Peptide Synthesis by Prior Thiol Capture. 4. Amide Bond Formation: The Effect of a Side-Chain Substituent on the Rates of Intramolecular O,N-Acyl Transfer

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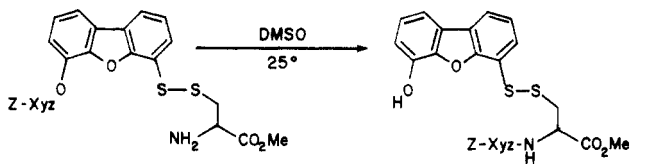
Received February 11, 1986

The effects of varying steric bulk of the side chain substituent of the acylating agent on the rate of the amide bond forming step of the dibenzofuran-based thiol capture strategy were determined from rates of intramolecular isomerization of methyl *S*-[4-(*N*-benzyloxycarbonyl-L-aminoacyloxy)-6-dibenzofuranylsulfenyl]-L-cysteinates to methyl (*N*-benzyloxycarbonyl-L-aminoacyl)-*S*-(4-hydroxy-6-dibenzofuranylsulfenyl)-L-cysteinates. The following aminoacyl derivatives were studied: Ala, Asn, Asp, Arg, Gly, Leu, Lys, Pro, Thr, and Val. Half times in Me_2SO at 25 °C of 2–4 h were observed for all cases except for Pro and Val, which are roughly an order of magnitude slower, and for Asp, which shows evidence of intramolecular general base catalysis by the neighboring carboxylate group. A steric rationalization for the anomalously slow proline transfer rate is proposed.

In earlier papers in this series we have reported convenient routes to medium-sized polypeptides linked as 4-acyloxy derivatives to a 6-mercaptodibenzofuran[1] and have demonstrated the efficiency of intramolecular O,N-acyl transfer from an unactivated phenolic ester to the amino function of a cysteine derivative, across a disulfide-bridged dibenzofuran template, 1 → 2.[2] In communications we have set an upper limit of 0.1–0.2% on the racemization or epimerization that occurs at the α-carbon of the acyl derivative during these processes,[3] and we have set boundaries on the extent of disulfide interchange that occurs during the acyl transfer step in Me_2SO.[4] In a manuscript in preparation, solvent and polarity effects on rates and yields for formation of the disulfide bond of 1 are described, and applications of the thiol capture strategy to practical peptide synthesis are reported. In this paper we focus on the effect on the rate of the acyl transfer 1 → 2 of varying the structure of the side chain substituent of the acylating agent.



1 2

Earlier we have reported acyl transfer experiments for dibenzofuran-linked phenolic esters involving L-cysteine methyl ester as captive nucleophile and acetyl or carbobenzoxy-L-alaninyl as acyl species. These experiments established pronounced catalysis by Me_2SO, which is the preferred solvent, a large rate-accelerating effect of electron-withdrawing 1-substituents, and a range of effective molarities of the amine nitrogen at the acyloxy carbon from 3 to 9 M, with values for the preparatively useful 1-H and 1-Cl derivatives of 4.6 and 5.7 M, respectively. It remained to define the scope of side chain steric and electronic effects for the rate of the acyl transfer reaction 1 → 2.

Generally speaking, in the more polar aprotic solvents like Me_2SO and with unassociated peptide fragments the rate of peptide bond formation is determined largely by the two substituents that neighbor the new bond, and the reactions that form dipeptides are therefore legitimate models for the entire class of peptide coupling reactions.[5,6] Steric effects of rates of dipeptide formation have been studied for a variety of phenyl esters,[5,7] and with these the significant rate-retarding effect at the acyl site is β-branching. The amino acids valine and isoleucine that are β-branched are more than an order of magnitude less reactive than the unbranched derivatives. Analogous data for the novel intramolecular acyl transfer reaction 1 → 2 are given in Table I.

Table I. Rates of Intramolecular O,N-Acyl Transfer 1 → 2 (Me_2SO, 25 °C)

subst Xyz	10^3k_2, min^{-1}	$t_{1/2}$, h
L-Ala-Gly	6.0	2.0
L-Ala	3.8	3.0
L-Leu	3.0	4.0
L-Pro	0.34	34
L-Val	0.23	51
L-Lys(ᵉZ)	3.6	3.2
L-Asn	3.7	3.1
L-Asn(Mbh)[a]	4.8	2.4
L-Asp(ᵝ*t*-Bu)	10.3	1.1
L-Asp	~100	~0.1
L-Arg(ᵍAns)[b]	6.4	1.8
L-Thr(O-*t*-Bu)[c]	4.5	2.5

[a] Mbh ≡ *p,p'*-dimethoxybenzhydryl. [b] Ans = 9-anthracenesulfonyl; half time for *N*-Boc-Arg(Ans) = 2.3. [c] Estimated from HPLC rate data for the isomerization of *S*-(Boc-Cys(Acm)-Met-Arg(Ans)-Thr(O-*t*-Bu)-O-DBF-S)-H-Cys-Gly-Gly-Ala-OH.

