10% ee of **2a** in reactions of 1-hexyne with ethyl diazoacetate; low product yields were obtained that reflected the significant dependence of carbenes derived from the action of this catalyst on substrate reactivity. The chiral $Rh_2(MEPY)_4$ catalysts appear to have unique design features[18] for asymmetric cyclopropenation that are not present in the semicorrin copper catalysts, and efforts are underway to optimize these features for further enhancement of percent ee's. We are also working toward establishing the absolute configuration of these chiral cyclopropene derivatives.

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Supplementary Material Available: Listings of experimental details for catalytic reactions and product characterization (4 pages). Ordering information is given on any current masthead page.

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Glycopeptide Binding Site Spied through Transferred Heteronuclear NOE: [1-^{13}C]Ac-D-Ala-D-Ala Bonded to Vancomycin and Ristocetin A

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The structure[1] and binding[2] of glycopeptide antibiotics[3] to cell wall analogue oligopeptides are mostly characterized by high-field 1D and 2D homonuclear NMR spectroscopy. On the basis of these studies it is safely proved that, among other interactions, a strong hydrogen-bonding network exists between the binding pocket of the antibiotic and the carboxylate anion at the C-terminal of Ac-D-Ala-D-Ala.[4]

In this communication, we present a direct method—the transferred heteronuclear NOE—for the assignment of intermolecular H-bonding.[5] The ^{13}C-{^{1}H} NOE method[6] alone has some inherent difficulties when biomolecules are investigated, since in the slow motion regime the theoretical maximum of the signal enhancement is only ca. 15%. The effect may be buried in the noise, especially in the presence of chemical exchange.

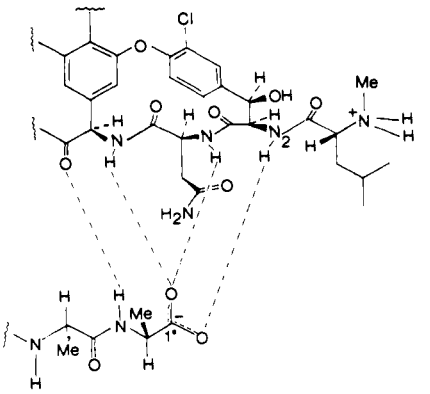

Figure 1. N-terminal region of vancomycin and the C-terminal part of a bacterial cell wall peptide analogue Ac-D-Ala-D-Ala. Intermolecular hydrogen bonds are indicated according to the structure of the complex.

To increase the sensitivity, we employed a [1-^{13}C]-labeled dipeptide as a guest with the hosts vancomycin or ristocetin A. Moreover, these compounds were dissolved in a cryogenic solvent DMSO-d_6:CCl_4 = 10:3 at 5 °C to ensure slow chemical exchange between the free and bonded states of the guest. We assume that the relevant structures of the complexes are maintained in the cryogenic media.[3b] The Ac-D-Ala-D-Ala concentration was 20 mM/L while the antibiotic concentration was held constant at 12.5 (vancomycin) or 20 mM/L (ristocetin A). It should be mentioned that the mixture also contained ca. 30% impurity arising from the inactive[2] D,L compound of the dipeptide, which resulted in a 16 ppb downfield shift in the ^{13}C NMR spectrum as compared to the active D,D component. When the D,D component is bonded to its host, the chemical shift of the labeled C-1 carbon is shifted downfield by more than 1 ppm! (The D,L compound may be used as an internal standard.)

Figure 1 shows the well-known partial structure of the heteroaggregate of vancomycin. A 2D ^{13}C-{^{1}H} NOE[6c] experiment indicated, surprisingly, a correlation between the *bonded* 2-NH of the antibiotic and the C-1 atom of the *free* dipeptide (Figure 2). This phenomenon can be explained by a chemical exchange mediated[7] ^{13}C magnetization transfer which follows the heteronuclear NOE. Since in the free state the C-1 signal is much sharper, it renders the detection of small NOE more feasible. ^{13}C-^{13}C EXSY[8] and the initial buildup rate of cross peaks verified the presence of exchange for both antibiotics and, in the case of ristocetin A, gave an estimate of 2 s for the average lifetime for the exchanging sites. In a 1D ^{13}C-{^{1}H} NOE experiment (Figure 3) we observed C-1 signals due to both bonded and nonbonded states. Semiquantitative evaluation (saturation efficiency is estimated to be 60%) gives approximately half of the theoretical maximum NOE. This fact suggests a short NH···CO distance (<250 pm) in accordance with predictions.[3c] In the case of ristocetin A, similar results are obtained; however, their interpretation is difficult since, besides the monomer, at least two other different

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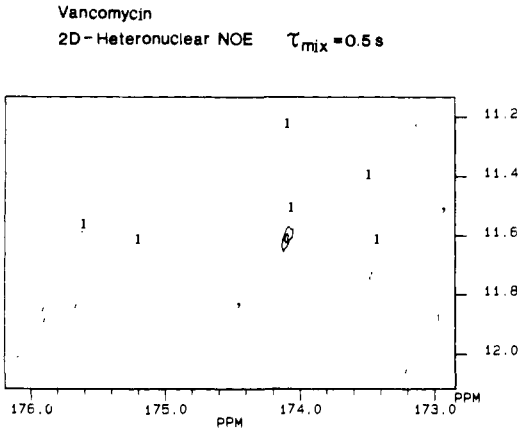

Figure 2. Two-dimensional ^{13}C-{^1H} NOE experiment[6] on a vancomycin/Ac-D-Ala-D-Ala complex described in the text. A Bruker WP 200 SY spectrometer was operated at 50.3 MHz for carbon detection. A total of 16 × 1536 transients were accumulated using a 0.8-s recycle delay and a 0.5-s mixing time.

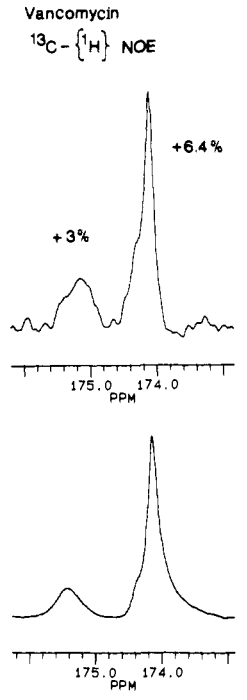

Figure 3. Top: One-dimensional ^{13}C-{^1H} NOE difference experiment as measured overnight on a vancomycin/Ac-D-Ala-D-Ala complex at 50.3 MHz. A recycle delay of 4 s was inserted to allow a complete decay of heteronuclear NOE built up during a 0.5-s (20 dB below 0.2 W decoupler power) presaturation 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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