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Toward a Stable α-Cycloalkyl Amino Acid with a Photoswitchable Cationic Side Chain

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ABSTRACT: The N-alkylated indanylidenepyrrroline (NAIP) Schiff base 3 is an unnatural α-amino acid precursor potentially useful for the preparation of semisynthetic peptides and proteins incorporating charged side chains whose structure can be modulated via Z/E photoisomerization. Here we report that the heteroallylic protons of 3 led to partial loss of ethanol accompanied by formation of the novel heterocyclic system 4 during attempted deprotection. We also show that the same protons catalyze the thermal isomerization of 3, making the light-driven conformational control concept ineffective for times longer than a few hours. These problems are not present in the previously unreported compound 5 where the acidic methyl group is replaced by an H atom. Therefore, 5, rather than 3, constitutes a promising prototype for the design of building blocks capable to modulate the electrostatic potential of a protein in specific locations via light irradiation.

INTRODUCTION

Photochemical switches are bistable compounds that can be interconverted between two different isomers (states) via light irradiation. The most widely used photoswitch is azobenzene that has been employed to control different biomolecular functions via a –N=N– double-bond photoisomerization interconverting its Z and E states. Owing to the significant change in molecular length (ca. 3.5 Å between the two p-carbons) but limited change in dipole moment (ca. 3 D) between its Z- and E-isomers, azobenzene is considered a mechanical rather than electrostatic switch. Literature examples of electrostatic photoswitches are compounds of the spiropyran-type that can be changed between a neutral state and a zwitterionic state via a photochemical ring-opening reaction. Such property has been employed to reversibly modulate α-helix–random coil conformational transitions and the activity of enzymes and channel proteins to achieve novel sensors.

N-Alkylated indanylidenepyrrroline Schiff bases (NAIPs) are novel biomimetic photochemical switches that replicate the efficient –C=C– double-bond photoisomerization occurring in visual pigments (e.g., the dim-light receptor rhodopsin). These molecules, which feature a permanently charged or zwitterionic pyrroline unit (see 1 and 2 in Figure 1), have been proposed to function both as mechanical and/or electrostatic switches. As illustrated in Figure 2A for 2, upon photoisomerization of the exocyclic double bond, the pyrroline unit undergoes a ca. 180° twisting yielding a spatial relocation of the charges and, in turn, a change in the orientation of the electric dipole moment with respect to the indanylidene framework. We have reported that the photolysis of 2 may induce a ca. 30 D dipole moment change which modulates the spectra of nearby fluorophores. Since NAIPs are photochromic compounds, irradiation at different wavelengths can be used to establish the isomer dominating the system photostationary state (see the histogram of Figure 2B) and implement the electrostatic switching concept.

It is apparent that the properties mentioned above provide the basis for achieving novel functional building blocks capable of modulating steric and electrostatic interactions in diverse macromolecular or supramolecular environments (see examples in ref 16). In spite of their interest for the preparation of novel photosensitive materials, 1, 2, and their analogues (see for instance ref 17) cannot be used in a block synthesis due to the lack of suitable functional groups for the coupling to a macromolecular or solid-state framework. For this reason, we have recently reported the preparation of 3, a NAIP switch...
featuring an indanylidene unit bearing protected amino and carboxylic groups at position $C_2'$. The NAIP switch constitutes an $\alpha$-cycloalkyl amino acid building block that could potentially be employed for the preparation of photoresponsive peptides or proteins. Indeed, methods for the incorporation of bulky $\alpha$-alkyl amino acid in synthetic peptides have been established. On the other hand, such incorporation requires, invariably, the deprotection of the amino and carboxyl functions.

In the next section we show that compound 3 is not a suitable building block for the preparation of photoresponsive peptides due to its chemical and thermal instability. With the aim to overcome the above drawbacks, we embarked on a combination of kinetic, synthesis, and structural studies whose main goal is represented by the successful preparation of the unnatural $\alpha$-cycloalkyl amino acid precursor 5.

RESULTS AND DISCUSSION

Nitrilium Cyclization and Formation of a Tetracyclic Byproduct. In the past, we have shown that an indanylidene nucleus can be successfully transformed into a polyconjugated iminium ion featuring a protonated or alkylated Schiff base function. Therefore, we envisioned that the same nitrilium cyclization chemistry could be employed to introduce the amino and carboxyl functional groups on the indanylidene portion of 1 giving access to a new class of unnatural $\alpha$-cycloalkyl amino acids suitable for peptide synthesis. Accordingly, we successfully prepared the NAIP switch 3: a protected $\alpha$-amino acid featuring a quaternary $\alpha$-carbon locked into a rigid indanylidene ring.

