sequences, is essential for cell replication and is implicated in severe diseases such as cancer[598] and Alzheimer's disease.[473] For example, Pin1 and Ess1 have been shown to interact with phosphoproteins, in particular those implicated in cell division. This specific binding may change protein conformation, catalytic activity, degradation, or location and constitute a novel signaling regulatory mechanism.[474] Pin1/Ess1 interact with the C-terminal domain of RNA polymerase II in vitro and in vivo and thus alter the interaction of polymerase with other proteins required for the transcription of genes.[599,600]

PPIases might also have antagonist biological functions: Yeast cyclophilin A and Ess1 have been shown to interact with the Sin3−Rpd3 histone deacetylase complex. Cyclophilin increases and Ess1 decreases disruption of gene silencing by the complex and thus modulate the activity of the complex by controlling the degree of acetylation of histones.[601]

4. Posttranslational Modifications of Proteins that Affect the PPIase Activity

Posttranslational modifications of the amino acid sequence of PPIases substrates also influence PPIase activity. As an example, phosphorylation of the Ser−Pro and Thr−Pro moieties affects the kinetics of both the catalyzed and uncatalyzed isomerization (see Table 9).[472,473] Phosphorylation-regulated amino acyl proline cis−trans isomerization by Pin1 has been shown to control the formation of paired helical fragments (PHF) which form the neurofibrillary tangles usually found in the brain of patients suffering from Alzheimer's disease and other neurodegenerative disorders. Glycosylation also influences the cis:trans ratio in model peptides and might be another way of regulating PPIase activity.[471]

5. PPIases and the Modulation of Protein–Protein Interactions

Due to their central role in the catalysis of protein folding and assistance in posttranslational processing, PPIases have been exclusively considered as enzymes for a long time, but much evidence suggests that PPIases have other functions, such as receptors and chaperones.[587,602]

Immunosuppression was the first established biological implication of hCyp-18 and FKBP-12 and, although it has been demonstrated that they do not exert their role through a catalysis of the cis−trans isomerization,[603] cis to trans interconversion of tertiary amide bonds is essential for tight binding of cyclosporin and FK506 (see part IVA.3 and Figure 16).[405,406] The same phenomenon was reported with FK506 which exclusively binds to hFKBP-12:[604] the ketoamide-pipecolinic motif exists as a single *cis*-isomer in the free state and isomerization to the *trans*-conformer is required for high-affinity interaction.[605] On the other hand, none of the PPIases are able to accelerate the slow isomerization process.

PPIases mediate the immunosuppression cell signaling pathway by interacting with an immunosuppressant (cyclosporin or FK506). The complex specifically interacts with calcineurin, a calcium-dependent phosphatase, and with calmodulin, a calcium-binding protein that is implicated in the regulation of many enzymes. Blockade of calcineurin interaction with NF-AT, a nuclear factor of activated T-cells, inhibits the activation of the transcription of the gene coding for interleukin-2. A distinct pathway has been delineated in the case of the rapamycin−FKBP-12 complex which causes a fall in ribosome activity.[606,607]

HFKBP-12 also specifically interacts with molecular channels, in particular, the ryanodine receptor calcium release channels (RyR−CRC) and stabilizes the channels in both their closed and open states[608] (Figure 28). Although there are many Xaa−Pro epitopes that might be able to bind to hFKBP-12, the PPIase activity is neither required for the interaction nor for RyR−CRC activity.[609] However, Ryr−CRC activity is affected by FKBP inhibitors such as rapamycin,[610] suggesting that the interaction with receptors takes place at the PPIase active site. HFKBP-12 and hFKBP-12.6 have been shown to interact with receptor protein kinase as well.[587]

Heterooligomeric nuclear receptors such as steroid hormone receptors contain multidomain PPIases (i.e., hFKBP-51, hFKBP-52) which directly interact with both the receptor and the chaperone heat-shock protein hsp90. In fact, the PPIases are not required for glucocorticoid receptor hetero complex assembly and folding of the hormone-binding domain;[587] however, hormone binding to the receptor causes substitution of hFKBP-51 by hFKBP-52 and concomitant recruitment of the transport protein dynein.[611] In the case of hFKBP-52, the interaction is regulated through the phosphorylation of a specific site of the PPIase.[612] The multidomain cyclophilin hCyp-40 has also been found in unactivated bovine estrogen receptor heterocomplex[587] and progesterone receptor complexes.[613]

TGF-β receptor promotes the association of two receptor components TβR-I and TβR-II which leads to the phosphorylation of TβR-I. HFKBP-12 binds to TβR-I via interaction with the FK506 binding pocket and inhibits receptor activation.[614] The interaction takes place at a specific Leu193−Pro194 site of TβR−I[615,616] and is inhibited by rapamycin. HFKBP-12 seems to act as a negative regulator of TβR-I internalization.[617−619]

PPIases also bind transcription regulators such as YY1 and therefore participate in the general transcription machinery.[492,620,621] HFKBP-52 exerts a transcriptional control by association with IRF-4, the interferon regulatory factor which plays an important role in gene expression in B- and T-lymphocytes, and causes a structural modification of IRF-4.[622] Other PPIases such as ESS1/Pin1 and hCyp-18 have been shown to regulate the transcription in particular by interacting with chromatin.[623] Very recently, Rycyzyn et al have shown that the intranuclear prolactin−cyclophilin B complex acts as a transcriptional inducer by interacting directly with Stat5, thereby enhancing Stat5 DNA-binding activity.[624]

Nuclear cyclophilin hCyp-18 also directly binds DNA in a zinc-dependent manner in macrophages and zinc cations inhibit its PPIase activity. Therefore, PPIases might directly recognize DNA specific sequences and hence might regulate the expression of certain genes.[625] Cyclophilin is also released by lipopolysaccharide-stimulated macrophages[626] and displays proinflammatory[627] and chemotactic activities.[628] In addition, hCyp-18 might mediate the HIV-1 infection of T-cells through a direct interaction with specific receptors located at the T-lymphocyte surface,[629] and this is only the first step of a larger implication of cyclophilin in the HIV-1 infectious process.

