A Proline Mimetic for the Design of New Stable Secondary Structures: Solvent-Dependent Amide Bond Isomerization of (S)-Indoline-2-carboxylic Acid Derivatives

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ABSTRACT: A thorough experimental and computational study on the conformational properties of (S)-indoline-2-carboxylic acid derivatives has been conducted. Methyl (S)-1-acetylindoline-2-carboxylate, both a mimetic of proline and phenylalanine, shows a remarkable tendency toward the cis amide isomer when dissolved in polar solvents. This behavior is opposite to the general preference of proline for the trans isomer, making indoline-2-carboxylic acid a good candidate for the design of different secondary structures and new materials.

INTRODUCTION

Conformational diversity is Nature’s way of disclosing important properties; therefore, a deeper understanding of the intrinsic conformational order of natural and unnatural amino acids is essential for the rational design of biomimetic compounds. Proline and phenylalanine are two important amino acids often involved, respectively, in protein conformational switching and aggregation phenomena. For proline and its analogues, the pioneering work of Wennemers’ group showed that a deep knowledge of the conformational properties of model peptides in different environments, especially for the cis/trans isomerization equilibrium, can lead to a number of applications in catalysis or in the synthesis of collagene-like materials, as a tool for polyproline II crystal structure resolution or in the use of oligoprolines in supramolecular assembled materials. Regarding phenylalanine, since the groundbreaking studies by Gazit about the aggregation properties of amyloid peptides, so many developments have taken place in nanobiotechnology and biomaterials that it seems appropriate to refer to more extensive reviews. The deeper comprehension and combination of the many factors that control the cis/trans isomerization and aggregation, like n→π* interactions, π–π stacking, or steric factors, have led to the study of many peptide analogues. For example, Tomasin’s group developed oxazolidinone-containing peptides, showing that the Phe-D-Oxd moiety is a privileged scaffold for controlling the formation of supramolecular materials, thanks to the preferential trans conformation of generic Xaa-D-Oxd bonds and the presence of phenylalanine. Moreover, they recently developed new soft materials based on l-DOPA, a psychoactive analogue of phenylalanine, and in collaboration with them we could spot through chiroptical techniques the elusive π-helix motif attained by oligomers containing pyroglutamic acid, a proline mimetic. (S)-Indoline-2-carboxylic acid ((2S)-Ind) is an interesting case, as it is both a mimetic of l-proline and l-phenylalanine. (2S)-Ind can also be seen as a phenylalanine with the side chain...
conformationally locked in a fixed orientation. Both these features make (2S)-Ind a good candidate for the investigation of its conformational properties. Contrary to the many studied proline analogues with substituents at position C4 and C5,39−43 pseudoprolines,44 or other bicyclic proline derivatives,45,46 (2S)-Ind cannot undergo stabilization or destabilization by ring puckering, as the 5-membered ring is intrinsically quasiplanar.

The biological importance of kinetic and thermodynamic quantities of prolinamide conformational interconversions is well demonstrated, for example, by the extensive work of Fischer's group.47−51 Cis/trans proline isomerization has indeed often been found as the rate-limiting step in the protein-folding processes. Regarding specifically (2S)-Ind, an earlier explorative computational study of Torras et al.52 on the N-acetyl-N′-methylamide derivative (Ac-(2S)-Ind-NHMe) investigated the structural role of the aromatic ring, highlighting that its presence further reduces the intrinsically low conformational flexibility of proline, giving higher preference for the cis state of the peptide bond involving the pyrrolidine nitrogen. However, the presence of a terminal secondary amide might have a strong influence on the conformation of the molecule, given the possibility of hydrogen bond formation. Consequently, in the conformational analysis of proline and its analogues, acetylated methyl esters or N-acetyl-N′,N′-dimethylamides are often preferred.53 For this reason we decided to investigate Ac-(2S)-Ind-OMe (1) and the dimer Ac-(2S)-Ind-(2S)-Ind-OMe (2) (Scheme 1) to explore both the methyl ester derivative and the amide bond in the dipeptide. Referring to Scheme 1, it was anticipated that in Ac-(2S)-Ind-OMe angle ϕ is fixed by the pyrrolidine ring; thus, the main degrees of conformational freedom of 1 are represented by the torsional angles ω and ψ. The amide angle ω is expected to assume values around 0° and 180° leading to cis and trans isomers, respectively, distinguished by NMR. Conversely, the rotational barrier for angle ψ is too low to be recognized by NMR, but it is still important for the overall conformational equilibrium.

