THE PRIOR THIOL CAPTURE METHOD FOR PEPTIDE SYNTHESIS

by

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TO MY PARENTS

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I wish to take this opportunity to thank Tina Freudenberger for the expeditious and efficient typing of this thesis.

To my parents this dissertation is dedicated. Without their love and support this goal would have been unattainable.

"There is nothing permanent

except change"

Herocletos

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NICHOLAS GEORGE GALAKATOS

Submitted to the Department of Chemistry at the Massachusetts Institute of Technology on February 21, 1984, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

New methodology was developed to address the problem of amide bond formation between medium to large-sized fragments. It is based on the principle of assembly by means of the high and selective reactivity of the thiol function rather than the weak affinity of an activated acyl carbonyl for an amino acid amine, and involves an intramolecular O,N-acyl migration which occurs via a twelve-membered atom assembly comprised of an O-acyl dibenzofuran and a cysteine disulfide.

Model acyl transfer assemblies were constructed by a thiol capture process entailing treatment of S-Scm, N-terminal cysteine residues with O-acyl dibenzofuran thiols: This reaciton was found to be quantitative and rapid at 10^{-4} M concentrations, and is best performed in hexafluroisopropanol, a solvent of exceptional solubilizing efficiency.

The dibenzofuranyl template was rationally designed, and once prepared efficiently and in large quantitites, was found to accommodate our methodology optimally: Captured N-terminal cysteine residues underwent rapid ($t_{1/2}$ as low as 10 min), and high-yield (>90%) intramolecular acylation with a representative array of amino acid esters.

Finally, a novel variation of solid-phase peptide synthesis was developed to allow the versatile construction of polypeptides functionalized at their C-termini as phenolic esters of dibenzofuran thiols: An octapeptide aryl thiol was efficiently generated by attachment of the template onto a solid support via an unsymmetrical disulfide bridge, followed by chain elongation and reductive liberation from the resin.

Thesis Supervisor: Daniel S. Kemp
Title: Professor of Chemistry

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INTRODUCTION

The science and art of peptide synthesis is rooted in several remarkable achievements of nineteenth-century science: notable among these are the development by Theodor Curtius of acyl azides as amide-forming reagents, and above all, the definition of the field of peptide and protein chemistry by the extraordinary series of methodological contributions of Emil Fischer.[1]

The modern era of peptide synthesis dates from the introduction in 1932 by Bergmann and Zervas of the carbobenzoxy group for reversible blocking of amino functions.[2] The fruits of this advance can be seen in du Vigneaud's synthesis in 1953 of the neurohypophyseal hormones oxytocin and vasopressin,[3] and these achievements are remarkable given the by modern standards primitive chemical and analytical methodologies available then.

Progress since that time has been extraordinary, and modern peptide synthesis now offers three approaches of great power and sophistication, each with an independent methodology and a corresponding set of strengths and limitations. These are solution-phase,[4] solid-phase,[5] and peptide synthesis by recombinant DNA.[6,7]

The oldest of these methods is synthesis in solution, usually carried out in a highly convergent manner and entailing isolation and purification of intermediates. In a convergent approach, which is the most efficient strategy for any synthesis, a number of small peptides is prepared by uncomplicated and efficient coupling reactions, and then

linked to form medium to large-sized fragments. The most notable recent achievements in classical solution-phase synthesis are the Yajima generation of ribonuclease A, completed in 1980,[8,9] and the preparation of human insulin by the Ciba Geigy group in 1974.[10]

Solid-phase synthesis was pioneered by Merrifield in 1962[11] and consists of linear chain elongation on a solid support. An amino acid or a C-terminal peptide acid is attached to a resin by its carboxyl function and chain elongation by repetitive acidolytic, neutralization and acylation cycles affords the resin-bound polypeptide which is then cleaved from the resin. Yamashiro's β-lipotropin[12] synthesis and the generation of gram-quantities of β-endorphin by Meienhofer at Hoffmann-La Roche,[13] represent some of the latest accomplishments in this field.

Finally, the most recent of the methods for peptide synthesis is non-chemical in nature and involves manipulations of recombinant DNA. Based on the discovery in 1973 that antibiotic resistance genes can be transferred to bacteria,[14] it entails the following operations: A synthetic, or isolated DNA sequence that codes for the desired peptide is introduced into a plasmid which is then transferred into a microorganism, usually a strain of *E. coli*. The organism is placed in culture, incubated, and the resulting *in vivo* produced peptide is isolated and purified. As a result of efforts by various academic and industrial groups, a variety of peptides produced *via* recombinant DNA techniques are now in hand: Human insulin is commercially available, and human growth hormone, interferon, and somatostatin are undergoing clinical tests.[15]

A summary of some of the most successful applications of these three methods to the synthesis of polypeptides is shown in Table 1.

Table 1: Recent Achievements in Peptide Synthesis

Solution-Phase	Solid-Phase	Recombinant DNA
Human Insulin, Rittel (1974)[10]	Ribonuclease A,[16,17] Merrifield (1969)	Human Somatotropin,[7] Goeddel (1980)
Ribonuclease A,[8,9] Yajima (1980)	Human β-lipotropin,[12] Yamashiro (1978)	Human Leukocyte Interferon,[18] Pestka (1980)

In spite of these impressive achievements, one should not be misled into concluding that any target polypeptide can be prepared efficiently by the available methodologies. Each new peptide of appreciable size poses a formidable and unpredictable challenge to the practicing peptide chemist, and as Wieland remarks,[19] "the manipulation in (peptide) synthesis becomes subject to such diversity, suspense and drudgery as is found with no other substances".

This statement accurately reflects the scope and limitations of peptide synthesis by existing methods. Although all three tactics described earlier possess an array of advantageous features, they each suffer from serious limitations that are inherent with the given synthetic strategy (Table 2).

For instance, although solution-phase synthesis of small peptides by stepwise elongation from the C-terminus is usually efficient, its efficacy decreases rapidly with fragments longer than 15-20 residues.[20] For larger peptides prepared in solution, fragment condensation is commonly employed. Again, complications arise when the chain length increases: Reaction rates become very slow in coupling peptides of more

Table 2: Scope and Limitations of the Existing Methods for Peptide Synthesis

	SOLUTION-PHASE	SOLID-PHASE	RECOMBINANT DNA
STRENGTHS	•convergent strategy •purification of intermediates possible •ready preparation of analogs	•speed •automation •ready preparation of analogs •no solubility problems	•speed •large scale production possible once methodology is established •no need for protective groups •no solubility problems
LIMITATIONS	•low coupling yields •solubility •requirement of protective group orthogonality •racemization risk •neighboring group participation reactions •protic solvents unsuitable •sluggish final deblocking	•linear strategy •sequence deletions •requirement of protective group orthogonality •excess of acylating agent required •sluggish final deblocking •purification hard •protic solvents unsuitable	•linear strategy •sequence alterations inefficient •purification hard •tolerates only small deviations from natural amino acid structures
BEST-SUITED FOR	•natural and unnatural small to medium-sized fragments	•natural and unnatural small to medium-sized fragments	•natural polypeptides in large scale

than 25-35 residues.[20] Frequently, condensation of large intermediate segments fails to generate more than trace amounts of products: Hofmann reports that in the attempted synthesis of ribonuclease T_1 only 3.8% of product was obtained from a reaction of a 24-peptide azide with a 57-peptide amine.[21]

The reasons for the low reaction rates are essentially two-fold: First, low solubility in common solvents, and second, low molar concentrations because of the high molecular weight of the large fragments. For example, molar concentrations were 0.03 M and 0.01 M, respectively in the coupling of the 24- and 57-peptide segments, i.e. approximately one-tenth the molarity in small peptide coupling.[35] Reaction rates at those concentrations may slow down to half-lives of a day, thus permitting irreversible intramolecular side reactions (e.g. racemization and neighboring group participation reactions), and pseudo-first-order solvolysis of the activated C-terminus. Frequently, molar concentrations may even be another order of magnitude lower due to severely limited solubility of large fragments in commonly used solvents such as DMF, DMSO, HMPA, or mixtures of these.[20]

In the successful recent solution-phase synthesis of ribonuclease A, Yajima employed a different strategy:[8,9] A large number of small segments (a total of thirty) ranging from di- to dodecapeptides was prepared by efficient classical methods, and then assembled one by one starting from the C-terminus. The use of excess acylating agent drove each coupling to completion, but with increased chain length it became necessary to increase the amount of excess, the number of coupling repeats and the reaction time. In each of the final two couplings,

thirty-fold excess of the acylating agents had to be employed in four consecutive runs over a period of seven days each for the reactions to be complete. However, despite the problems associated with the coupling of large fragments in solution, the utility of the solution phase approach in the preparation of short peptide segments should not be understated: Small to medium-sized sequences can be efficiently and rapidly prepared in pure form by this method.[20]

In solid-phase synthesis, no solubility problems are encountered if chain elongation is undertaken by the introduction of one amino acid residue per coupling cycle; in this respect, the solid-phase method appears to be more suitable for protein synthesis.[5] However, major limitations arise from features that are intrinsic to the approach, and in particular the lack of purification of resin-bound synthetic intermediates. The solid-phase methodology defies a basic tenet of classical organic synthesis, namely that of full purification and characterization of intermediates, and thus, in prinicple, it is acceptable only if all coupling and deprotection reactions are strictly quantitative.[22] In the opinion of one major contributor to this field, this has been held to limit the size of pure peptides accessible by this approach to fragments comprised of up to only ten amino acid residues.[23] For longer peptides, useful, if not very pure products might still be obtained, provided sufficiently high yields in the coupling and deprotection reactions are attainable. The demands however are very great: Stepwise assembly of a 30 residue polypeptide requires an average efficiency of better than 99% per cycle to give a crude product containing 75% of the desired sequence. At 98% efficiency, the yield drops to 56%.[24]

Unanticipated, but sequence-dependent deletions also contribute to complications even for small-sized fragments. An outstanding example is the decapeptide sequence 65-74 of acyl carrier protein, where amino acid incorporation after six residues fell to 50-60%, then rose seemingly to 100% for the next two, and finally dropped again to 30%.[25]

The inefficiency of existing coupling and deprotection procedures is further exacerbated by the generally harsh conditions required for the cleavage of the peptide from the resin at the end of the synthesis: Numerous side reactions and extensive decomposition of the peptide chain often result from this operation.[5,143]

Recently, improved efficiency in solid-phase syntheses was accomplished by the introduction of novel protective groups, resins and chain elongation protocols. For example, Sheppard and coworkers obtained higher yields with polyamide/$\underline{N}^{\alpha}$-Fmoc protocols than with polystyrene/$\underline{N}^{\alpha}$-Boc blocking in a variety of syntheses including substance P, human β-endorphin, and pancreatic trypsin inhibitor.[22] Moreover, Merrifields' work on benzyloxybenzyl polystyrene/$\underline{N}^{\alpha}$-Bpoc protocols has enabled reaction conditions to be made much milder than formerly.[26,27] These new tactics, together with optimized versions of existing protocols have advanced peptide synthesis by solid-phase to a highly operational level. Thus currently, a large number of biomedical research groups routinely employ solid-phase techniques for the preparation of medium-sized fragments ranging from ten to fifty amino acid residues.[28]

As an alternative to the chemical methodologies, peptide synthesis by recombinant DNA was conceived to address the problem of inexpensive mass production of essential hormones to be used as pharmaceuticals.[15]

Until recently, large quantities of these materials, were obtained either by isolation from animal tissue or by chemical synthesis. However, the enormous amount of effort required for the isolation approach, together with the prohibitively high cost of synthetic polypeptides, constitute both methods highly inefficient. In 1968 a study by Schally and Guillemin showed that isolation of less than one milligram of pure thyrotropin releasing hormone requires extraction of the hypothalami of 80,000 sheep[29] or 200,000 pigs.[30] Moreover, industrial synthesis (ca 1 kg/yr), although practiced on small peptides (e.g. oxytocin (nonapeptide), LHRH (decapeptide), and sometimes on larger fragments (e.g. secretin (27 residues)), is an unreasonable operation for peptides of chain length greater than 30 amino acid residues.[15]

At this point in time, most peptide chemists agree[15] that the recombinant DNA approach to the synthesis of polypeptides offers three major advantageous features: First, once the precise methodology is established, the production of the desired protein by genetically engineered bacteria is in principle limited only by the size of the fermenter. Second, the length of the peptide sequence should in theory be immaterial. And third, this method allows the production of proteins possessing human amino acid sequences, as opposed to the often pharmacologically unsuitable animal tissue analogs.