As shown in Scheme 1, the ethoxycarbonylated 5-methoxyindan-1-one, subjected to the action of N-bromosuccinimide and Amberlyst-15, gave almost quantitatively the brominated compound 6. The C-2 nitrogen introduction, easily accomplished via bromide displacement with sodium azide, followed by Staudinger reduction and trifluoroacetylation of the resulting primary amine, gave compound 7. The structure of the protected $\alpha$-amino acid featuring a quaternary stereocenter

Figure 1. Structures discussed in the present work. Compound 3 is an $\alpha$-cycloalkyl amino acid derivative of 1, and 4 is an undesirable tetracyclic synthesis byproduct obtained during the synthesis of 3. Compound 5 represents the main synthesis target.

Figure 2. Zwitterionic NAIP switch. (A) Schematic representation of an electrostatic switch based on the $Z/E$ isomerization of 2. The absorption maxima of the $E$- and $Z$-isomers in methanol solution are given in parentheses. The 180° rotation of the pyrrolinium unit will invert the dipole moment vector with respect to the indanylidene unit. The angle between the simulated dipole moment vectors of the $E$- and $Z$-isomers is from ref 14. The computed dipole moment values are 15.7 and 14.8 D, respectively. (B) Observed $E/Z$ photostationary state ratio of 2 as a function of the irradiation wavelength.
was confirmed by RX crystallography (see the Supporting Information).

The subsequent steps were devoted to the installation of the homoallyl acetamide group on the indan nucleus by exploiting the previously reported homoallyl rearrangement of cyclopropylindanol derivatives. Thus, the Grignard adduct exposed to the action of triflic anhydride in the presence of acetonitrile afforded compound. Dehydration of the secondary amide by using trimethylsilyl polyphosphate (PPSE) and the quenching of the resulting nitrilium ion on the internal olefin yielded the expected free base (Scheme 1) as a mixture of geometric isomers (E/Z 1:2).

While we demonstrated that the above protocol leads to the wanted Schiff base precursor, evidence for undesirable instabilities of the IP framework and E/Z ratio started to emerge. First, it was found that protons of the methyl group at δ = 2.27 ppm of underwent a fast exchange in CD3OD indicating a prototropic tautomeric equilibration of the Schiff base function with the corresponding enamine via the methyl group in vinyl position (see first line in Scheme 2). The acidity of the same methyl group was also held responsible for the transformation of the prepared mixture into the tetracyclic compound by simple elution through a column of silica gel. The structure of compound is confirmed by RX crystallography (see the Supporting Information). On the basis of these data, we assume that results from an intramolecular enamine acylation reaction whose mechanism is shown in Scheme 2. Notice that the equilibration between the E/Z steroisomers, as well as the tautomerization to the reacting enamine, are prerequisites for the acylation step.

In spite of the undesirable transformation described above, it was possible to prepare the geometric isomers of by flash chromatographic separation on silica gel conditioned with TEA.

Their treatment with methyl triflate gave the target isomers and 3Z (Scheme 3), whose geometrical identity was ascertained

Scheme 1. Synthesis of Free Base Protected α-Cycloalkyl Amino Acid 10

Scheme 2. Mechanism Assumed for the Formation of Tetracyclic Compound 11

Scheme 3. Methylation of Free Base NAIP Precursor 10
by NOESY experiment. These isomers were stable enough to be well-characterized; however, attempts to perform the hydrolysis of their ethoxycarbonyl groups, as a step toward the corresponding $\alpha$-amino acid, were completely unsuccessful. The only detectable material in the reaction mixture was the tetracyclic compound 4 that similarly to 11 could result from an intramolecular enamine acylation. The structure of compound 4 was demonstrated via RX diffraction analysis (see the Supporting Information). This finding reinforced the idea that, in order to access a photoresponsive amino acid that could be employed in peptide synthesis, it is necessary to access NAIP frameworks not bearing a methyl group (or any other alkyl group) in position C5 on the pyrroline ring. In other words, the behavior of both 3 and 10 indicated that the ethoxycarbonyl group at the C-2 indanylidene carbon suffered from the presence of hydrogens in heteroallylic position with respect to the C−C5≡N bond and that the presence of the methyl substituent on the pyrroline ring has, therefore, to be avoided.

To demonstrate our mechanistic hypothesis for the formation of 4, we prepared compound 12 where the methyl substituent on the pyrroline ring was replaced with a phenyl group. As expected, the absence of C−H bonds capable to conjugate with the C5≡N double bond led to the stable Schiff base. The preparation of 12 and of the final NAIP 13 corresponding to a protected $\alpha$-amino acid was accomplished by following the synthetic strategy used to prepare 10 but using...
benzonitrile instead of acetonitrile in the homoallylic rearrangement step (see Scheme 4).