### RESULTS AND DISCUSSION

We were pleased to see that the 1H NMR in CDCl3 of 1 showed well-separated signals, and through a complete bidimensional analysis it was possible to unambiguously assign each signal of the cis and trans isomers; the procedure is detailed in the Experimental Section. The capability to distinguish each signal is useful to study the physicochemical parameters of the equilibrium in solution. It was possible to evaluate the populations of cis and trans isomers, and consequently, the value of the cis-to-trans equilibrium constant K_{trans/cis} at 25 °C, through the ratio between the integrated areas of the two peaks corresponding to the Hα of the two isomers in the 1H NMR spectrum, found between 4.1 and 5.2 ppm (Figure 1 and Table 1).

![Scheme 1](https://example.com/scheme1.png)

Scheme 1. (Top) Cis−Trans Isomerization Equilibrium of the Amidic Bond in Ac-(2S)-Ind-OMe (1) and Major Torsional angles. (Bottom) Structure of Ac-(2S)-Ind-(2S)-Ind-OMe (2) Shown as the Major Cis−Cis Conformer, Relative to the Two ω Angles

![Figure 1](https://example.com/figure1.png)

Figure 1. 1H NMR in the Hα region of a 0.1 M solution of 1 in different solvents at 25 °C.

![Table 1](https://example.com/table1.png)

Table 1. K_{trans/cis} of Ac-(2S)-Ind-OMe (1) Obtained from 1H NMR Spectroscopy of 0.1M Solutions in Different Deutered Solvents at 298 K

| solvent   | K_{trans/cis} | δ (ppm) trans | δ (ppm) cis | ε_r |
|-----------|---------------|---------------|-------------|-----|
| CD3CN     | 0.87          | 5.17          | 4.91        | 8.9 |
| CD3Cl     | 0.63          | 5.10          | 4.93        | 37.5|
| CD2Cl2    | 0.47          | 5.15          | 5.22        | 32.7|
| DMSO-d6   | 0.31          | 5.04          | 5.04        | 46.7|

*Chemical shifts values of the Hα of the trans and cis conformer.**Solvent dielectric constant. *For CD3CN, K_{trans/cis} was calculated through the ratio between the integrated areas of the CH3 acetamide signals of the two isomers.

1). The preferred species in a 0.1 M CDCl3 solution of 1 is the cis conformer, with a corresponding K_{trans/cis} = 0.87. The conformational preference seems to be independent of the concentration, as the trans/cis ratio did not change analyzing 0.04 and 0.8 M solutions of 1 in CDCl3. More interestingly, we observed a marked influence of the solvent polarity on the equilibrium (Figure 1 and Table 1). More polar solvents favor the prevalence of the cis conformer. Another interesting phenomenon, also evident in Figure 1, is that the signal of the Hα in the cis conformer moves significantly to lower frequencies depending on the polarity of the solvent, while the Hα signal corresponding to the trans conformer is less affected.

As it can be seen in Table 1, by testing several solvents, the trans/cis ratio spans from 0.72 in benzene-d6 to 0.31 in DMSO-d6, depending on the dielectric constant (ε_r) of the solvent. This observation can be rationalized by assuming a larger dipole moment for the cis isomer than for the trans one, which results in
a stabilization of the cis conformation by more polar solvents.54 The experimental $K_{cis/trans}$ values were fitted against an empirical correlation of the type defined by eq SI4 (see the Supporting Information, SI), which describes a solvent-dependent conformational equilibrium where the solvent is seen as a uniform dielectric with relative permittivity $\varepsilon_r$.55 A very good fit was observed for all solvents, except benzene, demonstrating that the equilibrium is indeed controlled by the solvent reaction field, while benzene is possibly capable of specific interactions with the solute aromatic ring.

Comparing the measured $K_{cis/trans}$ with those reported for Ac-Pro-OMe, we can see that the nature of the solvent and the presence of the aromatic ring drastically affects the conformational equilibrium around the amide bond (Table 2).