For these reasons, the recombination of genetic material appears to be the method of choice for the synthesis of specific, naturally occuring peptide hormones in large quantities. However, if one asks the question, does this approach allow the investigation of biological activity and physicochemical properties of proteins by the study of closely related

sequences, the answer is probably not. Derivation of structure-activity functions requires the ready generation of analog sequences, usually containing unnatural amino acids, as was exemplified by the recently completed work on somatostatin by the Merck group.[31] Two major factors limit the applicability of the recombinant DNA methodology to this operation: First, chain elongation in vivo accommodates poorly any deviations from natural sequences, and tolerates only small alterations in the structure of the natural amino acids. And second, the generation of even simple analogs requires laborious total reformulation of the narrowly focused genetic protocols. The better understanding of the relation between structure and biological activity constitutes the most essential step towards the ultimate goal of producing tailor-made peptide pharmaceuticals.[32] The ready availability by peptide synthesis of hormone analogs serves such a purpose, and moreover permits the generation of unnatural proteins that are more potent than the original peptides (e.g. desaminooxytocin[33,34]).

Of the existing methodologies, none meets the requirements of speed, efficiency and versatility, needed for producing such analogs. The inherent limitations of the recombinant DNA approach were discussed earlier. Of the remaining two chemical methods, both solution-phase and solid-phase methodologies, although highly applicable in generating small-sized fragments, are increasingly inefficient as the peptide chain becomes longer. Perhaps, as Sheppard recently reflected, the answer may lie in a hybrid of solution and solid-phase, namely the "development of true solid-phase fragment condensation strategies."[22] Unfortunately, given the present limitations of convergent tactics, such an approach remains elusive.

CHAPTER I

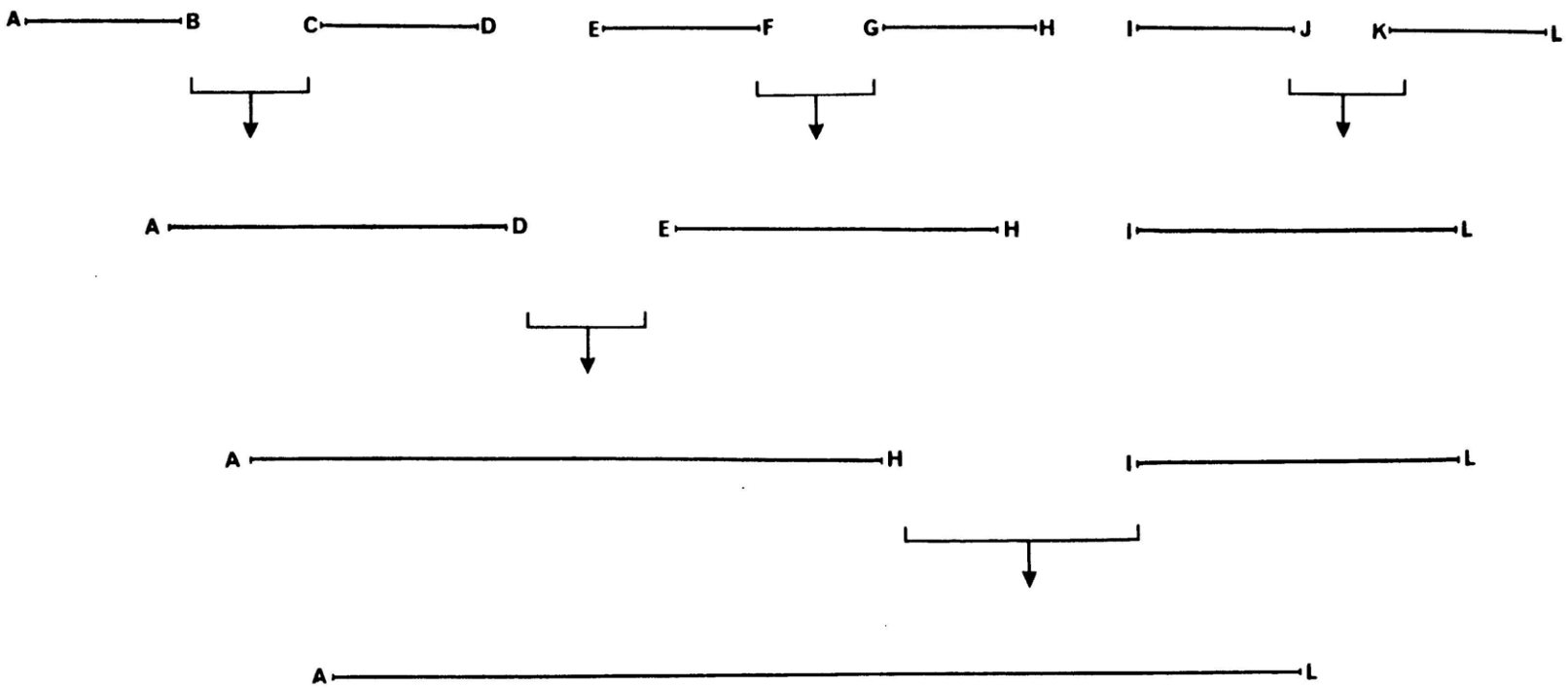
THE PRIOR AMINE CAPTURE PRINCIPLE

From the preceding discussion on the coupling issue, it is clear that the answer to the problem of versatile and efficient generation of polypeptides remains elusive. In nature, proteins are produced by ribosomes via a linear strategy, i.e. one amino acid residue is added at a time to the growing peptide chain. Hence, the classical solution-phase principle of segment condensation represents an operation that finds no precedent in a natural system. Quite conceivably then, the existing tactics may be the best that can be achieved by pure chemical means, owing to the limitations set by dilution and entropy.[36]

Given the problems encountered with the chemical methodologies, it is of great importance to realize that the remarkable achievements in the controlled biosynthesis of proteins using recombinant DNA protocols has been possible only because nature has provided DNA ligases, enzymes that can be used to join the ends of appropriately spliced large DNA segments. No equivalents of ligases for proteins are found in nature. However, the chemical equivalent of a protein ligase can be envisaged, and a hybrid methodology for polypeptide synthesis be proposed: medium-sized fragments would be prepared by the most efficient of solution or solid-phase protocols and then combined in a convergent fashion by means of a synthetic polypeptide "ligase" as the amide-forming reagent (Scheme I-1).

Segments AB, CD, EF, GH, IJ and KL of Scheme I-1 represent residues 10-15 amino acids long that can be rapidly and efficiently prepared by

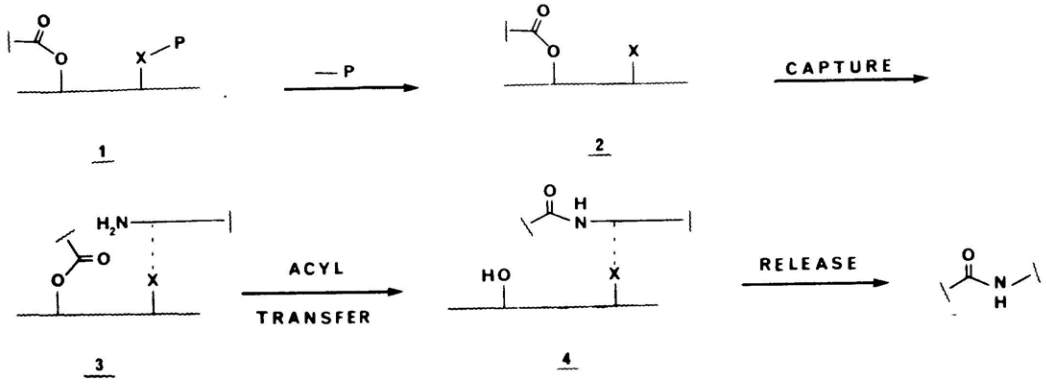
Scheme I-1



existing methodologies. These would be coupled in pairs using the polypeptide "ligase" to form AD, EH and IL, which in turn would undergo further condensation in two steps to afford the final protein.

For the last ten years, researchers in Professor Kemp's group have been engaged in the realization of such an unconventional strategy by employing the principle of prior amine capture[37,38,39] depicted in Scheme I-2:

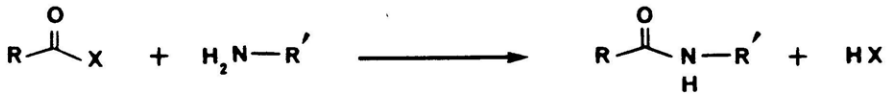
SCHEME I-2



Structure 1 symbolizes a weakly activated derivative that has been carried through a solution or solid-phase synthesis (as in Kenner's experiments where phenyl esters were used as carboxyl protective groups[40]). The function X of structure 2 is a capture site that is positioned in proximity to the acyl center and is capable of binding the amine component (also prepared by solution or solid-phase) to form 3 as a single species. Function XP of 1 represents a blocked form of the capture site. Intramolecular O,N-acyl transfer converts 3 to 4 and the reverse of the capture step releases the newly formed amide.