Thermal Isomerization. As anticipated above, an additional unfavorable feature of compounds 1, 2 and 3 is the thermal isomerization of the exocyclic C1′=C4 double bond. For instance, for 1 we have reported that, in the dark, a 1:1.4 1Z/1E photostationary state, generated via a 440 nm irradiation, evolves to 1:0.6 ratio in 24 h at 0 °C.11 At −40 °C, the same mixture only evolves to a 1.0:1.2 ratio indicating a barrier controlled change toward a thermal equilibrium dominated by 1Z. In order to clarify the origin of this process, we carried out kinetic studies taking 2 as a model system. As previously reported,14 the room-temperature 2Z/2E ratio in the dark is 1:0.01, and it is therefore fully dominated by 2Z. Irradiation with a 440 nm wavelength leads to a room temperature photostationary state with a 1.0:1.2 composition dominated by 2E; however, such a mixture reverts in the dark eventually leading to a reconstitution of the original composition (in more than 24 h).

The kinetic of the thermal re-equilibration process has been investigated by monitoring the 2E concentration as a function of time via spectrophotometric analysis after the composition of the starting photoequilibrated mixture was determined via HPLC analysis and found to be consistent with the data reported in ref 14. The thermal isomerization was spectrophotometrically monitored at regular times via absorption spectroscopy. The spectrophotometric data were then analyzed using the previously determined absorption coefficients14 for the Z and E isomers to determine the increase in the concentration of 2Z. The results of the measurements are reported in Figure 3A, which shows that the inverse of the concentration of the reacting 2E isomer fits the experimental data while the logarithm of the same concentration does not. This indicates a second order rather that the expected (for a net monomolecular isomerization) first-order kinetics. The same procedure was followed at different temperatures in the 276−313 K range to determine the rate constants. Figure 3B shows the corresponding Arrhenius plot whose linear correlation allows to determine a value of 12.1 kcal mol$^{-1}$ for the activation energy.

The observed second order kinetics is hypothetically explained on the basis of the reaction mechanism given in Scheme 5. This suggests that two molecules of 2E are required to achieve the observed rate. Taking into account the results of the previous subsection, which demonstrate the presence of acidic hydrogens in all Schiff bases prepared using the nitrilium cyclization route, we propose the initial formation of a transient dimer. One monomer in the dimer acts as a proton donor while the other as a proton acceptor. The proton is added to the exocyclic C1′=C4 double bond in such a way to produce a stable benzyl cation which is further stabilized by the electron-releasing methoxy substituent. In the cationic intermediate the twisting about the C1′−C4 single bond must be controlled by a small barrier leading to a fast Z/E equilibration. Back proton transfer then leads to reconstitution of the double bond with a 2Z stereochemistry.

Figure 4. Retrosynthetic analysis of compound 15.

Scheme 6. Synthesis of the NAIP 20
An evidence in favor of the above mechanism is based on the observation that the phenyl derivative 13 (see Scheme 4), lacking acidic hydrogens, not only does not undergo any cyclization but has a fully stable thermally E/Z ratio at room temperature.

**The Aldolic Approach to NAIPs.** Because of its chemical and conformational stability, compound 13 would constitute a first possible precursor of a α-cycloalkyl amino acid building block featuring a NAIP side chain. On the other hand, the bulky phenyl group located in an allylic position with respect to the light-responsive exocyclic double bond imposes serious steric impediment to the isomerization motion. For this reason, it is desirable to replace the original methyl group with a hydrogen atom that also results in the removal of hyperconjugating C−H bonds. In other words, we would like to prepare NAIPs featuring an unsubstituted pyrroline ring. The preparation of such compounds via the hitherto used nitrilium strategy appeared to be unachievable as the insertion of an alkyl or aryl (i.e., depending on the employed nitrile) substituent on the carbonyl group of 13 lacking acidic hydrogens, not only does not undergo any cyclization but has a fully stable thermally E/Z ratio at room temperature.

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Compound 15 (see Figure 4), featuring a polycyclic compound system, was selected as the target chemically stable NAIP amino acid precursor. We considered the retrosynthetic analysis of 15 disconnecting the indan and heterocycle moieties. After adjusting the oxidation state as in 14, the N-protected pyrrolidone could be disconnected from the indanone derivative 7. Accordingly, we conceived a synthesis route relying on the reactivity of the electrophile 7 with a nucleophile obtained via α-deprotonation of a suitable γ-lactam.

In order to test the feasibility of the proposed pivotal aldol reaction, we started employing the known indanone derivative 16 and the commercially available N-protected γ-lactam A. We found that the lithium enolate of A adds to the BF₃-activated carbonyl group of 16 giving the corresponding aldol 17, which then yields 18 by spontaneous dehydration in CHCl₃ (see Scheme 6). The structure of compound 18E was confirmed by RX diffraction analysis (see the Supporting Information). The next step led to the one-pot formation of the desired polycyclic aldimine 19, by reduction, cleavage of the N-Boc group and condensation to yield the E isomer. The free base 19E provided the NAIP 20E through N-quaternarization performed with methyl triflate. The geometry of the exocyclic double bond of 20E was inferred by NOESY experiment.