Table 2. Comparison of the $K_{cis/trans}$ of Ac-(2S)-Ind-OMe (1) and Ac-Pro-OMe in Different Solvents at 298 K

| Xaa         | CDCl₃ | DMSO-d₆ | C₆D₆ |
|-------------|-------|---------|------|
| Ac-Pro-OMe  | 3.6   | 3.8     | −4.9 |
| Ac-(2S)-Ind-OMe | 0.87  | 0.31    | 0.72 |

Importantly, contrary to most proline-mimetic compounds reported in the literature, 1 shows in solution an excess of the cis-amide isomer which increases with the solvent dielectric constant.

To confirm the importance of solvent polarity in the stabilization of the cis conformation of 1, we performed a series of titrations. We observed that adding sequential amounts of D₂O to a DMSO-d₆ solution of 1 did not affect at all the preference for the cis isomer, while adding portions of DMSO-d₆ to a solution of 1 dissolved in benzene-d₆ proportionally increased the population of the cis isomer. These experiments show that the conformational preference is dominated by the solvent polarity, as expected, rather than by solute–solvent hydrogen bonding.

With the aim of evaluating the thermodynamic parameters associated with the isomerization equilibrium in DMSO-d₆, we performed variable-temperature NMR experiments to be analyzed by the Van’t Hoff equation.53 As can be seen in Figure 2, the increase of temperature led to a coalescence of the cis/trans Hα signals around 5.2 ppm already at 60 °C, indicating a fast exchange between the two populations. However, the characteristic signal of the aromatic proton HAr₄ deshielded by the proximate acetamide group when the amido bond is in the cis conformation, remains anchored around 8.2 ppm, allowing the determination of $K_{cis}$ at different temperatures. By plotting ln $K_{cis}$ vs 1/T, $\Delta H^\circ$ and $\Delta S^\circ$ can be obtained (see the SI). The estimated values are $\Delta H^\circ = +3.10$ kcal/mol and $\Delta S^\circ = +8.2$ cal/molK for the cis-to-trans equilibrium (in DMSO). Thus, an enthalpy/entropy compensation effect seems to be at play for the considered conformational equilibrium.

Different methods have been proposed to measure the rate constants and the activation energy for the cis/trans isomerization.54,59 However, considering the unequal population between the two exchanging species, as well as similar spin–lattice relaxation time $T_1$ of the two species ($T_{1cis} = 2.39$ s; $T_{1trans} = 2.46$ s) we found the approach outlined by Perrin and Dwyer to be convenient.60 2D exchange spectroscopy (EXSY), which provides a map of the exchanging species, was used. This approach allows for the calculation of the rate constants for a chemical exchange process by measuring the cross-peak to diagonal peak intensity ratio. The ratio of the rate constants, obtained with the optimal mixing time found ($t_m = 0.03$ s), was $K_{cis/trans} = k_{cIS}/k_{tIS} = 0.31$. This value is fully consistent with that obtained from the Hα peak integral ratio at 25 °C (see above).

From the rate constants, we can also calculate the free energy of activation for the cis to trans and the trans to cis isomerization according to the absolute rate theory. The estimated values were $\Delta G^\circ_{cis} = 16.4$ kcal/mol and $\Delta G^\circ_{trans} = 15.7$ kcal/mol, which lie in the lower range of observed cis/trans barriers for N,N-disubstituted acetamides.51 NMR spectra of 1 acquired in CDCl₃ to the lower temperature of −60 °C did not influence at all the conformational equilibrium.

A well-recognized factor, which favors the trans conformer over the cis conformer in proline analogues, is the n→π* interaction between adjacent carbonyl groups.20,23 The nature of the terminal group can affect the equilibrium, as esters are more electrophilic than amides and act as better acceptors for the n→π* interaction.55 In our case, given the cis preference of 1, we speculated that other factors may overcome a possible n→π* interaction. Therefore, we expected that the presence of a tertiary amide as the acceptor group would not dramatically change the conformational preference. For that reason, we decided to synthesize compound 2 as model to confirm the preference of the cis conformer in an (S)-indoline-2-carboxylic acid derivative involved in a peptide bond (Scheme 1). Compound 2 is short enough to be a convenient model to investigate the impact of a tertiary amide in the backbone, avoiding the occurrence of cooperative effects which would show up in larger oligomers.

