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MICELLAR DIASTEREOSELECTIVITY - TRIPEPTIDE SUBSTRATES

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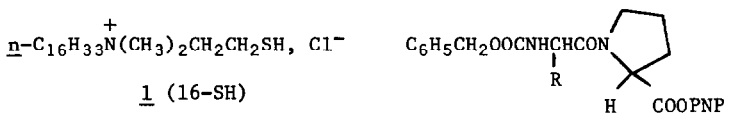
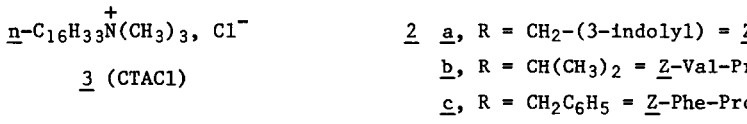
Summary. Kinetic stereoselectivities are reported for the micelle-catalyzed basic esterolyses of the four diastereomeric Z-(D or L)-Phe-(D or L)-Phe-(L)-Pro p-nitrophenyl esters.

Stereoselectivity is an underdeveloped area of micellar organic chemistry. Accordingly, much current attention is focussed on enantioselective micellar reactions between chiral nucleophiles and chiral substrates, especially amino acid esters.[2] In the course of studies designed to model reactions of proteolytic enzymes, we have observed substantial stereoselectivities attending the micellar cleavage of various dipeptide p-nitrophenyl (PNP) esters.[3,4] For example, at pH 8, micellar 16-SH (1) cleaved (LL)-N-carbobenzyloxy-Trp-Pro-PNP (2a) 5 times faster than the diastereomeric DL ester.[4] Similarly, LL/DL kinetic diastereoselectivities of 3.2 and 2.0 were found in the 16-SH micellar esterolyses of Z-Val-Pro-PNP (2b) and



n-$C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2SH$, Cl^-

1 (16-SH)



n-$C_{16}H_{33}\overset{+}{N}(CH_3)_3$, Cl^-

3 (CTACl)

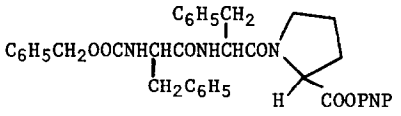
2 a, R = CH_2-(3-indolyl) = Z-Trp-Pro-PNP
b, R = $CH(CH_3)_2$ = Z-Val-Pro-PNP
c, R = $CH_2C_6H_5$ = Z-Phe-Pro-PNP

Z-Phe-Pro-PNP (2c), respectively.[4] In contrast, pH 8 micellar esterolyses in non-functional CTACl (3) were DL-stereoselective, exhibiting LL/DL rate constant ratios of 0.28 (2a), 0.10 (2b), and 0.35 (2c).[4,5]

We offered a rationale for the 16-SH LL-diastereoselectivity:[4] CPK models of the LL substrates, arranged in extended peptide conformations, exhibit "clefts" defined by their Pro and PNP moieties, and by the R groups of their variable amino acids. The CH_2 chain of 16-SH neatly fits into these clefts, poising the $CH_2CH_2S^-$ functionality (the active nucleophile in 16-SH/PNP ester cleavage[8]) slightly above and to the rear of the substrate's scissile carbonyl carbon; i.e., when LL-2a-2c are optimally arranged for hydrophobic bonding to 16-SH, the latter's thiolate moiety is optimally positioned for attack. In contrast, extended conformers of the

DL-dipeptide esters possess poorer binding sites for 16-SH because their R groups project away from their hydrophobic clefts. Binding of the DL substrates to 16-SH micelles should not orient them for optimal thiolate-carbonyl interaction; their cleavage by 16-SH should be less facile than cleavage of their LL isomers.