6. Immunophilins and Diseases

a. Immunophilins and the HIV-1 Infectious Process. Human immunophilins, cyclophilin hCyp-18, and FKBP-12 are also implicated in the infectious process of T-cells and macrophages by HIV-1, the pathogenic agent of AIDS (Figure 29).[630] HCyp-18 is suspected to catalyze a cis−trans isomerization of a Gly−Pro dipeptide in the V3 loop of the gp120 viral surface protein[631] and hence might regulate the interaction of gp120 with CCR5 receptors of the $CD4^+$ T-cells and subsequent viral infection.[632] HCyp-18 is also likely to play a role in cell recognition by interaction of a positively charged region of the protein with heparane sulfate located at the cell surface,[650] and hence might assist the gp120−CCR5 interaction.[631,632] This might be explained by the relocation of cyclophilin to the viral surface during maturation, whereas it is initially incorporated inside the virus core.[634]

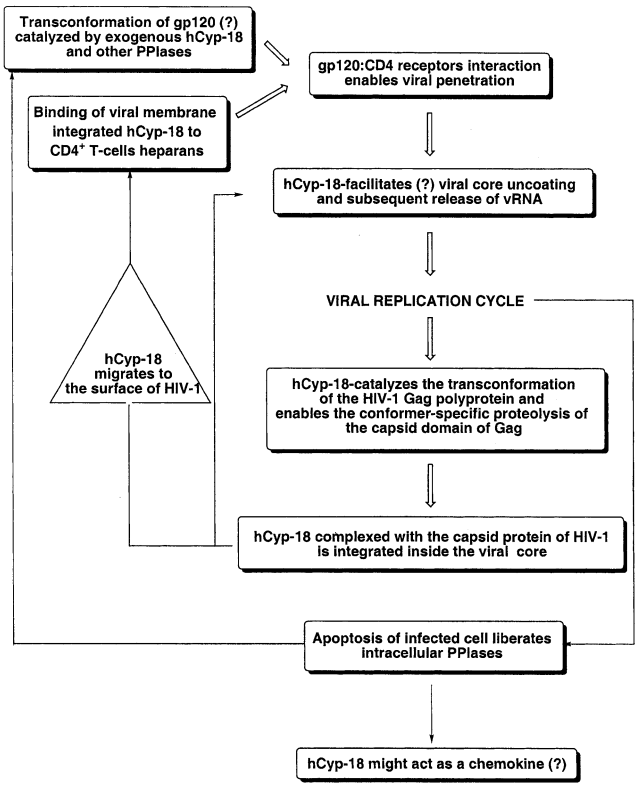


Figure 29. Multistep implication of hCyp-18 in the HIV-1 viral cycle.

There is much evidence that hCyp-18 not only modulates the gp120−CD4 interaction but also participates in the maturation of the infective virus. HCyp-18 specifically binds the Gag polyprotein from HIV-1.[635−638] X-ray and NMR structural data of hCyp-18 complexed with different fragments issued from the Gag polyprotein have shown that hCyp-18 forms a stable complex with both the immature Gag polyprotein and the capsid protein which comes from the posttranslational maturation of Gag.[589,594,639−646] HCyp-18 seems to be able to interact at several distinct sites on the Gag polyprotein which all contain a Gly−Pro motif.[423,589] Although hCyp-18 seems to interact closely with a single capsid protein, it is included inside a larger structural complex which participates in virion budding and stability of the viral core.[647,648] Binding is mediated by the central region of the CA domain of Gag which interacts with the hydrophobic pocket of cyclophilin[649] and requires Gag dimerization.[650] In vitro experiments have shown that depletion in hCyp-18 during virus core assembly[651,652] or treatment with cyclosporin A and analogues cause a large reduction of virus infectivity which cannot be reversed by exogenous cyclophilin.[653−657] In fact, hCyp-18 seems to destabilze the virus core to enable the release of viral RNA after infection of T-cells, although this hypothesis is still a matter of dis-

cussion.[658−660] However, the very recent resolution of the structure of the N-terminal fragment of Gag has demonstrated the formation of a β-hairpin located at the N-terminus of the CA domain which induces an approximatily 2 Å displacement of helix 6 and a concomitant move of the hCyp-18 binding site.[661] Therefore, the PPIase activity of cyclophilin seems to trigger the proteolytic cleavage of Gag, which induces structural changes inside the CA protein.[589]

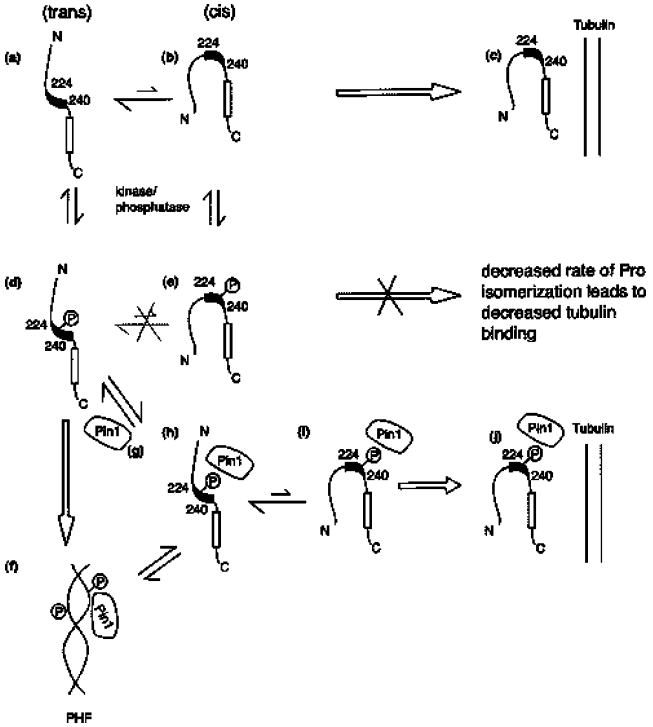
b. Implication of Pin 1 in Cancer and Apoptosis. Human Pin1, a member of the parvulin family of PPIases, is essential for the regulation of mitosis.[595] It catalyzes the cis−trans isomerization of the amino acyl−proline peptide bond in Ser(PO_3H_2)−Pro and Thr(PO_3H_2)−Pro moieties of polypeptides in both the folded and unfolded states with a much higher affinity than the corresponding nonphosphorylated sequences.[472,474,595,662] The preference for negatively charged Xaa−Pro dipeptides is a general characteristic of Pin1 which not only concerns the catalytic site: WW domains of Pin1 and its yeast homologue ESS1 bind Ser/Thr(PO_3H_2)−Pro sequences with a higher affinity than the catalytic site,[474,663] and the affinity can be enhanced by multiple phosphorylation of the repeated Ser−Pro sequence.[664,665] In *Xenopus laevis*, depletion in Pin1 is accompanied by hyperphosphorylation of proteins implicated in cell replication which causes a premature mitotic entry of the cells followed by apoptosis.[666] The cyclic undecapeptide cyclosporin A, which selectively inhibitits certain cyclophilins such as hCyp-18, and reduces the concentration of available cyclophilin also induces cancer[667] progression and apoptosis.[668] Although hCyp-18 has been shown to interact directly with chromatin,[623] the cancer induction pathway is not likely to be similar with that involving Pin1: CsA might induce TGF-β production which causes a malignant differentiation.[667]