The NMR analysis in DMSO-d₆ of the dimer Ac-(2S)-Ind-(2S)-Ind-OMe (2) demonstrated for both amido bonds a strong preference for the cis conformation, confirming this tendency also when a tertiary amide moiety is present as terminal substituent. In Figure 3, it is possible to appreciate the influence of solvent polarity in CDCl₃ and DMSO-d₆, while a complete attribution of NMR signals of the predominant cis–cis conformer of 2 in DMSO-d₆ is reported in the Experimental Section. Although compound 2 is too short to fold into a stable secondary structure, we expect the same preference for the cis geometry of the amide junction to be retained in longer oligomers of indoline-2-carboxylic acid, which will be the subject of future studies.

To investigate the cis/trans isomerization from a different perspective, a computational study was performed to determine the relative stability of the two isomers of 1 in various solvents.
Four different conformations (called C1, C2 and T1, T2, respectively) were considered, characterized by values of the ω dihedral of approximately 0 and 180 deg (C and T isomers), and of the ψ dihedral around +160 and ~20 degrees (1 and 2 conformers), respectively. The optimized geometries of the C1, C2 and T1, T2 conformers in DMSO are reported in Figure 4.

For each starting geometry, a full optimization was performed, followed by a frequency calculation to characterize the optimized geometries as minima and compute, for each conformer, its Gibbs free energy. The computed Gibbs free energies of the four conformers (i.e., with ω1, ω2, ψ1, and ψ2 (Scheme 1) are allowed to vary, yielding 16 potential conformers of which 15 corresponded to stable minima. In this case, too, we found the cis–cis conformers (i.e., with ω1 and ω2 ≈ 0°) to be the most stable ones, in good agreement with the experimental data.

Table 3. Computed Relative Free Energies of the Four Conformers of Ac-(2S)-Ind-OMe, in kcal/mol

| solvent  | C1  | C2  | T1  | T2  | K<sub>trans/cis</sub> |
|----------|-----|-----|-----|-----|----------------------|
| benzene  | 0.0 | 0.1 | 0.4 | 0.4 | 0.55                 |
| CHCl₃    | 0.0 | 0.0 | 0.4 | 0.3 | 0.52                 |
| CH₂Cl₂   | 0.1 | 0.0 | 0.5 | 0.4 | 0.53                 |
| CH₃OH    | 0.3 | 0.0 | 0.6 | 0.5 | 0.48                 |
| CH₃CN    | 0.3 | 0.0 | 0.6 | 0.5 | 0.48                 |
| DMSO     | 0.3 | 0.0 | 0.6 | 0.5 | 0.48                 |

