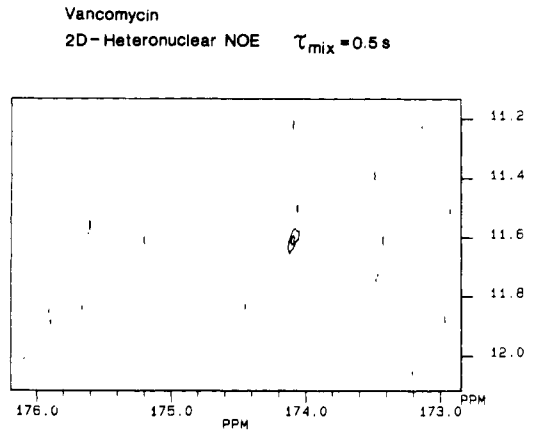

Figure 2. Two-dimensional ^{13}C-{^1H} NOE experiment[6] on a vancomycin/Ac-D-Ala-D-Ala complex described in the text. A Bruker WP 200 SY spectrometer was operated at 50.3 MHz for carbon detection. A total of 16 × 1536 transients were accumulated using a 0.8-s recycle delay and a 0.5-s mixing time.

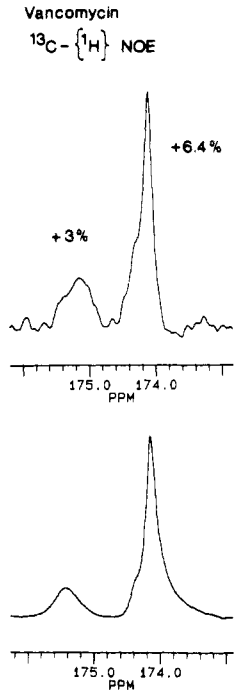

Figure 3. Top: One-dimensional ^{13}C-{^1H} NOE difference experiment as measured overnight on a vancomycin/Ac-D-Ala-D-Ala complex at 50.3 MHz. A recycle delay of 4 s was inserted to allow a complete decay of heteronuclear NOE built up during a 0.5-s (20 dB below 0.2 W decoupler power) presaturation of the 1-NH signal at 11.6 ppm. Bottom: Reference ^{13}C NMR spectrum of the complex. The strong sharp signal at 174 ppm corresponds to C-1 in the free state, while the smaller broad peak at 175.3 ppm represents the C-1 atom in bonded Ac-D-Ala-D-Ala.

dimeric forms of ristocetin A exist in this solution.[4d] Multiple dimerization was observed in the 125-MHz ^{13}C NMR spectra where the C-1 signal showed a "triplet" structure in the bonded state.

In conclusion, we found that heteronuclear NOE transferred to isotope-labeled guests may be an unique tool for locating the binding site of biomolecules like glycopeptides.

Acknowledgment. We thank the Hungarian Academy of Sciences for Grants OTKA 1144 and 1181.

Supplementary Material Available: Five figures including ^{13}C NMR spectra of the guest and ristocetin A, ^{13}C-{^1H} NOE and ^{13}C EXSY spectra, and buildup of EXSY peaks (6 pages). Ordering information is given on any current masthead page.

Continuous Fluorimetric Direct (Uncoupled) Assay for Peptidyl Prolyl Cis-Trans Isomerases

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Peptidyl prolyl cis-trans isomerases (PPIases)[1] catalyze the isomerization of the Xxx-Pro amide bond in peptides and proteins[2] and are abundant cytoplasmic receptors of immunosuppresive drugs.[3] The catalytic activity of PPIases is normally monitored spectrophotometrically by using the chymotrypsin-coupled assay of Fischer.[4,5] Because some PPIase substrates inhibit chymotrypsin[6] and other coupling enzymes degrade PPIases, we have developed a continuous, direct (uncoupled) PPIase assay based on intramolecular fluorescence quenching through collision.[7,8] When an *o*-aminobenzoyl (Abz) fluorophore and either a *p*-nitrophenylalanine (Phe(p-NO_2)) or a C-terminal *p*-nitrobenzyl (Bzl(p-NO_2)) quencher are incorporated into a proline-containing substrate, the fluorescence of Abz is suppressed by frequent intramolecular collisions with the quencher in the cis Xxx-Pro conformation of the peptide. Isomerization of the Xxx-Pro bond from cis to trans causes an increase in fluorescence due to a decrease in collisional quenching. To develop an uncoupled assay, the ratio of cis to trans Xxx-Pro conformers must differ from the equilibrium established in aqueous media. We have discovered that Xxx-Pro substrates dissolved in solutions of LiCl/TFE (or THF) exist predominantly in the cis conformation.[4h] When a LiCl/TFE solution of substrate is added to a biological buffer, the Xxx-Pro cis/trans conformational equilibrium typical for aqueous media is restored, and the enzymatic catalysis of this process can be monitored.