The successful preparation of 19 and 20 prompted the extension of the aldolic approach to the indanone derivative 7 so as to obtain a photomodulable α-cycloalkyl amino acid precursor potentially useful, after deprotection, for peptide synthesis (Scheme 7). We found that 7 features a diminished electrophilic character of the indanone carbonyl, maybe due the presence of the secondary trifluoroacetamide group and/or steric congestion around the reactive center which renders partially incisive the BF₃-activating role. However, apart from a lower yield in the aldolic reaction and the need of promoting dehydration with the use of a Tf₂O−DMAP system, we smoothly reproduced all steps. Interestingly, we observed that, for both 14 and 18, the multistep one-pot process leading to the cyclic aldimine 15 and 19 yielded a single geometric isomer. The pure free base 15Z, treated with methyl triflate, gave the NAIP switch 5Z whose geometry was ascertained by NOESY experiment.

**Spectral and Photochemical Characterization.** The UV spectra of a mixture of 3E/Z, 13E/Z, and 20E and 5Z were recorded, and their photochemical behavior was explored. The solution has been irradiated at room temperature in an NMR Pyrex tube at different wavelength until reach the photostationary state. The E/Z isomer ratio at the photostationary state has been calculated by integration of distinctive ¹H NMR signals. All compounds undergo Z→E and E→Z photoisomerization displaying a photomodulable stationary state. Below we report the photostationary states achieved using three different wavelength in the 350−450 nm range for NAIP 3, 5 and 20. Notice that in all cases (see Tables 1−3) we were able to invert the E/Z isomer ratio which resulted in a dominant E isomer for 3 and 5 and a dominant Z isomer for 20 upon irradiation with low wavelength values. This indicates that the <i>λ</i><sub>max</sub> value of the 3Z, 5Z, and 20E must be

Scheme 7. Synthesis of NAIP 5Z
blue-shifted with respect to the \( \lambda_{\text{max}} \) value of the alternative isomer. (Notice that the backbone and, consequently, the \( \pi \)-system geometry of \( 3Z, 5Z \), and \( 20E \) are consistent. The \( E \) label for the dominating isomer of compound 20 is only due to a change in the substituent priorities). NAIP 13 featuring the bulk phenyl group at C5 has also been irradiated at room temperature in a NMR pyrex tube at different wavelength (\( \lambda = 380, 430, 480 \text{ nm} \)) and the reaction followed by \( ^1 \text{H} \) NMR. After irradiation at 380 nm we see a photostationary state containing only one of two geometric isomers (the one already presents in greater amount in the initially mixture). The irradiation at 480 nm shifts the mixture equilibrium toward the other geometric isomer indicating that the bulk phenyl group does not represents an impediment to the photoisomerization. This result is consistent with the demonstrated ability of crowded alkenes to photoisomerize.\(^{34} \)

As expected, in contrast with compound 3, the \( 13, 20, \) and \( 5 \) E/Z ratio achieved after 24 h irradiation in CDCl\(_3\) is stable when the solution was maintained in the dark and at room temperature.

**Difficulties in Achieving a Deprotected \( \alpha \)-Cycloalkyl Amino Acid with a NAIP Side Chain.** The simultaneous and selective cleavage of the protective groups of the NAIP switch \( SZ \) to obtain the corresponding \( \alpha \)-amino acid was attempted via both hydrolysis or reaction with different nucleophiles. Regrettably, the attempted hydrolysis in acid (HCl, triflic acid) and basic (LiOH, \( \text{K}_2\text{CO}_3, \text{NaOH} \)) conditions at 0 °C and room temperature using various solvents mixture (THF/H\(_2\)O, MeOH/H\(_2\)O, CH\(_3\)CN/H\(_2\)O) highlighted an unusual stability of the protective groups which did not react. During the same treatment, we observed no change in the structure of compound \( SZ \). However, a total decomposition of the starting material was observed when using reflux conditions. The same lack of reactivity was demonstrated with nucleophiles (NH\(_3\) material was observed when using reflux conditions. The same hydrolysis or reaction with different nucleophiles.

Further studies aiming to uncover an effective protocol for removing the protecting groups are presently being carried out in our laboratory.