For the sake of completeness, we carried out geometry optimizations in various solvents also on compound 2 (see the SI). In the dimer, angles ω1, ω2, ψ1, and ψ2 (Scheme 1) are allowed to vary, yielding 16 potential conformers of which 15 corresponded to stable minima. In this case, too, we found the cis–cis conformers to be the most stable ones, in good agreement with the experimental data. In the attempt to rationalize the observed behavior of (S)-indoline-2-carboxylic acid derivatives, we estimated all of the possible interactions that could influence their conformational preferences by using natural bond orbital (NBO) analysis. We first verified the extent of the n→π* interaction for compound 1, in comparison with Ac-Pro-OMe which prefers the amide trans conformation. All the typical geometrical indicators of the n→π* interaction (C==O···π* distance, C=O−C Bürgi–Dunitz trajectory, pyramidalization of ester C=O) as well as the overlap between the n and π* COO orbitals, were similar for the two compounds in their trans conformations (see the SI). This finding suggested that the different conformational behavior is not related to a different extent of the n→π* interaction. In fact, the main geometrical difference between Ac-(2S)-Ind-OMe (1) and Ac-Pro-OMe is not in the reciprocal arrangement between the amide and ester groups but in the fact that in the former compound the N-acetyl moiety lies in the same plane as the phenyl ring, as shown in Figure 5. A possible reason for the higher stability of the cis isomer of Ac-(2S)-Ind-OMe (1) is a subtle combination between steric and electrostatic effects involving the N-acetyl moiety. In the trans isomer of Ac-(2S)-Ind-OMe, a steric repulsion between CH₃ and H₄Ar is detectable, which is relieved both in the cis isomer (between CH₃ and CH₂) and in Ac-Pro-OMe (between CH₃ and CH₂). Conversely, the cis isomer allows for a stabilizing attraction between C==O and H₄Ar, although this latter effect is less important than anticipated. Atom charges calculated with natural population analysis reveal a slight increase of H₄Ar charge (by 10% in CHCl₃) passing from the trans to the cis isomer of Ac-(2S)-Ind-OMe, which might be underestimated by calculations. From the computed dipole moments in various solvents of the cis and trans isomers, calculated as the Boltzmann average of the dipoles of the two conformers for each isomer, it appears that there is no definite prevalence of either isomer as the more polar one. This is due to the combination of the local dipoles allied with the amide and ester moieties, which in the four isomers arrange in different ways as depicted in Figure 4, yielding an overall dipole with variable direction and intensity. It is clear that, being the populations of the four conformers all very similar, and the respective dipole moments very variable, subtle changes in the populations may affect the average dipole moment substantially. This may be the reason why polarizable continuum solvent models seem not fully adequate to quantitatively describe the solvent-dependent conformational equilibrium of Ac-(2S)-Ind-OMe (1).
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comparison with the lowest-energy conformer of Ac-Pro-OMe.
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indicative of a C–H-aromat.O==C interaction in the cis isomer. At
the same time, however, the atom charge on C==O oxygen is the
same (within 0.5% in CHCl3) for the two isomers. Moreover,
atom charges are only slightly affected when switching from
CHCl3 to DMSO; for example, the C==O oxygen negative
charge increases by ~2% (absolute value) in the more polar
solvent, while the H atomic charge increases by ~1%. So, although
the calculations hint at a possible C==H:O==C interaction, we
must conclude that they do not highlight a clear solvent
dependence for this interaction.

CONCLUSIONS
The collected experimental and computational evidence unambiguously demonstrated that (S)-indole-2-carboxylic acid
derivatives, when involved in amide bonds, have a strong
preference for the cis conformation, especially in polar solvents.
It is interesting to note how these results confirm the findings of
Siebler et al.15 on the importance of dipole moment for proline
derivatives isomerization, although in our case the effect of the
polarity of the solvent is much more pronounced. Besides the
influence of the dipole moment on the conformational
equilibrium, a combination of steric and electrostatic
interactions may contribute to stabilizing the amide cis
genometry. Considering that small differences at a molecular
level transposes to big changes of macroscopic properties, these
findings could lead to the synthesis of longer oligomers forming
stable polypeptide I like structures, or the use in peptide
sequences of (S)-indole-2-carboxylic acid as a preferential
scaffold to form β-hairpins, or as a conformational constrain for
the design of new organo-catalysts.