The prior amine capture principle was developed to advance and utilize a "ligase" for polypeptides, and was conceived first, by examination of the rate expression for the conventional amide-forming reaction, and second, by elaboration of the thought-provoking experiments of Brenner[41] and Wieland.[42]

Amide-bond formation, as performed by classical methods, is a bimolecular reaction involving the nucleophilic nitrogen of a peptide amine and the electrophilic carbon of an activated acyl derivative.[36]



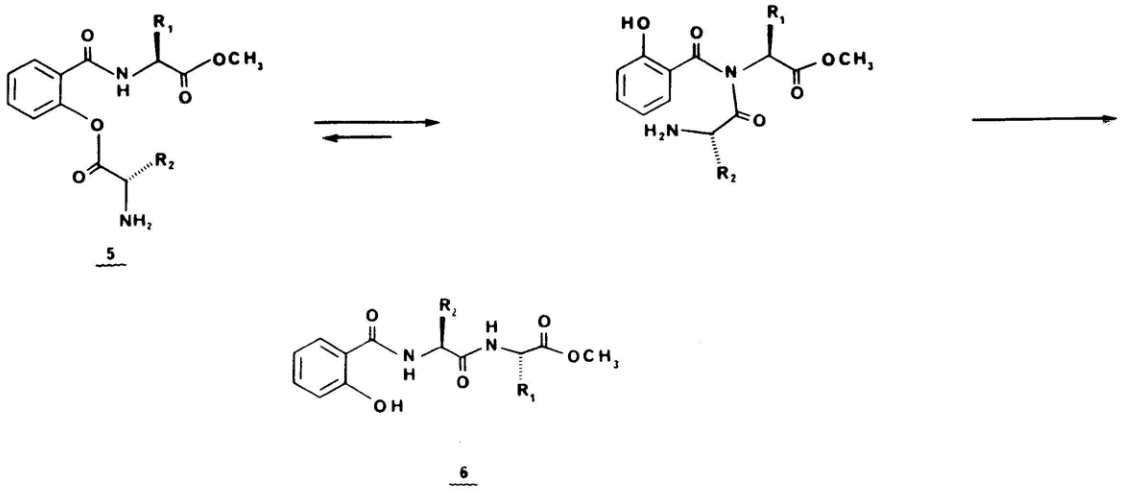
$$\text{rate of amide formation} = k[RCOX][R'NH_2]$$

$$\text{rate of intramolecular side reactions} = k'[RCOX]$$

Since conventional amide-bond formation is a bimolecular process and the reactions leading to racemization (e.g. azlactone formation) and neighboring group participation (e.g. lactam formation from Arg, His, dehydration of C-terminal Asn, Gln) are all unimolecular, the dilution that results from the increased molecular weight of the fragments to be combined naturally leads to the preferential formation of side products. This point is well exemplified by Hofmann's ribonuclease T_1 synthesis,[21] discussed in the Introduction, where low molar concentrations of reactants (0.03 M acyl azide and 0.01 M amine) and slow acylation rates resulted in the poor coupling yield (3.8%) between a 24- and a 57-amino acid fragment. Thus, given the inefficiency of the existing methods for coupling large fragments in solution, the peptide chemist is faced with the problem of finding, and then combining two needles in a haystack.

The foundations of the prior amine capture strategy were laid by early examples of facile intramolecular acyl transfer resulting in the formation of amide bonds. Brenner's experiments on salicylamide models, performed more than 25 years ago,[41] although did not address the needles-in-haystack problem, nevertheless directed attention to the highly efficient character of amide bond formation by intramolecular acyl transfer (Scheme I-3). Similarly, rapid S,N-acyl migration with S-acetyl-cysteine was observed by Wieland as early as 1953.[42]

SCHEME I-3



For R_1=H, R_2=Bz, 5 → 6: 94%

Provided that the reaction sequence outlined in Scheme I-1 can be realized in a suitable chemical system, the prior amine capture strategy offers the following intrinsic advantages:[36]

1. The relatively weak affinity of the peptide amine towards the acyl group is replaced by the strong attraction between the yet unspecified site X and the amine component.
2. Assuming that the O,N-acyl transfer proceeds efficiently, the

acyl derivative 2 need only be mildly activated (thus prohibiting side-chain acylation). In effect, the enthalpic activation of the electrophilic carbon of a conventional bimolecular coupling is replaced by an entropic activation resulting from the close proximity of the amine nitrogen to the acyl carbon of 3.

3. Since the amide-forming reaction occurs intramolecularly, it is expected to follow first-order kinetics. Thus, 90% yields are expected in approximately one-third the time in half-lives required to bring a bimolecular reaction to completion.

4. Racemization is unlikely to be a problem if the prior amine capture strategy is employed: Since azlactone formation results in cleavage of the ester bond, subsequent recombination of dilute solutions of the two molecules to form racemized product is a highly unlikely event (needles-in-haystack principle).

5. Provided the capture step involves a compatible linking reaction, protic solvents known to break unproductive tertiary association of peptide fragments and to favor random coil conformations, can be used.

Realization of these advantages requires that two very challenging problems be solved: First, a capture process must be found that occurs rapidly, quantitatively, selectively and at high dilution (10^{-3} to 10^{-4} M). Second a molecular geometry must be generated that would allow rapid intramolecular acyl transfer between the amine and acyl functions of 3 (Scheme I-2).

A. Capture: Three options exist for the capture process:[36] First, non-covalent binding, i.e. attachment by hydrogen bonding or van der

Waals association involving large surface areas of site X of 2. Second, enzymatic, i.e. utilization of the semisynthetic principle,[43] and third, capture by covalent bond formation between site X and the N-terminal peptide.

The first option suffers from present lack of understanding of the relation between structure and binding specificity. Attempts by various prominent synthetic and physical organic chemists to prepare enzyme analogs in order to study such interactions have not as yet resulted in any major breakthroughs.[32]

The enzymatic approach would be patterned after the semisynthetic principle:[43] Since no natural enzyme is known to function as a ligase for peptides, researchers have resorted to "tricking" proteases to reverse their natural activity in order to form amide bonds. However, it is difficult to see how a general method can be developed from the existing examples, each of which required considerable experimental optimization to minimize proteolysis concurrent with amide-bond formation.

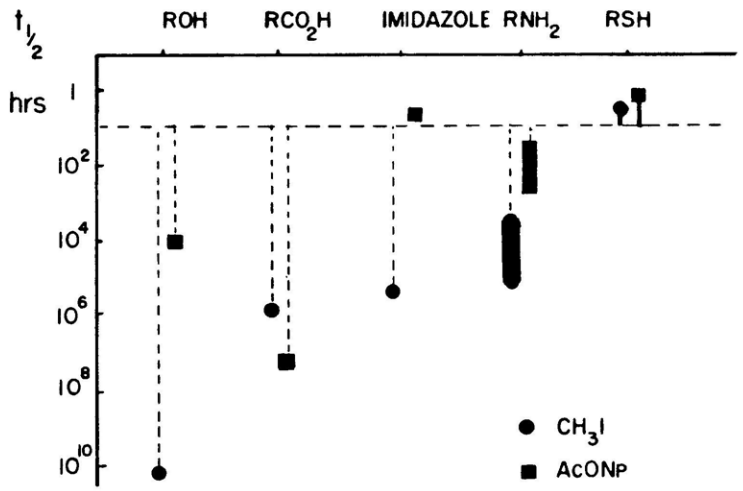
The last option is the most appealing. Within the framework of covalent linkage, four possibilities exist: Trapping of the peptide fragment at site X by its N-terminal nitrogen, capture with the C-terminal carboxyl group or the hydroxyl function of serine and tyrosine, attachment by the imidazole nitrogen of histidine or the π-electron system of tryptophan, and finally, capture with the nucleophilic thiol group of cysteine.

Carboxylic and alcoholic oxygens are insufficiently nucleophilic for any rapid and selective process that can be envisaged (Figure I-1). On

the other hand, the imidazole nitrogen of His causes overactivation of the system: it is well known that acyl-imidazoles acylate peptide chains indiscriminately.[44]

Of the remaining two possibilities, the trapping of the peptide by its N-terminal amino function was examined in these laboratories first,[36-39,46,47] whereas in the last four years attention has been directed towards the thiol capture approach.[48-51]

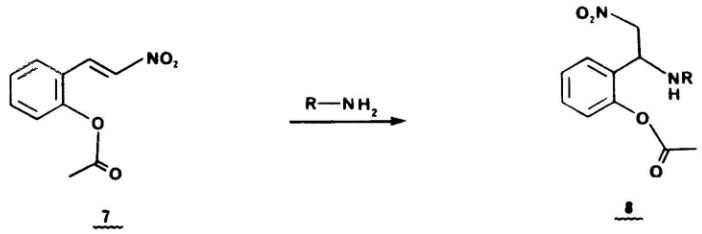
FIGURE I-1: Second-order Nucleophilic Reactivity at 0.0001 M Concentrations of Reactants.[45]



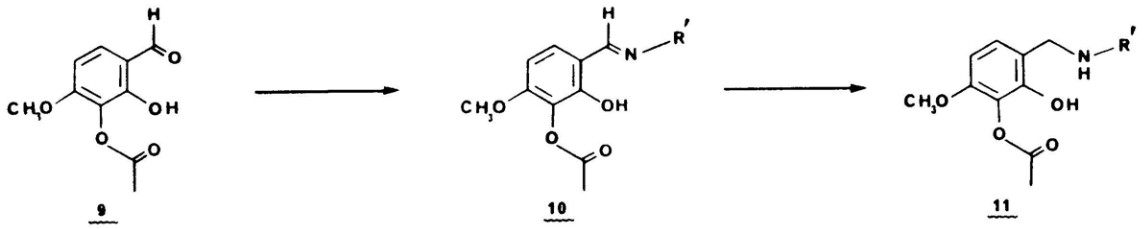
Although amino acid amines are relatively poor nucleophiles, they are known to undergo rapid Michael additions to sufficiently activated conjugated systems, and to generate imines with aldehyde carbonyls. These approaches to prior amine capture were investigated in these

laboratories by Vellaccio and Grattan and the results have been recently reviewed by Kemp.[36] A representative sample of the scope and limitations of these tactics is shown below.

Thus, Vellaccio found that reaction of nitrostyrene 7 with various amino acid amines affords quantitative yields of Michael adducts:[38]



Similary, Grattan showed that rapid and quantitative imine formation occurs upon treatment of salicylaldehyde 9 with amino acid amines. Product 10 could be readily reduced to generate secondary amine 11:[39]



In both cases, species 8 and 11 represent capture products of type 3 (Scheme I-2), where function X is the conjugated double bond of 7 and the aldehyde carbonyl of 9.

Although these models demonstrated efficient capture, the rate constants observed were not adequate for efficient reaction at high dilution. Moreover, 8 and 11 proved to be problematic in the acyl

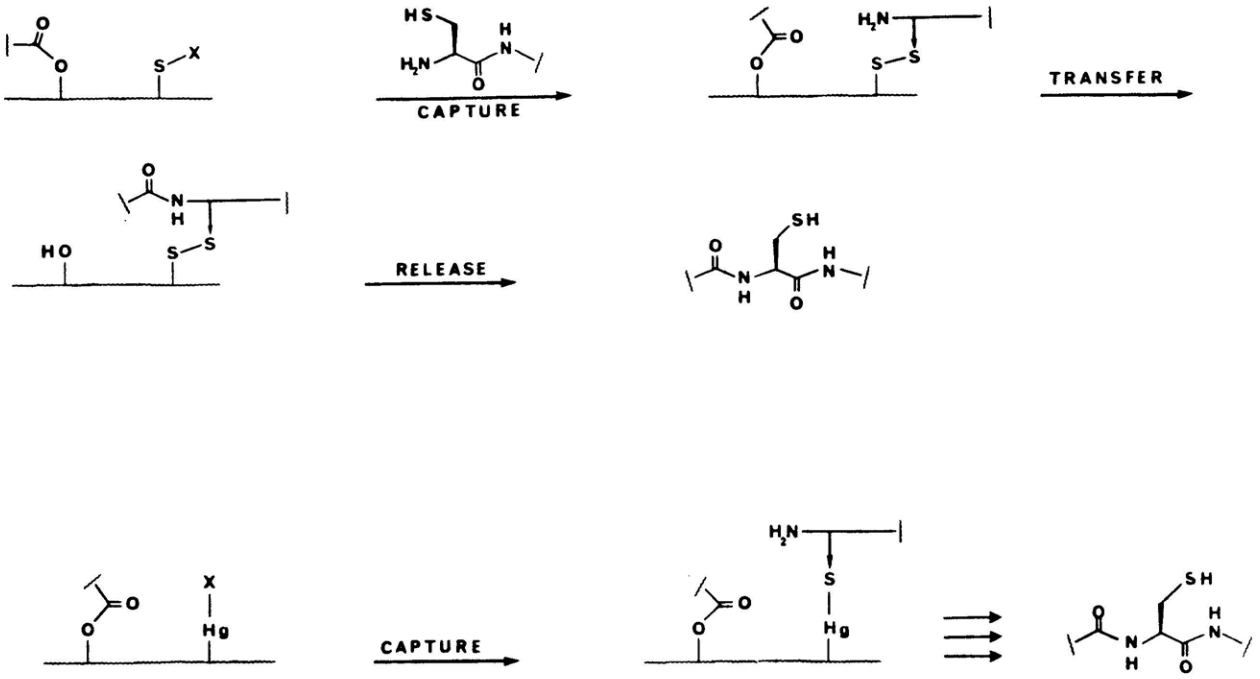
transfer step (see discussion that follows). Hence, another approach seemed warranted, since these problems appeared to be intrinsically related to the use of a terminal amine as the reactive site.