**CONCLUSIONS AND PERSPECTIVES**

The \( Z/E \) photoisomerization of NAIPs provides the basis for the development of electrostatic light-driven switches to be inserted in specific positions of a synthetic peptide or protein backbone in the form of unnatural residues featuring a quaternary \( \alpha \)-carbon and, consequently, a conformationally rigid (with respect to the local backbone) side chain. The change in the local electrostatic field following the photochemically driven ca. 180° rotation of the pyrrole unit could, in principle, be exploited to control different structural interactions such as salt bridges, protonation states and hydrogen bonds in nearby residues (for a recent study of the effect of the change in electrostatic field on protein functions see ref 35). At the same time, these changes may be followed by looking at the fluorescence or absorption of an internal reporter (e.g., the 3-methylinodole side chain of tryptophan) as previously proposed.\(^{14} \)

Until now, no NAIP-based compounds had been reported featuring both thermally stable states (i.e., constant \( Z/E \) isomer ratios) at room temperature and the presence of functional groups allowing for the incorporation of the switch in a peptide chain. We have demonstrated above that it is indeed possible to achieve such desired structures in the form of protected \( \alpha \)-cycloalkyl amino acids (structure \( S \)). Because of the similarity between the quaternary carbon environment of \( S \) and a local peptide backbone, the data of Table 3 indicate that the control of the \( Z/E \) ratio via irradiation with light of different wavelength should also be effective in a protein environment. Further studies aiming to uncover an effective protocol for removing the protecting groups are presently being carried out in our laboratory.

**EXPERIMENTAL METHODS**

**Synthesis and Structure Analysis.** *Experimental Data.* General: melting points were measured with a hot-stage apparatus. The \( ^1 \text{H} \) and \( ^13 \text{C} \) NMR spectra were recorded with an instrument operating at 200.13 and at 50.33 MHz, respectively, or with an instrument operating at 400.13 and at 100.62 MHz, respectively. Chemical shifts are reported in part per million from internal TMS. Mass spectra were recorder in the positive or negative ion mode with an instrument by using electrospray ionization. All solvents were previously dried according to standard procedures. Analytical TLC was performed on silica gel 60 F254 plates. Flash column chromatography was carried out on silica gel 60 (0.040–0.063 mm). Irradiation was made with a Rayonet apparatus. *Ethyl 2-Bromo-5-methoxy-1-oxo-2,3-dihydro-1H-indene-2-carboxylate (6).* A solution of 5-MeO-1-indanone (5.00 mmol, 810 mg) in diethyl carbonate (20 mL) was added dropwise to a stirred suspension of Na\(_2\)SO\(_4\) and concentrated under reduced pressure. The crude residue (5.00 mmol), N-bromosuccinimide (5.25 mmol, 935 mg), and Amberlyst-15 (3.75 g) in ethyl acetate (50 mL) was stirred at rt for 30 min. After completion of the reaction, as indicated by TLC, the reaction mixture was filtered and washed with ethyl acetate. The combined organic extracts were dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure.

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The crude residue (5.00 mmol) was used to obtain \( 5Z \). The small residue (5.00 mmol) was used to obtain \( 5E \). The small residue (5.00 mmol) was used to obtain \( 5Z \). The small residue (5.00 mmol) was used to obtain \( 5E \). The small residue (5.00 mmol) was used to obtain \( 5Z \). The small residue (5.00 mmol) was used to obtain \( 5E \).

**Table 1. Changes of the Photostationary-State Composition for Compound 3**

<table>
<thead>
<tr>
<th>( \lambda ) (nm)</th>
<th>Z</th>
<th>E</th>
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<td>350</td>
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<tr>
<td>400</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>0.34</td>
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**Table 2. Changes of the Photostationary-State Composition for Compound 20**

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<th>Z</th>
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<td>394</td>
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<tr>
<td>440</td>
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</table>

**Table 3. Changes of the Photostationary-State Composition for Compound 5**

<table>
<thead>
<tr>
<th>( \lambda ) (nm)</th>
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</tr>
<tr>
<td>440</td>
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</table>