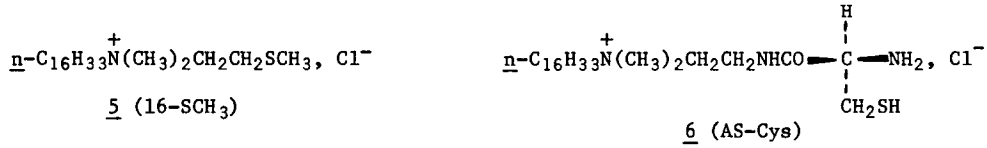
To challenge this rationale, we have now prepared four diastereomeric tripeptide esters, Z-(D or L)-Phe-(D or L)-Phe-(L)-Pro-PNP (4), and subjected each one to several micellar PNP cleavage reactions. Substrates 4 were generally synthesized by mixed anhydride coupling of



4

Z-(D or L)-Phe to (D or L)-Phe-(L)-Pro-PNP[4] in THF (-15°) using isobutylchloroformate/N-methylmorpholine.[9] The tripeptide esters were purified by crystallization from acetone/i-PrOH/H_2O (LLL, LDL) or $CHCl_3/Et_2O$ (DLL, DDL), and characterized[10] by satisfactory elemental analysis (CHN), homogeneous tlc behavior, and structurally-consistent nmr spectra.

Kinetic studies were performed in pH 8 micellar solutions of CTACl (3), 16-SCH_3 (5),[11] AS-(L)-Cys (6),[12] and 16-SH (1).[8] Slower reactions (in micellar 3 or 5) were followed by release of p-nitrophenoxide ion in a conventional spectrophotometer (see Table for conditions). Faster reactions (in micellar 1 or 6) were monitored at 400 nm with a Durrum stopped-flow



5 (16-SCH_3)

6 (AS-Cys)

spectrophotometer: an HCl-acidified (pH ~ 3) 8 x 10^{-3} M aqueous surfactant solution of 4 x 10^{-5} M substrate was mixed with an equal volume of 0.04 M (μ = 0.10, KCl) "pH 8" phosphate buffer, previously adjusted to pH ~ 10.3 by addition of 1 N NaOH. The final reaction pH was 8.0 ± 0.05, and post-mixing reactant concentrations were "standard" (see Table). Least-squares pseudo-first-order rate constants for p-nitrophenoxide release were conventionally evaluated from strip-chart or photographic records; good first order kinetics (r > 0.999) were observed over > 80% of reaction, and the reproducibility of k_ψ was better than 4.5% for duplicate reactions. Rate constants are gathered in the Table.

Several important observations and conclusions derive from the data. (a) The kinetic potency of micellar 16-SH (pK_a ~ 7.3[8]) depends on deprotonation to the nucleophilic 16-S^- form;[8] accordingly, micellar 16-SCH_3, which cannot readily afford 16-S^-, is kinetically equivalent to non-functional micellar CTACl. (b) The stereoselectivity patterns characteristic of the Z-AA-(L)-Pro-PNP dipeptide substrates[4] persist with the diastereomeric tripeptides: in micellar CTACl (or 16-SCH_3) DLL and LLL substrates cleave[5] less rapidly than DDL or LDL substrates, whereas, in micellar 16-SH (or AS-Cys), all the esterolyses are orders of magnitude faster, and

Table. Kinetics of the Micellar Cleavage of Z-Phe-Phe-(L)-Pro-PNP[a]

Surfactant	k_ψ (sec^{-1}) for diastereomers of 4			
	LLL	DLL	LDL	DDL
CTACl (3)	0.0038	0.0047	0.013	0.0076
16-SCH_3 (5)	0.0031	0.0037	0.011	0.0069
AS-Cys (6)[b]	2.3	5.4	2.1	1.5
16-SH (1)[c]	9.0	13.4	5.3	4.3

[a]Conditions: [surfactant] = 4.0×10^{-3} M, [4] = 2×10^{-5} M, pH 8.0, 0.02M phosphate buffer, μ = 0.05 (KCl), 25°C. [b]80% free SH. [c]100% free SH.

the kinetic (thiolate nucleophilic) cleavage order is reversed, DLL or LLL > DDL or LDL. The chirality of the amino acid adjacent to Pro thus determines the gross kinetic stereoselectivity in thiolate (L > D) or non-functional (D > L) micelles for both dipeptide and tripeptide esters. (c) Examination of CPK molecular models of the four diastereomeric tripeptide esters, in the light of the substrate cleft/$(CH_2)_{15}$ interaction model, predicts that the extended peptide conformation of DLL-4 should interact most effectively with the CH_2 chains of 16-SH or AS-Cys; only the DLL substrate features a "cleft" which is lined exclusively with C and H (optimal for hydrophobic bonding to the surfactant and consequent optimal functional group alignment). The three other diastereomers have one or more heteroatomic regions within their "clefts". The data reveal that DLL-4 is, indeed, cleaved most rapidly by micellar 16-SH or AS-Cys.