As shown in Figure I-1, thiols, and especially thiolates, are among the most nucleophilic species known. Towards alkyl halides, the thiolate anion is the most reactive of nucleophiles (with the exception of RSe^-),[52] and similar reactivity is displayed towards activated disulfides.[53] Moreover, the affinity of thiols towards mercuric ions is well documented: Simpson[54] determined the association constant for cysteine and phenylmercuric chloride to be $10^{15.8}$. It is therefore natural to conclude that the thiol function of cysteine is the obvious candidate for capture by a side-chain. Since cleavage of alkyl thioethers, which would result from trapping of a thiol by an alkyl halide, is a problematic process, two strategies can be considered as primary candidates for study (Scheme I-4):

The first involves the formation of an unsymmetrical disulfide linkage as a means of attachment to the "ligase", and the other, the trapping of an N-terminal cysteine thiol as the mercaptide. In both cases, function X of Scheme I-4 represents a leaving group. The problem of final release of the newly formed peptide from the coupling reagent can be easily solved by the use of an array of well-precedented mild conditions.[65]

However, by selecting the thiol capture approach, a serious limitation is imposed on the versatility of the prior amine capture strategy: Only N-terminal cysteine residues can be trapped. An obvious question to ask then is how severely does this choice limit the synthetic objectives.

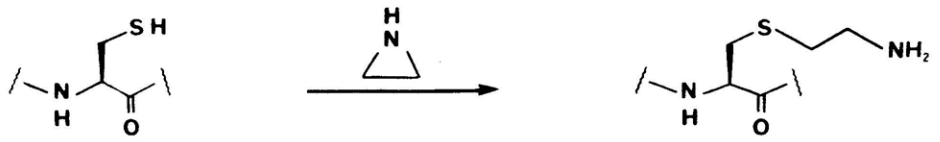
SCHEME I-4



The cysteine content of proteins varies widely. In a tabulation of more than 200 peptides, a range of zero to fifteen mole per cent was noted, with a mean value in the range of 3-5%.[55] For optimal use of our strategy, cysteines must be spaced every 15-20 amino acid residues.[28] Although many proteins may approximate this behavior, a sizable fraction does not. Therefore, an alternative approach is needed for their synthesis.

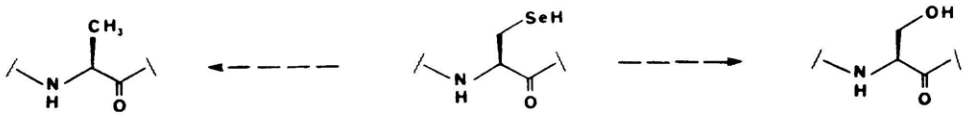
Possiblities exist for extending the thiol capture methodology to include methionine, lysine, alanine, serine and valine. Thus, employment of homocysteine, followed by methylation generates methionine. Similarly, reaction of cysteine-containing peptides with aziridine

affords an unnatural amino acid residue that is an isostere of lysine, and is mistaken as such by trypsin:[56,57]



Selenoethers are known to react with Raney nickel to afford hydrocarbons,[58,59] while alkyl phenyl selenoethers produce alkyl halides upon treatment with halogens or methyl iodide.[60]

These literature precedents provide hope that selenocysteine, shown by Walter to be compatible with the operations of peptide synthesis,[61] and expected to behave equally well or even better than cysteine in the capture step, can be converted to alanine or serine at a late step of the synthesis. Similar reactivity is expected by the selenium analog of penicillamine: Deselenation would generate a valine residue.



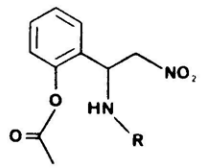
In conclusion then, of the four possible capture systems described above, the most promising appears to be the one involving the trapping of cysteine by its side-chain. However, the first step towards the establishement of practicality requires the discovery of a lead structure that demonstrates efficient intramolecular O,N-acyl transfer.

B. Intramolecular Acyl Transfer. As described earlier, the feasibility of amide-bond formation by intramolecular acyl transfer

across small-sized rings was established by Brenner[41] and Wieland.[42] Recent attempts by Ugi and coworkers to utilize this general principle have not been encouraging: it is not clear that the interaction of an isonitrile with an imminium salt provides greater affinity than the one between an amine and an acyl center.[62]

Unlike the capture problem the question of efficacy of the acyl transfer reaction is not related to functional group manipulation, but instead revolves around choices of molecular geometries. In effect then, our task was one that has many of the attributes of drug design.[48] Like many hypotheses for fit at drug receptor sites, our design principles were formulated gradually by hypotheses based on examination of stereo-models and preliminary experiments, since these rules were intended primarily to allow selections from hundreds of potentially efficient acyl transfer systems. Once a few structures which permit transfer were found, we proceeded to improve them by small structural modifications.[48]

In the initial studies undertaken in Professor Kemp's laboratory, the captured atom was the amine nitrogen of an N-terminal peptide, such as for 8 and 11.[38,39] Although early on it was realized that clean capture at high dilution was unlikely, the study was performed to test the possibility of acyl transfer across unstrained small-sized rings:



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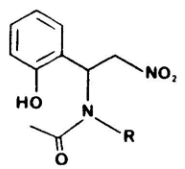

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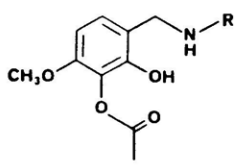

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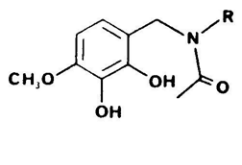


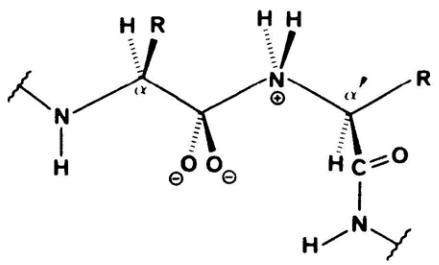
13

For 8, O,N-acyl transfer across a 6-membered ring was rapid for H_2N-R = ethyl glycinate (k_{intr} = 2 x 10^{-2} min^{-1} in CH_3CN). However, it was found that an anomalously large steric effect operates on this model: the observed rate ratios for Gly/Ala of 100 and Gly/Val of 1000,[38] far exceed the values of Gly/Ala = 4 and Gly/Val = 10 found for the intermolecular aminolyses of p-nitrophenyl esters.[63]

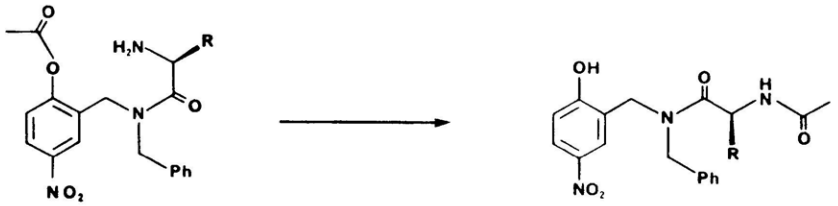
Further examination of similar models did not result in any substantial improvements, and inspection of likely transition states revealed the steric problem to be intrinsic to the 6-membered ring transition state.[36] The consistently negative nature of these observations prompted the development of a design principle that would allow acyl migrations across rings larger than 6 atoms. It was hoped that by transfering the acyl function across carefully-designed medium or large-sized rings, the anomalous steric effects would be overcome.[46,47]

The structural requirements for such systems were two-fold:[47] First, the rate-determining transition state for acyl transfer should have tetrahedral character at both the acyl carbon and the amine nitrogen, and second, the orientation of the $^{\alpha}$C-CO and N-$^{\alpha'}$C bonds must be trans-anti about the forming C-N linkage, in accordance with the model of p-nitrophenyl ester aminolyses[63] (Figure I-2).

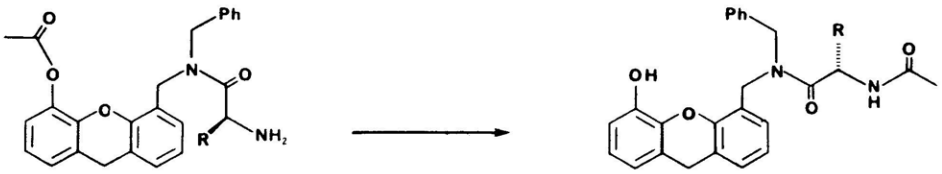
FIGURE I-2: Acyl Transfer Transition State



Structural candidates for these second-generation templates were chosen by inspection of Dreiding and CPK space-filling models of tetrahedral intermediates for acyl transfer. Practicality not withstanding, systems 14 and 16 were prepared solely to test the feasibility of intramolecular O,N-acyl migration across 9- and 12-membered assemblies.[47]



14 15



16 17

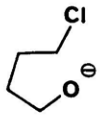
A solution of 14 in DMSO isomerized quantitatively at 25°C to 15 with half-lives of seconds, and with Gly/Ala/Val rate ratios of 1.0/0.8/1.4. The much less activated xanthene esters 16 exhibited half-lives of 8, 10 and 61 min for Gly, Ala and Val, respectively. These results are quite impressive in view of the fact that transfer across 9- and 12-membered rings were at-first-sight implausible and unprecedented.

The degree of efficiency of these intramolecular acyl migrations was estimated by the principle of effective local concentration (ELC). ELC as defined by Kirby,[64] is the concentration of an external nucleophile of similar structural class that is required to achieve an intermolecular

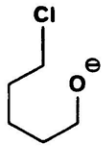
rate equal to the rate of the intramolecular reaction. The value of ELC for a given reaction can be obtained by dividing the first-order rate constant for the unimolecular reaction by the second-order rate constant of the appropriate bimolecular process:

$$ELC = \frac{k_{intr}}{k_2}$$

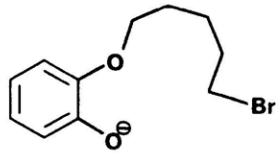
Some examples of the dramatic variation of ELC with ring size are shown below:[64]



6×10^4 M



280 M



0.05 M

For the intramolecular O,N-acyl transfer, an effective local concentration of 1 M for the free amine of structure 3 (Scheme I-2) would seem realistic.[36] Provided the acyl migration proceeds rapidly, and the actual peptide concentration is of the order of 10^{-3} to 10^{-4} M, the prior amine capture method would have affected a local concentration increase of at least three orders of magnitude over the respective bimolecular reaction.