EXPERIMENTAL SECTION
General Information and Materials. All nonaqueous reactions
were run in oven-dried glassware under a positive pressure of argon,
with exclusion of moisture from reagents and glassware, transferring
solvents and liquid reagents with hypodermic syringes. The glassware
has been dried with a heating gun under vacuum and allowed to cool
under argon. Anhydrous solvents and liquid reagents were obtained
using standard drying techniques. Solid reagents were of commercially
available grade, used without further purification and when necessary,
stored in a controlled atmosphere and/or at ~20 °C. (S)-Indoline-2-
carboxylic acid was purchased from abcr GmbH. Reactions were
monitored by thin-layer chromatography using Merck silica gel 60 F254
plates. Visualization of the developed chromatogram was performed by
UV absorbance, aqueous potassium permanganate, or iodine. Flash
coloratography was performed using Sigma-Aldrich silica gel 60,
particle size 40–63 μm, with the indicated solvent system. NMR
spectra, unless otherwise specified, were recorded on Bruker Avance
DRX 400, 401.36 MHz for 1H and 100.92 MHz for 13C. Chemical shifts
are reported in ppm with the deuterated solvent signal as the internal
standard. Data are reported as follows: chemical shift, integration,
multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn =
quintet, m = multiplet and br = broad), and coupling constant in hertz.
All 13C spectra were obtained with complete proton decoupling.
Structural assignments were made with additional information from
gCOSY, gHSQC, gHMBC, and NOESY experiments. 2D-EXSY
(exchange spectroscopy) experiments, based on 2D-ROESY
(rotating-frame Overhauser enhancement spectroscopy) sequence,
and NOESY experiments were recorded on a VARIAN INOVA 600 MHz
instrument. The optimized 2D map was recorded by using a relaxation
time of 15 s, a mixing time of 0.03 s, 128 increments of 4 transients of
2X, and each was collected. HPLC-ESI-QToF full-scan analyses
analyses (FIA) were carried out with a 1200 Infinity HPLC
(Agilent Technologies, USA), coupled with a quadrupole–time-of-flight
tandem mass spectrometer (6530 Infinity Q-ToF; Agilent
Technologies) through a Jet Stream ESI interface (Agilent). Mass Hunter
Workstation Software (B.04.00) was used to control the HPLC and the
mass spectrometer, for data acquisition, and for data analysis.
Methyl (S)-1-Acetylindoline-2-carboxylate Ac-(2S)-Ind-OMe
(1). (S)-Indole-2-carboxylic acid (H-Ind-OH) (2.8 g, 17.2 mmol)
dissolved in 280 mL of methanol and cooled to 0 °C with an ice
bath. To this suspension was added dropwise thionyl chloride (1.87
mL, 25.7 mmol, 1.5 equiv). The reaction mixture was stirred for 1 h and
allowed to reach room temperature. Afterward, the resulting solution
was heated at 70 °C and allowed to stir at reflux for 16 h. After being
cooled at room temperature, the reaction mixture was concentrated
under reduced pressure. The residue was dissolved in ethyl acetate
(ETOAc) and washed with an aqueous saturated solution of sodium
bicarbonate (NaHCO3) three times. The combined organic layers were
washed with brine, dried over Na2SO4, and concentrated under reduced
pressure. The resulting crude material was purified by flash
coloratography on silica gel using 10 to 50% ETOAc in hexane to
give the desired product (H-(2S)-Ind-OMe) as a white solid in an 82%
yield (2.5 g, 14.1 mmol). 1H NMR (400 MHz, CDCl3) δ = 7.12–6.99
(m, 2H), 6.79–6.68 (m, 2H), 4.39 (dd, J = 10.2, 5.5 Hz, 2H), 3.76 (s, 3H),
3.36 (m, 2H).
In a round-bottom flask, 510 mg (2.9 mmol, 1 equiv) of H-(2S)-Ind-
OMe was dissolved in 25 mL of dry dichloromethane and the resulting
solution stirred at 0 °C. A catalytic amount (0.5%) of 4-
dimethylaminopyridine (DMAP) and 1.60 mL (11.5 mmol, 4 equiv)
of triethylamine (TEA) were added to the solution. Acetic anhydride
(2.2 mL, 23 mmol, 8 equiv) was then added dropwise, and after 15 min,
the reaction mixture was warmed at room temperature and let stirring
for 16 h. The reaction mixture was then concentrated under reduced
pressure, and the residue was dissolved in dichloromethane, acidified
with HCl 1 M, and washed three times with dichloromethane. The
combined organic layer was washed over Na2SO4, filtered, and
concentrated under reduced pressure. The resulting crude product was
purified by flash chromatography on silica gel using 10 to 50% ETOAc in
hexane to give the desired product as a white solid in an 85% yield (3.58
mg, 2.45 mmol). TLC Rf: 0.6 (Hex/ETOAc = 7:3), Mp: 65 °C. 1H NMR
(400 MHz, CDCl3) δ = 8.21 (d, J = 8.1 Hz, 1Htrans), 7.28–7.12 (m, 2Htrans
+ 3Htrans), 7.03 (t, J = 7.7 Hz, 1Hcis + 1Htrans), 5.17 (d, J = 10.7 Hz,
1Htrans), 4.91 (d, J = 10.7 Hz, 1Hcis), 3.77 (s, 3Hcis), 3.73 (s, 3Hcis),
3.62 (dd, J = 16.6, J = 10.7 Hz, 1Hcis), 3.47 (dd, J = 16.6 Hz, J = 10.7 Hz,
3.18 (m, 2Htrans), 2.91 (s, 3Hcis), 2.8 (s, 3Htrans), 2.31 (m, 2Htrans), 2.29 (m, 2Htrans), 2.17 (s, 3Hcis).
with DCM and washed with HCl 1M, aqueous saturated sodium
Na2SO4, dichloromethane. The combined organic layers were dried over
1H NMR (400 MHz, DMSO-<sup>d<sub>6</sub></sup>) δ = 9.0 Hz, 0.25H), 2.52 (s, 2.25H) and 2.19 (s, 0.75H).
In a two-neck round-bottom flask, 210 mg (1.02 mmol, 1 equiv) of Ac-<sup>−</sup>S)-Ind-OMe (181 mg, 1.02 mmol, 1 equiv) was added, and the reaction mixture was heated at reflux for 16 h before being allowed to cool to room temperature. The reaction mixture was then diluted with DCM and washed with HCI 1M, aqueous saturated sodium bicarbonate, and brine. The organic layer was then dried over Na2SO4 and evaporated under reduced pressure. The resulting solid was purified by flash chromatography on silica gel using 10−50% EtOAc in hexane. The desired product was obtained as white solid in an isolated yield of 46% for solubility issues (168 mg, 0.46 mmol). TLC Ref = 0.6 (DCM:MeOH = 9:1), M.P. = 175 °C (dec) 1H NMR (400 MHz, CDCl3) δ = 8.35−8.14 (m, 1H), 7.36−7.11 (m, 5H), 7.11−6.95 (m, 2H), 5.87−5.75, 5.51−4.54, 5.34−5.21 and 5.12−4.92 (m, 4 m, 2H), 3.95−3.00 (m, 7H), 2.47 and 2.18 (couple of d, 3H). 1H NMR characterization of prevailing cis−cis species (>70%) in the stereo-processes occurring between the two species, one e
The term r accounts for unequal populations and is defined as
where I<sub>LT</sub> and I<sub>T</sub> are the peak intensities of two exchangeable resonances in the EXSY, I<sub>L</sub>T and I<sub>T</sub>L are the intensities of the exchange cross peaks, and τ<sub>n</sub> is the mixing time. X<sub>c</sub> and X<sub>t</sub> are the mole fractions of the cis and trans forms. The choice of the mixing time τ<sub>n</sub> is critical. Kinetic effects on the cross-peak intensities will be too small to measure accurately, if it is too short. However, the effects will be so large as to be insensitive to the kinetic parameters, if it is too long. The optimum mixing time should be chosen to minimize the error in the rate constant and an approximate expression was shown to be