Pin1 also increases the transcription of several β-catenin target genes such as cyclin and c-Myc. β-Catenin levels have recently been correlated with Pin1 concentration: overexpression of Pin1 in human breast cancer causes an increase of β-catenin concentration. Furthermore, Pin1 levels affect the stability of β-catenin in vivo. Pin1 might regulate the turnover and subcellular localization of β-catenin by interfering with its binding to APC, a protein that is encoded by a tumor-suppressor gene.[669]

c. Pin1 and Neurodegenerative Disorders. Alzheimer disease is the most common and best investigated neurodegenerative disorder associated with the formation of paired helical fragments (PHF) of τ protein which forms neurofibrillary tangles. The τ protein contains Thr^{231}−Pro^{232} and Ser^{235}−Pro^{236} sequences that are phosphorylated in PHF. A very small fraction of τ protein exists as a folded conformer with a *cis*-Pro isomer in the [224−240] region. Only this *cis*-conformer is able to bind tubulin. Pin1 accelerates the rate of cis → trans isomerization and thus increases τ binding to tubulin. Hyperphosphorylation of the τ protein, which has been recognized as a major feature of neurodegeneration, might decrease the rate of uncatalyzed trans-to-cis isomerization of the Ser−Pro peptide bond and hence inhibit the binding of *cis*-τ to tubulin as depicted in Scheme 41. This process may be reversed by Pin1, which specifically interacts with Ser(PO_3H_2)−Pro, catalyzes the trans-to-cis interconversion, and assists the interaction of phosphorylated τ with tubulin. However, the interaction of Pin1 with hyperphosphorylated PHF (either via its catalytic site or its WW domain)[663,665] leads to an increase of Pin1 sequestring, and therefore, concentrations of soluble Pin1 fall dramatically.[473] Other neurodegenerative disorders such as Pick's disease, corticobasal degeneration, and supranuclear palsy are associated with an increase in hyperphosphorylated τ protein and the formation of PHF.

Other PPIases, in particular hFKBP-12, are also found in very high concentrations in the central nervous system (up to 50-fold compared with immune

Scheme 41. Schematic Representation of Possible Conformational and Binding Interactions of τ[a]



[a] The τ protein is drawn with the residues 224−240 highlighted and the tubulin binding domain as a boxed region. In solution τ (224−240) exists as a random coil ensemble, some conformers of which contain trans Pro [(a) represented as an extended conformation, >90%] and others cis Pro [(b) represented as a folded conformation, <10%]. A very small fraction of conformers, perhaps accessible via cis Pro intermediates, adopts a conformation suitable for binding to tubulin (c). On phosphorylation (d), the cis−trans equilibrium is slowed in rate (but not extent). The conformational rearrangements necessary for selection of a binding conformer are thus slowed sufficiently that binding to tubulin is reduced. The free phosphorylated peptide (d) thus becomes more available for PHF formation (f). Free Pin1 (g) is able to bind either to phosphorylated PHF (f) or phosphorylated soluble τ (h). In the latter case, it speeds up prolyl isomerization to the cis form (i), thereby leading to enhanced selection of binding conformations (j), explaining how Pin1 can reverse the lack of binding seen for phosphorylated τ. In Alzheimer disease, hyperphosphorylation leads to increased PHF (f), increased sequestering of free Pin1 and hence reduced soluble Pin1 for facilitating prolyl isomerization (h to i), also resulting in reduced tubulin binding by τ (j). Reprinted with permission from Daly, N. L.; Hoffmann, R.; Otvos Jr., L.; Craik, D. J. *Biochemistry* **2000**, *39*, 9039−9046. Copyright 2000 American Chemical Society.

system). However, though phosphorylation levels of the protein GAP-43 are affected by FKBP-12 inhibitors that induce neurone regeneration, the exact mechanism is still a black box.[606]

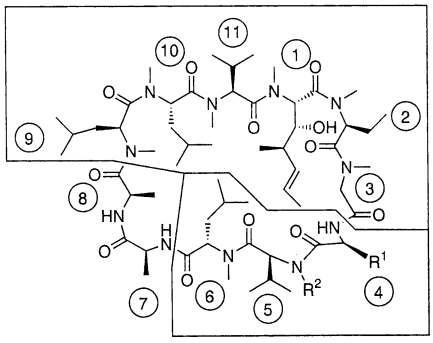
Prion diseases such as bovine spongiform encephalopathy, scrapie of sheep, kuru and Creutzfeldt-Jakob disease, all are characterized by amyloid deposits (scrapies) in certain regions of brain.[670] The scrapies are constituted by agregated PrP^{Sc}, an isoform of the PrP^{c} protein. There are marked differences in the secondary structures of PrP^{c} and PrP^{Sc}: PrP^{c} shows a 42% α-helical content and only 3% β-sheet whereas PrP^{Sc} has 30% α-helical and 45% β-sheet contents. PrP^{Sc} self-agregates and is able to initiate $PrP^{c} \rightarrow PrP^{Sc}$ transition of other soluble PrP^{c} molecules. PrP^{Sc} displays a proteinase-resistant core which is absent in PrP^{c}, and therefore, agregation and formation of scrapies cannot be reversed.[671] The implication of one or several proline residues has been proposed to account for these conformational changes, and Pro101 of the prion protein has been clearly implicated in the formation of β-sheet fibrils.[672,673] Proline isomerization at Pro166 has also been demonstrated to be rate limiting in the folding of the Ure2 protein isolated from *Saccharomyces cerevisiae* which contains a N-terminal glutamine/asparagine-rich prion domain (PrD) and propagates by a mechanism that is very similar to those of the 254 residue prion protein.[674] Therefore, isomerization of Pro166 might induce the alignment of accessible prion domains, which is not favored in the native dimer, and the subsequent amyloid formation. These results suggest that cis−trans isomerization of a peptidyl proline bond might initiate large conformational changes in the prion protein and might be the molecular base of $PrP^{c} \rightarrow PrP^{Sc}$ transition. The implication of chaperone, in particular PPIases, in this process is still unclear.