With the exception of data for proline, valine, and the aspartic acid derivatives, the half lives of the table are grouped about a value of ca. 3 h, and from experience with other amino acid esters one can likely generalize this result to include 17 of the 20 amino acid residues. A more quantitative comparison with intermolecular aminolysis is given in Figure 1 in which the logarithms of the rate constants of Table I are plotted as functions of the logarithm of the analogous rate constant for the coupling of the *p*-nitrophenyl ester Z-L-Xyz-ONp with H-L-Ala-OMe in DMF.[5] The slope of the resulting line is 1.02, and in five of the six cases, the correlation is good, indicating that

(1) Kemp, D. S.; Galakatos, N. G. *J. Org. Chem.* **1986**, *51*, 1821. In press; Kemp, D. S.; Leung, S.-L.; Kerkman, D.; Galakatos, N. In *Peptides—1980*; Brunfeldt, K., Ed.; Scriptor: Copenhagen, 1981; pp 191–195.
(2) Kemp, D. S.; Galakatos, N. G.; Bowen, B.; Tan, K. *J. Org. Chem.* **1986**, *51*, 1829.
(3) Kemp, D. S.; McBride, B., submitted for publication.
(4) Kemp, D. S.; Fotouhi, N., submitted for publication.
(5) Kemp, D.; Choong, S.-L.; Pekaar, J. *J. Org. Chem.* **1974**, *39*, 3841.
(6) Kemp, D. S.; Bernstein, Z.; McNeil, G. *J. Org. Chem.* **1974**, *39*, 2831.
(7) Khurgin, Y. I.; Dimtrieva, M. G. *Tetrahedron* **1965**, *21*, 2305. Pless, J.; Boissonnas, R. A. *Helv. Chim. Acta* **1963**, *48*, 1609.

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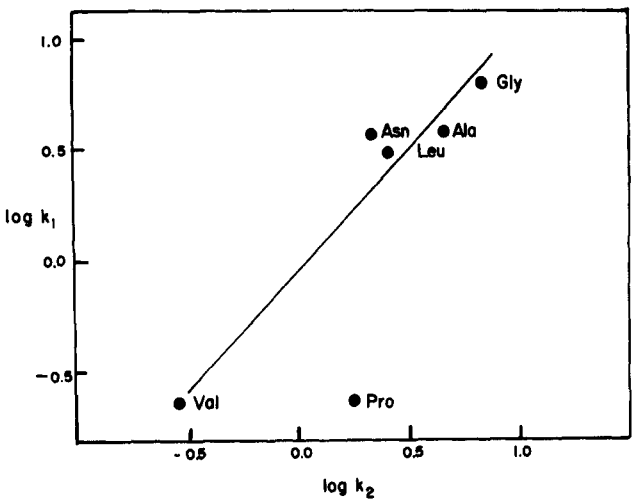


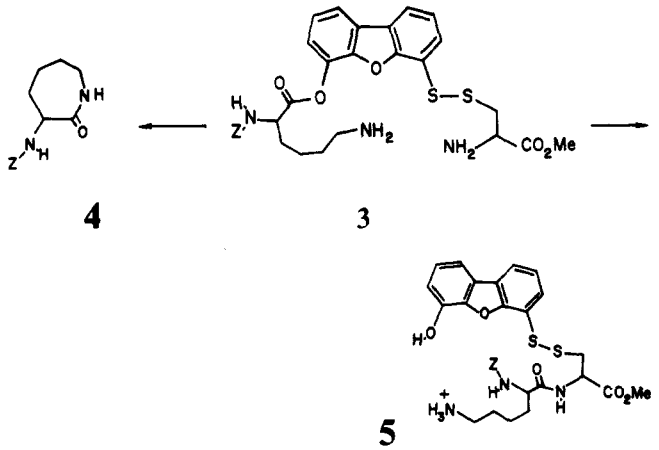
Figure 1. Relative steric sensitivities of intramolecular and intermolecular aminolysis reactions. k_1 is the rate constant for the reaction 1 → 2, 25 °C, Me_2SO; k_2 is the rate constant for the reaction Z-Xyz-ONp + H-Ala-OMe → Z-Xyz-Ala-OMe + HONp, 30 °C, DMF, where ONp is the *p*-nitrophenyl ester.

a nearly identical steric sensitivity is seen for the two reactions.

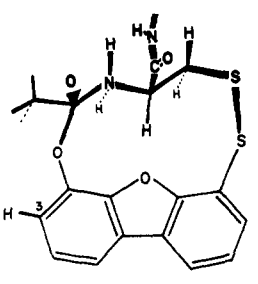
The point for proline is seen to show a large negative deviation, and in fact for the reaction 1 → 2 for Xyz = Pro, the rate is 5.8 times slower than would be expected from intermolecular reactions of simple proline activated esters. For the intramolecular transfer reaction there are clearly three cases, Val, Pro, and by analogy, Ile for which the rate is expected to be slow and the use of a more highly activated acyl derivative may be appropriate.[2] Strikingly, opposing inductive and steric effects render threonine derivatives as reactive as the simple amino acids that lack β-branches.