\( \delta_c: 1.17 (s, J = 7.2 \text{ Hz}, 3 \text{H}), 3.52 (d, J = 18.4 \text{ Hz}, 1 \text{H}), 3.81 (s, 3 \text{H}), 4.06 (d, J = 18.0 \text{ Hz}, 1 \text{H}), 4.16 (q, J = 7.2 \text{ Hz}, 2 \text{H}), 6.81–6.88 (m, 2 \text{H}), 7.64 (d, J = 8.4 \text{ Hz}, 1 \text{H}). \)
Ethyl 5-Methoxy-1-(5-methyl-2H-pyrrol-4(3H)-ylidene)-2,2-trifluoroacetamido)-2,3-dihydro-1H-indene-2-carboxylate (1). A trimethylsilyl polypephosphat (PSP) solution, prepared by heating at reflux for 1 h a mixture of PBr3 (3.50 mmol, 497 mg) and hexamethyl disilane (4.91 mmol, 1.04 mL) in CCl4 (5 mL), was added to 9 (0.35 mmol, 150 mg) at rt. The reaction mixture was heated at reflux for 5 h, cooled to room temperature, diluted with CH2Cl2 and quenched with NaOH 2 N. The aqueous layer was extracted with CH2Cl2 over Na2SO4 and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel conditioned with TEA and ethyl acetate as eluent to give 90 mg (63%) of product 10 as yellow oil. 1H NMR (CDCl3, 400 MHz) δH: 1.06 (t, J = 7.2 Hz, 3H, E isomer), 1.14 (t, J = 7.2 Hz, 3H, Z isomer), 2.13 (s, 3H, Z isomer), 2.27 (s, 3H, E isomer), 2.51–2.64 (m, 2H, mix E/Z), 3.00–3.06 (m, 2H, 4H, mix E/Z), 3.36–3.66 (m, 4H, mix E/Z), 3.72–4.08 (m, 4H, mix E/Z), 3.78 (s, 6H, mix Z/E), 4.13 (q, J = 7.2 Hz, 2H, E isomer), 4.21 (q, J = 7.2 Hz, 2H, Z isomer), 6.73–6.84 (m, 4H, E/Z mix), 7.31 (d, J = 8.4 Hz, 1H, E isomer), 7.50 (d, J = 8.4 Hz, 1H, Z isomer), 7.75 (s, 1H, E isomer), 7.92 (s, 1H, Z isomer). ESI-MS, m/z: [M + H] + = 411, [M + Na +] = 433, [M − H] − = 409. Anal. Calc. for C20H23F3N3O7S: C, 56.07; H, 5.41; N, 6.54.

(E/Z)-Ethyl 5-Methoxy-1-(5-methyl-2H-pyrrol-4(3H)-ylidene)-2,2-trifluoroacetamido)-2,3-dihydro-1H-indene-2-carboxylate (11). Compound 11 was recovered quantitatively as a yellow-orange oil. 1H NMR (CDCl3, 400 MHz) (mix 1:1 Z/E) δH: 1.11 (t, J = 7.2 Hz, 3H, Z), 1.11 (t, J = 7.5 Hz, 3H, E), 2.56 (s, 3H, E), 2.72 (s, 3H, Z), 3.13 (d, J = 16.6 Hz, 1H, E), 3.18–3.30 (m, 4H, mix E/Z), 3.40 (d, J = 16.6 Hz, 1H, Z), 3.52 (s, 3H, E), 3.54 (s, 3H, Z), 3.77 (d, J = 16.6 Hz, 1H, E), 3.90 (d, J = 16.6 Hz, 1H, Z), 4.10–4.30 (m, 6H, mix Z/E), 4.63–7.00 (m, 4H, mix E/Z), 7.51 (d, J = 9.7 Hz, 1H, Z), 7.54 (d, J = 9.2 Hz, 1H, E), 8.52 (s, 1H, E), 8.90 (s, 1H, Z). 13C NMR (CDCl3, 100 MHz) δC: 13.51 (Z/E), 17.30 (E), 17.43 (Z), 28.33 (H), 32.03 (Z), 37.63 (E), 37.90 (Z), 43.18 (E), 43.58 (Z), 55.70 (Z/E), 57.68 (Z), 58.12 (E), 63.46 (E), 63.57 (Z), 69.36 (Z), 71.67 (E), 70.19 (Z), 109.18 (Z), 109.44 (E), 114.43 (E), 114.84 (Z), 115.41 (q, J = 287 Hz, Z/E), 127.94 (E), 128.12 (Z), 129.28 (E), 129.63 (Z), 130.92 (E), 131.31 (Z), 149.74 (Z), 150.36 (E), 151.19 (Z), 151.65 (E), 156.60 (q, J = 38 Hz, Z), 156.70 (q, J = 38 Hz, E), 163.57 (Z), 163.45 (E), 166.57 (E), 166.72 (E). ESI-MS, m/z: [M + Na]+ = 425. Anal. Calc. for C22H24F6N2O7S: C, 45.99; H, 4.21; N, 4.88. Found: C, 46.14; H, 4.20; N, 4.89.

2,2-Trimethoxy-N-(8-methoxy-5-oxo-1,2,3,5,5a,6-hexahydroinden[l,2-]ethyl-5a-y-acetamide (12). Compound 12 was obtained after chromatography on SiO2 or treatement with LiOH of mixture of 12E/Z. The residue was purified by flash column chromatography on silica gel (ethyl acetate) to give 95 mg (63%) of product 12 as yellow solid (mp >200 °C). Anal. Calc. for C22H24F6N2O7S: C, 45.99; H, 4.21; N, 4.88. Found: C, 46.14; H, 4.20; N, 4.89.