G. From PPIase Inhibitors to Novel Therapeutics

PPIases have numerous biological functions beyond protein folding and immunosuppression.[492,546,550] Therefore, there is a considerable interest in selectively inhibiting certain PPIases for AIDS therapy, treatment of neurodegenerative diseases and cancer, and screening of PPIases inhibitors is very likely to yield novel therapeutics.[494,606] This is a formidable task in view of the variety of immunophilins and biological involvements of PPIases. The main difficulties arise from the multiple involvement of a given PPIase (i.e., cyclophilin hCyp-18) and from the overlapping of distinct PPIases for a given function which allows either to complement (i.e., hCyp-18/FKBP-12) or to amplify the effects of a depletion (i.e., hCyp-18/Pin1).

Most PPIase ligands have been derived from the cyclosporin and FK506 series, and many chemical modifications have been carried out to modulate their selectivity and affinity, in particular, their immosupressive activity. Crystallographic data as well as structure−activity relationship studies[675] have allowed the delineation of the cyclophilin- and calcineurin-binding moieties.[676,677] Series of modified CsA derivatives **177** and **178**[678,679] (Chart 15) have shown interesting anti-HIV-1 activities, but the

Chart 15. Cyclosporin A (CsA) 75 and Two Non-Immunosuppressive Analogues 177 and 178 that Inhibit Cyclophilin hCyp-18[a]



75 R^1 =  R^2 = H IC_{50} (hCyp-18) = 190 nM RI = 100%

177 R^1 =  R^2 = H IC_{50} (hCyp-18) = 114 nM RI < 0.1%

178 R^1 =  R^2 = Bn IC_{50} (hCyp-18) = 280 nM RI < 0.1%

[a] Immunosuppressive effect of compounds **177** and **178** is below 0.1% relative to that of CsA. These analogs have been obtained via either a Leu4Ile chemical modification of CsA or a N-alkylation of the nitrogen of residue 5. The motifs interacting either with hCyp-18 (top frame) or calcineurin (bottom frame) have been delineated.

therapeutic use of cyclosporin derivatives is marred by numerous side-effects such as liver and kidney toxicity,[680] induction of apoptosis,[668] and stimulation of growth of existing cancers.[667] Another limitation arises from the synthetic difficulties encountered in the systematic modification of cyclosporins.[680−682] An interesting derivatization of cyclosporin by selective N-alkylation at N5 has provided several compounds with promising anti-HIV-1 activity[683] (Chart 15).

Modified fragments of Gag (compounds **179**−**185**) also exhibited affinities equivalent to that of the entire capsid protein[423,589,631] and efficiently inhibited hCyp-18 with highly selectivity[406] (Table 14). In particular, Gag[86−91] was modified to give a pentapeptide that binds hCyp-18 with a higher affinity ($K_d = 3 \pm 0.5\ \mu M$) than the entire capsid protein ($K_d = 16 \pm 4\ \mu M$) (Figure 30).[424]

Chemical modification of macrolides such as FK506 (compound **186**) and rapamycin is more difficult due to the nonpeptidic nature of the inhibitor, but modification of the tricarbonyl moiety,[684] ring contraction,[685] and side-chain alkylation[686,687] have yielded active compounds that inhibit FKBPs independently of their immunosuppressive activity[688] (Figure 31).

As stated previously, the tricarbonyl moiety has been identified as a common motif in FKBPs inhibitors isolated from natural sources. Consequently, truncated FK506 analogues containing a ketoamido-pipecolinyl moiety display interesting properties that have been exploited for the development of new therapeutics.[606] The ketone carbonyl group of the ketoamide motif is perpendicular to the planar amide bond and hence seems to mimic the rotating amide[689,690] (see Scheme 37B). Low molecular weight FK506 derivatives[691] have given rise to the development of a large series of ketoamido,[692,693]

Table 14. Sequence and Biological Activity in Test-Tube Assays of a Set of Peptides Derived from the Capsid Domain of the Gag Polyprotein and the V3 Loop of the Gp120 Protein from HIV-1[a]

no.	peptides	sequences	K_i (μM)
179	CA[81−117]	H−DRLHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGW−OH	8.3 ± 0.8
180	CA[81−105]	Ac−DRLHPVHAGPIAPGQMREPRGSDIA−NH_2	180 (IC_{50})
181	CA[86−92]	Ac−VHAGPIA−NH_2	710 (IC_{50})
182		$(CH_3)_2CH-CH_2CO-$HAGPIA−NH−Bn	3 ± 0.5 (K_d)
183	CA[153−172]	Ac−IRQGPKEPFRDYVDRFYKTL−OH	<1
184	CA[214−228]	H−MMTACQGVGGPGHKA−OH	0.35 ± 0.07
185	V3	H−CNTRKSIHIGPGRAFYTTGE−OH	0.33 ± 0.03 (K_d)

[a] The one letter code has been used for clarity, and the canonic GP dipeptide which interacts at the cyclophilin active site has been underlined.

