Minimal Acylase-Like Peptides. Conformational Control of Absolute Stereospecificity

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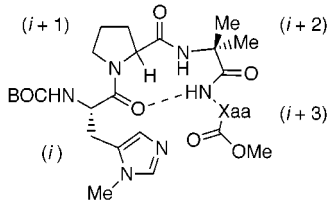
Selective interactions of chiral substrates with proteins are of central interest to molecular recognition and biological catalysis. Enzymes that catalyze enantioselective reactions have also achieved a prominent place in synthetic organic chemistry due to their frequently excellent efficiencies.[1] At the heart of the selectivity of these catalysts is the ability of the protein to process one enantiomer of a given substrate while the other remains inert. The basis for enantiomer discrimination is frequently attributed to the inherent chirality of the enzyme.[2] Of note, however, is that while useful mnemonic devices have been derived for particular enzymes to predict which substrates will give good selectivities, detailed mechanistic understanding of the basis for selectivities has been difficult to acquire.[3,4] One consequence is that the design of enzymes that catalyze reactions with the opposite enantioselectivity remains a significant challenge.[5] One approach involves the total chemical synthesis of enantiomeric enzymes with all D-amino acids.[6] An alternative strategy is the synthesis of new peptides which have D-amino acids placed at key positions so that *diastereomeric* peptides with *enantiomeric* selectivity profiles result. In this approach, the goal becomes the synthesis of diastereomeric peptides with pseudo-enantiomeric shapes and functional group presentation.

We are studying synthetic peptides that catalyze enantioselective reactions, including acyl transfer, with the goal of developing synthetically useful enzyme mimics that also shed light on the mechanistic basis for peptide-based stereoselectivity.[7] We have initially focused on a class of synthetic peptides that can adopt a well-defined conformation in a hydrophobic medium. As shown below, the tetrapeptides **1** and **2** represent interesting functional group ensembles for this purpose since they possess a catalytically active alkylimidazole substructure[8,9] within a sequence that is biased toward the adoption of the illustrated β-turn conformation.[10] We conjectured that incorporation of enantiomeric proline residues in the (i + 1) position of peptides **1** and **2** would cause each new peptide to adopt complementary conformations that might confer opposite selectivity during acylation reactions (e.g., eq 1).[11] Therefore, a single stereochemical change within the tetrapeptide sequence was projected to confer a reversal of selectivity. Thus, we sought to demonstrate that the proline stereogenic center could orchestrate the sense of enantioselectivity within a set of diastereomeric catalysts.

1 (i + 1) = L-Pro
2 (i + 1) = D-Pro



The synthesis and evaluation of peptides with the general structures **1** and **2** has resulted in a validation of this hypothesis. A group of 10 tetrapeptides was synthesized[12] and evaluated as catalysts for the kinetic resolution of (±)-*trans*-2-*N*-acetamidocyclohexanol (**3**, eq 1). Reactions were run at 25 °C in toluene solution under conditions employing 2−5 mol % of catalyst relative to substrate. Reactions were allowed to proceed for 6−12 h and then quenched by addition of methanol. As the data in Table 1 show, those peptides containing an L-proline residue in the (i + 1) position (**1a**−**1e**) each resulted in preferential acylation of (*S*,*S*)-**3** to give the product with s (k_{fast}/k_{slow})[13] values of up to 5.7. In contrast, when peptides **2a**−**2e** (containing D-proline in the (i + 1) position) were employed, the opposite enantiomer of substrate underwent preferential reaction. In addition, in these cases substantially more selective catalysis was observed, with catalyst **2a** affording recovered (*S*,*S*)-**3** in 98% ee at 58% conversion (s = 28). Thus, catalysts with L-Pro in the (i + 1) position preferentially acylated (*S*,*S*)-**3**; catalysts with D-Pro in the same position processed (*R*,*R*)-**3**.

A second trend within this series of peptides is the matching of the proline stereogenic center with that of the amino acid residue in the (i + 3) position. For example, within the D-proline series, the two peptides with L-configurations at the (i + 3) position, **2a** and **2c**, were the most selective (**2a**, s = 28; **2c**, s = 21). Furthermore, peptide **2e**, with Gly in the (i + 3) position, afforded a selectivity factor of 14, which suggests that the presence of an L-residue at position (i + 3) is advantageous for the kinetic resolution of

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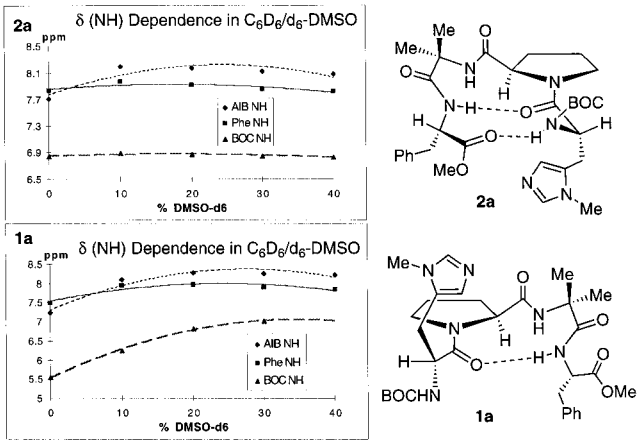
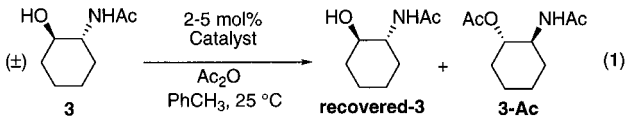


Figure 1. Solution NMR data for **2a** and **1a** and projected conformations.