2,2-Trimethoxy-N-(8-methoxy-5-oxo-1,2,3,5,5a,6-hexahydroinden[l,2-]ethyl-5a-y-acetamide (12). Compound 12 was obtained after chromatography on SiO2 or treatement with LiOH of mixture of 12E/Z. The residue was purified by flash column chromatography on silica gel (ethyl acetate) to give 95 mg (63%) of product 12 as yellow solid (mp >200 °C). Anal. Calc. for C22H24F6N2O7S: C, 45.99; H, 4.21; N, 4.88. Found: C, 46.14; H, 4.20; N, 4.89.
tert-Butyl 3-(5-Methoxy-2,2-dimethyl-2,3-dihydro-1H-inden-1-ylidene)-2-oxopyrrolidine-1-carboxylate (18E/Z). A 1 M solution of lithium hexamethyldisilazide (LiHMDS) in anhydrous THF (1.80 mL, 1.80 mmol) was added at −78 °C under nitrogen atmosphere to N-Boc-2-pyrroldione A (1.50 mmol, 278 mg) dissolved in anhydrous THF (5 mL). After 1 h, a solution of the compound 16 (1.65 mmol, 314 mg) and BF₃·EtO (1.65 mmol, 206 μL) in anhydrous THF (8 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h, NH₄Cl (s.s.) was added, and the crude was extracted in EtO. The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure and the residue purified by flash column chromatography on silica gel (3:7, ethyl acetate/petroleum ether). The obtained compound 17 poured in CHCl₃ underwent spontaneous dehydration (checked by ESI-MS) to afford 18 (410 mg, 76%) as a white solid (1:1 mixture of E/Z isomers).

(E)-tert-Butyl 3-(5-Methoxy-2,2-dimethyl-2,3-dihydro-1H-inden-1-ylidene)-2-oxopyrrolidine-1-carboxylate (18E). White solid. Mp: 170–173 °C. ¹H NMR (CDCl₃, 400 MHz) δ: 1.24 (s, 6H), 1.54 (s, 9H), 2.94 (s, 2H), 3.00 (t, J = 7.1 Hz, 2H), 3.76 (t, J = 7.1 Hz, 2H), 3.82 (s, 3H), 6.78–6.80 (m, 2H), 7.42 (d, J = 8.4 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ: 26.74, 27.39, 28.16, 29.66, 43.36, 50.98, 55.38, 82.11, 109.47, 113.47, 119.98, 128.06, 133.53, 143.04, 150.49, 151.05, 161.47, 167.02. ESI-MS, m/z: [M + Na⁺] = 380, [2M + Na⁺] = 737. Anal. Calcd for C₂₁H₂₇NO₄: C, 70.50; H, 7.62; N, 3.94. Found: C, 70.79; H, 7.58; N, 3.94.

(E/Z)-(2-(Ethyloxy)carbonyl)-2-(2,2,2-trifluoroacetamido)-2,3-dihydro-1H-inden-1-ylidene)-methyl-5-phenyl-3,4-dihydro-2H-pyrilum (13E/Z). Methyl trifluoromethanesulfonate (0.19 mmol, 22 μL) was added to a solution of 12E/Z (0.19 mmol, 100 mg) in anhydrous benzene (5 mL). The product of reaction was recovered quantitatively as yellow oil. ¹H NMR (CDCl₃, 400 MHz) (mix Z/E) δ: 1.07 (t, J = 7.0 Hz, 3H, major), 1.08 (t, J = 7.1 Hz, 3H, minor), 2.97–3.08, 3.12–3.23 (m, 2H, major), 3.00–3.38 (m, 2H, minor), 3.15 (d, J = 17.0 Hz, 1H, minor), 3.23 (s, 3H, major), 3.43 (d, J = 16.3 Hz, 1H, major), 3.54 (d, J = 17.0 Hz, 1H, minor), 3.61 (s, 3H, major), 3.70 (d, J = 16.3 Hz, 1H, major), 3.70 (s, 3H, major), 3.74 (s, 3H, minor), 3.98–4.25 (m, 3H, major), 4.11–4.30 (m, 3H, minor), 4.45–4.60 (m, 1H, minor), 4.72–4.80 (m, 1H, major), 6.12 (dd, J₁ = 8.9 Hz, J₂ = 2.4 Hz, 1H, major), 6.41 (dd, J₁ = 8.9 Hz, J₂ = 2.4 Hz, 1H, minor), 6.60 (d, J = 2.4 Hz, 1H, major) 6.76 (d, J = 1.4 Hz, 1H, minor), 6.95–7.03 (m, 3H, minor), 7.40–7.70 (m, 5H, major), 7.63–7.81 (m, 5H, minor), 8.21 (bs, 1H, minor and major). ¹³C NMR (CDCl₃, 100 MHz) δ: 13.41 (minor and major) and 28.97 (minor), 33.25 (minor), 39.01 (minor and major), 42.47 (major), 45.65 (minor), 55.49 (major), 55.78 (minor), 58.91 (minor), 59.24 (major), 63.71 (minor), 64.35 (major), 67.39 (minor), 70.92 (major), 108.34 (major), 109.35 (minor), 113.92 (major), 115.32 (minor), 115.41 (q, J = 288 Hz, minor and major), 125.79 (minor and major), 126.72 (major), 126.82 (minor), 128.72, 129.59, 130.49, 130.66, 131.03 (minor and major), 133.15 (minor), 133.30 (major), 148.21 (minor), 149.56 (major), 155.83 (q, J = 37 Hz, minor and major), 156.07 (minor), 157.33 (major), 163.21 (major), 164.13 (minor), 168.48 (minor and major), 170.09 (minor and major). ESI-MS, m/z: [M⁺] = 487. Anal. Calcd for C₁₁H₇F₅NO₂·S·C: 50.94; H, 4.12; N, 4.40. Found: C, 50.76; H, 4.10; N, 4.41.