Rapid acyl transfer and freedom from intramolecular side reactions are of special concern for those amino acids that bear nucleophilic side chain groups five or six atoms removed from the acyl carbon.[8] We have not studied the most nucleophilic of these cases, histidine, which will almost certainly require imidazole-blocking. The remaining cases are aspartic acid, asparagine, and arginine, which are found to give clean intramolecular transfer, 1 → 2, and except for aspartic acid derivatives to react at rates close to the average, as seen in Table I. Not surprisingly ε-Z-lysine behaves unexceptionally as well.

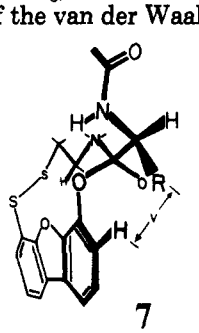
Since neighboring group participation to generate seven-membered rings is generally much less facile than that observed with five- or six-membered cases,[9] it was of interest to examine the dibenzofuranyl ester **3**, which lacks a side chain protective group on the lysine ε-amino function. Two amino groups can thus compete in this molecule for the weakly activated phenolic ester function. When the bis(hydrochloride) salt of **3** (0.005 M in Me_2SO) is treated with 2 equivalents of triethylamine, the ε-lactam of N^{α}-Z-L-lysine **4** was formed in 90% yield. Treatment with 1 equiv of base results in the formation of a 2:1 mixture of lactam and the product of O,N-acyl transfer, the unsymmetrical disulfide of 4-hydroxy-6-mercaptodibenzofuran and Z-L-Lys-L-CysOMe **5**. In this case the greater effective local concentration and higher intrinsic nucleophilicity of the lysine ε-amino group is nearly balanced by the higher concentration of the more weakly basic cysteine α-amino group. This result shows that the intramolecular O,N-acyl transfer across a twelve-membered ring that was refined by a rational design process can be nearly competitive with a more conventional cyclization to form a seven-membered lactam. It also demonstrates the need to protect the ε-amino groups of lysine residues that are to be linked to cysteines by thiol capture.



The anomalously slow acyl transfer rate for 1 → 2 where Xyz = proline deserves further comment. A useful perspective for rationalizing this result begins with the geometry **6** previously proposed[2] for the intramolecular acyl transfer reaction. From **6** for example it is apparent that

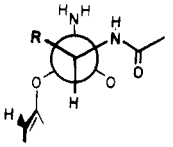


existence of an unstrained ring requires that the *S* configuration at the L-cysteine α-carbon induces an *R* configuration at the acyl carbon, at which chirality is defined in the transition state and tetrahedral intermediate by the relative dispositions of the C–O^- and C–OAr bonds. It is also apparent that the dihedral angle at the Ar–O bond is constrained to roughly 90°, with the result that the dibenzofuran 3-hydrogen intrudes into the space normally dominated by the groups attached to the α-carbon of the acyl function, as seen more readily in **7**. From models one can estimate the H–H distance V of **7** as 2.9 Å for R = H and ca. 2.1 Å for R = CH_3, or in the latter case well within the repulsive part of the van der Waals potential curve.[10]



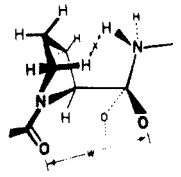
For acyl amino acid derivatives other than proline the major effect of interactions of the 3-dibenzofurano hydrogen is a favoring of one staggered rotomer about the α-C–C acyl bond, shown in Newman projection in **8**. For

(8) Inman, J. K. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1981; Vol. 3, pp 260–278.
(9) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183.
(10) Allinger, N. L. *Adv. Phys. Org. Chem.* **1976**, *13*, 1.

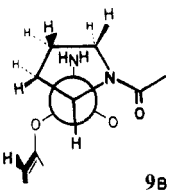


8

proline derivatives undergoing the reaction 1 → 2, the conformational population about the α-C–C acyl bond appears to offer no unstrained choice. One effect of the introduction of the pyrrolidine ring is to constrain the Ramachandran dihedral angle φ to the range of ca. −60°,[11] which has the effect of creating severe steric interactions between C–O⁻ and C=O oxygens (distance *W* of **9**, estimated at 2.9 Å) and CH and NH hydrogens (distance *X* of **9**, estimated at 1.5 Å). Changes in conformation of the pyrrolidine ring lessen but do not remove these destabilizing contacts.

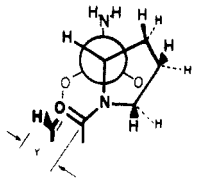


9A



9B

By contrast, rotamers **10** and **11** encounter the special constraint of the 3-dibenzofuran hydrogen (distance *Y* of **10** is ca. 2.5 Å; distance *Z* of **11** is in the range of 1.5–1.8 Å). Effectively, the dual rigidities of the aryl ester and pyrrolidine groupings conspire to retard acyl transfer in the proline case.



