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HIGHLY ENANTIOSELECTIVE AMIDE LIGATION BY PRIOR THIOL CAPTURE

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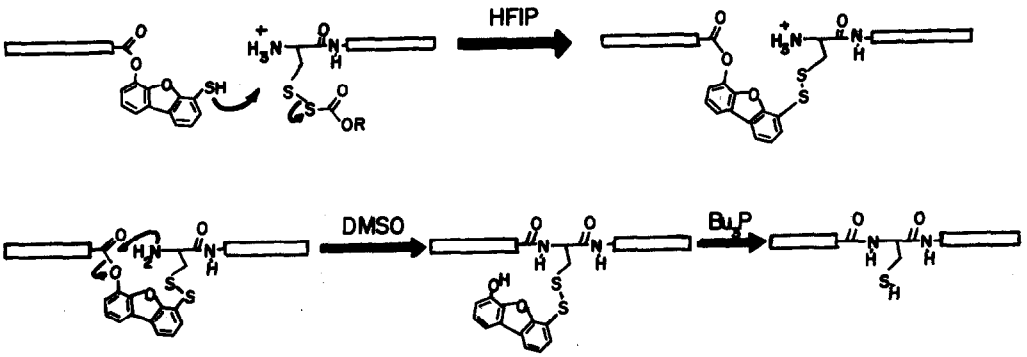
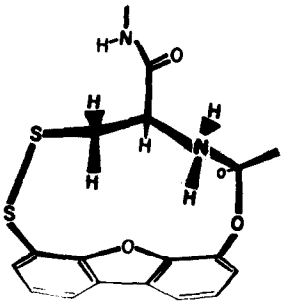
Abstract: The mixed disulfides from (R,S)-1-(Cbz-L-alanyloxy)-9-mercapto-phenoxathiin-10-sulfoxide and H-L-Cys-OMe undergo O,N-acyl transfer in DMSO at 23°C with rate constants of 7.0 h^{-1} and 0.048 h^{-1}, and a rate ratio of 146; EM values of 0.2 M and 0.0015 M.

We have previously described a thiol capture strategy for the ligation in protic media of N-terminal Cys-bearing peptides that lack side-chain protective groups.[1,2] In this and in the accompanying paper we report progress toward new classes of templates for the amide bond forming step of thiol capture.



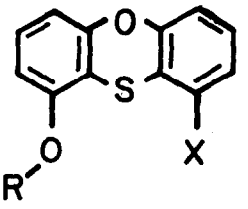
Thiol capture ligation requires a spacing element or template between the C-acyl carbon and the thiophile that permits efficient intramolecular O,N-acyl transfer from an ester with relatively low acylating potential, i.e., an unactivated phenyl ester. Previously we have described tricyclic o-acyloxy o'-mercapto-diphenyl ethers that define a series of structurally 'tuned' templates, complementary to the geometry of one conformation of the transition state for intramolecular acyl transfer.[3] A dibenzofuran with an effective molarity (EM) of 5-10 M for the peptide-forming O,N-acyl transfer reaction proved optimal and has been used to achieve practical and clean coupling between medium-sized partially blocked and unblocked peptide fragments.[2] Because of the small scope for redesign of the diphenyl ether framework, we sought new templates that are structurally more versatile and more efficient.

1

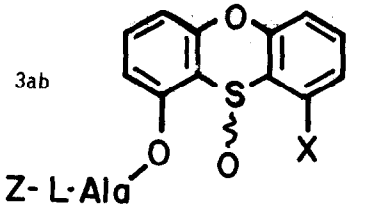


Molecular mechanics calculations suggest that the tetrahedral intermediate 1 and by analogy, the transition state for dibenzofuran-templated O,N-acyl transfer is strained by ca 4 kcal/mol. Retaining the approximate geometry of this transition state while relieving its strain was a first priority. A second was design of a template that carries an optimally oriented general base, which should result in a roughly fifty-fold rate increase, based on previous results with aspartate derivatives. A third was the study of chiral templates that can discriminate between acyl transfer to L and D-cysteine residues. Rate data for diastereomers should increase the incisiveness of molecular modeling by permitting tuning of the parameter set.