tert-Butyl 3-(5-Methoxy-2,2-dimethyl-2,3-dihydro-1H-inden-1-ylidene)-2-oxopyrrolidine-1-carboxylate (18E/Z). A 1 M solution of lithium hexamethyldisilazide (LiHMDS) in anhydrous THF (1.80 mL, 1.80 mmol) was added at −78 °C under nitrogen atmosphere to N-Boc-2-pyrroldione A (1.50 mmol, 278 mg) dissolved in anhydrous THF (5 mL). After 1 h, a solution of the compound 16 (1.65 mmol, 314 mg) and BF₃·EtO (1.65 mmol, 206 μL) in anhydrous THF (8 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h, NH₄Cl (s.s.) was added, and the crude was extracted in EtO. The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure and the residue purified by flash column chromatography on silica gel (3:7, ethyl acetate/petroleum ether). The obtained compound 17 poured in CHCl₃ underwent spontaneous dehydration (checked by ESI-MS) to afford 18 (410 mg, 76%) as a white solid (1:1 mixture of E/Z isomers).
tert-Butyl 3-(2-(Ethoxycarbonyl)-5-methoxy-2,2,2-trifluoroacetamido)-2,3-dihydro-1H-inden-1-ylidene)-1-oxopyrrolidine-1-carboxylate (14Z). A 1 M solution of lithium hexamethyldisilazane (LHMDS) in anhydrous THF (1.80 mmol, 1.80 mL) was added at −78 °C under nitrogen atmosphere to N-Boc-2-pyrrolidinone (1.50 mmol, 278 mg) dissolved in anhydrous THF (5 mL). After 1 h, cold compound 7 (1.65 mmol, 569 mg) and BF₃·Et₂O (1.65 mmol, 206 µL) were added, and stirring was continued for 3 h at the same temperature. NH₄Cl (s.s.) was added, and the mixture was extracted with EtO. The combined organic layers were dried over Na₂SO₄ and concentrated under vacuum gave a residue which was purified by flash column chromatography on silica gel (at the first dichloromethane elution). Then the residue was treated with TEA to give 213 mg (28% over two steps) of compound 14Z as white solid. Mp: 157−160 °C. H NMR (CDCl₃, 400 MHz) δH: 1.22 (t, J = 7.2 Hz, 3H), 1.51 (s, 9H), 3.03−3.07 (m, 2H), 3.41 (J = 16.0 Hz, 1H), 3.75−3.80 (m, 3H), 3.83 (s, 3H), 4.21−4.26 (m, 2H), 6.82 (s, 1H), 6.88 (dd, J1 = 8.8 Hz, J2 = 2.0 Hz, 1H), 7.46 (d, J = 8.8 Hz, 1H), 8.29 (s, 1H). 13C NMR (CDCl₃, 100 MHz) δC: 13.92, 24.19, 28.05, 43.51, 55,50, 62.48, 66.43, 82.85, 108.95, 114.86, 115.93 (q, J1 = 8.8 Hz, 1H), 120.98, 126.80, 132.44, 147.48, 149.03, 150.20, 155.47 (q, J2 = 37 Hz, 1H), 158.27 (q, J3 = 286 Hz), 125.50, 131.81, 134.73, 162.33, 167.44, 170.22. ESI-MS, m/z: [M + Na+] = 535. Anal. Calcld for C₂₄H₂₇F₃N₂O₇:C , H, N, 4.99.

**Kinetic Measurements.** Absorption spectra were recorded on an HP 8453 diode-array spectrophotometer. The sample irradiation was carried out using a Xenon lamp equipped with a monochromator for the selection of the excitation wavelength (380 or 440 nm). The thermal reaction was followed keeping the sample in dark conditions in the range temperature range 276−313 K. An Oxford Instruments (Abingdon, UK) cryostat was used for the temperature control. The chromatographic analysis has been performed by use of a Waters apparatus equipped with analytical Symmetry C18 (4.6 × 250 mm; 5 µm) or Prontosil 200-3 C30 (4.6 × 250 mm; 3 µm) columns and a UV Vis diode array detector.


