Peptide Synthesis by Prior Thiol Capture. 1. A Convenient Synthesis of 4-Hydroxy-6-mercaptodibenzofuran and Novel Solid-Phase Synthesis of Peptide-Derived 4-(Acyloxy)-6-mercaptodibenzofurans†

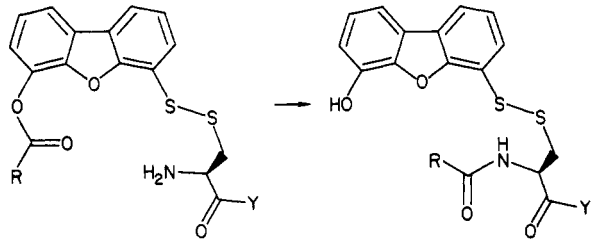
D. S. Kemp* and Nicholas George Galakatos

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received March 26, 1985

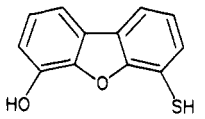
Peptide bond formation by intramolecular acyl transfer across a rigid template is outlined. The template precursor 6-hydroxy-4-mercaptodibenzofuran (3) is obtained from 4-methoxydibenzofuran by metalation with *n*-butyllithium, oxidation with elemental sulfur, and demethylation. Routes to 4-(acyloxy)-6-mercaptodibenzofurans 4 are reported. Esters 4 derived from simple urethane N-blocked α-amino acids are conveniently prepared by direct acylation of 6-hydroxy-4-(methoxycarbonyldithio)dibenzofuran (12), followed by reduction with tri-*n*-butylphosphine. Esters 4 derived from the C-terminal carboxyl groups of peptides are prepared by a novel variant of conventional solid-phase peptide synthesis in which the chain elongation steps are carried out on the 4-((α-aminoacyl)oxy)-6-dibenzofuranyldithio function linked through the disulfide to a polymeric support. At the completion of the elongation steps, release of 4 from the resin is achieved by reduction of the disulfide bond with tri-*n*-butylphosphine.

Conventional amide bond-forming reagents are frequently unreliable when applied to the convergent coupling of large peptide fragments.[1] During the past decade this problem has prompted us to seek an unconventional coupling method in which amide bond formation occurs intramolecularly and is preceded by a covalent capture step, which in our thiol capture strategy involves the sulfur atom of a peptide bearing an N-terminal cysteine residue.[2] In our most promising tactic the capture step generates a disulfide of structure 1 which undergoes intramolecular O,N-acyl transfer to generate the amide 2.



1 2

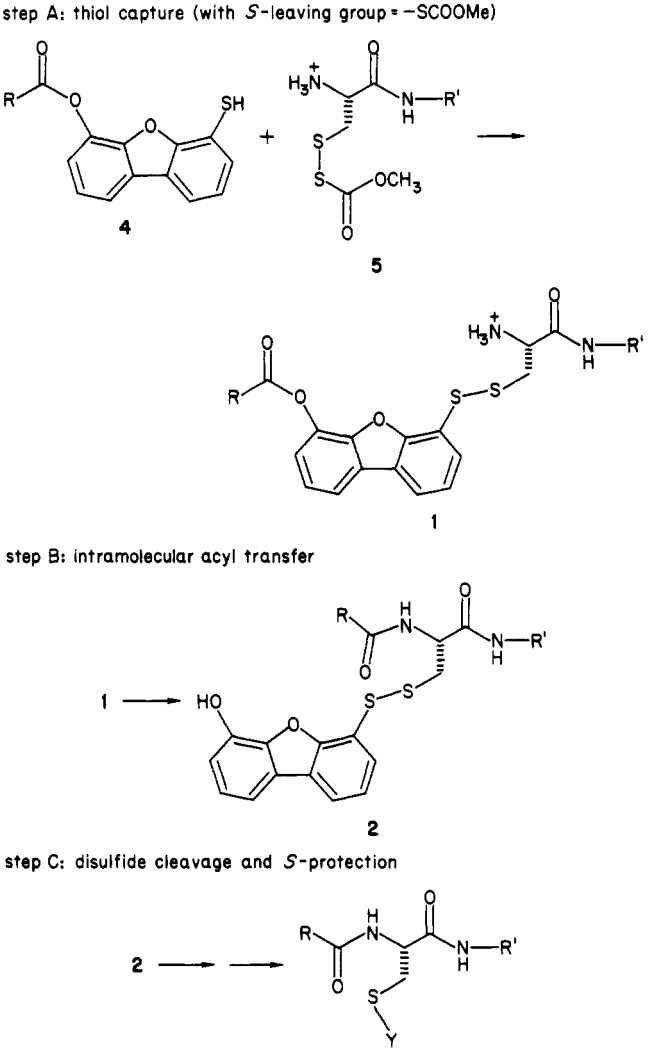
This is the first of a series of detailed reports on the thiol capture strategy. In it we describe a practical synthesis of 6-hydroxy-4-mercaptodibenzofuran (3) as well as a novel form of solid-phase peptide synthesis that permits ready generation of medium-sized polypeptides functionalized at their C-termini as phenolic esters of 3. As seen in Scheme I these are the natural precursors of disulfides 1.



3

The rationale,[2] major features,[2] and precedents[3] for thiol capture have been reported previously as was the design principle[4] we have employed in selecting the dibenzofuran function as a template for intramolecular acyl transfer.[5] Brief reports have outlined the efficiency of the acyl transfer reaction 1 → 2 and its application to the synthesis of a dihydrosomatostatin derivative.[6] In the accompa-

Scheme I. Thiol Capture Strategy with a Dibenzofuran Template



† Abbreviations: The following standard abbreviations are used in this paper: Boc = *tert*-butoxycarbonyl; Bpoc = 2-*p*-biphenylyl-2-propyloxycarbonyl; DCC = dicyclohexylcarbodiimide; DIEA = diisopropylethylamine; HFIP = 1,1,1,3,3,3-hexafluoro-2-propanol; OSu = *O*-ester of *N*-hydroxysuccinimide; Scm = methoxycarbonylsulfenyl; TEA = triethylamine; TFA = trifluoroacetic acid; Trt = triphenylmethyl.

(1) Meienhofer, J. *Biopolymers* **1981**, *20*, 1761. Romovacek, H.; Dowd, S. R.; Kawasaki, K.; Nishi, N.; Hofmann, K. *J. Am. Chem. Soc.* **1979**, *101*, 6081.
(2) Kemp, D. S. *Biopolymers* **1981**, *20*, 1793.

0022-3263/86/1951-1821$01.50/0

nying paper we report a proof of structure for **3** in the context of structure–activity study of the template. Papers in preparation will describe the sulfur capture chemistry and the application of the overall strategy to peptide coupling reactions.

Background

A brief restatement of the issues underlying the selection of reactions for Scheme I will facilitate discussion. This scheme is ultimately intended to be used for couplings at the late stages of fragment condensation syntheses of large peptides and proteins that contain cysteine residues at their natural abundance, spaced five to twenty amino acid residues apart. Appropriate fragments **4** bearing the dibenzofuran template will be prepared by solid-phase synthesis, purified, and then linked by the three-step reaction sequence of the scheme. (Generalizations of the thiol capture protocol to proteins of more varied structure will be discussed in later reports.)

In the first step of Scheme I the reaction of a (sulfenylcarbomethoxy)-functionalized thiol with a second, free thiol is used to form an unsymmetrical disulfide. This chemistry[7] has been successfully applied to peptide problems by Hiskey[8] and by Kamber.[9] The advantages of the prior thiol capture strategy over conventional amide bond formation stand or fall with the characteristics of step A, which must occur cleanly and very rapidly at submillimolar concentrations in protic solvents or solvent mixtures that maximize solubilities of peptides and proteins and minimize their tendency toward association. Nearly as important is the intramolecular efficiency of the acyl transfer step B. Provided the effective molarity[10] of the amine of **1** at the ester carbonyl is high, a very weakly activated phenolic ester can be employed and most reactive side-chain functions of R and R′ can be left unprotected.

As reported elsewhere,[5,6] capture in the unusual protein-solubilizing solvent hexafluoro-2-propanol and acyl transfer with the dibenzofuran template meet these conditions. Details and optimal procedures for these steps will be reported subsequently. Here we focus on convenient preparative routes to esters **4**.

Synthesis of 6-Hydroxy-4-mercaptodibenzofuran (3). Convenient routes to 4-functionalized dibenzofurans were developed over 40 years ago by H. Gilman and coworkers,[11] who noted that metalation of dibenzofuran by alkyllithium reagents occurs regiospecifically at the 4-position owing to the directing effect on the furanoid oxygen. Thus Gilman prepared 4-hydroxydibenzofuran (**6**), the intermediate required for our synthesis, by a reaction of 4-lithiodibenzofuran with oxygen in the presence of *n*-butylmagnesium bromide.[12] In our hands this preparation reliably generates crude **6** in 50–60% yield (Scheme II) based on dibenzofuran. A second lithiation of the methyl ether **7** followed by reaction with elemental sulfur[13] forms the thiol **8**, convertible to the desired **3** by demethylation.