When ELC studies were performed with 14 and 16, the values obtained were 0.6 M and 18 M respectively,[47] indicating that the structural characteristics of 16 fit the design principles of an efficient acyl transfer model. Hence, although only a model, 16 thus established the feasibility of intramolecular O,N-acyl transfer across large-sized rings.

The experience obtained thus far was applied to the formulation of a set of design principles for the system that was the most likely candidate for practicality: the thiol capture-derived assembly, described in Scheme I-4; these are as follows:[48]

1. All bond angles and bond distances should have normal values (i.e. the C-S-S-C dihedral angle is approximately 90° and the C-Hg-S bond angle is 180°).

2. Single bonds should have staggered orientations of substituents.

3. The van der Waals interactions should be minimal.

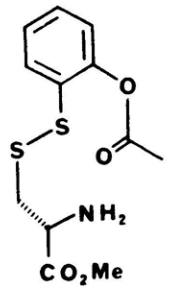
4. The leaving group at the acyl site should be a phenoxide ion.

5. The acyl carbon and the amine nitrogen are assumed to be tetrahedral, and the $^{\alpha}$C-CO and $^{\alpha'}$C-N bonds should assume all trans-anti orientation about the forming C-N bond.[63]

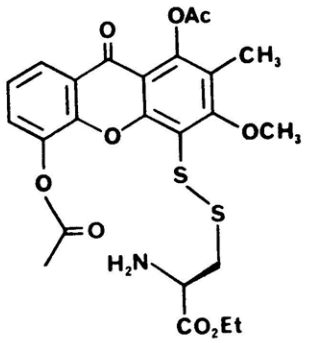
6. The spacer that connects the thiol capture site and the phenolic ester should be an exceptionally rigid unit.

Inspection of molecular models and evaluation of preliminary experiments indicated that templates 18 and 19 could accommodate the design features for the disulfide system, whereas 21, and to a lesser degree 20, are consistent with those of the mercaptide assembly. Accordingly, See-Lap Leung and Daniel Kerkman of these laboratories set out to synthesize these systems.[50,51]

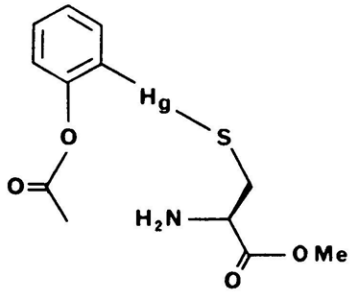
Species 18 was conveniently generated by reaction of the methoxycarbonylsulfenyl (Scm) derivative of 1-mercapto-2-hydroxybenzene with BocCysOCH$_3$, followed by acidolytic deprotection and amine liberation. At concentrations of ca. 10^{-4} M, this system exhibited moderately slow intramolecular O,N-acyl transfer ($t_{1/2}$ = 8 h in DMSO).[50]



18



19



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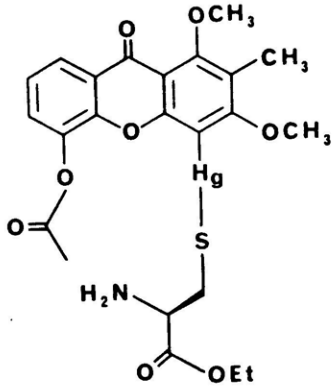
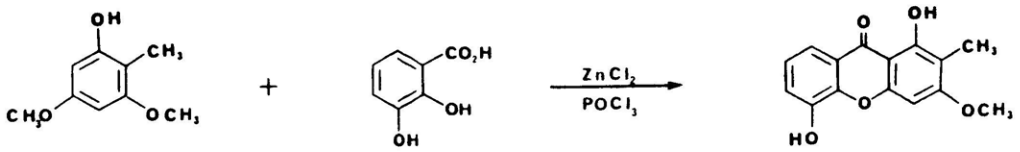


21

Xanthone 19 was much more efficient: Half-lives in DMSO and DMF at 10^{-4} M concentrations were 2.7 h and 28 h respectively (25°C). From the second-order rate constant for the bimolecular aminolysis of 1,3-dimethoxy-2-methyl-5-acetoxyxanthone with ethyl glycinate the ELC for the intermolecular reaction was calculated as being 0.6 M,[50] almost one-half of the desired value of 1 M.[36] However, 19 is of little practical use, since its synthesis proved to be exceptionally troublesome: Condensation of phenol 22 with benzoic acid 23 afforded 24, which in turn was carried through nine steps to afford 19 in only 0.3% overall yield (Scheme I-5).[65]

The mercury models were not as promising: Structure 20 showed no evidence of acyl transfer in DMSO over 50 h.[66] Species 21 underwent a

Scheme I-5



22 23 24

moderately slow acyl migration ($t_{1/2}$ = 8 h in DMSO; ELC = 0.6 M), but the electron-rich character of the xanthone system caused concommitant demercuration.[51]

These preliminary studies thus established feasibility. It remained to refine and extend these results to achieve practical acyl transfer. Hence at the outset of this thesis four goals were set:

1. The in-depth investigation of the thiol capture reaction that results in unsymmetrical disulfide formation: A protocol should be developed where this process is rapid, quantitative, selective, efficient at very low concentrations (10^{-3} to 10^{-4} M), and applicable to both globally and minimally protected peptide fragments. This is discussed in Chapter II.

2. The development of a new acyl transfer system based on thiol capture *via* an unsymmetrical disulfide: Its design should follow from the lead cases described above; its synthesis must be short, efficient and applicable to large-scale preparations and its acyl transfer efficiency (ELC) should be greater than that of 19 by at least a factor of three. This is described in Chapter III.

3. The demonstration of the practicality of the prior amine capture principle as applied to the thiol capture methodology: This should entail, first, the development of solution and solid-phase protocols that would accommodate the strategy; second, the synthesis of peptide fragments utilizing these new methods, and finally, the realization of the overall scheme by segment condensation *via* intramolecular acyl transfer. This is described in Chapter IV.

4. The preparation and testing of acyl transfer models containing mercury at the capture site. This is discussed in Appendix III.

CHAPTER II

THE THIOL CAPTURE REACTION

As it was discussed in Chapter I, the first priority in this thesis was the development of an efficient process for trapping N-terminal cysteine peptides as unsymmetrical disulfides by "ligase"-type synthetic templates. Such a thiol capture reaction, if successful, would constitute the first step of a novel approach for peptide synthesis in which the amide bond is formed intramolecularly between the amino function of the trapped cysteine and the mildly activated acyl center of the template.[36]

Realization of a practical form of thiol capture requires that the following four requirements are met:

1. The capture reaction should be exceptionally selective and must proceed quantitatively, rapidly and at very low concentrations (10^{-3} to 10^{-4} M).

2. The process should be applicable to both globally protected and free N-terminal cysteine peptides.

3. For maximal versatility, the solvent(s) employed should be capable of dissolving a wide array of proteins and must disfavor unproductive intermolecular association, i.e. β-pleaded sheets, between fragments.

4. In its optimal form the thiol capture reaction should avoid extremes in pH and allow the employment of aqueous mixtures needed for the solubilization of minimally protected segments.

Unsymmetrical disulfides can be generated by the addition of an oxidant to a simple mixture of two different thiols. However, this

approach usually results in the generation of one unsymmetrical and two symmetrical disulfides in the statistical molar ratio of 2:1:1.[53a]

$$\underset{(A)}{4\ RSH} + \underset{(B)}{4\ R'SH} \xrightarrow{(O)} \underset{(AA)}{RS-SR} + \underset{(BB)}{R'S-SR'} + \underset{(AB\ +\ BA)}{2\ RS-SR'}$$

In theory,[53a] but also in practice,[67] the yield of the unsymmetrical disulfide can be improved if one component is applied in excess, provided the thiols combined are of similar nature and possess minimal conformational and steric effects. This is shown in Table II-1.

TABLE II-1: Yield of unsymmetrical disulfide bond formation as a function of molar ratio of reacting thiols[53a]

Molar ratio (A:B)	% unsymmetrical disulfide (AB + BA)[a]
1:1	50
1:2	67
1:3	76
1:4	80
1:5	83
1:10	91

[a]Based on A.

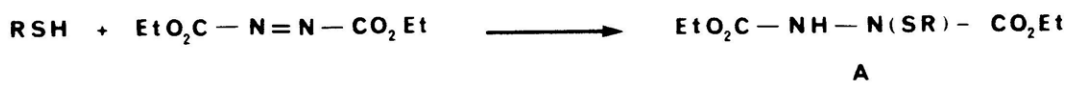
A better approach involves prior activation of one of the two thiols, as shown in Scheme II-1.

Scheme II-1

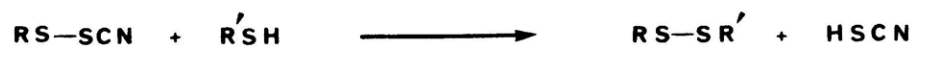
$$RSH + R'S-X \longrightarrow RS-SR' + HX$$

In the literature, attempts to generate conveniently activated derivatives of thiols have been mostly unsuccessful and only few such species have enjoyed practical use. Thus, sulfenyl chlorides (X = Cl),[68] sulfenyl amines ($X = NH_2CH_2R$)[69] and sulfenyl imides ($X = N(CO_2R)_2$)[70] have not attained any importance owing to the harsh conditions required for their generation. Similar problems were also encountered in the cases of thiosulfates ($X = SO_3$),[71] S-alkyl thiosulfonates ($X = SO_2R$)[72] and S-alkyl thiosulfinates (X = SOR).[73]

The elegant activation method of Mukaiyama[74] was also problematic. Although the reaction proceeds under mild and neutral conditions, product mixtures are often obtained due to concommitant disulfide interchange occuring during the activation step.