Table 1. Selectivities in Kinetic Resolutions of (±)-3 with Catalysts 1 and 2



catalyst	% conversion (Based on **3**)	(*s*)	% ee of **3**	% ee of **3-Ac**
1a Xaa = L-Phe	56	3.0	44 (*R*,*R*)	34 (*S*,*S*)
1b Xaa = D-Phe	71	5.7	89 (*R*,*R*)	36 (*S*,*S*)
1c Xaa = L-Val	61	3.4	54 (*R*,*R*)	35 (*S*,*S*)
1d Xaa = D-Val	63	4.3	65 (*R*,*R*)	39 (*S*,*S*)
1e Xaa = Gly	57	3.5	50 (*R*,*R*)	38 (*S*,*S*)
2a Xaa = L-Phe	58	28	98 (*S*,*S*)	73 (*R*,*R*)
2b Xaa = D-Phe	57	14	89 (*S*,*S*)	66 (*R*,*R*)
2c Xaa = L-Val	61	21	99 (*S*,*S*)	63 (*R*,*R*)
2d Xaa = D-Val	62	9.2	88 (*S*,*S*)	55 (*R*,*R*)
2e Xaa = Gly	63	14	97 (*S*,*S*)	57 (*R*,*R*)

3 with catalysts of this general structure. Within the L-proline series, D-residues were slightly preferred at the corresponding positions (**1b**, $s = 5.7$; **1d**, $s = 4.3$).

The mechanistic basis for the turnover in selectivity observed for catalysis by peptides **1** versus peptides **2** is under investigation. However, the opposite enantioselectivities may be correlated to the specific conformations of the peptides in solution. For purposes of evaluating the specific impact of the proline stereogenic center, we undertook a direct comparison of the conformations of the most selective catalyst **2a** and its corresponding diastereomer **1a**, employing NMR and IR techniques.[14]

Peptide **2a** exists as a single conformation in C_6D_6 solution. The chemical shift dependences of the NH groups as a function of increasing Lewis base concentration[15] were measured and representative data are shown in Figure 1. Nearly invariant chemical shifts for the Phe(NH) and the His(NH) are observed for peptide **2a** when it is dissolved in C_6D_6 with various concentrations of d_6-DMSO. In contrast, the Aib(NH) shows a significant downfield shift as the DMSO concentration increases. Similar observations result from a variable temperature study of the chemical shifts of the NH groups of peptide **2a** when dissolved in C_6D_6.[16] Whereas the Phe(NH) and His(NH) groups exhibit smaller temperature coefficients ($-d\delta/\Delta T = 0.009$ and 0.015, respectively), the Aib(NH) reveals a stronger dependence ($-d\delta/\Delta T = 0.022$). These data support a β-hairpin conformation for **2a**, with intramolecular (Phe)C=O···H−N(His) and (His)C=O···H−N(Phe) hydrogen bonds, as shown in Figure 1. Furthermore, evaluation of the ROESY spectrum of **2a** reveals a number of critical NOE's. In particular, the Pro(Cα-H) and the Aib(NH) are in close proximity, consistent with a Type II′ β-turn for **2a**. Of note is that this assignment is consistent with the folding of short peptides containing a D-Pro as a central residue for β-hairpin nucleation.[17]

In contrast, peptide **1a** exists as a 4:1 mixture of conformers in C_6D_6 solution at 25 °C. (Coalescence is observed in d_6-DMSO at 80 °C.) NMR experiments analogous to those described above have revealed that the major conformation of **1a** possesses a unique intramolecular hydrogen bond corresponding to (His)C=O···H−N(Phe). The other two amide NH groups do not appear to be involved in intramolecular H-bonding. In addition, as in the case of **2a**, a strong NOE is observed between the Pro(Cα-H) and the Aib(NH). These data point to the major conformation of **1a** being that of a Type II β-turn in solution.[18]

Evaluation of the proposed structures on the basis of these data suggests that diastereomeric peptides **1a** and **2a** are approximate mirror images of one another in terms of their local structure in the β-turn region (Type II vs Type II′, respectively; See Figure 1, structures **1a** and **2a**). The fact that they also exhibit complementary chiral selection in reactions of enantiomers suggests that these peptides also exhibit complementary features in the transition states for their corresponding acyl transfer reactions. One possibility is that the pseudo-enantiomeric β-turns dispose the nucleophilic side chain on opposite faces of the β-turn (**1a**) or β-hairpin (**2a**) architecture. Allylic strain[19] necessitates that in the case of **1a**, the imidazole group be disposed syn to the L-Pro(C=O). On the other hand, with **2a** the nucleophilic imidazole should be disposed on the opposite face of the plane defined by the D-Pro five-membered ring. The implications of such conformational differences on the transition state architectures responsible for the complementary enantioselectivity are the subject of ongoing investigations in our laboratory.

In summary, we have demonstrated that the absolute stereochemical configuration of a proline residue located at the ($i + 1$) position of a tetrapeptide acylation catalyst can orchestrate the stereochemical course of a kinetic resolution. Exchange of an L-Pro residue for a D-Pro not only affords catalysts that selectively process the opposite enantiomer of a given substrate, but also results in a dramatic increase in absolute selectivity. Extension of these concepts to obtain diastereomeric peptides which provide even higher degrees of complementary enantiomeric selection for a range of substrates is the focus of our current efforts.

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Supporting Information Available: Characterization data for all compounds and experimental details for their preparation (10 pages).

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