Scheme II. Synthesis of 6-Hydroxy-4-mercaptodibenzofuran

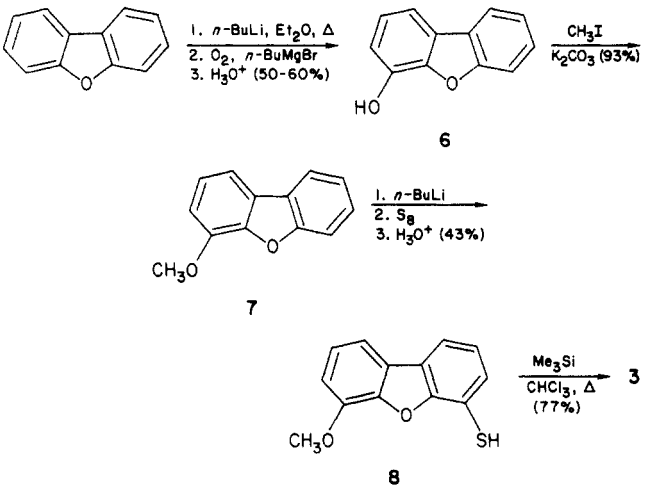


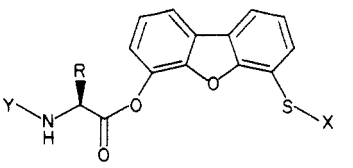
Table I. 4-(Acyloxy)-6-SScm-dibenzofurans 9

amino acid		yield (%)	mp (°C)
Z-L-Ala	**9a**	94	131
Z-L-Leu	**9b**	98	127
Boc-L-Ala	**9c**	80	148
Boc-L-Phe	**9d**	74	91
Boc-L-Leu	**9e**	71	112
Bpoc-L-Phe	**9f**	84	64 dec
Bpoc-L-Tyr(O-*t*-Bu)	**9g**	65	70

The yield and ease of purification of **8** vary markedly with the conditions of lithiation. Optimal results are seen with THF in a temperature range of −28 to −32 °C.[14] Higher temperatures or the use of diethyl ether as solvent results in contamination of **8** with positional isomers. (Structural evidence establishing the 4,6-substitutional assignment for **3** is given in the accompanying paper.) Although we have observed direct conversion of **6** to **3** by reaction with 2 equiv of *n*-butyllithium in THF followed by oxidation with sulfur, the four-step procedure of Scheme II appears to be more convenient.

S-Blocked, O-Acylated Derivatives of 3

A first step toward preparation of peptide derivatives of structure **4** (Scheme I) is convenient synthesis of O-acylated derivatives of **3** in which the reactive thiol function is masked by a readily cleavable blocking group. We explored the symmetrical disulfide of **3** as a possible precursor but have found the *S*-sulfenylcarbomethoxy derivatives **9** to be much more convenient for this purpose.



9, X = Scm
10, X = H
11, X = CH_3

Reaction of **3** with ScmCl gives **12** which can be conveniently acylated by an *N*-urethane-blocked amino acid

(3) Ugi, I. In *The Peptides, Analysis, Synthesis, Biology*, Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 365–381. Wieland, T.; Bokelman, E.; Bauer, L.; Lang, H.,; Lau, H.; Schäfer, W. *Liebigs Ann. Chem.* **1953**, *583*, 129. Brenner, M.; Zimmermann, J.; Wehrmüller, J.; Quitt, P.; Hardtmann, A.; Schneider, W.; Beglinger, U. *Helv. Chim. Acta* **1957**, *40*, 1497.

(4) Kemp, D.; Kerkman, D.; Leung, S.-L.; Hanson, G. *J. Org. Chem.* **1981**, *43*, 490.

(5) Kemp, D.; Leung, S.-L.; Kerkman, D.; Galakatos, N. In *Peptides-1980*; Brunfeldt, K., Ed., Scriptor: Copenhagen, 1981; pp 191–195.

(6) Kemp, D.; Galakatos, N., Bolin, D. In *Peptides: Synthesis, Structure, Function*, Rich, D., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; pp 73–75.

(7) Brois, J.; Pilot, J.; Barnum, H. *J. Am. Chem. Soc.* **1970**, *92*, 7629.

(8) Hiskey, R.; Li, C.; Vunnam, R. *J. Org. Chem.* **1975**, *40*, 3697. Hiskey, R.; Muthukumaraswamy, N.; Vunnam, R. *J. Org. Chem.* **1975**, *40*, 950.

(9) Kamber, B. *Helv. Chim. Acta* **1973**, *56*, 1370.

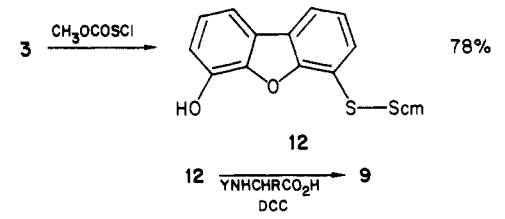
(10) Kirby, A. *Adv. Phys. Org. Chem.* **1980**, *17*, 183.

(11) Gilman, H.; Cheney, L.; Willis, H. *J. Am. Chem. Soc.* **1929**, *61*, 951. Gilman, H.; vanEss, P. *Ibid.* **1939**, *61*, 1365. Gilman, H.; Young, R. *Ibid.* **1944**, *66*, 1884.

(12) Gswend, H.; Rodriguez, H. *Org. React. (N.Y.)* **1979**, *16*, 1–360.

(13) Janczewski, M.; Maziarzyk, H. *Rocz. Chem.* **1977**, *51*, 891.

(14) Experiments conducted by Dr. M. Kolovos in these laboratories.



and dicyclohexylcarbodiimide to yield species **9** (Table I). These weakly activated phenolic esters are generally crystalline, easily purifiable, and stable to storage at 4 °C.

Clean and nearly instantaneous cleavage of the Scm blocking group occurs when **9** is treated with an equivalent of tributylphosphine in dioxane–water at 25 °C.[15] Al-

$$\mathbf{9} + n\text{-}Bu_3P + H_2O \xrightarrow[H_2O]{\text{dioxane}} \mathbf{10} + n\text{-}Bu_3PO + COS + MeOH$$

though the resulting thiols **10** ≡ **4** are stable in dilute solution, they are best used immediately after formation, since slow O,S-ester equilibration is observed in concentrated solution under neutral or basic conditions.

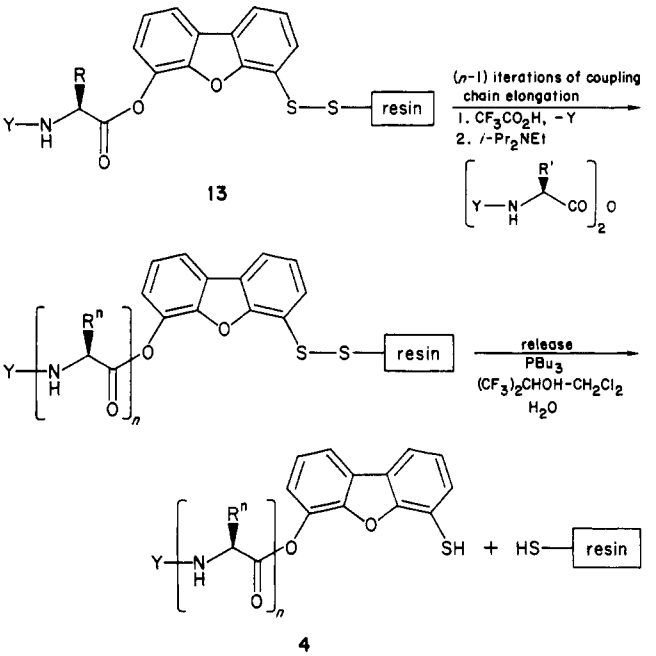
The above discussion applies only to the synthesis of esters derived from urethane-blocked derivatives of single amino acids. If a similar route is applied to the synthesis of the C-terminal esters of polypeptides, side reactions such as azlactone formation intrude, and low yields of impure products are usually obtained.[16] For this reason a "backing off" strategy[17] is often used to prepare phenolic esters of peptides: acidic removal of an N-blocking group from an amino acid ester such as **9** generates an amine salt which is converted to its conjugate base in the presence of a highly reactive peptide or amino acid derived acylating agent.

We demonstrated the feasibility of this approach by one example. When **11a** (Y = Boc, R = CH_3) was treated with trifluoroacetic acid–anisole, followed by Boc-Gly-L-Phe-OSu (3 equiv) in dichloromethane containing diisopropylethylamine (1 equiv) for 13 h, a high yield of the corresponding tripeptide ester **11b** (Y = Boc-Gly-L-Phe, R = CH_3) was isolated. This result establishes the resistance of the phenolic ester function **9** to conditions of deblocking, amine release, and acylation similar to those used in solid-phase peptide synthesis.

Synthesis of Peptide Esters 4 on a Polystyrene Support. By far the simplest tactic for the formation of medium-sized peptides functionalized at their C-termini as 4-(acyloxy)-6-mercaptodibenzofurans would be a form of solid-phase peptide synthesis in which the dibenzofuran template serves as a linkage between the growing peptide chain and its insoluble support. This would permit use of solid-phase peptide synthesis to prepare the range of sizes of products for which it is most reliable.[18]

Although isolated precedents exist in the literature,[19,20] the use of even a weakly activated phenolic ester as the site of attachment for the repetitive operations of a solid-phase synthesis involves risk. The inertness of the 4-(acyloxy)dibenzofuran function under these conditions had to be established carefully, especially in view of reports that neighboring ether functions can act as weak catalysts for the aminolysis and hydrolysis of phenolic esters.[21]

Scheme III. Solid-Phase Peptide Synthesis on a Dibenzofuran Template, Resin-Bound by a Disulfide Bond[a]



[a] Y = Boc or Bpoc.