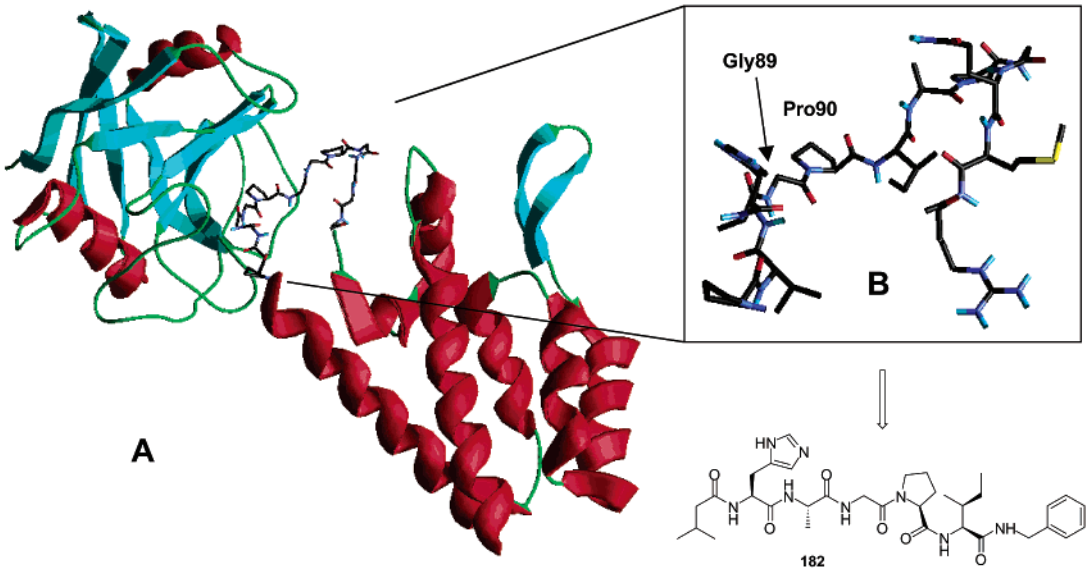


Figure 30. Crystal structure of hCyp-18 bound to the amino-terminal domain of HIV-1 capsid (1AK4) at 2.36 Å resolution (represented using the PDB Viewer Software 3.7b2, Glaxo-Wellcome). From ref 646. The complex (A) is represented in ribbons except the flexible loop CA[85−97] from the capsid domain (backbone representation). A detailed representation of the loop is given (B) as well as the structure of a modified peptide derived from CA[87−92] (compound **152**) that binds hCyp-18 more efficiently than the entire capsid protein. Reprinted with permission from ref 424. Copyright 2000 American Chemical Society.

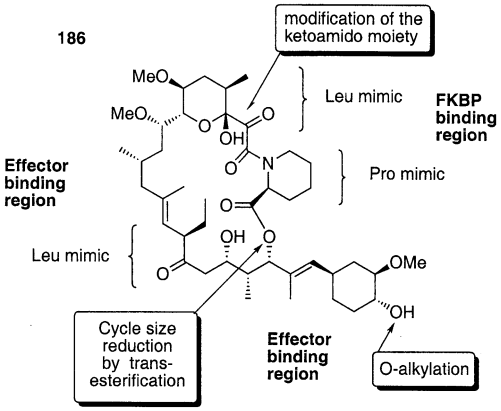


Figure 31. The FKBPs inhibitor FK506 (compound **186**). The FKBP binding and effector binding regions have been delineated. Possible modifications of the natural molecule have been mentioned (arrows and frames).

β-keto,[694] sulfonamido,[695] and ureido[696] analogues (compounds **187**−**191**) which have displayed interesting anti-neurodegenerative and neuroregenerative properties[697−699] (Chart 16). The single ketoamido-containing pseudopeptide directed toward cyclophilins is compound **192** which has been proposed as a PPIase transition state inhibitor, although biochemical assays have shown that it is equally efficient in inhibiting hFKBP-12 than hCyp-18.[509] In further attempts, ketoamido-proline derivatives have shown lower activities and selectivities with hCyp-18.[511]

The phosphonamidyl-proline-containing pseudopeptide **193** (Chart 17) has displayed a micromolar affinity and a very high selectivity toward hCyp-18, since it did not affect the hFKBP-12 PPIase activity. In turn, deprotection of the phosphonamidyl moiety into a negatively charged phosphonamidate results in a complete lack of inhibition, although a significant affinity is preserved.[511] In contrast, the phosphinyl-proline equivalent is not a transition state mimic (compound **194**), putatively due to the absence of the pyrrolidine nitrogen, although other causes such as steric hindrance and conformational restraints may be consided[700] (Chart 17). Cyclophilins are highly specific for the proline pyrrolidine ring which is deeply inserted inside the hydrophobic recognition site and so are not very permissive to ligand modification in the P1−P1′−P2′ region[496] (Figure 32). This was also confirmed with 3- and 4-fluoroproline-containing peptides which are poor inhibitors of hCyp-18.[500] These results likely explain the limited chemical diversity of cyclophilin inhibitors, whereas screening of librairies and de novo design have led

Chart 16. Structures of Truncated FK506 Analogues 187−191

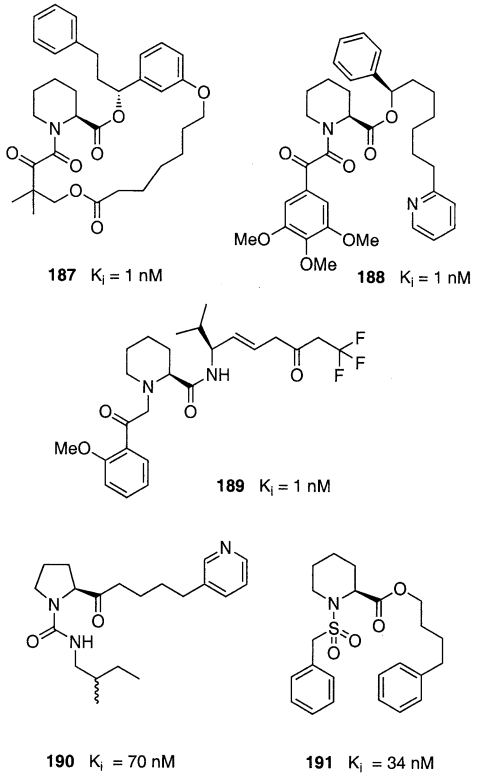
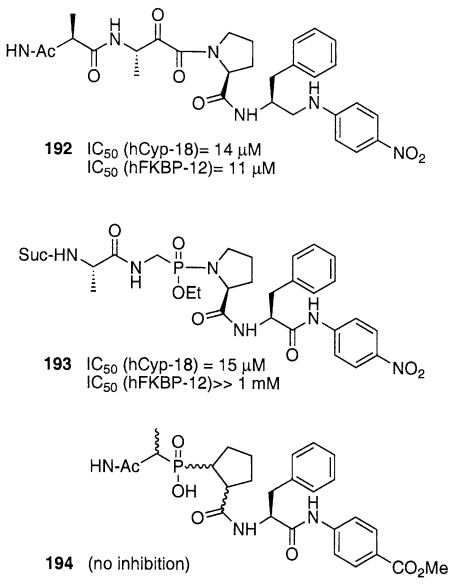


Chart 17. Structures of Pseudopeptides 192 (Suc−Ala−Alaψ(COCO−NH)Pro−Phe−*p*Na), 193 (Suc−Ala−Alaψ(PO(OEt)−NH)Pro−Phe−*p*NA) and 194 (Suc−Ala−Alaψ(P(O)OH−CH)Pro−Phe−*p*NA)



to the emergence of a variety of active compounds inhibiting FKBPs.