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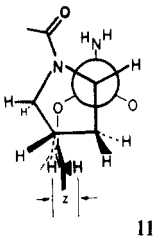
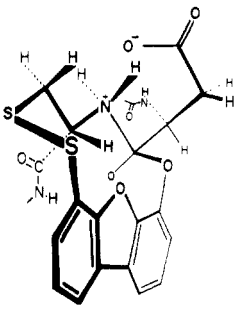


11

The extraordinarily rapid acyl transfer reaction that is seen when the side chain carboxyl group of aspartic acid is unprotected is almost certainly the result of the formation of an intramolecular salt bridge and/or the formation of a strong intramolecular hydrogen bond. As we have noted previously,[2] the large solvent effect that is seen for the general reaction 1 → 2 (Me_2SO > DMF > MeCN) is consistent with the appearance in the transition state of a solvent molecule that is strongly hydrogen bonded to the NH^+ function and that assists the transfer of a proton from nitrogen to the phenolic oxygen. Substitution of $CH_2CO_2^-$ for the R group of **8** generates a structure **12** in which the required hydrogen bond can be generated internally, and at least formally, **12** exemplifies a principle of salt-bridge assistance that has been recently suggested by Young.[17] The rate acceleration that is seen for the Asp case challenges us to redesign the framework of the acyl-transfer template to incorporate a hydrogen bonding site within the general framework of the dibenzofuran or other acyl-transfer template. Casual inspection of structures **6** and **7** shows that such redesign is a nontrivial objective. Work along these lines is in progress and will be reported subsequently.

(11) Karle, I. L. In *The Peptides*; Gross, E.; Meienhofer, J., Eds.; Academic: New York, 1981; Vol. 4, pp 36–46. Creighton, T. E. *Proteins*; W.H. Freeman and Co.: New York, 1984, p 164.



12

Summary. With the exception of aspartic acid and its esters, the β-branched amino acid derivative valine (and by analogy isoleucine) and the cyclic imino acid proline, the natural amino acids have been shown directly or by analogy to undergo intramolecular O,N-acyl transfer 1 → 2 with half times in the range of 2–4 h in Me_2SO at 25 °C, which is suitable for synthetic work. The sluggish reactivity of Val and Ile is consistent with the hindered character of these amino acids and is in accord with their behavior in intermolecular coupling reactions. The behavior of Pro appears to be unique to the reaction 1 → 2 and can be rationalized by an examination of the steric environment of the probable transition state for the reaction. If Xyz of **1** is an aspartic acid residue bearing a free carboxylate function, the rate of acyl transfer is at least 10 times faster than expected on simple electronic grounds, and this finding can be rationalized in terms of intramolecular assistance of the N,O proton transfer that must accompany reaction in an aprotic solvent.

Experimental Section

IR spectra were recorded on a Perkin-Elmer Model 283-B spectrometer. High-resolution ^1H NMR and ^{13}C NMR spectra were obtained on either a Bruker WM-250 or a Bruker WM-270 instrument. Chemical shifts are reported in ppm downfield from Me_4Si and splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Low-resolution, high-resolution, and field-desorption mass spectra were recorded on Varian MAT-44, Varian CEC-110, and Finnigan MAT-731 mass spectrometers, respectively. UV spectra were taken on a Perkin-Elmer Model 330 UV–Vis spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

Analytical thin-layer chromatography was performed on glass precoated silica gel 60 plates (Merck F-254) using solvent systems (A) $CHCl_3$–EtOAc (9:1) or (B) neat CH_2Cl_2. Compounds were visualized by UV absorption (254 nm), phosphomolybdic acid, 1% ninhydrin in a 9:1 EtOH–CF_3CO_2H mixture (primary and secondary amines). Preparative layer chromatography was performed on Analtech GF 1000-μm and GF 2000-μm silica gel plates and flash chromatography on silica gel 60 (230–400 mesh) using 100% CH_2Cl_2 as eluent.

HPLC was performed on a Waters system consisting of two Model 6000-A pumps, a model 680 automated gradient controller, a model U6K injector, a Model 440 dual channel UV detector (280, 254 nm), an extended wavelength module (229, 214 nm), and a Model 730 data module. HPLC runs were conducted in the reverse-phase mode on Whatman Partisil columns.