The use of a phenolic ester as anchoring group for the peptide chain precludes choice of the fluorenylmethyloxycarbonyl (Fmoc) group for N^{α}-protection, since this group is routinely cleaved by large excesses of secondary amines such as piperidine.[22] However, the standard choice of trifluoroacetic acid cleavable *tert*-butoxycarbonyl (Boc) for N^{α}-blocking and functionalized benzyl groups for side-chain functions[23] should be compatible with our system, as should the alternative of *tert*-butyl-derived side-chain blocking groups and 2-*p*-biphenylyl-2-propyloxycarbonyl (Bpoc) for N^{α}-protection.[24] (Owing to the mildness of the deblocking conditions that it allows, the latter scheme is particularly attractive to us, and we have used it frequently in recent unpublished applications.)

The attachment of the peptide ester-bearing dibenzofuran template to a polymer is a critical issue. Conceptually, attachment by means of an unsymmetrical disulfide (Scheme III) offers many appealing features, as well as potential problems resulting from possible lability of the disulfide bond. The major finding of this paper is the development of a versatile and reliable form of solid-phase peptide synthesis based on the reaction sequence of Scheme III.

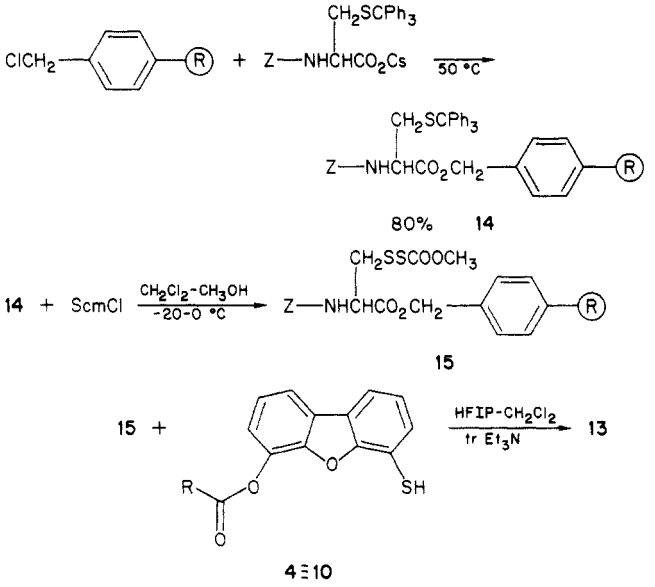
Great versatility should follow from the inertness of unsymmetrical disulfide functions to the relatively mild acids and bases employed in Scheme III. For example, if the blocking groups are Bpoc at the N^{α}-site (cleaved with 0.5% trifluoroacetic acid in dichloromethane) and Boc and O-*t*-Bu at the side chains, then either selective removal of N^{α}-Bpoc groups or removal of all protective groups should

(15) Harpp, D.; Granata, A. *J. Org. Chem.* **1980**, *45*, 271.
(16) Kovacs, J.; Kisfaludy, L.; Ceprini, M. *J. Am. Chem. Soc.* **1967**, *89*, 183.
(17) Iselin, B.; Schwyzer, R. *Helv. Chim. Acta* **1960**, *43*, 1760. Goodman, M.; Steuben, K. *J. Org. Chem.* **1962**, *27*, 3409.
(18) Atherton, E.; Gait, M.; Sheppard, R.; Williams, B. *Bioorg. Chem.* **1979**, *8*, 351.
(19) Kenner, G.; Seely, J. *J. Am. Chem. Soc.* **1972**, *94*, 3259. Hudson, D.; Kenner, G. W.; Sharpe, R.; Szelke, M. *Int. J. Pept. Prot. Res.* **1979**, *14*, 177.
(20) Rivaille, P.; Roulais, D.; Merle, M.; Gauton, J.; Pidoux, E., Milhaud, G. In *Peptides 1978*; Siemion, I., Kupryszewski, G., Eds.; Wroclaw University Press: 1979; pp 519–521. Buckle, M.; Epton, R.; Marr, G. *Polymer* **1980**, *21*, 481.
(21) Bankowski, K.; Drabarek, S. *Rocz.-Chem.* **1972**, *46*, 607.
(22) Carpino, W.; Han, G. *J. Am. Chem. Soc.* **1970**, *92*, 5748. Chang, C.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1978**, *11*, 146.
(23) Barany, G.; Merrifield, R. In *The Peptides: Analysis, Synthesis, Biology*, Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, Part A, pp 1–284.
(24) Wang, S.; Merrifield, R. *Int. J. Pept. Protein Res.* **1969**, *1*, 235.

be possible without disturbing the resin linkage. Alternatively, since phosphines do not cleave Bpoc, Boc, or O-*t*-Bu functions under neutral conditions, phosphine treatment should result in release of a fully blocked peptide derivative from the resin. The latter possibility may allow purification and characterization of the peptide–template unit followed by reattachment to a resin and continuation of a solid-phase synthesis.[26]

Realization of Scheme III requires a simple, reliable method for constructing an unsymmetrical disulfide from a resin-bound sulfur derivative and N$^{\alpha}$-blocked amino acid esters of dibenzofuran thiols **4**. The Scm route to unsymmetrical disulfides[7,8] was a natural synthetic choice; however the literature suggests difficulties in applying this reaction to polymer-bound thiols.[25] In our hands low and irreproducible yields resulted from attempts to combine dibenzofuran-4-thiols with thiol or trityl thioether-functionalized resins that had been activated with ScmCl. As we will report elsewhere, both the formation of Scm derivatives and their reactions with thiols involve solvation by protic species. We believe that in part our early failures to generate resin-linked disulfides resulted from the inhomogeneity of functional sites on a chloromethylated polystyrene, their tendency to induce demixing of binary solvent pairs, and the low polarity of many functionalized sites.

A successful protocol for forming the resin-bound disulfide **13** involves a three-step sequence, beginning with displacement of the benzyl chloride sites of commercial 1% cross-linked chloromethylated polystyrene beads by the cesium salt of Z-L-Cys(Trt)-OH. The resulting ester **14** is combined with ScmCl to form the S-activated species **15**, which is then allowed to react with 4-(acyloxy)-6-mercaptodibenzofuran **10** in dichloromethane–hexafluoro-2-propanol with or without a trace of triethylamine. Overall yields of **13** from **14** are in the range of 40–60%, which gives an acceptable loading level of 0.15–0.3 mmol of peptide per gram of resin.



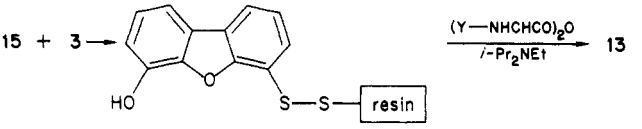
Treatment of resin with hot pyridine followed by Volhard titration allows indirect determination of the yield of **14**. Overnight reaction at 50 °C in DMF with the bulky salt Z-L-Cys(Trt)-OCs leaves 20% of pyridine-labile chloromethyl sites unchanged. (Subsequent reaction with Z-Gly-OCs results in substitution at a further 10% of the less reactive sites.) The hindered character of the first nucleophile together with the requirement of a protic environment for the other reactions of the attachment sequence restricts sites of disulfide attachment to regions of the resin that are relatively open and accessible to polar molecules. (Since we rely on a phosphine-induced disulfide cleavage to release **4** from the resin, contamination of product by species formed by subsequent reactions at residual chloromethyl groups is not likely.) It should also be noted that the combined cysteine, disulfide, and dibenzofuran functions constitute a long spacer that should further increase the accessibility of the α-amino sites of the growing peptide chain to external reagents.[27]

Scheme IV. Solid-Phase Synthesis Steps in Chain Elongation

1. wash 3 × 2 min CH_2Cl_2 (10 mL)
2. deblock[a] 1 × 5 min TFA–CH_2Cl_2 (10 mL)
   2 × 15 min TFA–CH_2Cl_2 (10 mL)
3. wash 5 × 2 min CH_2Cl_2 (10 mL)
4. acylation[b] 1 × 10 min 0–25 °C (10 mL)
   $(Y\text{-Aaa-O})_2O$, 0.1–0.2 M, 3–4 equiv + 2–3 equiv of DIEA in CH_2Cl_2
5. wash 3 × 2 min CH_2Cl_2 (10 mL)
6. wash 3 × 2 min dioxane (10 mL)
7. wash 4 × 2 min CH_2Cl_2 (10 mL)

[a] With N$^{\alpha}$-Boc, 50% TFA; with N$^{\alpha}$-Bpoc, 0.5% TFA + 1% thioanisole. [b] Monitored by ninhydrin test and phosphine release (see text and Experimental Section).

In the above procedure, a presynthesized 4-(acyloxy)-6-mercaptodibenzofuran (**10**, Y = Boc or Bpoc) is allowed to react with the resin **15**. For most cases we have found that an alternative is simpler. Resin **15** is first combined with 4-hydroxy-6-mercaptodibenzofuran (**3**). The free phenolic function of the resulting **16** is then acylated by 3 to 4 equiv of a symmetrical anhydride of an N$^{\alpha}$-blocked amino acid in the presence of diisopropylethylamine in dichloromethane. After 30 min less than 1% of **16** remains; significantly this residual **16** resists a subsequent attempted acylation with acetic anhyride.