Several other immunophilin inhibitors including thioamide **195**,[361] nonisomerizable amino acyl-proline surrogates **197**−**199**,[701−706] constrained ground state **200**, and transition state **201** analogues inhibitors[707,708] or the reactive 5-fluoroproline **202**[709] have been tested as well and interact with hCyp-18 or FKBP-12 in the micromolar range (Chart 18). Very recently, pentapeptides containing either the D-Ser-(PO_3H_2)-Pro moiety or the Ser(PO_3H_2)-ψ[CS−N]Pro motif (respectively, compounds **196a** and **196b**) have been shown to be the first peptide inhibitors of Pin1 which display IC_{50} values in the low micromolar range with full reversibility.[710] The inhibitor containing a D-Ser residue exhibits remarkable stability against the phosphatase activity of cell lysate.[710]

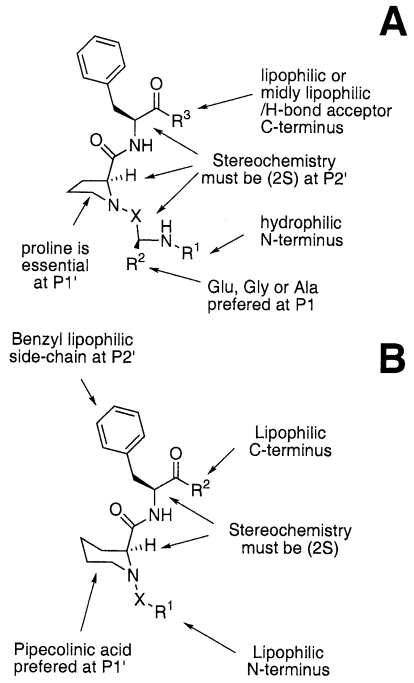


Figure 32. Summary of the main features of "ideal" hCyp-18 ligand (A) or hFKBP-12 ligand (B).

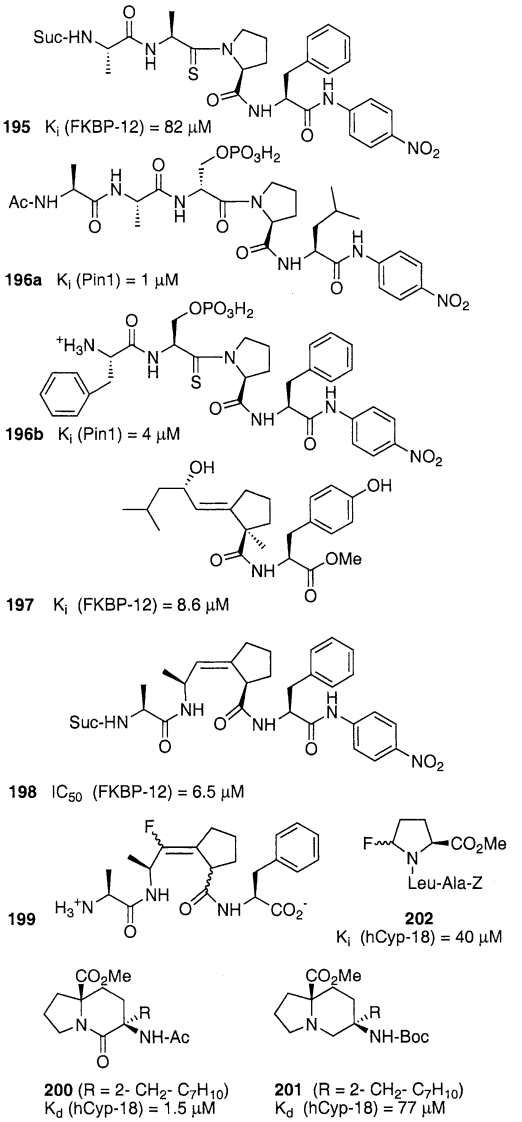
Juglone **203a** has been found to inhibit parvulin-like enzymes such as *E. coli* parvulin[485] and human Pin1[711] in a selective and irreversible way by quenching two cysteine thiols of the active site. The basic cause of inactivation seems to be a partial unfolding of the PPIases following the covalent attachement of two molecules of naphthoquinone.[485] Juglone **203a** and plumbagin **203b** also block cancer progression in the rat[711] putatively by inhibiting transcription.[712] Cycloheximide derivatives **204** and **205** inhibit FKBPs and, less efficiently, parvulin. They display an interesting neuroregenerative activity. Compound **205** features a reduced cytotoxicity.[713] Bicyclic compounds **206**, which might make van der Waals contacts similar to those observed with the pipecolinic motif, display interesting affinities.[714] Very recently, the dimedone amino acid derivative **207**[715] and phenylenediamine-based compound **208**[716] have been patented as cyclophilin-binding compounds (Chart 19).

Other PPIase inhibitors such as cymbimicin A,[717] sangliferin A,[718] and cyclolinopeptides[719,720] have been reported in the literature, and particular synthetic efforts should be made to generate molecular diversity starting from these scaffolds.

V. Cis–Trans and Cisoid–Transoid Isomerism around Single Bonds

Although free rotation about a σ-bond (in particular C−C bond) is permitted, preferential torsional angles (anti, $\omega = 180°$; gauche, $\omega = \pm 60°$), which correspond

Chart 18. Structures of Pseudopeptides 195 (Suc−Ala−Alaψ(CS−NH)Pro−Phe−*p*Na), 196a (Ac−Ala−Ala−D-Ser(PO_3H_2)−Pro−Leu−*p*NA) and 196b (Phe−Ser(PO_3H_2)−ψ[CS−N]Pro−Phe−*p*NA)[a]

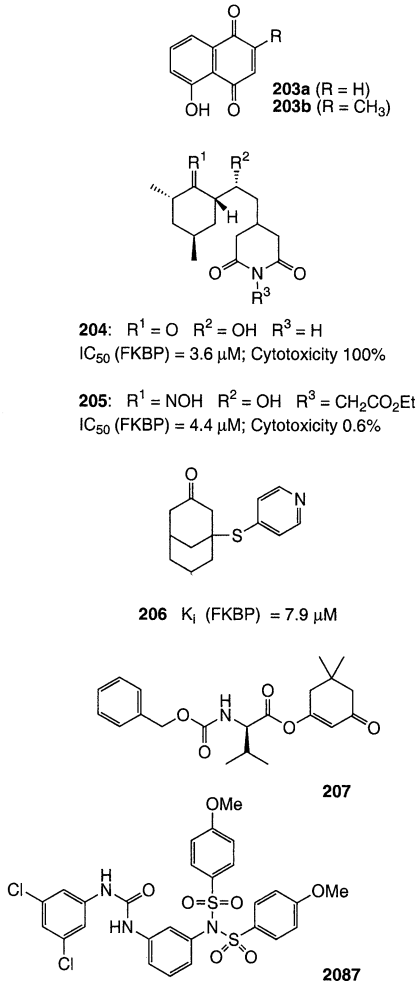


[a] Alkene-containing peptidomimetics **197**−**199** and *cis*-proline ground-state and transition-state analogues (respectively, **200** and **201**); 5-fluoroproline-containing peptide **202**.