Syntheses of Abz-Gly-Ala-Pro-Phe(p-NO_2)-NH_2 **1** and Abz-Ala-Ala-Pro-Phe-$NHCH_2$Bzl(p-NO_2) **2** were carried out by using standard protocols,[9] and the purified peptides were characterized

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(7) Fluorogenic substrates quenched by intramolecular collision are reviewed in the following: (a) Yaron, A.; Carmel, A.; Katchalski-Katzir, E. *Anal. Biochem.* **1979**, *95*, 228-235. A general mathematical treatment of quenching by molecular interaction and complex formation can be found in the following: (b) Vaughan, W. M.; Weber, G. *Biochemistry* **1970**, *9*, 464-473.

(8) The pair *o*-aminobenzoyl/*p*-nitrophenylalanine was previously used for the assay of peptidyldipeptide hydrolase[7a] and, recently, in fluorogenic substrates of HIV-1 proteinase: Toth, M. V.; Marshall, G. R. *Int. J. Peptide Protein Res.* **1990**, *36*, 544-550.

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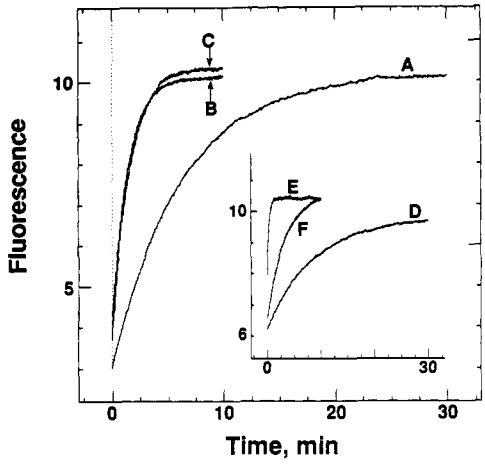
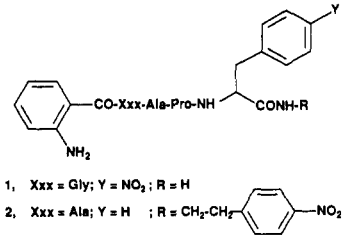


Figure 1. Fluorescent peptidyl prolyl cis–trans isomerization assays using 3.0 μM **1** (curves A–C) and 0.6 μM **2** (curves D–F, inset) at 0.0 ± 0.1 °C: A, uncatalyzed; B, 540 nM human recombinant cyclophilin (hrCyp)[12]; C, 940 nM human recombinant FKBP12 (hrFKBP12).[13] Inset: D, uncatalyzed; E, 11 nM hrCyp; F, 47 nM hrFKBP12. The instrument settings for all assays were identical so that the fluorescent intensities for **1** and **2** could be compared (λ_{ex} = 337 nm, λ_{em} = 410 nm). Assay buffer composition was 25 mM Hepes/NaOH, pH 8.0, 50 mM NaCl; volume of assays was 2.0 mL.

by several analytical methods.[10] The combination of *o*-aminobenzoyl and *p*-nitrobenzyl groups was selected for collisional fluorescence quenching;[7a] the absence of a spectral overlap between the absorption band of the *p*-nitrobenzyl group and the emission spectrum of the *o*-aminobenzoyl group precluded quenching by resonance energy transfer.[11]