The crucial nature of the chirality at the adjacent amino acid in determining these micellar diastereoselectivities is illustrated by certain rate constant ratios. From the Table, $k_\psi^{LLL}/k_\psi^{LDL}$ = 1.7 and $k_\psi^{DLL}/k_\psi^{DDL}$ = 3.1 (for 16-SH cleavage), bracketing the analogous ratio k_ψ^{LL}/k_ψ^{DL} = 2.0 for the Z-Phe-Pro-PNP dipeptide substrates.[4] A similar correspondence emerges when the 16-SH tripeptide kinetic stereoselectivities are examined relative to stereoselectivities in CTACL:

$$\frac{(k_\psi^{LLL}/k_\psi^{LDL})_{16\text{-SH}}}{(k_\psi^{LLL}/k_\psi^{LDL})_{CTACl}} = 5.9 \qquad \text{and} \qquad \frac{(k_\psi^{DLL}/k_\psi^{DDL})_{16\text{-SH}}}{(k_\psi^{DLL}/k_\psi^{DDL})_{CTACl}} = 5.0,$$

once again bracketing the analogous ratio, $[(k_\psi^{LL}/k_\psi^{DL})_{16\text{-SH}}/(k_\psi^{LL}/k_\psi^{DL})_{CTACl}]$ = 5.7, observed for Z-Phe-Pro-PNP.[4]

Although the micellar tripeptide diastereoselectivities are strongly dependent on chirality at the adjacent amino acids,[13] they can be modulated by chirality variation at the amino acid once-further-removed from Pro (Table). The smaller rate constant ratios $(k_\psi^{DLL}/k_\psi^{LLL})_{16\text{-SH}}$ = 1.5 and $(k_\psi^{LDL}/k_\psi^{DDL})_{16\text{-SH}}$ = 1.2 suggest, however, that chirality variation at the remote amino acid

ffects diastereoselectivity less than chirality variation at the adjacent amino acid of these ripeptide substrates.

The foregoing results and analysis are largely in keeping with our suggested model for iastereoselectivity in nucleophilic functional micellar esterolyses.[4] We are extending our tudies to other dipeptide and tripeptide substrates.

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5) The CTACl micellar reactions probably all proceed via base-catalyzed intramolecular displacement on the Pro ester carbonyl by the nitrogen atom of the adjacent amino acid residue, with formation of a diketopiperazine.[6] We have proven this mechanism for 2a;[7] substrate 2b is under study. The intramolecular diketopiperazine formation is DL-stereoselective for steric reasons.[7]

6) Cf., M. Goodman and K. C. Stueben, J. Am. Chem. Soc., 84, 1279 (1962).

7) R. A. Moss and Y-S. Lee, to be published.

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9) LLL-4 was prepared by mixed anhydride coupling of Z-Phe-Phe to Pro-PNP.

10) LLL-4, mp 124-125°C, $[\alpha]_D^{24}$ - 43.1° (c = 1.0, dioxane (for all rotations)); LDL-4, mp 136.5 138°C, $[\alpha]_D^{23}$ - 15.4°; DLL-4, (glass) mp 85-89°C, $[\alpha]_D^{23}$ - 47.4°; DDL-4, mp 103-105°C, $[\alpha]_D^{24}$ - 17.4°.

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12) R. A. Moss, T. J. Lukas, and R. C. Nahas, J. Am. Chem. Soc., 100, 5920 (1978).

13) The R group of the adjacent amino acid is a key element of the "floor" of the hydrophobic cleft of the LL dipeptide and (D or L)LL tripeptide substrates, In extended conformers of DL dipeptide or (D or L)DL tripeptide substrates, this R group points away from the cleft, and is replaced by polar, heteroatomic units which are less suitable for hydrophobic bonding to the surfactant chain.

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