Tetrahydrofuran and *p*-dioxane were obtained dry and peroxide-free by distillation from sodium benzophenone ketyl. Dimethylformamide (DMF) was dried over molecular sieves (Linde 4A), then distilled from ninhydrin in vacuum, and stored in brown bottles at 4 °C over sieves. Trifluoroacetic acid was fractionally distilled from P_2O_5 and then redistilled from anhydrous L-valine. Reagent grade CH_2Cl_2, $CHCl_3$, and CH_3CN were dried over molecular sieves (Linde 4A); methanol was dried over Linde 3A sieves. Methyl *N*-*tert*-butoxycarbonyl-*S*-(methoxycarbonylthio)-L-cysteinate (Boc-L-Cys(Scm)-OMe) was prepared by the procedure of Hiskey et al.[12] and recrystallized from hexane,

mp 76–78 °C (lit. 78 °C). Triethylamine (TEA) and diisopropylethylamine (DIEA) were distilled first from ninhydrin and then from sodium and stored in sealed ampules at −20 °C; tri-*n*-butylphosphine (PBu_3) and dicyclohexylcarbodiimide (DCC) were fractionally distilled in vacuo and stored under N_2 at 4 °C.

Preparation of 6-(Methoxycarbonyldithio)-4-(benzyloxycarbonylaminoacyloxy)dibenzofurans [4-(Z-L-Xyz-O)-6-DBF-S-Scm]. As reported previously[1] a mixture of 1 equiv each of 6-(methoxycarbonyldithio)-6-dibenzofuranol,[1] dicyclohexylcarbodiimide, and Z-L-Xyz-OH was contained in a suitable solvent such as dichloromethane, dioxane, tetrahydrofuran, or chloroform. After 12 h, the mixture was filtered, and the filtrate was extracted with aqueous $KHSO_4$, $NaHCO_3$, and water, then dried, and evaporated. The residue was crystallized directly or purified by preparative layer chromatography. The following *N*-benzyloxycarbonyl amino acids were esterified in this manner.

L-Ala: mp 130–131 °C, 70%, from benzene–hexane. Anal. Calcd for $C_{25}H_{21}NS_2O_7$: C, 58.70; H, 4.14; N, 2.74; S, 12.54. Found: C, 59.58; H, 4.56; N, 2.81; S, 12.09.

L-Val: mp 127–128 °C, 66%, from ethyl acetate–hexane. Anal. Calcd for $C_{27}H_{25}NS_2O_7$: C, 60.10; H, 4.67; S, 11.88. Found: C, 59.94; H, 4.77; S, 11.52.

L-Lys (^{t}Boc): mp 114–118 °C, 73%, from chloroform–hexane.

L-Lys(^{t}Z): mp 138–140 °C, 48%, from THF–hexane. Anal. Calcd for $C_{36}H_{34}N_2S_2O_9$: C, 61.52; H, 4.87; N, 3.98; S, 9.13. Found: C, 61.60; H, 4.94; N, 3.94; S, 9.15.

L-Asn(mbh) ≡ N^β-(4,4′-dimethoxybenzhydryl)-L-asparagine: mp 186–188 °C, 42%, from THF–petroleum ether. Anal. Calcd for $C_{40}H_{36}N_2S_2O_9$: C, 64.06; H, 4.65; N, 3.59; S, 8.31. Found: C, 64.09; H, 4.94; N, 3.64; S, 7.94.

L-Asn (by trifluoroacetic acid treatment of the above substance): 120 °C dec, 76%.

L-Ala-Gly: mp 164–165 °C, 61%, from ethyl acetate–cyclohexane. Anal. Calcd for $C_{27}H_{24}N_2S_2O_8$: C, 57.03; H, 4.25; N, 4.92; S, 11.28. Found: C, 56.99; H, 4.31; N, 4.95; S, 11.30.

L-Leu: mp 126–127 °C, 75%, after PLC and hexane trituration.

L-Pro: mp 58–61 °C, 50% after flash chromatography. Anal. Calcd for $C_{27}H_{23}NO_7S_2$: C, 60.32; H, 4.31; N, 2.61; S, 11.93. Found: C, 60.19; H, 4.58; N, 2.54; S, 11.73.

L-Asp(O-*t*-Bu): mp 92–93 °C, after flash chromatography and crystallization from ethyl acetate–hexane. Anal. Calcd for $C_{30}H_{29}NO_9S_2$: C, 58.91; H, 4.78; N, 2.29; S, 10.48. Found: C, 58.81; H, 4.92; N, 2.27; S, 10.58.

General Procedure for the Preparation of Methyl *S*-[4-(*N*-Benzyloxycarbonyl-L-aminoacyloxy)-6-dibenzofuranylthio]-L-cysteinates 1. As previously reported,[2] cleavage of the methoxycarbonyldithio function to a thiol was carried out by treatment of an above-prepared 6-(methoxycarbonyldithio)-4-(benzyloxycarbonylaminoacyloxy)dibenzofuran in dioxane–water or hexafluoroisopropyl alcohol–water mixture with an equivalent of tri-*n*-butylphosphine. Lyophilization yielded the 4-(*N*-benzyloxycarbonylaminoacyloxy)-6-dibenzofuranthiol, which was usually combined without purification with an equivalent of Boc-L-Cys(Scm)-OMe[12] in a water–hexafluoroisopropyl alcohol–acetonitrile mixture to yield the *N*-Boc-Cys derivative of 1, which could usually be purified by preparative layer chromatography but which was occasionally used without purification, after evaporation of the solvent. The *N*-Boc-Cys derivative of 1, Xyz = Arg(Ans) was more conveniently prepared by reaction of the resin-linked unsymmetrical disulfide[1] of 4-hydroxy-6-mercaptodibenzofuran with Z-Arg(Ans)-OH (6 equiv) and DCC (5 equiv) in dichloromethane containing 4-(*N*,*N*-dimethylamino)pyridine (0.2 equiv). After the standard washing steps,[1] the 4-(acyloxy)-6-mercaptodibenzofuran was liberated by treatment with triethylphosphine (2 equiv) in dichloromethane–hexafluoroisopropyl alcohol (5:1) for 5 min; reaction with Boc-L-Cys(Scm)-OMe was carried out as described above.