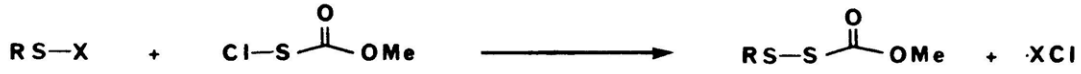
Exceptions to these unreliable approaches are the methodologies associated with sulfenyl thiocyanates and thiocarbonates. First reported by Lecher in 1922[68] and later reinvestigated by Hiskey,[75] reaction of mercaptans and thioethers (e.g. S-trityl, S-isopropyloxymethyl) with thiocyanogen reliably generates sulfenyl thiocyanates that can be trapped by thiols to afford unsymmetrical disulfides:[76]



Although this approach has been successfully utilized in many syntheses of unsymmetrical cysteine derivatives,[77] it suffers from some limitations: sulfenyl thiocyanates are known to decompose in the

presence of amines, are unstable in amide solvents such as DMF, and induce side reactions when employed on arginine-containing peptide fragments.[75]

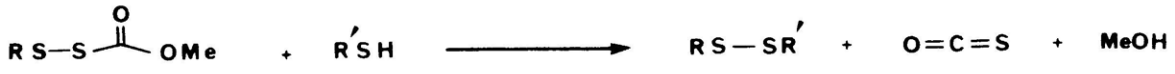
In a fashion similar to RS-SCN, sulfenyl thiocarbonates can be generated by treatment of thiols or thioethers (e.g. S-trityl, S-acetamidomethyl, S-benzhydryl) with methoxycarbonylsulfenyl chloride (ScmCl):[79,80]



25

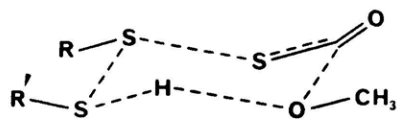
X = H, Tri, Acm, Bzh

However, in contrast with the thiocyanate derivatives, Scm compounds of type 25 are stable to storage, resistant to many deblocking and coupling conditions, and can be readily converted to unsymmetrical disulfides when treated with free thiols:[78-84]



25

This reaction, first discovered by Brois in 1970,[70] was postulated on slender evidence to proceed in an S_N2 fashion via the cyclic transition state 26:[78]



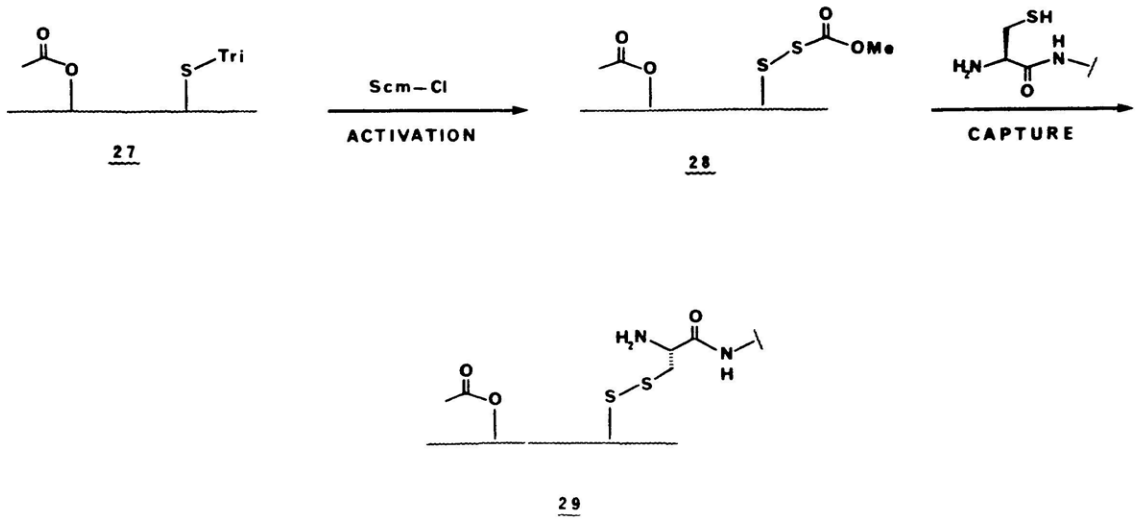
26

Essentially quantitative yields are reported for the reactions of ethyl-SScm with benzyl mercaptan, thiophenol and 1-mercaptopropane.[78]

Applications to peptides were first explored by Kamber[80] and Hiskey,[79] whereas an impressive demonstration of the utility of the Scm functionality comes from Kamber's total synthesis of human insulin: Cysteine residues at A-20 and B-19 were bridged in 77% isolated yield by condensation of an Scm-activated cysteine tetrapeptide with the thiol function of a cysteine dipeptide.[80]

Of the existing methods for unsymmetrical disulfide formation, the Scm approach appeared to be the most suitable for the prior thiol capture strategy. The reaction of an arene thiol with a cysteine Scm derivative would provide the closest analogy to the above precedents. However, the alternative capture of a cysteine peptide bearing a free thiol function by an arene Scm moiety offered some tactical advantages, and we chose to explore it first. The following general sequence outlines this latter approach (Scheme II-2).

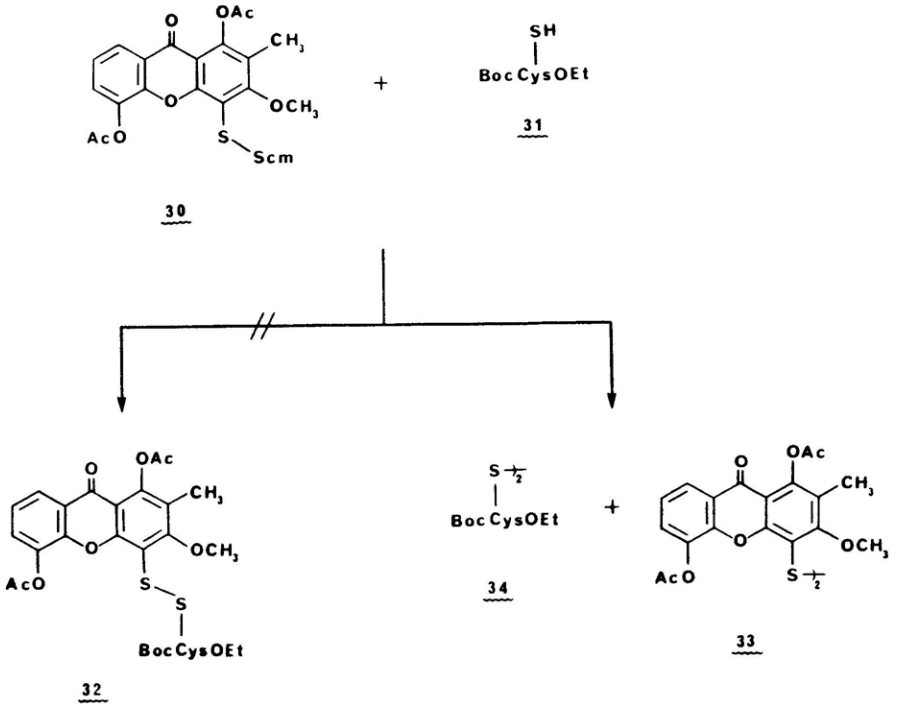
Scheme II-2



27 28 29

An S-trityl or S-acetamidomethyl-protected acetoxy template, e.g. 27, would be activated with ScmCl to generate species 28 which could be purified. Thiol capture of an N-terminal cysteine amine at the activated capture site of 28 would then afford unsymmetrical disulfide 29 which would undergo intramolecular O,N-acyl transfer as described in Chapter I.

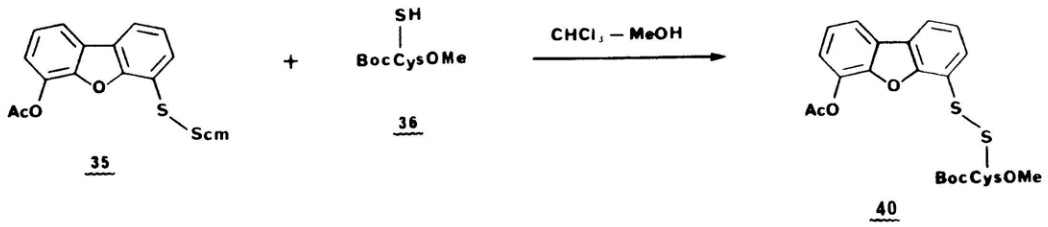
One of the first attempts to realize Scheme II-2 in these laboratories gave disappointing results: Kerkman found that reaction of the aryl-SScm derivative 30 (prepared by treatment of the corresponding aryl thiol with ScmCl) with ethyl N^{α}-Boc-cysteinate, afforded only symmetrical disulfides 33 and 34:[65]



In view of the efficiency and selectivity of the Scm reactions in the hands of Hiskey and Kamber, this result is somewhat surprising. However, it should be noted that Kerkman's target disulfide 32 is highly

unsymmetrical in both the steric and electronic sense.

In this thesis, capture of methyl N^{α}-Boc-cysteinate, 36, by the acetylated dibenzofuran template 35, performed under Kamber's conditions,[80] afforded the mixed disulfide 40 in only 38% yield.



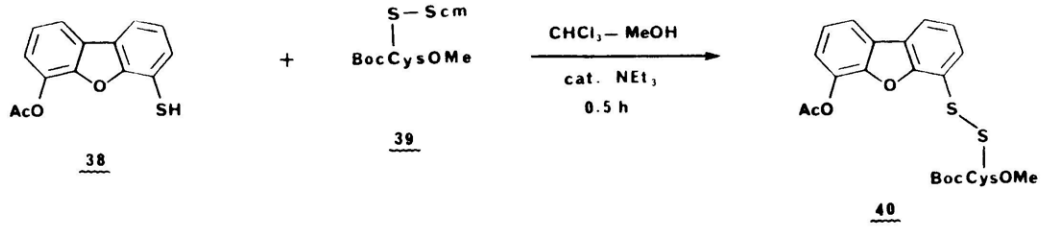
In an effort to study the effect of aryl electron density and improve the yields of the capture reaction, Stanley Dranginis in these laboratories prepared a variety of electron-rich and electron-deficient model aromatic Scm derivatives, and studied their reaction with methyl N^{α}-Boc-cysteinate[85] (Table II-2).

Table II-2: Effect of electron density on unsymmetrical disulfide formation via the Scm strategy

Aryl-SScm	Temperature	Solvent	% unsymmetrical disulfide
phenyl	25°C	$CHCl_3$-MeOH (1:1)	73
p-Cl-phenyl	25°C	$CHCl_3$-MeOH (1:1)	60
p-NO_2-phenyl	25°C	$CHCl_3$-MeOH (1:1)	trace
p-OCH_3-phenyl	25°C	$CHCl_3$-MeOH (1:1)	78
p-OCH_3-phenyl	25°C	hexafluoroisopropanol	75
p-OCH_3-phenyl	-45°C	$CHCl_3$-MeOH (1:1)	79

As shown in Table II-2, electron-withdrawing substituents suppress unsymmetrical disulfide formation, but electron-donating groups (e.g. methoxy) do not appear to effect the product distribution.

Having fallen short of our goal to achieve quantitative unsymmetrical bond formation by the convenient treatment of an Scm-activated template with a cysteine thiol, in this thesis we pursued the more lengthy, but literature precedented alternative of realizing thiol capture by allowing an arene thiol to react with an Scm-functionalized N-terminal cysteine residue. Thus, treatement of acetoxydibenzofuran thiol 38 (prepared by reduction of 35) with BocCys(Scm)OMe, 39 (prepared from 36 by reaction with ScmCl), generated the desired mixed disulfide 40 in 87% isolated yield:

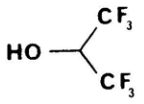


This reaction, when followed by HPLC, indicated quantitative conversion of 38 to 40, suggesting that the major requirement of efficiency for thiol capture was met.

The discovery of a synthetic approach that allows the high yield generation of unsymmetrical disulfides marked the turning point of this thesis. However, this accomplishment would have little practical use unless we could demonstrate that the thiol capture reaction, performed as in the conversion of 38 to 40, could be affected in solvents that solubilize large peptide fragments.

Mixtures of methanol and aprotic non-polar solvents such as chloroform have invariably been employed by others[78-80] to affect unsymmetrical disulfide bond formation by the Scm protocol. Clearly, these media are not suitable for the practical realization of the prior thiol capture strategy, since solvents of extremely high solubilizing power are needed for the dissolution of large peptide fragments. However, the employment of methanol by Hiskey and Kamber was encouraging in that it suggested that a medium of protic character is needed for clean thiol capture. Therefore, if we could demonstrate that a protic solvent of exceptional solubilizing power is compatible with the Scm strategy, then we would have greatly resolved the question of applicability of our methodology to the synthesis of polypeptides.

Although phenols[86] and cresols[87] have been successfully employed as efficient solvents for large peptide fragments, we were attracted to an even better possibility: Polyfluorinated alcohols, and in particular hexafluoroisopropanol (HFIP), 41.