16

Both the disulfide and the phenyl ester functions of **13** might be expected to show lability, and the versatility and utility of Scheme III depends on our demonstrating chain elongation conditions that result in clean (>99+%) acylation of a peptide amine while leaving the disulfide and phenyl ester functions intact. Lukenheimer and Zahn have noted that resin-bound cystine-containing peptides are inert to 10% triethylamine in dichloromethane or DMF as well as to hydrogen bromide in trifluoroacetic acid,[25] and the Ciba-Geigy group has made similar observations for cysteine peptides in solution.[28] In accord with their findings we have not observed disulfide cleavage in any step of Scheme III prior to phosphine treatment. By contrast, the phenolic ester function of **13** shows some

(25) Kullmann, W.; Gutte, B. *Int. J. Pept. Protein Res.* **1978**, *12*, 17. Lukenhiemer, W.; Zahn, H. *Liebigs Ann. Chem.* **1970**, *740*, 1.
(26) Wieland, T.; Lowalter, J.; Birr, C. *Liebigs Ann. Chem.* **1970**, *31*, 47. Mitchell, A.; Erickson, B.; Ryabtsev, M.; Hodges, R.; Merrifield, R. *J. Am. Chem. Soc.* **1976**, *98*, 7357.
(27) For discussion of the spacer concept, see ref 23, pp 31 and 59; Atherton, E.; Clive, D.; Sheppard, R. *J. Am. Chem. Soc.* **1975**, *97*, 6584. Arshady, R.; Atherton, E.; Clive, D.; Sheppard, R. *J. Chem. Soc., Perkin Trans. 1* **1981**, 529.
(28) Reference 9; see also: Riniker, B.; Brugger, M.; Kamber, B.; Sieber, P.; Rittel, W. *Helv. Chim. Acta* **1969**, *52*, 1058.

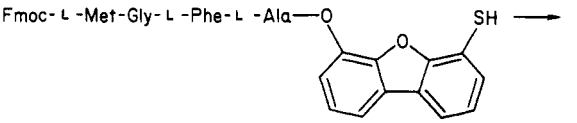
base-catalyzed lability in the presence of protic solvents or catalysts for acyl interchange such as *N*-hydroxysuccinimide. These problems are minimized by the protocol of Scheme IV in which dichloromethane, dioxane, and/or dimethylformamide are used throughout as solvents, and the α-amino group is liberated from its salt by treatment with diisopropylethylamine in the presence of a symmetrical anhydride. Acylation in the presence of this hindered base was first introduced by Meienhofer and co-workers to minimize the extent of amine protonation by carboxylic acid released from the anhydride during acylation.[29]

After each acylation step the progress of the synthesis is monitored by ninhydrin assay for residual free amino groups[30] and by treatment of a small portion of resin with phosphine, followed by HPLC assay of the liberated peptide. A negative ninhydrin test is almost always observed if an assay is performed 3 min from the start of acylation. Detection of byproducts after phosphine cleavage is facilitated by the high extinction coefficient of the dibenzofuran function at ca. 280 nm; thus, cleavage of the phenyl ester bond can determined by monitoring the amount of **3** that is released after phosphine treatment.

In order to optimize reaction conditions of Scheme IV we studied syntheses and properties of **17** and two of its resin-bound precursors, **13** (Y = Boc-Gly-L-Phe, R = CH_3) and **13** (Y = H-Gly-L-Phe, R = CH_3). When the latter species was allowed to remain in swollen state in dichloromethane for several hours, a slow fragmentation of the phenyl ester occurred as evidenced by release of **3** after reaction of the resin with phosphine. The experiment suggests that resin-bound free amines are labile and should be acylated as soon as they are liberated from their salts, as in Scheme IV. Fragmentation of the ester linkage is also seen if 2-propanol is used in one of the wash steps of the protocol for chain elongation. Most probably, traces of alcohol are retained by the resin-bound peptide and effect transesterification in the basic or acidic steps. A lability in the presence of alcohols has been noted in previous studies of solid-phase synthesis using phenyl esters as anchors.[20]

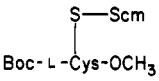
Formation of diketopiperazines after the amine is liberated in the second chain elongation step is a special concern with our phenyl ester linkage. We have found that only 1–2% of diketopiperazines are formed if the concentration of acylating agent is high (0.1–0.3 M), and the amine is liberated only in its presence. Acylation by agents less reactive than symmetrical anhydrides or with very hindered amino acids may conceivably result in greater problems with this side reaction. (Detection of **3** by HPLC assay after phosphine cleavage of a test sample allows capping of **16** by reaction with acetic anhydride prior to the next chain elongation step.)

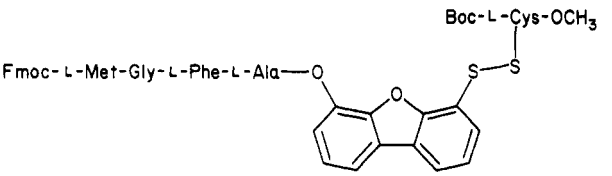
An alternative solution to diketopiperazine formation is acylation with an N^{α}-blocked dipeptide active ester as used by Meienhofer and co-workers with the Fmoc protocol.[29] To explore this option we have used the *N*-hydroxysuccinimide (HOSu) ester of Boc-Gly-L-Phe-OH to acylate **13** (Y = H, R = CH_3). An important side reaction seen in our protocol with HOSu esters is transesterification of the phenyl ester linkage by free HOSu that is released as the acylation proceeds. In model experiments **13** (Y = Boc-Gly-L-Phe, R = CH_3) was dissolved in dichloromethane containing 0.05 M HOSu, with or without an equivalent of tertiary amine. In the former case, 4% of phenyl ester cleavage occurred in 4 h; in the latter, 9% of cleavage was seen in 20 min. Suppression of transesterification to an acceptible level (0.5–2%) results if high concentrations of active ester (0.1–0.3 M) are used for short reaction times. Under optimized conditions the tetrapeptide derivative **17** was obtained without detectable peptide-derived impurities and was converted to **18** essentially quantitatively.[31]



17

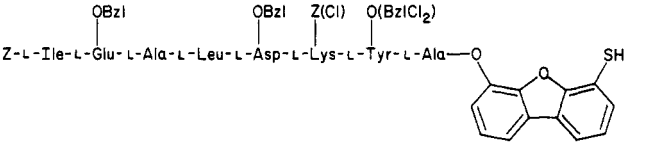
\+





18

As a first realistic test of the methodology of Scheme IV we selected the synthesis of the octapeptide derivative **19**, the choice of the side-chain blocking groups being dictated more by availability of amino acid derivatives than by consonancy with our long range plans in this area. Application of our methodology to this synthesis was uneventful and yielded **19** in a crude yield of >95% based on the initial alanine loading. The purity by HPLC was 84%, and the major impurity was 4-acetoxydibenzofuran-6-thiol, formed prior to the initiation of the solid-phase synthesis. The cleanness and rapidity of the synthesis compared favorably with those of other solid-phase synthetic protocols that we have used.



19

Elsewhere we will report studies designed to test the scope of the capture and acyl transfer steps of the thiol capture strategy. In the course of these investigations we have successfully and routinely used the solid-phase methodology described here for synthesis of a variety of medium-sized peptides including examples containing the troublesome amino acids asparagine and arginine. Particular points of convenience and novelty that characterize Scheme IV include the short reaction times required for complete acylation of resin-bound amine, convenient assays of homogeneity through phosphine cleavage, and the potential (which we have not yet explored) for reattachment by resin–disulfide linkage of purified fragments. Although the disulfide linkage was developed to meet the special needs of the thiol capture strategy, its unusual versatility suggests other applications in solid-phase peptide synthesis.

In this paper we have reported a direct and convenient preparative route to medium-sized peptide fragments es-

(29) Meienhofer, J.; Waki, M.; Heimer, E.; Lambros, T.; Makofske, R.; Chang, C.-D. *Int. J. Pept. Protein Res.* **1979**, *13*, 35.

(30) Kaiser, E.; Colescott, R.; Bossinger, C.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595.

(31) mp 163.5–166 °C dec; Amino acid analysis: Met, 1.02 (1); Gly, 1.08 (1); Phe, 0.99 (1); Ala, 0.98 (1); FD mass spectrum: *m/e* 1078 (M^+).

terified at their C-termini with the thiol capture template 4-hydroxy-6-mercaptodibenzofuran. In accompanying and subsequent papers on amide bond formation by prior thiol capture we report our studies with the capture and acyl transfer steps, racemization studies for all steps of the reaction sequence, determination of the effect of amino acid side-chain substituents on the rates of intramolecular acyl transfer, and applications of the thiol capture methodology to peptide synthesis.

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; b, broad. Low resolution, high resolution, and field desorption mass spectra were recorded on Varian MAT-44, 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. Amino acid analyses were conducted on a Glenco MM-60 microcolumn analyzer; hydrolysates were prepared in evacuated Reacti-Therm tubes (Pierce) at 110 °C for 30 h in either 6 N HCl (free peptides) or a 2:1:1 12 N HCl–HOAc–phenol mixture (resin-bound peptides). Values for Cys were not determined.

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), and Ellman's reagent (thiols).[32] 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 6,000-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 under the following conditions: system D: liquid phase, 85% methanol–1% acetic acid–14% water on a 5/25 ODS-1 column; system F: liquid phase, 80% methanol–20% water on a 10/25 ODS-1 column. Flow rate: 1 mL/min. Data are reported as the retention time (t_R) in min, followed by relative integration when applicable.