Dissolution of the *N*-Boc-Cys derivatives of 1 in trifluoroacetic acid for 1 h at 0 °C followed by evaporation to dryness, evaporation with two portions of toluene, and trituration of the resulting oil with ether yielded the trifluoroacetate salt of 1 as a white powder. In the case of 1, Xyz = Asp(O-*t*-Bu), the salt was best prepared directly from the thiol by reaction with 1.2 equiv of $Cl^-H_2{}^+$Cys(Scm)OMe in HFIP; 1, Xyz = Asp, was prepared by treatment of the resulting salt with TFA for 30 min at 0 °C, followed by evaporation.

Rate Determination for the Intramolecular Acyl-Transfer Reaction: 1 → 2. Freshly prepared trifluoroacetate salts of 1 were dissolved in Me_2SO-d_6 and treated with an equivalent of triethylamine (concentrations ranging from 3.3×10^{-2} to 2.1×10^{-3} *M*). The reaction was followed by 250-MHz ^{1}H NMR at ambient temperature (25 °C). To initiate a run, a sample of the TFA salt was weighed in an NMR tube and then dissolved in a measured volume of deuterated solvent, and the appropriate amount of triethylamine was added. The tube was shaken to effect complete solution of the reactants, and the reaction was monitored by taking ^{1}H NMR spectra at regular intervals. Time zero was taken at the point when the amine base was added.

The half-time for the reaction was defined as the time when the integration of the dibenzofuran C_7-H (δ 7.0) was equivalent to one-quarter of the integration of the benzylic protons of the benzyloxycarbonyl group (δ 4.9–5.1). Portions of the NMR spectra of the Pro derivatives of 1 and 2 were difficult to resolve, owing to peak doubling attributable to urethane rotomers. For this reaction, the half-time was defined as the time when the integration of the cysteine amide NH (δ 8.5) was equivalent to one-quarter of the integration of the benzylic protons of the benzyloxycarbonyl group.

Reaction yields were calculated at t = 40 h according to (1) % yield = 2(integration of C_7H)/(integration of Bzl protons).

In most cases identity of product was established by recovery of product by evaporation, treatment with tri-*n*-butylphosphine or dithiothreitol to liberate 4-hydroxy-6-mercaptodibenzofuran and Z-L-Xyz-L-Cys-OMe and characterization of the latter by comparison with an authentic sample. In the case of 1, Xyz = Asp, in addition to normal product characterization, the HPLC trace of the reaction mixture was examined for presence of the cyclic anhydride of Z-Asp-OH; no trace of this substance was found.

4-(*N*-Benzyloxycarbonyl-L-alanyloxy)-6-dibenzofuranthiol. To a suspension of 92.4 mg (0.181 mmol) of 4-(*N*-benzyloxycarbonyl-L-alanyloxy)-6-(methoxycarbonyldithio)dibenzofuran[1] in 1.4 mL of 4:1 dioxane–water was added under N_2 46 μL (0.185 mmol) of tri-*n*-butylphosphine, and the mixture was warmed until all solid had dissolved, then cooled, and lyophilized. The resulting residue (117 mg) was triturated with 3.5 mL of cold acetonitrile, filtered, washed with 2 × 0.5 mL of acetonitrile, and dried to yield 47 mg of white crystals; 62%; mp 158–160 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.75 (3 H, d, J = 7 Hz), 4.03 (1 H, s), 4.76–4.90 (1 H, m), 5.12–5.24 (2 H, m), 5.42 (1 H, br d, J = 8 Hz), 7.23–7.45 (7 H, m), 7.74 (1 H, dd, J = 8.1 Hz), 7.83 (1 H, dd, J = 8 Hz).

Anal. Calcd for $C_{23}H_{19}O_5NS$: C, 65.54; H, 4.54; N, 3.32; S, 7.61. Found: C, 65.70; H, 4.73; N, 3.27; S, 7.41.