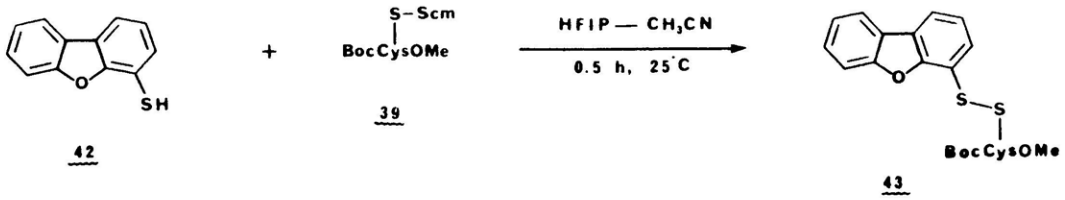
41

First prepared by Middleton[88] at Du Pont, HFIP has been found to possess an extraordinary capacity to dissolve macromolecules by breaking tertiary structures. This property presumably stems from the exceptionally high hydrogen-bonding ability of this solvent, which results from the inductive effect exerted on the hydroxyl function by the two trifluromethyl groups.[89] Although HFIP has been utilized in the dissolution of polymers such as polyesters and polyacrylonitriles,[90] it has enjoyed

limited use in peptide chemistry, and only in cases where other solvents were either unsuitable or incompatible with the given operation. Thus, Phillips employed HFIP to dissolve ribonuclease,[90] and Blout utilized it for UV and CD studies of polypeptides.[91,92] Finally Kamber used HFIP as a convenient medium in his efforts to differentiate between the S-Acm and S-Tri funcionalities in their reaction with iodine.[93]

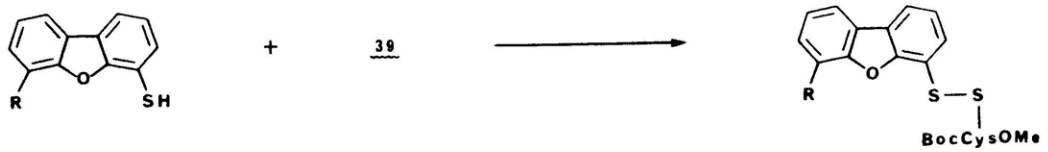
Hexafluoroisopropanol is one of the most suitable media for the thiol capture reaction when the process is performed on polypeptides. In addition to its known ability to inhibit unproductive secondary association of polypeptides,[90] HFIP is freely miscible with water,[90] thus allowing the use of aqueous mixtures when high-polarity media are needed to solubilize peptide fragments or accelerate reaction rates. Moreover, hexafluoroisopropanol has a boiling point of only 58°C,[90] which allows rapid and complete evaporation, in contrast with the commonly used high boiling polar aprotic solvents such as DMF, DMSO, and HMPA. The unique nature of HFIP is pointedly summarized by Blout as follows:[91] "We have found that hexafluoroisopropanol has many of the desired properties of an ideal polypeptide solvent. It dissolves a wide variety of non-ionic polypeptides, as well as most proteins. It is more transparent than other common polypeptide solvents... Preliminary work has also demonstrated that it is useful in NMR studies of polypeptide conformation."

The compatibility of HFIP with the Scm moiety was successfully tested by the reaction of 39 with 4-mercaptodibenzofuran, 42 (prepared by treatment of 4-lithiodibenzofuran with elemental sulfur[108]). The thiol capture was rapid, did not require a base catalyst, and afforded the mixed disulfide 43 in 87% isolated yield:



42 + 39 → 43

Finally, the generality of our approach was further documented by allowing thiols 61 and 45 to react with the Scm activated cysteine 39. A summary of all capture results is shown in Table II-3.



61: R = OH
45: R = OAlaZ

46: R = OH
47: R = OAlaZ

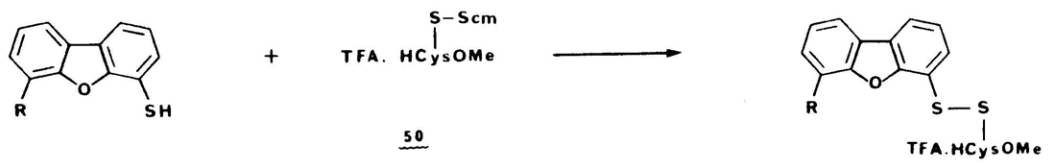
Table II-3: Thiol capture on cysteine 39

R-Dbf-SH[a]	Conditions[b]	Product, % yield
42: R=H	HFIP-$CHCl_3$ (22:1), 0.5 h	43: 83%
42: R=H	HFIP-CH_3CN (4:6), 0.5 h	43: 87%
61: R=OH	MeOH, cat. NEt_3, 1 h	46: 87%
38: R=OAc	MeOH-$CHCl_3$, cat. NEt_3, 0.5 h	40: 87%
45: R=OAlaZ	HFIP, 0.5 h	47: 90%
48: R=OAlaGlyZ	MeOH-DMF (2:1), 1.5 h	49: 22%

[a]Dbf: dibenzofuran substituted at C-4 and C-6. [b]All reactions were performed at 25°C.

Having established conditions for the efficient capture of N-protected cysteine derivatives (e.g. 39), we directed our attention to the more challenging problem of trapping N-terminal cysteine peptides possessing a free amino functionality. Demonstration of the feasibility of such process would allow realizaiton of a semisynthetic scheme in which Scm-activated cysteine peptides bearing unblocked N-termini would be captured by the thiol function of the template and then immediately allowed to undergo acyl transfer.

Although no literature precedent could be found for such an operation, Hiskey reports that the Scm moiety is resistant to the acidolytic conditions required for the deblocking of the Boc protection.[79] Thus, treatment of 39 with neat anhydrous trifluoroacetic acid at 0°C generated the TFA salt of methyl S-(Scm)-L-cysteinate, 50, in quantitative yield. Although initial thiol capture experiments on 50 in DMF were discouraging, the Scm-activated cysteine salt was found to react cleanly and rapidly in HFIP at 7 x 10^{-3} M concentration with an equimolar amount of template thiol to afford unsymmetrical disulfides as crystalline solids in 81-96% isolated yields (Table II-4). The identity of the captured cysteine salts was confirmed by comparison with the authentic materials generated by TFA-mediated removal of the blocking group from the corresponding N^{α}-Boc derivatives.



50

Table II-4: Thiol capture on cysteine salt 50

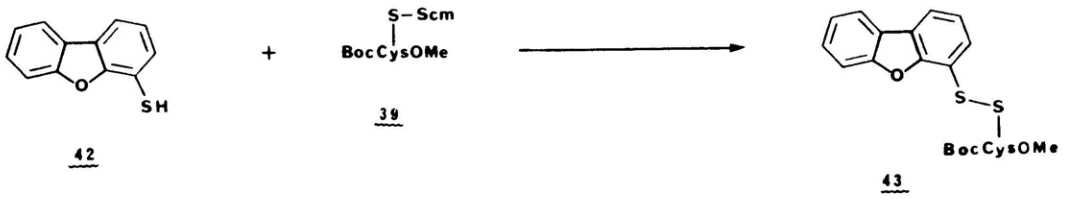
R-Dbf-SH	Conditions	Unsymmetrical disulfide
42: R=H	DMF, 20 min, 25°C	51: 32%
42: R=H	HFIP-$CHCl_3$ (5:1), 0.5 h[a]	51: 81%
38: R=OAc	HFIP, 0.5 h[a]	52: 82%
45: R=OAlaZ	HFIP, 0.5 h[a]	53: 96%

[a]0°C to room temperature.

Following this last result, we focused our attention on the last and perhaps most critical unanswered issue of this chapter, namely that of applicability of the thiol capture reaction at very low concentrations of reactants.

As discussed earlier, studies on simple systems indicated that the powerful polypeptide solvent HFIP can be successfully employed as the medium for thiol trapping. Given the incompatibility of protic solvents with the classical amide-forming methods, and the requirement of the prior thiol capture principle that medium to large-sized fragments be linked by an SS bond prior to intramolecular O,N-acyl transfer, this result represents a breakthrough. However, irrespective of the capacity of any solvent to dissolve macromolecules, it would be unreasonable to assume that in a real-life situation the concentrations of the peptide aryl thiol and the Scm-activated N-terminal cysteine fragment would ever exceed 10^{-3} or 10^{-4} M.[28] For this reason we undertook a kinetic study that we hoped would resolve the question of the effect of high dilution, and help us measure rate constants, investigate solvent effects, study base catalysis, and formulate a mechanism for the thiol capture reaction.

As a convenient model for this undertaking, we selected the reaction of thiol 42 with BocCys(Scm)OMe, 39:



42 39 43

Reactions were carried out in HFIP-CH_3CN-H_2O mixtures at ambient temperature under pseudo-first-order conditions at ca 4 x 10^{-4} M thiol concentration and ca 9 x 10^{-3} M in 39. The processes were monitored by HPLC at 280 nm (Figure II-2) and followed by the disappearance of 42 with time. Linear dependence of the pseudo-first-order rate constant on thiol concentration was noted in all cases, suggesting that the rates of these reactions are simply dependent on the product of thiol and cysteine concentrations (Figure II-1). Data are presented in Table II-5.