2



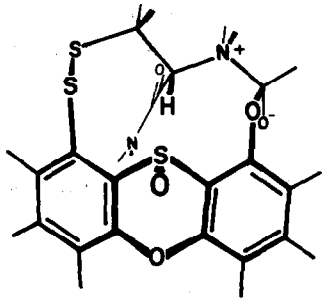
3ab



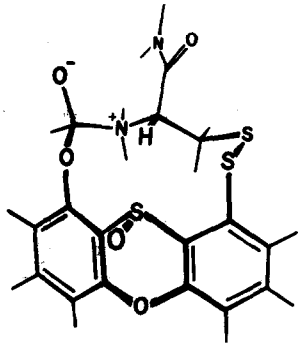
The phenoxathiin derivatives 2 and **3ab**, X = H-L-Cys(S)-OMe, provide a particularly interesting target series. The distances between the substituent oxygen and sulfur atoms, as well as those between the attached ring carbons, are very similar for 4,6-dibenzofuran and 1,9-phenoxathiin, suggesting that a transition state geometry like that of **1** should apply to both, with the significant difference that the nonplanar, pleated character of the phenoxathiin ring system is expected from modeling to reduce transannular van der Waals strain. The diastereomeric sulfoxides **3ab** allow a first examination of enantioselectivity for these O,N-acyl transfer reactions, as well as providing a small tuning of the van der Waals radius and partial charge of sulfur.

Elsewhere we have reported a convenient synthesis of the Scm-masked thiols corresponding to **2** and **3ab** as well as a baseline separation of the diastereomers **3ab**, X = SS-CO-OMe, by preparative HPLC.[4] Conversion to the unsymmetrical cysteine disulfides was carried out by Bu_3P cleavage of the Scm functions, followed by reaction with Boc-L-Cys(Scm)-OMe and cleavage of the t-butoxycarbonyl group with trifluoroacetic acid.[5] The second order rate constants for the reactions of **2** and **3ab**, X = H,[6] with excess H-L-Cys(Bzl)-OMe in DMSO at 23±1°C were calculated from HPLC measurements under pseudo first order conditions. The

amine concentration was varied by six-fold (0.05–0.30 M), yielding rate constants of 0.80 $M^{-1}h^{-1}$ for 2 R = Ac, X = H and 0.44 $M^{-1}h^{-1}$ for 2 R = Z-L-Ala, X = H, with no evidence for curvature. Reaction of the sulfoxide 3ab, X = H, as a 1:1 mixture of SO diastereomers in DMSO at 23±1°C with an equimolar amount of H-L-Cys(Bzl)-OMe was followed by HPLC. A rate difference of only 15–20% was detected for the diastereomers, and the rate constant was calculated as an average, 32 $M^{-1}h^{-1}$. The conversion of the phenoxathiin to its sulfoxide is therefore seen to result in a 32/0.44 = 70-fold rate increase. If the Hammett ρ value for this reaction is 2.6 (measured for the analogous reaction of a dibenzofuran ester[1]), an expected rate ratio of 66 is calculated from σ^- values[7] of 0.7 for MeSO– and 0.0 for MeS- functions. Clearly no proton transfer or hydrogen bonding interactions of the sulfoxide group are required to explain the enhanced reactivity of 3ab X = H.



4a



4b

Intramolecular acyl transfer rates for 2, X = H-L-Cys(S)-OMe and for the more reactive diastereomer of 3ab X = H-L-Cys(S)-OMe were monitored in DMSO-d_6 at 23±1°C (ca. 2 mM) by ^{1}H NMR spectoscopy.[8] For the acetyl derivative 2 R = Ac, X = H-L-Cys(S)-OMe a rate constant of 0.31 h^{-1} was obtained, corresponding to an EM of 0.31/0.80 = 0.39 M; for the Z-L-Ala derivative 2,R = Z-L-Ala, X = H-L-Cys(S)-OMe the corresponding values are 0.13 h^{-1} and 0.30 M. The Z-L-Ala derivative of the more reactive sulfoxide 3 X = H-L-Cys(S)-OMe showed values of 7.0 h^{-1} and 0.22 M. These EM values are thus more than an order of magnitude below those observed for dibenzofuran-mediated acyl transfer, clearly demonstrating that calculated internal strain estimates only one factor that contributes to the observed efficiency of this intramolecular reaction.