Tetrahydrofuran and *p*-dioxane were obtained dry and peroxide-free by distillation from sodium benzophenone ketyl. Dimethylformamide (DMF) was dried over molecular sieves (Linde 4 Å), then distilled from ninhydrin under vacuum and stored in brown bottles at 4 °C over sieves. Trifluoroacetic acid was fractionally distilled from P_2O_5, then redistilled from anhydrous *L*-valine. Reagent grade CH_2Cl_2, $CHCl_3$, and CH_3CN were dried over molecular sieves (Linde 4 Å); methanol, over Linde 3 Å sieves.

Dibenzofuran and iodotrimethylsilane (Me_3SiI) were purchased from Aldrich. Solutions of *n*-butyllithium (*n*-BuLi) in *n*-hexane were obtained from Alfa and titrated prior to use.[33] Triethylamine (TEA) and diisopropylethylamine (DIEA) were distilled first from ninhydrin, 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; methoxycarbonylsulfenyl chloride (ScmCl) was prepared according to published procedures[34] and purified by spinning-band distillation.

Dichloromethane used in solid-phase peptide synthesis was distilled from phosphorus pentoxide, stored over Linde 4 Å molecular sieves, and passed through a column of basic alumina immediately before use. Protected amino acids were purchased from Chemalog.

4-Hydroxydibenzofuran (6). To dibenzofuran (118.9 g, 0.71 mol) in anhydrous diethyl ether (1.30 L) was added dropwise (30 min) at room temperature under N_2 *n*-butyllithium (0.82 mol) in *n*-hexane (341 mL), and the mixture was heated to reflux for 15 h. The resulting yellow-green suspension was then allowed to cool to room temperature and was used immediately in the next step.

To a flame-dried, 5-L, four-necked flask equipped with a mechanical strirrer was added anhydrous diethyl ether (1.20 L). The vessel was cooled to −78 °C and the solvent was saturated with dry O_2. To the cooled solvent mixture was added simultaneously and over a period of 35 min the solution of lithiodibenzofuran in Et_2O and fresh *n*-butylmagnesium bromide (1.15 mol) in diethyl ether (600 mL). The mixture was then allowed to warm up slowly to room temperature and was stirred for 12 h with a slow flow of oxygen.

The resulting white suspension was then cooled to 0 °C and cautiously acidified with 6 N HCl until both phases became clear. The aqueous phase was extracted with Et_2O, and the combined organic layers were washed with 1 N HCl and extracted with 1 N NaOH. The alkaline phases were combined, acidified to pH 1 with concentrated HCl, and extracted with Et_2O. The organic layers were combined, washed with water and brine, dried ($MgSO_4$), and evaporated to yield a tan solid residue (73.7 g, 56%), mp 96–98 °C. Recrystallization from hexane afforded pure **6** as white needles (55.3 g, 42%): mp 97–99 °C (lit.[11] mp 98.5–99.5 °C); TLC R_f 0.43 (A); HPLC t_R 4.50 (D); IR (KBr) ν_{max} 3400–3010 (b), 1630, 1600, 1503, 1435 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.59 (1 H, b s, Ar OH), 7.01 (1 H, dd, J = 8, 1 Hz, C_3-H), 7.20 (1 H, t, J = 8 Hz, C_2-H), 7.34 (1 H, td, J = 8, 1 Hz, C_8-H), 7.44 (1 H, td, J = 8, 1 Hz, C_7-H), 7.49 (1 H, dd, J = 8, 1 Hz, C_1-H), 7.58 (1 H, dm, C_6-H), 7.92 (1 H, dm, C_9-H); mass spectrum, m/e (relative intensity) 184 (M^+, 100), 155 (24), 128 (41), 102 (29), 92 (39), 63 (46), 51 (60), 39 (26).

4-Methoxydibenzofuran (7). To crude **6** (63 g, 0.34 mol) in acetone (2.40 L) was added anhydrous potassium carbonate (72 g, 0.52 mol). The suspension was heated to 40 °C, methyl iodide (182 mL, 2.9 mmol) was added in one portion, and the mixture was heated to reflux for 11 h. The resulting suspension was then allowed to cool to room temperature, the solvent was evaporated, and the residue was dissolved in CH_2Cl_2–water. The layers were separated, the aqueous phase was extracted with CH_2Cl_2, and the organic layers were combined, washed with 1 N NaOH, water, and brine, dried ($MgSO_4$), and evaporated. The resulting brown oily residue was distilled under vacuum (bp 150 °C (0.8 mmHg)) to afford pure **7** as a colorless oil that solidified on standing (62 g, 93%): mp 50.5–51.0 °C (lit.[11] mp 52 °C); TLC R_f 0.69 (A); HPLC t_R 6.52 (D); ^1H NMR ($CDCl_3$) δ 4.04 (3 H, s, Ar OCH_3), 6.95 (1 H, d, J = 8 Hz, C_6-H), 7.24 (1 H, t, J = 8 Hz, C_2-H), 7.33 (1 H, t, J = 8 Hz, C_8-H), 7.44 (1 H, t, J = 8 Hz, C_7-H), 7.52 (1 H, d, J = 8 Hz, C_1-H), 7.62 (1 H, d, J = 8 Hz, C_6-H), 7.91 (1 H, d, J = 8 Hz, C_6-H); mass spectrum, m/e (relative intensity) 198 (M^+, 100), 183 (41), 155 (72), 127 (49), 101 (16), 77 (24).

4-Mercapto-6-methoxydibenzofuran (8). To **7** (17.2 g, 86.8 mmol) in tetrahydrofuran (130 mL) contained in a three-necked flask equipped with a low temperature thermometer and cooled under N_2 to −28 °C was added dropwise (20 min) *n*-butyllithium (101 mmol) in *n*-hexane (46.0 mL). The resulting orange-colored solution was stirred at −28 °C under N_2 for 2.5 h to yield a white suspension which was stirred under these conditions for an additional 1 h. To the mixture was then added in one portion elemental sulfur (Baker; sublimed, N.F.; 3.52 g, 110 mmol). The temperature of the reaction rose to 0 °C, and the white precipitate dissolved to yield a clear purple-colored solution. The mixture was then allowed to warm up slowly to room temperature (2.5 h), and the resulting light-orange clear solution was cooled to 0 °C, carefully acidified with 10% HCl (35 mL), and poured into ice-cold 10% HCl. The aqueous phase was extracted with Et_2O, and the organic layers were combined, washed with water, and extracted with 0.5 N NaOH. The alkaline phases were combined, washed with Et_2O, and acidified to pH 1 with concentrated HCl.

(32) Glaser, C.; Maeda, H.; Meienhofer, J. *J. Chromatogr.* **1970**, *50*, 151.

(33) Winkle, M.; Lansinger, J.; Ronald, R. *J. Chem. Soc., Chem. Commun.* **1980**, 87.

(34) Weiss, W. Ger. Pat. 1 224 720, Farkenfabriken Bayer A. G., 1966. Mühlbauer, E.; Weiss, W. Ger. Pat. 1 568 632, Farkenfabriken Bayer A. G., 1969. Zypancic, B. *Synthesis* **1975**, 169.

The aqueous mixture was then extracted with CH_2Cl_2, and the organic layers were combined, washed with water, dried ($MgSO_4$), and evaporated to afford a white solid residue possessing a strong thiol odor (14.4 g, 72%). HPLC analysis of the crude product indicated that it was free of positional isomers and symmetrical disulfides. Recrystallization from CH_2Cl_2–pentane yielded pure **8** as odorless white shiny crystals (8.6 g, 43%): mp 101–102 °C; TLC R_f 0.62 (A); HPLC t_R 7.19 (D), disulfides appear at 11.6 min; ^1H NMR ($CDCl_3$) ν_{max} 3005, 1628, 1585, 1497, 1410 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.98 (3 H, s Ar OCH_3), 4.02 (1 H, s, Ar SH), 6.90 (1 H, dd, J = 8 Hz, C_7-H), 7.15 (1 H, t, J = 8 Hz), 7.18 (1 H, t, J = 8 Hz), 7.29 (1 H, dd, J = 8, 1 Hz, C_3-H), 7.42 (1 H, dd, J = 8 1 Hz, C_9-H), 7.63 (1 H, dd, J = 8, 1 Hz, C_1-H); mass spectrum, m/e (relative intensity) 230 (M^+, 100), 215 (39), 187 (60), 158 (23), 115 (74).

Anal. Calcd for $C_{13}H_{10}O_2S$: C, 67.83; H, 4.35; S, 13.91. Found: C, 67.80; H, 4.44; S, 14.17.