\[
t_{m,\text{opt}} \sim \frac{1}{T_1 + k_{\alpha-\alpha} + k_{\beta-\beta}}
\]

We found that τ<sub>n</sub> = 0.03 s is the closest to the optimal value.

1H NMR Characterization of Prevailing Cis−Cis Species (>70%) of 2 in DMSO-d<sub>6</sub> on a 600 MHz Instrument. The main species of Ac-Ind-Ind-OMe (2) in DMSO-d<sub>6</sub> was fully characterized by comparison of analysis by COSY and HSOQC maps and homonuclear dipolar correlations in ROESY map (see the SI). The prevailing conformer was identified as the cis-cis one on the basis of the significant ROE detected between the more intense singlets at 2.05 and 3.73 ppm due to the methyl protons of the acetyl moiety and the ester function, respectively. Therefore, among NMR signals of H<sub>c</sub> of the conformer mixture (5.00−6.00 ppm), the two more intense ones (doublet of doublets) at 5.24 and 5.27 ppm were assigned to the two units of the cis−cis species. In particular, the high-frequency signal (5.27 ppm) was attributed to the H<sub>r</sub> of the residue with the N-acetyl terminal substituent, which is in spatial proximity of the methyl group of acetyl moiety (ROE constraint). Accordingly, to the above said stereochemical assignment, no ROEs between aromatic protons and H<sub>r</sub> of methyl ester terminal residue were detected. Starting from each H<sub>r</sub>, the two diastereotopic protons of their adjacent methylene were assigned (3.29/3.78 ppm for the N-acetyl residue and 3.34/3.76 ppm for the methyl ester residue) by means of their scalar correlations. H<sub>r</sub> of each residue was identified on the basis of the ROE effects produced by the methylene protons. Resonances of aromatic protons of each residue were assigned on the basis of their scalar correlations, starting from H<sub>r</sub> of each residue. NMR characterization data for the cis−cis species are collected in the SI.