Methyl *N*-*tert*-Butoxycarbonyl-*S*-[6-(4-benzyloxycarbonyl-L-alanyloxy)dibenzofuranylthio]-L-cysteinate and Trifluoroacetate Salt of Methyl *S*-[6-(4-Benzyloxycarbonyl-L-alanyloxy)dibenzofuranylthio]-L-cysteinate (1B). A solution of 33.4 mg (79.8 μmol) of the above-prepared thiol in 1.5 mL of 14:1 hexafluoroisopropyl alcohol–chloroform was added 26.1 mg (80.3 μmol) Boc-L-Cys(Scm)-OMe in 0.7 mL of hexafluoroisopropyl alcohol. After 0.5 h of being stirred under nitrogen, the solvent was evaporated and the residue dried to yield 82.6 mg of solid, purified by preparative layer chromatography (1000 μm; eluent, 9:1 $CHCl_3$–EtOAc) to give 46.7 mg of solid: 90% mp 133–135 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.40 (9 H, s), 1.80 (3 H, d, J = Hz), 3.67 (3 H, s), 5.18 (2 H, m), 7.28–7.42 (8 H, m), 7.70 (1 H, dd, J = 8.1 Hz), 7.83 (1 H, dd, J = 8.1 Hz), 7.92 (1 H, dd, J = 8 Hz, 1 HO).

Anal. Calcd for $C_{32}H_{34}N_2O_9S_2$: C, 58.70; H, 5.23; N, 4.28. Found: C, 58.68; H, 5.41; N, 4.17.

Treatment of 27.9 mg of the above product with 0.6 mL trifluoroacetic acid for 1 h at 0 °C followed by evaporation, distillation three times with toluene in vacuum, drying, and trituration with 1:1 ether–petroleum ether gave 1B as its trifluoroacetate salt: 23 mg, 81%; mp 85–88 °C; ^{1}H NMR (270 MHz, Me_2SO-d_6) δ 1.57 (3 H, d), 3.67 (3 H, s), 5.07(2 H, s), 7.14–7.56 (8 H, m), 7.78 (1 H, d, J = 8 Hz), 8.18 (2 H, m), 8.22 (1 H, d, J

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= 8 Hz); field desorption mass spectrum, m/e 555 (M^+ – Tfa). Substitution of HCl in dioxane for TFA gave the HCl salt, mp 170 °C dec.

Product Characterization: Identification of Z-L-Ala-L-Cys-OMe after Completion of the O,N-Acyl Transfer 1B → 2B. In an NMR tube was dissolved 16.7 mg (28.3 μmol) of the above-prepared HCl salt dissolved in 0.4 mL of Me_2SO-d_6 [0.07 M salt] and treated with 4.0 μL of triethylamine. As O,N-acyl transfer occurred at 25 °C, the benzyl methylene resonance in the ^1H NMR spectrum shifted from δ 5.12 to 5.03, and a signal at δ 7.07 attributable to a proton ortho to a free phenol appeared. At 30 h the ratio of areas of the δ 7.07 and 5.12 + 5.03 peaks had reached the expected value of 0.5.

The solution was taken up in 20 mL of ethyl acetate, washed with 5% $KHSO_4$ (2 × 5 mL), water (2 × 5 mL), dried, filtered, and evaporated. The resulting oil was taken up in 0.3 mL of methanol and treated with 8 mg of dithiothreitol (52 μmol) and 2 μL of 1 N KOH for 3 h at 25 °C. Analytical thin-layer chromatography showed two major spots corresponding to 4-hydroxy-6-dibenzofuranthiol and Z-L-Ala-L-Cys-OMe, identical R_f with that of an authentic sample.[13]

σ-L-(*N*-Benzyloxycarbonylamino)caprolactam (4). Following the procedure of Boyle et al.,[14] 201 mg (1.57 mmol) of α-L-aminocaprolactam was prepared from L-lysine methyl ester hydrochloride and dissolved in 7 mL of water. Sodium bicarbonate was added to bring the pH to 8, and the solution was cooled as 300 μL (2.1 mmol) of benzyloxycarbonyl chloride was added dropwise. After 3 h the mixture was extracted with 3 × 20 mL of ethyl acetate, and the combined extracts were washed as follows: 10 mL of water, 2 × 10 mL of 5% $KHSO_4$, water, and brine. Then the mixture was dried and evaporation. The residue was recrystallized from benzene–hexane to yield 113 mg (27%) of fluffy crystals: mp 145–148 °C; ^1H NMR (270 MHz, Me_2SO-d_6) δ 1.2–1.8 (m, 6 H), 3.13 (m, 2 H), 5.02 (s, 2 H), 7.03 (d, 1 H), 7.35 (s, 5 H), 7.78 (m, 1 H).

Anal. Calcd for $C_{14}H_{18}O_3N_2$: C, 64.11; H, 6.92; N, 10.67. Found: C, 64.17; H, 6.98; N, 10.69.