4-Mercapto-6-hydroxydibenzofuran (3). To **8** (3.52 g, 15.3 mmol) in $CHCl_3$ (23.0 mL) was added in one portion at room temperature under N_2 iodotrimethylsilane (11.0 mL, 77.3 mmol), and the mixture was heated to reflux for 42 h. The resulting dark-red, clear mixture was allowed to cool to room temperature and was then poured into MeOH (170 mL). The partially decolorized solution was stirred for 5 min, and then the mixture was evaporated to dryness. The red solid residue was dissolved in CH_2Cl_2–H_2O, the layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The organic layers were combined, washed successively with 10% $Na_2S_2O_3$, water, and brine, then dried ($MgSO_4$), and evaporated to afford a yellow powder (3.18 g, 96%), mp 130–135 °C. Recrystallization from CH_2Cl_2 at −20 °C yielded pure **3** as a pale odorless fluffy solid (2.55 g, 77%): mp 133.5–135.5 °C; TLC R_f 0.20 (A); HPLC 5.03 (D); IR ($CHCl_3$) ν_{max} 3670, 3600, 1600, 1500, 1410 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.90 (1 H, b s, Ar SH), 5.80 (1 H, b s, Ar OH), 7.04 (1 H, dd, J = 8, 1 Hz, C_7-H), 7.22 (1 H, td, J = 8, 1 Hz), 7.23 (1 H, td, J = 8, 1 Hz), 7.36 (1 H, dd, J = 8, 1 Hz, C_3-H), 7.47 (1 H, dd, J = 8, 1 Hz, C_9-H), 7.72 (1 H, dd, J = 8, 1 Hz, C_1-H); mass spectrum, m/e (relative intensity) 216 (M^+, 100), 187 (41), 155 (10), 115 (41), 55 (61).

Anal. Calcd for $C_{12}H_8O_2S$: C, 66.65; H, 3.73; S, 14.83. Found: C, 66.56; H, 3.81; S, 14.79.

4-(Methoxycarbonyldithio)-6-hydroxydibenzofuran (12). To **3** (1.02 g, 4.72 mmol) in methanol (10.0 mL) was added in one portion with vigorous stirring ScmCl[9] (0.50 mL, 5.45 mmol). Heat was evolved, and the clear yellow-green mixture was stirred at room temperature under N_2 for 2 h. The solvent was then evaporated, and the residue was dried under vacuum and evaporated 3 times with CH_3CN to yield a viscous oil (1.6 g). Flash chromatography afforded pure **12** as a colorless oil that solidified on standing (1.13 g, 78%). Trituration with a 1:1 mixture of Et_2O and hexane afforded a white powder (1.01 g, 70%): mp 106–108 °C; TLC 0.44 (A); HPLC t_R 5.95 (D); IR ($CHCl_3$) ν_{max} 3560 (b), 3005, 1732, 1600, 1409, 1135 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.90 (3 H, s, SScm), 6.89 (1 H, b s, Ar OH), 7.06 (1 H, dd, J = 8, 1 Hz, C_7-H), 7.21 (1 H, t, J = 8 Hz), 7.29 (1 H, t, J = 8 Hz), 7.42 (1 H, dd, J = 8, 1 Hz, C_9-H), 7.68 (1 H, dd, J = 8, 1 Hz, C_3-H), 7.87 (1 H, dd, J = 8, 1 Hz, C_1-H); mass spectrum, m/e (relative intensity) 306 (M^+, 17), 247 (6), 216 (19), 184 (32), 155 (23), 115 (+4), 59 (100); high resolution mass spectrum, calcd for $C_{14}H_{10}O_4S_2$ 306.0021, found 306.0047.

Anal. Calcd for $C_{14}H_{10}O_4S_2$: C, 54.89; H, 3.29; S, 20.93. Found: C, 54.89; H, 3.42; S, 21.07.

Representative Procedure for the Acylation of 9. 4-(Methoxycarbonyldithio)-6-(*N*-*tert*-butyloxycarbonyl-L-alanyloxy)dibenzofuran (9c). To **12** (1.43 g, 4.67 mmol) in CH_2Cl_2 (15.0 mL) was added in one portion *N*-Boc-*L*-alanine (973 mg, 5.14 mmol) in the same solvent (20.0 mL), and the solution was cooled to 0 °C. To this was added in one portion DCC (1.20 g, 5.83 mmol), and the mixture was stirred first at 0 °C for 2 h and then at room temperature for an additional 16 h. The resulting white suspension was cooled to 0 °C, and the DCU formed was collected by filtration and washed with chilled CH_2Cl_2. The filtrates were combined, diluted with CH_2Cl_2, washed with ice-cold sodium citrate buffer (0.5 M in citric acid, pH 3.5), ice-cold $NaHCO_3$, and water, dried ($MgSO_4$), and evaporated. Purification of the residue (2.91 g) by flash chromatography yielded **9c** as a white solid (1.77 g, 80%), mp 146–148 °C. Alternatively, recrystallization of the crude product from EtOAc afforded **9c** as white needles in 71% yield: mp 148–149 °C; TLC R_f 0.57 (A); HPLC t_R 8.70 (D); ^1H NMR ($CDCl_3$) δ 1.49 (9 H, s, Ala Boc), 1.78 (3 H d, J = 7 Hz, Ala CH_3), 3.89 (3 H, s, SScm), 4.74–4.85 (1 H, m, Ala CH), 5.27 (1 H, b d, J = 8 Hz, NH), 7.27–7.39 (3 H, m), 7.76 (1 H, dd, J = 8, 1 Hz), 7.82 (1 H, dd, J = 8, 1 Hz), 7.92 (1 H, dd, J = 8, 1 Hz, C_1-H).

Anal. Calcd for $C_{22}H_{23}O_7NS_2$: C, 55.33; H, 4.85; N, 2.93; S, 13.43. Found: C, 55.53; H, 5.01; N, 2.94; S, 13.69.

4-(Methoxycarbonyldithio)-6-(*N*-benzyloxycarbonyl-L-alanyloxy)dibenzofuran (9a): TLC R_f 0.57 (A); ^1H NMR ($CDCl_3$) δ 1.80 (3 H, d, J = 7 Hz, Ala CH_3), 3.85 (3 H, s, SScm), 4.80–4.91 (1 H, m, Ala CH), 5.17 (2 H, s, bzl), 5.23 (1 H, bd, J = 8 Hz, NH), 7.29–7.37 (8 H, m), 7.73 (1 H, dd, J = 8, 1 Hz), 7.81 (1 H, dd, J = 8, 1 Hz), 7.91 (1 H, dd, J = 8, 1 Hz, C_1-H); FD mass spectrum, m/e 511 (M^+).

Anal. Calcd for $C_{25}H_{21}O_7NS_2$: C, 58.70; H, 4.14; N, 2.74; S, 12.53. Found: C, 58.92; H, 4.29; N, 2.70; S, 12.74.

4-(Methoxycarbonyldithio)-6-(*N*-benzyloxycarbonyl-L-leucyloxy)dibenzofuran (9b): TLC R_f 0.64 (A); HPLC t_R 7.48 (F); ^1H NMR ($CDCl_3$) δ 1.12 (6 H, m, Leu *i*-Pr), 1.78–2.13 (3 H, m), 3.86 (3 H, s, SScm), 4.78–4.87 (1 H, m, Leu CH), 5.17 (2 H, s, bzl), 5.38 (1 H, b d, J = 8 Hz, NH), 7.30–7.38 (8 H, m), 7.75 (1 H, dd, J = 8, 1 Hz), 7.82 (1 H, dd, J = 8, 1 Hz, C_1-H).

4-(Methoxycarbonyldithio)-6-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyloxy)dibenzofuran (9d): TLC R_f 0.27 (B); HPLC t_R 13.15 (D); ^1H NMR ($CDCl_3$) δ 1.45 (9 H, s, Phe Boc), 3.35–3.62 (2 H, m, Phe bzl), 3.89 (3 H, s, SScm), 4.95–5.10 (1 H, m, Phe CH), 5.18 (1 H, b d, J = 8 Hz, NH), 7.21–7.43 (8 H, m), 7.45 (1 H, dd, J = 8, 1 Hz), 7.82 (1 H, dd, J = 8, 1 Hz), 7.94 (1 H, dd, J = 8, 1 Hz, C_1-H); FD mass spectrum, m/e 553 (M^+).

Anal. Calcd for $C_{28}H_{27}NO_7S_2$: C, 60.74; H, 4.92; N, 2.53; S, 11.58. Found: C, 60.94; H, 5.00; N, 2.48; S, 11.82.

4-(Methoxycarbonyldithio)-6-(*N*-*tert*-butyloxycarbonyl-L-leucyloxy)dibenzofuran (9e): TLC R_f 0.60 (A); HPLC t_R 6.50 (F); ^1H NMR ($CDCl_3$) δ 1.11 (6 H, m, Leu *i*-Pr), 1.49 (9 H, s, Leu Boc), 1.84–1.91 (1 H, m), 1.99–2.10 (2 H, m, Leu CH_2), 3.89 (3 H, s, SScm), 4.70–4.78 (1 H, m, Leu CH), 5.12 (1 H, bd, J = 8 Hz, NH), 7.29–7.40 (3 H, m), 7.75 (1 H, dd, J = 8, 1 Hz), 7.81 (1 H, dd, J = 8, 1 Hz), 7.93 (1 H, dd, J = 8, 1 Hz, C_1-H).

Anal. Calcd for $C_{25}H_{29}O_7NS_2$: C, 57.79; H, 5.62; N, 2.69; S, 12.34. Found: C, 58.03; H, 5.82; N, 2.66; S, 12.30.