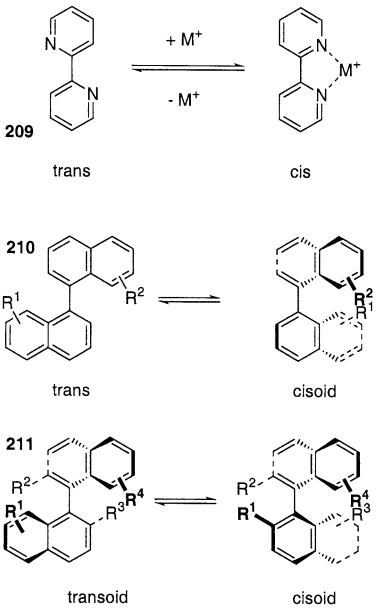
to energy minima, restrict the conformation of most of the molecules. However, conformational preferences may be more restrained in the case of severely crowded chains and when backbone valence angles are smaller than tetrahedral (e.g., thioethers, disulfides, ...) and, in some cases, energy of interconversion may be high. Some additional effects (long-range interactions or charges) are also able to orientate the conformational preference to *cisoid*- or *transoid*-isomers.[722]

The thermodynamics and use of cis−trans and cisoid−transoid isomerisms of biaryl compounds **209**−**211** (Scheme 42) have been detailed in many excellent reviews[723,724] and have played a critical role in organic and inorganic chemistries, supramolecular chemistry, as well as in the emergence of the concept of foldamer.[725] *Cisoid*- and *transoid*-isomers are also found in oligomers such as oligo(*m*-phenylethenylene)

Chart 19. Structures of Non-Peptide Inihibitors of PPIases



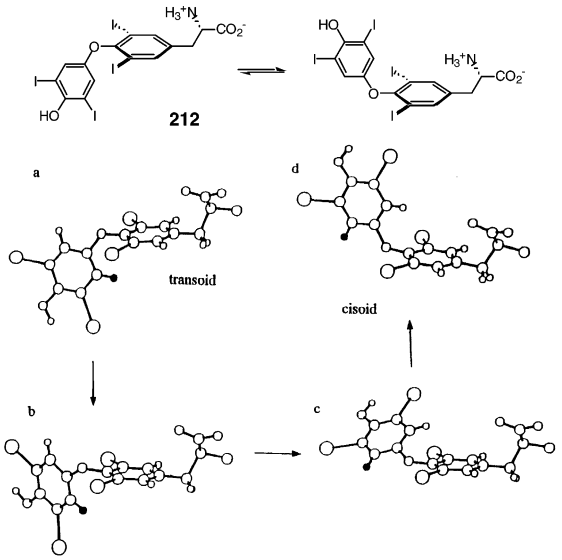
Scheme 42. Examples of Cis−Trans, Cisoid−Trans, and Cisoid−Transoid Isomerism in Biaryl Compounds 209−211



in which cisoid-to-transoid inversion results in a change of packing behavior and hence in dramatic modifications of the properties.[726]

Isomerism may also occur about two σ-bond connecting either a carbon or a heteroatom to two aryl moieties. Such isomerism has been observed with thyroid hormones that exist as a mixture of *cisoid*- and *transoid*-conformers (Scheme 43). The barrier of

Scheme 43. Sketch of the Interconversion from *transoid*- to *cisoid*-thyroxine by Cooperative Rotation around ϕ and ϕ' Torsional Angles[a]

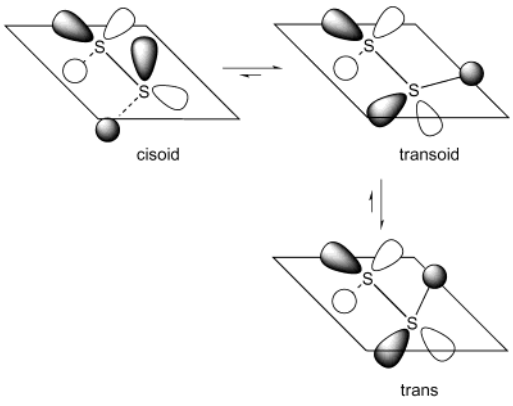


[a] Adapted from Duggan, B. M.; Craik, D. J. *J. Med. Chem.* **1997**, *40*, 2259.

interconversion of thyroxine via the concerted rotation of ϕ and ϕ' angles is pretty high (8.7−8.9 kcal mol^{-1}); therefore, both isomers are observed by high-field NMR spectroscopy and isomerization can be monitored by variable temperature experiments.[727] Conversion from the *cisoid*- to the *transoid*-isomer is critical for the binding to the thyroid hormone receptor and the subsequent triggering of conformational changes inside the receptor.[728]

In simple disulfides, the conformational freedom around the C−S−S−C moiety is mainly limited by electronic interactions of the sulfur lone pairs (Scheme 44).[729] Although the *transoid*-isomer is by far the

Scheme 44. Cisoid−Transoid Isomerism about a Disulfide Bond



most abundant (ω about 110°), many different conformations are found in acyclic compounds and particular strains can favor the *cisoid* and *rectangular* conformations, in particular, in the case of methylcyclophane analogues.[730] The *cis*-conformation is only observed in particular cases since it implies an unusually long S−S bond (2.1 Å in the case of $TMS_3C-S-S-CTMS_3$). Trisulfides (C−S−S−S−C) are also found to adopt a preferential *transoid*-(helical)-conformation.[731]

In peptides and proteins, the *cisoid*-conformation is poorly represented and always corresponds to a restriction of the conformational space of the peptide backbone.[732,733] In most cases, especially in severely constrained peptides (i.e., cyclic peptide **213**), disulfide conformations are intermediary between *cisoid*- and *transoid*-isomers due to additional restraints and hence do not evolve (Figure 33).[734]