The less reactive of the two diastereomeric phenoxathiin sulfoxides 3ab X = H-L-Cys(S)-OMe required isotopic dilution analysis[9] at ca. 10^{-5} M to permit accurate estimation of the rate constant, which is 0.048 h^{-1}, corresponding to an EM value of 0.0015 M and a rate ratio for the sulfoxide diastereomers of 146. The intramolecular O,N-acyl transfer reaction with the more reactive sulfoxide is therefore highly enantioselective for L vs. D-cysteine.

This impressive difference appears at first consistent with our model for the transition state geometry, since a principal source of destabilization of the cyclic intermediate is transannular nonbonded interaction between the Cys α-H and the template ring heteroatoms. However, molecular modeling suggests that unlike the case of 1 the phenoxathiin transition

state can accomodate two distinct conformations that are nearly isoenergetic: **4a**, with an 'M' configuration of the cysteinyl backbone, and **4b**, with a 'W' geometry. For a given chirality of sulfoxide, these alternatives require opposite Cys chirality. If **4a** and **4b** are truely isoenergetic and both accessible, no enantioselectity can be expected! Evidently more subtle factors are operative in determining this reaction path, such as a correlation between the geometries at the acyl carbon of starting materials and transition states.

If the preferred geometry is **4b**, modification of the phenoxathiin nucleus to include a functional intramolecular general base should be feasible. Which transition state is in fact preferred therefore has considerable significance for future design experiments. This point can be settled by determination of the absolute configuration of the phenoxathiin sulfoxides, and experiments to this end are in progress.

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REFERENCES

1. Kemp, D. S., Biopolymers, 1981, 20, 1793; Kemp, D.S.; Galakatos, N.G.; Bowen, B.R.; Tan, K., J. Org. Chem., 51, 1829 (1986).
2. Fotouhi, N.; Galakatos, N.G.; Kemp, D.S., J. Org. Chem., 54, 2803 (1989).
3. Kemp, D.S.; Carey, R.I.; Dewan, J.; Galakatos, N.G.; Kerkman, D.; Leung, S-L., J. Org. Chem., 54, 1589 (1989).
4. Kemp D.S.; Buckler, D.R., J. Org. Chem., 54, 3647 (1989).
5. For **2** R - Ac, X = Boc-L-Cys(S)-OMe TLC R_f = 0.40 (2:1 hexane/EtOAc); **2** R = Z-L-Ala X = Boc-L-Cys(S)-OMe TLC R_f = 0.29 (2:1 hexane/EtOAc); HRMS: (M+H)+ Calcd for $C_{23}H_{26}N_7S_3$: 524.0871; Found: 524.0872; **3ab** X = Boc-L-Cys(S)-OMe HPLC RP C_{18} 40 % MeCN/60% 0.1 % TFA), t_r = 9.70', 11.72', MS (70ev) 702(M^+), 686 (M^+ -16), HRM (M+H)+ Calcd for $C_{32}H_{35}N_2O_9S_3$: 687.1505; Found: 687.1510.
6. **2** X = H, R = Ac or Z-Ala and **3ab** X = H were prepared from 1-hydroxyphenoxathiin or the 10-oxide using standard acylations with Ac_2O (mp 75-76°C, TLC R_f= 0.63 (2:1 hexane.EtOAc), MS(70 ev) 258(M^+), 216(M^+ -16)) or Z-(L-Ala-O)$_2$O (mp 93.5-95°C, TLC R_f 0.65(10:1 $CHCl_3$/EtOAc), MS (70ev) 421(M^+), 313,216); **3ab** X = H, HPLC (RP C_{18} 40% MeCN/60% 0.1% TFA t_r = 11.62' 12.62', MS (70ev) 427 (M^+), 421 (M^+-16), 322.
7. Leffler, J.E.; Grunwald, E. "Rates and Equilibria of Organic Reactions," Wiley, New York, 1963, pp 173 and 211.
8. Rate constants were calculated from 4-10 data points through a half life (R>0.99); the integrated area of a characteristic ester resonance was compared with an internal standard.
9. HPLC separation[4] insures diastereomeric purity of esters **3ab** X = H-Cys(S)-OMe ([LL + DD] vs [LD + DL]). Acyl transfer runs involving ^{14}C-labeled **3** (9.24 μCi/mM) were quenched with Et_3P/N_2H_4 and diluted with 30 mg Z-L-Ala-L-Cys(H)-OMe which was reisolated, after purification by prep HPLC and crystallization to constant activity.

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