Isomerization of the above substrates was followed by monitoring the increase in fluorescence at 410 nm upon excitation at 337 nm. Substrate **1** or **2** was dissolved in a 470 mM solution of LiCl in TFE, which increased the population of the cis isomer to 50–70% as determined by NMR spectroscopy. The increased initial cis substrate concentration produced a 1.7–2.5-fold enhancement in fluorescence upon isomerization to 90% trans (Figure 1). Assays were conducted at 0 °C to minimize the uncatalyzed thermal isomerization (Figure 1). At this temperature, the rate constant k_c for the uncatalyzed cis to trans isomerization was 2.2 × 10^{-3} s^{-1} for **1** and 1.2 × 10^{-3} s^{-1} for **2**, with activation free energies of 19.3 and 19.5 kcal/mol, respectively. Under subsaturating conditions, the disappearance of excess cis conformer (concentration [C] at time t) is described by the integrated eq 1 derived in the supplementary material. $[S]_0$ is the total substrate

$$[C] = [C]_0 e^{-(k_c' + k_c + k_t' + k_t)t} + \frac{k_t' + k_t}{k_c' + k_c + k_t' + k_t}[S]_0(1 - e^{-(k_c' + k_c + k_t' + k_t)t}) \quad (1)$$

concentration, $[C]_0$ is the initial cis conformer concentration, k_c and k_t are the thermal rate constants for cis → trans and trans → cis isomerization, and k_c' and k_t' are apparent first-order rate constants defined as $[E]_0 k_{cat}^{(c)}/K_m^{(c)}$ and $[E]_0 k_{cat}^{(t)}/K_m^{(t)}$, respectively. $[E]_0$ is the enzyme concentration; upper indexes (c) and (t) in k_{cat} and K_m refer to cis → trans and trans → cis catalysis, respectively.

The continuous spectrofluorometric direct assay for PPIases can be used to detect new PPIases and new PPIase inhibitors; for example, inhibition of cyclophilin by CsA is easily detected. Analogues of substrates **1** and **2** should facilitate determining the substrate specificity of PPIases, particularly in the C-terminal region which cannot be characterized by reported coupled assays.

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Supplementary Material Available: Derivation of the initial rate equation (eq 1) and the procedure for the synthesis of peptide **1** (6 pages). Ordering information is given on any current masthead page.

(9) Peptide **1** was synthesized by solid-phase techniques using a Fmoc approach and the "PAL" handle (Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hundson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730–3743) for establishing the C-terminal peptide amide. Peptide **2** was synthesized by classical solution methods (Bodanszky, M.; Bodanszky, A. In *Practice of Peptide Chemistry*; Springer-Verlag: New York, 1984) using N^{α}-*tert*-(butyloxycarbonyl)-protected (Boc) amino acids.

(10) Peptides **1** and **2** were characterized by high-resolution fast atom bombardment mass spectrometry (HR-FABS calcd for $C_{26}H_{32}N_7O_7$ (**1**) 554.2363, found 554.2344; $C_{35}H_{42}N_7O_7$ (**2**) 672.3148, found 672.3129) and by HPLC on a Vydac C-18 column (4.6 × 250 mm): linear gradient over 20 min of CH_3CN/0.036% TFA and H_2O/0.045% TFA from 1:19 to 4:1, flow rate 1.2 mL/min, detection at 214 and 254 nm; single peak at t_R = 12.9 min (**1**), t_R = 18.0 min (**2**). The ^1H NMR spectra of **1** and **2** were consistent with the structures.

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Keto Boronate Reduction: A Novel Method for High 1,3-Relative Asymmetric Induction†

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Although the methods for establishing 1,3-asymmetric induction in systems possessing heteroatoms at both stereocenters of interest are legion, there exist but a handful of approaches to 1,3-asymmetric induction in compounds where a heteroatom does not comprise one of the substituents of the stereodirecting center.[3] A general method for accomplishing this type of transformation is described herein.

Recent investigations in our laboratories have employed novel intramolecular conformational control elements to achieve high relative asymmetric induction in carbonyl addition reactions.[4] In the present study, 2-alkyl-4-keto 1-boronate esters are showcased as substrates wherein both conformational control and facial

† Dedicated with great warmth and respect to Professor Herbert C. Brown on the occasion of his 80th birthday.

(1) Alfred P. Sloan Foundation Fellow, 1987–1991.

(2) National Institutes of Health Postdoctoral Fellow, 1991–1993.

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