4-(Methoxycarbonyldithio)-6-(*N*-*p*-biphenylylisopropyloxycarbonyl-L-phenylalanyloxy)dibenzofuran (9f): TLC R_f 0.64 (A); HPLC t_R 11.87 (F); ^1H NMR (CD_2Cl_2) δ 1.72 (3 H, s, Bpoc CH_3), 1.76 (3 H, Bpoc CH_3), 3.29–3.46 (2 H, Phe Bzl), 3.89 (3 H, s, SScm), 4.85–5.10 (1 H, m, Phe CH), 5.44 (1 H,bd, J = 8 Hz, NH), 7.19–7.63 (17 H, m), 7.78 (1 H, d, J = 8 Hz), 7.88 (1 H, d, J = 8 Hz), 8.01 (1 H, d, J = 8 Hz, C_1-H); FD mass spectrum, m/e 692 (M^+).

Anal. Calcd for $C_{39}H_{33}O_7NS_2$: C, 67.71; H, 4.81; N, 2.02; S, 9.27. Found: C, 67.55; H, 4.98; N, 2.05; S, 9.15.

4-(Methoxycarbonyldithio)-6-(*O*-*tert*-butyl-*N*-*p*-biphenylylisopropyloxycarbonyl-L-tyrosyloxy)dibenzofuran (9g): TLC R_f 0.62 (A); HPLC t_R 18.46 (F); ^1H NMR (CD_2Cl_2) δ 1.33 (9 H, s, Tyr *O*-Bu-*t*), 1.74 (3 H, s, Bpoc CH_3), 1.78 (3 H, s, Bpoc CH_3), 3.35–3.60 (2 H, m, bzl), 3.87 (3 H, s, SScm), 4.78–4.95 (1 H, m, Tyr CH), 5.49 (1 H, bd, J = 8 Hz, NH), 7.01 (2 H, d, J = 9 Hz), 7.18 (1 H, d, J = 8 Hz), 7.28–7.63 (13 H, m), 7.79 (1 H, d, J = 8 Hz), 7.87 (1 H, d, J = 8 Hz), 8.00 (1 H, d, J = 8 Hz, C_1-H); FD mass spectrum, m/e 764 (M^+).

Anal. Calcd for $C_{43}H_{41}O_8NS_2$: C, 67.61; H, 5.41; N, 1.83; S, 8.39. Found: C, 67.42; H, 5.59; N, 1.76; S, 8.41.

Procedure for Solid-Phase Peptide Synthesis. For the repetitive steps of solid-phase peptide synthesis, a glass-frit-equipped reaction vessel constructed from Merrifield's prototype was used.[35] Stirring was carried out by an 11 rpm 360° rotation of the vessel about its midpoint. Positive nitrogen pressure was used for filtration. Qualitative ninhydrin assay followed the literature procedure.[30] Solvents and reagents for solid-phase synthesis were purified as described earlier. Volhard titration was carried out by a literature procedure.[36]

(35) Gisin, B.; Merrifield, R. *J. Am. Chem. Soc.* **1972**, *94*, 3102.

Polystyrene-Linked Benzyl Ester of *S*-Trityl *N*-Benzyloxycarbonyl-L-cysteine (14). The diethylammonium salt of Z-L-Cys(Trt)-OH[37] (1.85 g, 3.24 mmol) was converted to the acid by partitioning between a 0.5 M pH 3.5 citrate buffer and dichloromethane. After washing, drying, and evaporating the latter, the residue was dissolved in a 3:1 ethanol–water mixture (20 mL) and treated with cesium carbonate[38] to pH 7.6. The residue from evaporation was thrice dissolved in 15 mL of DMF and concentrated to dryness in vacuum. The resulting salt was dissolved in 26 mL of DMF with warming and added to 3.0 g of chloromethylated polystyrene (2.5 mmol of chlorine; Chemalog 1% cross-linked, assayed by Volhard titration). (We have used both 2% and 1% cross-linked resin but have found the latter to give more consistently reproducible results.) The mixture was stirred gently at 50 °C under nitrogen for 18 h and then was filtered through a sintered funnel and washed with 3 × 25 mL of each of the following solvents in sequence: 5:1 DMF–water, DMF, 5:1 DMF–water, EtOH, DMF, EtOH, dichloromethane. The residue was collected and dried under vacuum for 24 h to yield 3.9 g of white solid **14** (81% reaction by Volhard titration).

Polystyrene-Linked Benzyl Ester of SScm-Functionalized *N*-Benzyloxycarbonyl-L-cysteine (15). The *N*-Z-L-Cys-(Trt)-*O*-functionalized resin **14** prepared as described above (550 mg, 0.37 mmol bound Cys) was suspended in 5 mL of dichloromethane, chilled to −18 °C, and stirred gently as 0.45 mL (5.0 mmol) of methoxycarbonylsulfenyl chloride (ScmCl, Fluka) was added. The suspension was allowed to warm to 0 °C over 60 min and then treated with 0.45 mL of MeOH, after which the mixture was allowed to reach 25 °C over 2.5 h, filtered, and washed with 4 × 10 mL of the following: dichloromethane, 1:1 dichloromethane–MeOH, dichloromethane. The Scm-functionalized resin **15** was used immediately in a dibenzofuran anchoring reaction.

Polystyrene-Linked Benzyl Ester of *S*-(4-Hydroxy-6-dibenzofuranthio)-*N*-benzyloxycarbonyl-L-cysteine (16). The reaction of the Scm-functionalized resin with the dibenzofuran thiol was carried out in a Schlenk filter tube (Ace Cat. No. 7761) connected by a pair of take-off tubes to the Ace-Burlitch inert atmosphere system (Ace Cat. No. 7818). Typically 2.0 g (1.34 mmol) of *S*-trityl-functionalized resin was converted to SScm functionalized resin as described above, and the resulting white solid was transferred to the Schlenk filter tube with the help of 20 mL of dichloromethane. A positive nitrogen pressure was used to force excess solvent through the fritted disc leaving the solvent-swollen resin above the frit. To this was added 666 mg (3.1 mmol) of 4-hydroxy-6-mercaptodibenzofuran (**3**) dissolved in 16 mL of a dichloromethane–hexafluoroisopropyl alcohol–dioxane–water mixture (20:20:20:1). The suspension was agitated and the solvent evaporated by a slow upward flow of nitrogen through the frit for 100 h. The resin was then filtered by means of positive nitrogen pressure and washed with 10-mL portions of dichloromethane (3 × 2 min), dioxane (3 × 2 min), and dichloromethane (4 × 2 min), then dried in vacuum to yield 1.91 g of **16** as a tan solid.

To determine the loading level of the resin a sample of **16** (36.6 mg) was suspended in 1 mL of dioxane–water (9:1) and treated under nitrogen, with stirring, with 50 μL (200 μmol) of tri-*n*-butylphosphine for 2 min at 25 °C. Analysis of an aliquot by reverse phase HPLC (280 nm detection) and comparison of peak area with that of a standard peak established the anchoring yield as 59%, based on cysteine content, and the loading level as 0.37 mmol/g of resin (dry weight).

Polystyrene-Linked Benzyl Ester of *S*-(4-[*N*-*tert*-Butoxycarbonyl-L-alanyloxy]-6-dibenzofuranthio)-*N*-benzyloxycarbonyl-L-cysteine (13, Y = Boc, R = CH_3). Method A. By Reaction of 15 with 10 (Y = Boc, R = CH_3). A solution of **9c** (Y = Boc, R = CH_3) (277 mg, 0.580 mmol) in a 9:1 dioxane–water mixture (5 mL) was degassed and treated under nitrogen with 147 μL (0.590 mmol) of tri-*n*-butylphosphine for 5 min. The solvent was evaporated to dryness under vacuum, and the residue was dissolved in 4 mL of degassed dichloromethane and transferred by syringe to **15** which had been prepared from 550 mg of **14** (0.37 mmol) and preswollen under nitrogen in a Schlenk tube with degassed dichloromethane. Residual thiol was transferred with 6 × 1 mL portions of HFIP; these were pooled and added to the resin. The solvent was slowly evaporated by a slow stream of nitrogen over 48 h. The resulting resin was washed thrice with 10-mL portions of each of the following: dichloromethane, dioxane, dichloromethane. The filtrates were pooled and analyzed by HPLC. The resin was dried under vacuum to yield **13** (Y = Boc, R = CH_3) (555 mg) of white solid. The filtrates contained 63% of the thiol **10c** and the resin contained 0.24 mmol of disulfide-linked **10c** per g of resin.

Method B. By Acylation of 16. A sample of resin **16** (495 mg) was washed with 4 × 10 mL of dichloromethane (2 min/wash) and then was treated for 10 min with shaking with a chilled (0 °C) solution obtained by adding 0.20 mL (1.15 mmol) of diisopropylethylamine to the filtrate obtained after 45 min at 0 °C from a mixture of 703 mg of Boc-L-Ala-OH (3.71 mmol) and 370 mg of dicyclohexylcarbodiimide (1.80 mmol) in 6 mL of dichloromethane. The resulting resin was washed twice (10 mL for 2 min) with dichloromethane, and the acylation step was repeated for 10 min with a freshly prepared solution of symmetrical anhydride. The resulting resin was washed with 10-mL portions of the following solvents: dichloromethane (3×), dioxane (3×), dichloromethane (6×). After drying, the resin was obtained as a light brown solid, wt 570 mg. Phosphine cleavage demonstrated less than 1% of **16** remained.