1H NMR Signals of 1, Cis and trans stereoisomers of 1 were attributed on the basis of NOESY analysis (see the SI). Even though the majority of dipolar interactions suffered from exchange processes occurring between the two species, one effect was selective for the trans one. In particular, in CDCl<sub>3</sub>, H<sub>r</sub> at 5.17 ppm produced NOE at 2.17 ppm (acetyl group), which is due to exchange processes, but not at 2.48 ppm, which is its acetyl group. On the contrary, H<sub>r</sub> at 4.91 ppm gave NOEs at both frequencies of acetyl groups. Therefore, H<sub>r</sub> centered at 5.17 and 4.91 ppm must be attributed to trans and cis isomer, respectively. Starting from the frequency of H<sub>r</sub> and based on the analysis of scalar correlations and integration, the complete assignment of cis and trans isomer was obtained (see the SI).

Optimization of the Timing Mix for EXSY Experiments. The rate constants and the activation energy for the cis/trans isomerization were determined in DMSO-d<sub>6</sub> from 2D exchange spectroscopy (EXSY) which provides a map of the exchanging species. Considering the greatly unequal populations of the two exchanging species, as well as similar spin−lattice relaxation time T<sub>1</sub> of the two species (T<sub>1</sub> <sub>c</sub> = 2.39 s; T<sub>1</sub> <sub>t</sub> = 2.46 s) we found the approach outlined by Perrin and Dwyer convenient. This approach allows for calculation of rate constant for chemical exchange by knowing the diagonal peak to cross-peak intensity ratio. For a simple two-site exchange, the total exchange rate k = k<sub>α-α</sub> + k<sub>β-β</sub> is given by the equation

\[
k = \frac{1}{\tau_n} \ln r + \frac{1}{\tau_m} - \frac{1}{r - 1}
\]
Solvation effects have been accounted for using the integral equation formalism formulation of the polarizable continuum model (IEF-PCM). Natural bond orbital (NBO) analysis was run with NBO version 3. All of the DFT calculations have been performed using the Gaussian 16 suite of programs. HPLC Analysis of 1 and 2. A 1000 ppm solution was prepared in DMSO and then further diluted in MeOH to ca. 50 ppm and injected in the chromatographic system. The separation was performed on an Agilent Zorbax Extend C18 Rapid resolution HT column (50 × 2.1 mm, 1.8 μm particle size). The injection volume was 1 μL, and the column temperature was 30 °C. Separation was obtained by using a gradient of 0.1% formic acid in water (elucent A) and 0.1% formic acid in acetonitrile (elucent B) programmed as follows: 90% A for 2 min, followed by a linear gradient to 50% B in 9 min, then to 70% B in 3.3 min, finally to 90% B in 3.7 min held for 12 min at 90% B. Re-equilibration time for each analysis was 13 min. The chromatographic runs were performed at a flow rate of 0.2 mL/min. The eluents were all HPLC-MS grade, Sigma-Aldrich. The MS acquisition was performed in full scan, and the Jet Stream ESI operating conditions were: drying gas (N₂, purity >98%): 350 °C, nozzle voltage 1000 V, skimmer 65 V, octapole RF 750 V. The Jet Stream ESI operating conditions were: drying gas flow rate of 0.2 mL/min. The eluents were all HPLC-MS grade, Sigma-Aldrich. The MS acquisition was performed in full scan, and the Jet Stream ESI operating conditions were: drying gas (N₂, purity >98%): 350 °C at 10 L/min; capillary voltage 4.5 kV; nebulizer gas 35 psig; sheath gas (N₂, purity >98%): 375 °C at 11 L/min. High-resolution mass spectra were acquired in the range 100–3200 m/z in high-resolution positive mode. The fragmentor was kept at 175 V, nozzle voltage 1000 V, skimmer 65 V, octapole RF 750 V. The mass axis was calibrated prior analyses using the Agilent tuning mix HP0321 (Agilent Technologies) prepared in acetonitrile and water.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00184.

Full characterization of the synthesized compounds, NMR experiments, and calculation details (PDF)

FAIR data, including the primary NMR FID files, for compounds 1 and 2 (ZIP)

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