Dimethyl Bis[N^{α},N^{ϵ}-bis(benzyloxycarbonyl)-L-lysyl]-LL-cystinate. A solution of 108 mg (316 μmol) of LL-cystine dimethyl ester dihydrochloride[15] and 70 μL of *N*-methylmorpholine (630 μmol) were combined in 1.5 mL of DMF to which 327 mg (640 μmol) of Z-L-Lys(ᵉZ)-OSu (freshly prepared from Z_2-L-Lys-OH, *N*-hydroxysuccinimide, and dicyclohexylcarbodiimide) was added. After 14 h at 25 °C, the mixture was diluted with 50 mL of ethyl acetate and 10 mL of 5% $KHSO_4$, the layers were separated, and the organic phase was washed with 5% $KHSO_4$, 5% $NaHCO_3$, and water. The solid obtained after drying and evaporation was recrystallized from tetrahdyrofuran–hexane to give 220 mg of a white powder, mp 165–170 °C.

Anal. Calcd for $C_{52}H_{64}N_6S_2O_{14}$: C, 58.85; H, 6.08; N, 7.92; S, 6.04. Found: C, 58.80; H, 6.01; N, 7.83; S, 6.16.

Methyl *N*-Benzyloxycarbonyl-L-alanylglycyl-L-cysteinate. A. From the Cystine Derivative. To a solution of 194 mg (0.57 mmol) LL-cystine dimethyl ester dihydrochloride[15] in 4.8 mL dimethylformamide was added 1 equiv of triethylamine at 0 °C. To the resulting suspension were added 339 mg Z-L-Ala-Gly-OH[16] (1.21 mmol), 280 mg of 1-hydroxybenzotriazole (1.83 mmol), and 311 mg of dicyclohexylcarbodiimide (1.51 mmol). After 15 min at 0 °C and 24 h at 25 °C, the suspension was cooled, treated with 0.15 mL of 50% aqueous acetic acid for 10 min, filtered, and evaporated. The residue was dissolved in 5 mL of ethyl acetate, cooled to 0 °C, and filtered, and the filtrate was washed: 5% $KHSO_4$, 5% $NaHCO_3$, water, and brine. Drying and evaporation followed by recrystallization from ethyl acetate gave *N*,*N*′-bis-(benzyloxycarbonyl-L-alanylglycyl)-LL-cystine dimethyl ester, 257 mg, 57%, mp 123–125 °C. Reduction with dithiothreitol in methanol (2 h, KOH catalyst) gave 76% of the title compound, mp 106–108 °C.

B. From 2A. Acylation of 6-hydroxy-4-dibenzofuranyl disulfide with Z-L-Ala-Gly-OH by a procedure analogous to that described in A gave 6-(*N*-benzyloxycarbonyl-L-alanylglycyl-oxy)-4-dibenzofuranyl disulfide, 39%, mp 188–190 °C. Reduction of the disulfide linkage by dithiothreitol as described in A gave 6-(Z-L-Ala-Gly-O)-DBF-4-SH, 73%, mp 134–136.5°. To a solution of 62 mg (0.19 mmol) of this substance in 1 mL of 2:1 methanol–DMF was added a solution of 57 mg (0.2 mmol) of Boc-L-Cys-(Scm)-OMe in 1 mL of methanol. After 2 h the solution was poured into 100 mL of 5% $KHSO_4$, and the precipitate was washed, dried, and purified by preparative layer chromatography (2000 μm; $CHCl_3$–EtOAc). Trituration with ether–petroleum ether gave the *N*-Boc derivative of **1A**: 30 mg, 22%; mmp 153–155 °C; field desorption mass spectrum, m/e 712 (M^+). Treatment with dry hydrogen chloride in dioxane for 20 min followed by lyophilization and drying in vacuum gave the hydrogen chloride salt of **1A**, methyl *S*-(6-benzyloxycarbonyl-L-alanylglycyloxy-4-dibenzofuranylthio)-L-cysteinate. Neutralization of this salt by partitioning between ice-cold 1% aqueous potassium carbonate and chloroform followed by separation, drying, and evaporation gave **1A** as an oil in 84% yield.

A solution of 8.0 mg (13 μmol) of **1A** in 0.4 mL of Me_2SO-d_6 was evaporated in high vacuum after 40 h at 25 °C. The residue (**2A**) was dissolved in 0.6 mL of methanol containing 2.2 mg of dithiothreitol (14 μmol) and 1 μL/N KOH. After 2 h at 25 °C the solution was added to 0.1 N HCl at 0 °C and extracted with dichloromethane. The extracts were washed with water, dried, and evaporated. The resulting 8.5 mg of residue was purified by preparative layer chromatography (1000 μm, 98:10:2 chloroform–methanol–acetic acid) to yield pure Z-L-Ala-Gly-L-Cys-OMe, 3.9 mg, 76%, mp 104–107 °C, identical by TLC, ^1H NMR, and mixture mp with the sample prepared in A: ^1H NMR (250 MHz, Me_2SO-d_6) δ 1.22 (3 H, d, J = 7 Hz), 3.65 (3 H, s), 3.76 (2 H, m), 5.0 (2 H, m), 7.36 (5 H, br s); mass spectrum (70 eV), m/e 397 (M^+).

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