Representative Solid-Phase Synthesis. Preparation of 4-[*N*-Benzyloxycarbonyl-L-isoleucyl-L-glutamyl(γ-benzyl)-L-alanyl-L-leucyl-L-aspartyl(β-benzyl)-L-lysyl(*N*ε-2-chlorobenzyloxycarbonyl)-L-tyrosyl(*O*-2,6-dichlorobenzyl)-L-alanyloxy]-6-mercaptodibenzofuran (19). A. Synthesis. A 1.0-g sample of resin **13** (Y = Boc, R = CH_3) containing 0.25 mmol of Boc-L-Ala was treated with 9 mL 0.8 M acetic anhydride in dichloromethane for 10 min, at which point 0.2 mL of diisopropylethylamine was added. After 5 min, the resin was washed with 4 × 10 mL dichloromethane and subjected to the chain elongation protocol of Scheme IV.

B. Acylation. To a solution of the *N*-urethane-blocked amino acid (5.00 mmol) in 8 mL of dichloromethane was added at 0 °C dicyclohexylcarbodiimide (2.43 mmol), and the mixture was stirred at 0 °C for 45 min and filtered. The collected urea was washed with 1 mL of ice-cold dichloromethane, and the combined filtrates were cooled to 0 °C and treated with 0.50 mL (2.86 mmol) of diisopropylethylamine. The resulting solution was immediately transferred to the amine-bearing resin. After each acylation step, a sample of the resin was treated with tri-*n*-butylphosphine as described above and the resulting solution was analyzed by HPLC. Two major products were observed after each cycle, 4-acetoxy-6-mercaptodibenzofuran (11 ± 2%) and the peptide-bearing template **4** (86 ± 2%). The resin from the seventh acylation step was dried under vacuum to yield a light yellow solid, 1.12 g, a sample of which was subjected to acid hydrolysis and amino acid analysis: Ala 2.08 (2); Tyr 0.99 (1); Asp 0.81 (1); Leu 0.99 (1); Glu 0.77 (1); Ile 0.96 (1).

A sample of 208 mg of this resin was preswelled in 1.3 mL of dichloromethane, then treated under nitrogen with 23 μL (0.16 mmol) of triethylphosphine and 1.3 mL of hexafluoroisopropyl alcohol (HFIP). After 15 min the suspension was filtered and washed 5 times with alternate portions of HFIP and dichloromethane. The combined filtrates were evaporated to dryness and the residue was suspended and evaporated 3 times under vacuum with dioxane to remove phosphorus-containing species. The resulting derivative **19**, 63 mg, a white powder, was used for HPLC and elemental analysis; HPLC (UV, 280 nm, Whatman Partasil 10/25 ODS-3 reverse phase C18 column, 1% aqueous acetic acid–2-propanol 1:3, 0.5 mL/min) showed **19** as a cleanly separated major peak with slight tailing at 12.2 min (rel area 84%). The major impurity, identified by coinjection, was 4-acetoxy-6-mercaptodibenzofuran (9.2 min, rel area 14%) which was carried through the synthesis as a constant fraction of the product, as noted in the above paragraph.

Anal. Calcd for $C_{91}H_{100}N_9O_{19}SCl_3 \cdot 3H_2O$: C, 60.18; H, 5.88; N, 6.94. Found: C, 60.30; H, 6.05; N, 6.43.

Acknowledgment. Financial support from NIH Grant GM 15453 is gratefully acknowledged. We thank Dr. C.

(36) Stewart, J.; Young, J. In *Solid Phase Peptide Synthesis*; Freeman: San Francisco, 1969; pp 55–58.
(37) Zervas, L.; Photaki, I. *J. Am. Chem. Soc.* **1962**, *84*, 3887.
(38) Gisin, B. F. *Helv. Chim. Acta* **1973**, *56*, 1476.

Costello of Prof. K. Biemann's laboratory for high resolution and field desorption mass spectra, N. Fotouhi for optimization of experimental conditions for the solid-phase protocol, and Dr. M. Kolovos for optimization of the conversion of 7 to 8.

Registry No. 3, 101697-54-5; 6, 19261-06-4; 7, 41799-27-3; 8, 101697-55-6; **9a**, 101697-56-7; **9b**, 101697-64-7; **9c**, 101697-65-8; **9d**, 101697-66-9; **9e**, 101697-67-0; **9f**, 101697-68-1; **9g**, 101697-69-2; **11a**, 101697-57-8; **11b**, 101697-70-5; **12**, 101697-58-9; **17**, 101711-51-7; **18**, 101697-61-4; **19**, 101697-62-5; ScmCl, 26555-40-8; BOC-L-Ala-OH, 15761-38-3; Z-L-Cys(Trt)-OH·NHEt$_2$, 53308-88-6; BOC-L-Cys(Scm)-OMe, 53907-23-6; BOC-Gly-L-Phe-OSu, 101697-71-6; dibenzofuran, 132-64-9.

Peptide Synthesis by Prior Thiol Capture. 2. Design of Templates for Intramolecular O,N-Acyl Transfer. 4,6-Disubstituted Dibenzofurans as Optimal Spacing Elements

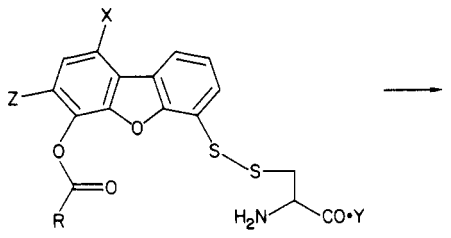
D. S. Kemp,*[†] Nicholas G. Galakatos,[†,‡] Benjamin Bowen,[†] and Kenneth Tan[‡]

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and The Arthur Scott Laboratory of Chemistry, Reed College, Portland, Oregon 97202

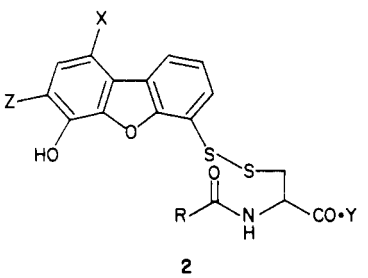
Received March 26, 1985

A central feature of the strategy for amide bond formation by prior thiol capture is an intramolecular acyl transfer across a template that links the phenolic ester function of one peptide with an unsymmetrical disulfide involving the side chain of the N-terminal cysteine residue of a second peptide. The structures of 4-hydroxy-6-mercaptodibenzofuran (**3**) and 4-hydroxy-6-mercaptophenoxythiin (**4**) are established by ^{1}H NMR spectra of deuterated dibenzofuran and phenoxythiin derivatives. On the basis of the criterion of effective molarity, a dibenzofuran template for intramolecular acyl transfer is shown to be approximately 2 orders of magnitude more efficient than a phenoxythiin. An effective local concentration of ca. 5 M and a Hammett ρ value of 2.6 are observed for the intramolecular acyl-transfer reaction **1** → **2**.

In the accompanying paper we have described general features of a strategy of prior thiol capture, which provides a new methodology for forming amide bonds between medium to large sized peptide fragments.[1] A key step in this strategy is the intramolecular O,N-acyl transfer reaction **1** → **2**, which is facilitated by the selection of a



1



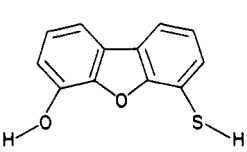
2

X = H, Cl, NO_2; Z = H, Cl

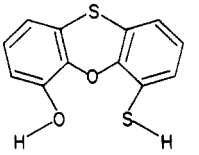
4,6-substituted dibenzofuran as an optimal linking element.[2] In earlier report[3] we have described our general approach to the design of molecular frameworks that facilitate intramolecular acyl transfer, and in that report we have noted advantages of large, rigid templates over smaller frameworks that result in acyl transfer through intermediates containing the more orthodox five- or six-membered rings.

In this paper we give a brief account of the reasoning and experiments that have led us to a selection of a dibenzofuran as a template for the acyl-transfer step of the thiol capture strategy. An unambiguous proof of structure is given for **3** and **4**, key precursors in our optimization experiments, and the acyl-transfer reaction **1** → **2** is defined by proof of intramolecularity and studies of the effects of solvent and substituents on its rate.

Structural Characterization of 3 and 4. In the accompanying paper we report a synthesis of **3** from 4-methoxydibenzofuran by metalation with *n*-butyllithium followed by oxidation with elemental sulfur and demethylation. Gilman and co-workers established[4] that dibenzofuran itself undergoes preferential metalation at the 4-position owing to the directing effect of the furanoid oxygen, and in the course of their work they assigned the structures of 4-hydroxy and 4-methoxydibenzofuran unambiguously.[5] We have noted that when lithiated 4-methoxydibenzofuran gives a mixture of lithio derivatives, presumably as a result of competitive coordination by the



3



4

[†] Massachusetts Institute of Technology.
[‡] Reed College.

(1) Kemp, D. S. *Biopolymers* **1981**, *20*, 1793.
(2) Kemp, D.; Leung, S.-L.; Kerkman, D.; Galakatos, N. In *Peptides–1980*; Brunfeldt, K., Ed., Scriptor: Copenhagen, 1981; pp 191–195.
(3) Kemp, D.; Kerkman, D.; Leung, S.-L.; Hanson, G. *J. Org. Chem.* **1981**, *46*, 490.
(4) Gilman, H.; Cheney, L.; Willis, H. *J. Am. Chem. Soc.* **1929**, *51*, 951.
(5) Gilman, H.; van Ess, P. *J. Am. Chem. Soc.* **1939**, *61*, 1365. Gilman, H.; Swiss, J. *Ibid.* **1944**, *66*, 1884.

0022-3263/86/1951-1829$01.50/0