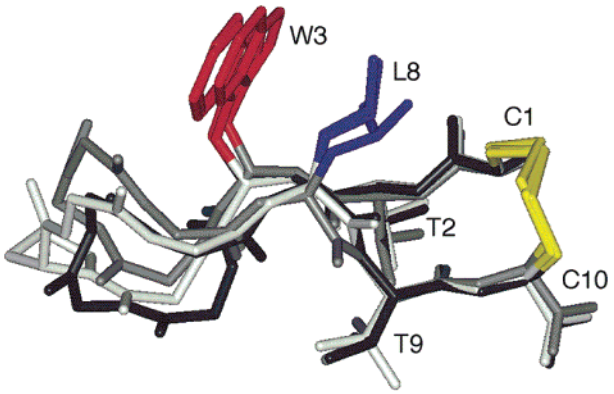


Figure 33. Minimized mean structures of peptides Ac−Cys−Thr−Trp−Glu−Gly−Asn−Lys−Leu−Thr−Cys−NH_2 (gray), Ac−Cys−Thr−Trp−Glu−Asn−Gly−Lys−Leu−Thr−Cys−NH_2 (black), Ac−Cys−Thr−Trp−Glu−D-Pro−Asn−Lys−Leu−Thr−Cys−NH_2 (white). For clarity, nonproline side-chains of the four turn residues are not shown. Reprinted with permission from ref 734. Copyright 2001 American Chemical Society.

VI. Conclusion and Perspectives

It is often difficult to reduce different and complex processes to a simple concept. This is the case with the cis−trans isomerization of organic molecules since isomerization may concern olefins, diazene and related compounds as well as pseudo-double bonds such as amides. Moreover, there is a vast number of cis−trans isomerization pathways, including deconjugation, doublet inversion, heterolytic and homolytic bond breaking as well as molecule splitting and reassembling. However, all these reactions result in a lasting but in most cases, reversible modification of the molecule geometry which causes changes in its physical, chemical, and biological properties. Could some general rule be drawn from the numerous example of cis−trans isomerization reported in the literature? In particular, what might be the relationship existing between polyenes and amides which are found in biomolecules?

The free enthalpy difference $\Delta G°$ between *Z*- and *E*-isomers is about 4 kcal mol^{-1} in retinal and typically around 2 kcal mol^{-1} for peptide bonds. The energy barrier to isomerization is much lower in the case of pseudo-double bonds such as amides ($\Delta G^{\ddagger} \sim$ 20 kcal mol^{-1}) than for olefins ($\Delta G^{\ddagger}$ is 90−170 kcal mol^{-1}). Consequently, the two conformations may coexist, but interconversion of olefins requires higher energies to occur than amides. This implies that, in

biomolecules, olefins isomerize mainly via photochemical processes that do not directly affect the cell constituants. In the absence of light, radical generators, or appropriate enzymes, C=C bonds without electron-withdrawing substituents are stable and do not isomerize. Although the high energy barrier limitates the rate of cis−trans isomerization in peptides, the rate of uncatalyzed interconversion is not negligible depending on temperature and solvent. Although enthalpy differences of secondary and tertiary amides are small, the entropy loss in the trans → cis isomerization is smaller in Xaa−Pro sequences than in nonprolyl peptides. In proteins, the environment proximate to and remote from the isomerizing peptide bond as well as olefins plays a crucial role. Furthermore, the *cis*- and *trans*-isomers are overwhelmed by favorable interaction with spatially neighboring groups. Protein chromophores such as retinal, the *p*-coumaryl moiety in PYP, the GFP chromophore and phytochromophores are embedded inside the protein structure. Therefore, they are highly sensitive to the stereoelectronic and steric effects induced by the protein backbone and the residues side chains that twist the polyene chain and thus decrease the energy barrier to isomerization. This has been exemplified by the specific light-driven isomerization of retinylidene that occurs at $C^{11}=C^{12}$ in rhodopsin and at $C^{13}=C^{14}$ in bacteriorhodopsin. Constraints in proteins account for the specific isomerization of specific proline residues and some secondary amide bonds, which exist almost exclusively as the *trans*-isomer may isomerize from trans to cis. In most cases, particular strains may explain this preference, as demonstrated in the case of the Gag-polyprotein which possesses three Gly−Pro sequences but is specifically isomerized at $Gly^{89}-Pro^{90}$. Isomerization also induces small to large molecular reshaping especially in proteins and membranes and thus triggers signal transduction. Although the succession of molecular events that cause isomerization may be light, heat, free radicals or protein−protein interaction, all of them result in local shape changes and intra/intermolecular interactions which are relayed by the protein scaffold. Therefore, local cis−trans interconversion plays a central role in the control of protein folding and cell life multiplication and communication as well as in the transduction of exogenous signals.

Amino acyl-proline isomerization is also involved in the induction of diseases such as AIDS, neurodegenerative diseases including Alzheimer's disease and BSE, and certain cancers. Consequently, cyclophilins and FKBPs, originally considered as simple receptors of immunosuppressive molecules, seems to be implicated in a number of biological processes beyond immunosuppression as well as several pathologies, and have been identified as potential targets for novel therapeutics. The catalytic mechanism of isomerization by cyclophilins and FKBPs implies a H-bonding which causes a deconjugation of the Xaa−Pro amide bond. In parvulins, especially human Pin1, a cystein residue of the active site is suspected to attack the amide carbonyl group and to disrupt the amide conjugation. The elucidation of both their specificity and catalytic mechanism gave important information for the design of novel inhibitor devoid of immunosuppressive effects.

Curiously, cis−trans isomerization of biomolecules has not been considered as a crucial step of life for a long time, yet its importance is amply illustrated by the unprecedented research efforts that have been made in the last 20 years to understand, control, and exploit this process. Consequently, all drug design and SAR must take cis−trans isomerization into account for the development of new ligands and inhibitors.

Although diazenes are not usually found in biomolecules, proteins and peptides may be either grafted or cyclized with diarylazo moieties to give light-responsive peptides and proteins. The diarylazo motif readily isomerizes upon light irradiation in a completely reversible way and allows the design of light-tunable peptides and proteins. Impressive advances have been made in the field of isomerizable polymers and oligomers and cis−trans interconversion is presently acknowledged as one of the molecular bases of supramolecular chemistry. There is no doubt that the better understanding of the molecular basis of this phenomenon will open the way to novel applications in molecular engineering, in particular, for the design of more sensitive and cooperative biosensors, very-high-capacity data storage in switchable memories, more efficient molecular motors and shuttles, and innovative applications in bioelectronics. Cis−trans isomerization is also a simple way, originally found by nature, of increasing molecular diversity, and has been proposed as a basis for the generation of librairies by dynamic combinatorial chemistry.

VII. References

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