FIGURE II-1: Calculation of k_ψ for run #2, Table II-5

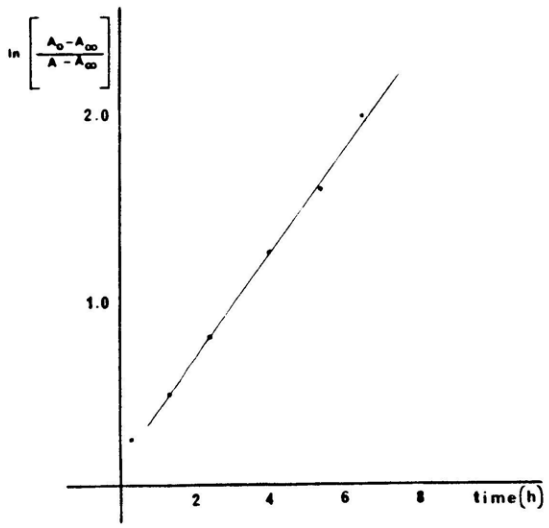


Figure II-2

Thiol Capture Reaction: Run #2, Table II-5

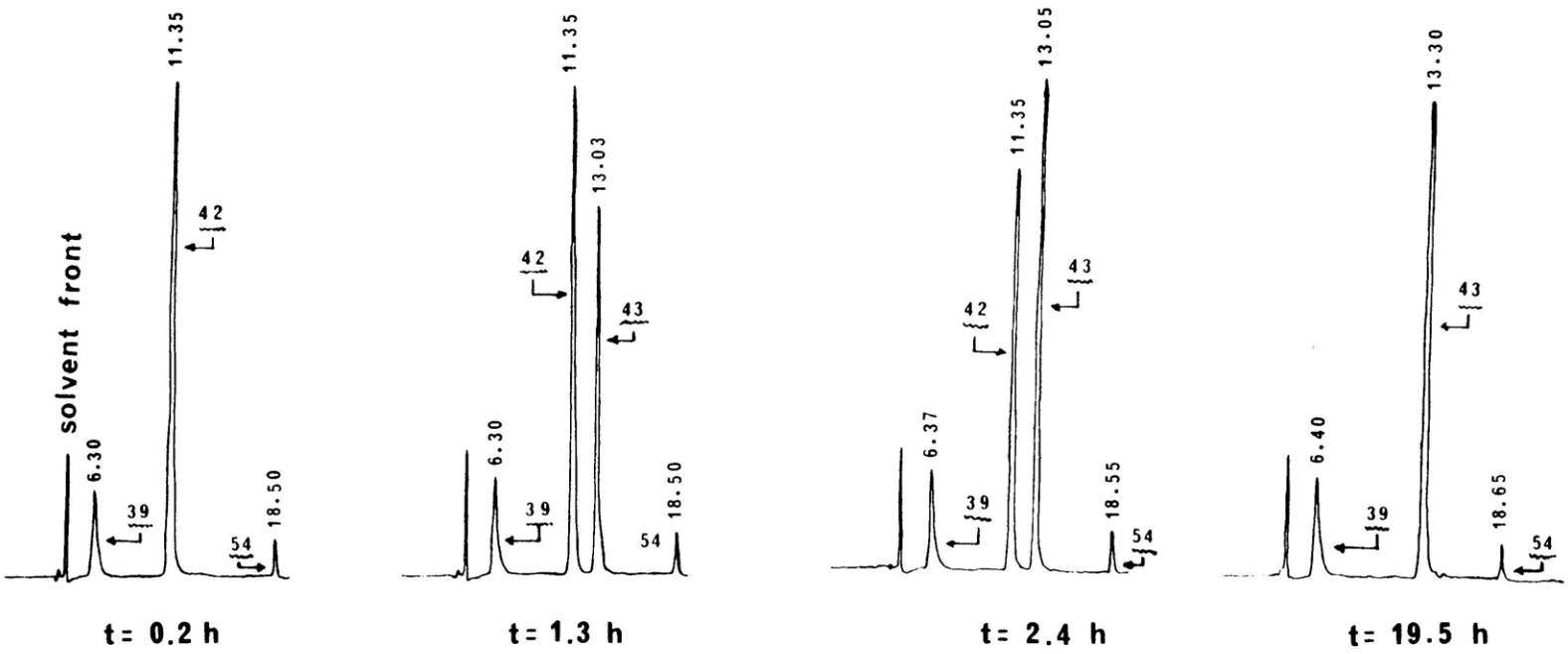


TABLE II-5: Kinetics of unsymmetrical disulfide bond formation between thiol 42 and Scm-cysteine 39 at 25°C.

Run	Solvent	[42]	[39]/[42]	Base[a]	[base]/[42]	$t_{1/2}$ (h)	k_2 ($h^{-1}M^{-1}$)	%$(ArS)_2$[b]
1	HFIP-CH_3CN (91:9)	8.57×10^{-4} M	22	-	-	15	2.5	10
2	HFIP-CH_3CN (87:13)	4.20×10^{-4} M	21	TEA	1.04	2.5	31	0
3	HFIP-CH_3CN (87:13)	4.20×10^{-4} M	21	DIEA	1.03	2.1	37	0
4	HFIP-CH_3CN (87:13)	4.31×10^{-4} M	21	DTBMP	1.01	2.2	36	2
5	HFIP-CH_3CN (87:13)	4.31×10^{-4} M	21	TEA	4.1	0.53	146	1
6	HFIP-CH_3CN (87:13)	4.31×10^{-4} M	21	TEA	10.1	reaction over within 1 h		2
7	HFIP-CH_3CN-H_2O (82:9:9)	4.44×10^{-4} M	21	-	-	2.0	36	1
8	HFIP-CH_3CN-H_2O (78:13:9)	4.27×10^{-4} M	21	TEA	1.02	reaction over within 0.5 h		0
9	HFIP-CH_3CN-H_2O[c] (55:9:36)	4.50×10^{-4} M	21	-	-	reaction over within 4 min		0

[a]Abbreviations: TEA = triethylamine, DIEA = diisopropylethylamine, DTBMP = 2,6-di-tert-butyl-4-methylpyridine. [b] % of 4-dibenzofuranyldisulfide, 54, after 6 half-lives. [c]Heterogeneous mixture.

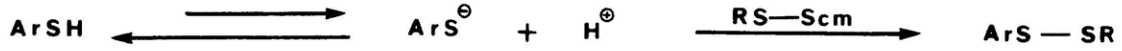
The data shown in Table II-5 indicate that two factors appear to affect the rate of the capture reaction: base catalysis and solvent polarity.

Tertiary amine bases seem to catalyze the thiol capture process in a first-order fashion. Noteworthy is also the fact all three amines employed induced approximately equal acceleration in rate, despite their inherent difference in basicity and steric bulk. It is particularly significant that 2,6-di-tert-butyl-4-methylpyridine is an efficient catalyst since nucleophilic participation of the amine in undesired processes, such as disulfide interchange, cannot occur with this species. Triethylamine has a pK_a of 10.6[94] and diisopropylethylamine is expected to exhibit approximately the same basicity. The much more hindered pyridine derivative should be substantially less basic and its pK_a value is assumed to be one or two units lower than that of 2,6-dimethylpyridine (DMP: $pK_a = 6.0$[94]). The observation that all three bases catalyze the reaction essentially to the same extent suggests that the catalytic species is not the amine, but instead the quartenary salt resulting from acid-base reaction of the amine with hexafluoroisopropanol.

Moreover, there appears to be a pronounced dependence of the reaction rate on solvent polarity: A fifteen-fold acceleration in rate was observed when water was introduced in the reaction mixture to the extent of 9% aqueous constitution. This result is in accordance with the magnitude of rate changes observed in similar reactions when simpler alcoholic solvents were diluted with water.[95]

These two factors, and especially the striking dependence of rate on solvent polarity are inconsistent with the concerted mechanism proposed

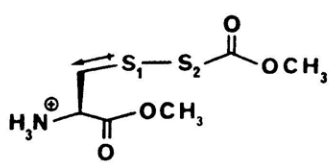
by Brois,[78] and instead support a stepwise bimolecular mechanism involving the aryl thiolate anion as the reactive species:



In one experiment, the reaction of equimolar amounts of 42 and 39 was studied at 4.44×10^{-4} M concentration in each reactant, in a solvent mixture consisting of HFIP-CH_3CN-H_2O (55:9:36 volume ratio). The observed half-life for thiol capture was 0.33 h, corresponding to a second order rate constant $k_2 = 6.8 \times 10^3\ h^{-1}M^{-1}$. Only 1% of symmetrical disulfide 54 was formed after ten half-lives. This last result demonstrates that thiol capture can be achieved efficiently, rapidly and at very low concentration of reactants.

Attempts to perform a kinetic study on the condensation of 4-mercaptodibenzofuran with the TFA salt of methyl S-(Scm)-L-cysteinate, presented us with the welcome problem of not being able to measure the rate by HPLC because the reaction was too fast: When a solution of 42 in HFIP-CH_3CN (91:9) was treated with an equimolar amount of the cysteine salt (4.54×10^{-4} M each), the reaction went to completion within 5 min and afforded the unsymmetrical disulfide 51 in 94% yield (6% of symmetrical disulfide was observed at that point).

This result can be rationalized by examination of the electronic nature of the cysteine salt:



The strong inductive effect of the ammonium ion creates electron deficiency at S_1. The electrophilic character of that atom is thus enhanced, and attack by the aryl thiolate anion proceeds rapidly to afford the unsymmetrical disulfide.

The conditions employed for this last reaction were by no means optimal: Significant rate acceleration was observed when the thiol capture reaction was performed in aqueous media (Table II-5).

In summary then, four points were established in this chapter:

1. Conditions were found under which thiol capture proceeds quantitatively, rapidly and at very low concentrations. Kinetic data were obtained to support these assertions, and a mechanism for the reaction is proposed.

2. The reaction was shown to be applicable to both N-protected and free N-terminal cysteine.

3. The solvent selected for the process, i.e. HFIP, known to possess exceptional solubilizing efficiency and to inhibit unproductive secondary association of peptide fragments, is consistent with our methodolgy and provided the best results.

4. Hexafluoroisopropanol can accommodate water to form polar media. Aqueous mixtures of HFIP caused acceleration in the rate of the thiol capture reaction and thus rendered the use of base catalysts obsolete.

Thus, having established the feasibility of an efficient thiol capture process, we proceeded to search for a template geometry that would allow rapid intramolecular O,N-acyl transfer. This is discussed in Chapter III.

CHAPTER III

INTRAMOLECULAR ACYL TRANSFER

The potential advantage of thiol capture, is the assembly of fragments by means of high and selective reactivities of the thiol function, rather than by the weak affinity of an activated acyl carbonyl for the amine function.[36] As discussed in Chapter I, two points must be established before such an approach can attain any practical utility: First, the capture step should be efficient at low concentrations of fragments to be combined; and second, a geometry must be found that permits rapid intramolecular acyl transfer with an acylating agent possessing a degree of activation equivalent to that of a simple phenyl ester (used as a protective group by Kenner[40]).

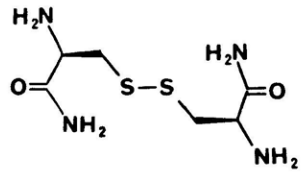
The problem of thiol capture was successfully resolved by employment of the methoxycarbonylsulfenyl (Scm) group as the activating species for unsymmetrical disulfide formation (Chapter II). In this Chapter, the solution to the second issue will be discussed.

The significance of a well-chosen geometry for a template assembly that would accommodate an intramolecular O,N-acyl transfer can only be underscored by the realization that the α-amino group of cysteine is an inherently poor nucleophile, since nucleophilicity toward an acyl carbon usually correlates directly with basicity for amines of a given structural class.[96]

Cysteine is the least basic of the amino acids. In a compilation of pK_a values for α-amino groups of the twenty naturally occuring residues, Greenstein and Winitz note that only proline has a pK_a value greater than

10, namely 10.6; four have values lower than 9, i.e. His (8.97), Lys (8.90), Asn (8.80), and Cys (8.33)[97]; the remaining eleven have pK_a's ranging from 9.00 to 10.00.

Moreover, amide substitution at the C-terminus causes decrease in basicity of the N-terminal amine by ca. 1.5 pK_a units. For instance, glycine, in its zweterionic form, has a pK_a of 9.60, whereas glycylglycine has a value of 8.17.[97] Most pertinent to our problem, L-cystine diamide, 55, has a reported pK_a value of only 6.69.[98] This weakly basic character has the agreeable consequence that the acyl transfer reaction can be run under conditions of effectively neutral pH, but it nevertheless underscores the need for an efficient intramolecular acyl transfer mechanism.

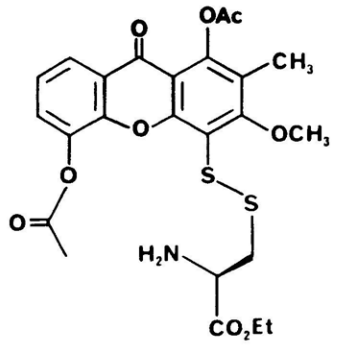


54

As described in Chapter I, examination of molecular models, formulation of preliminary design principles for template geometry, and actual testing of promising structural candidates, led to the discovery by Kerkman that the xanthone framework of 19 accommodated most of the requirements for a successful acyl transfer system.[48]

Assembly 19 underwent intramolecular acyl transfer across a 12-membered ring relatively cleanly and at a reasonably fast rate ($t_{1/2}$ = 2.7 h).[48] However, as discussed in Chapter II, this system suffered from

two major limitations: low acyl transfer efficiency (ELC = 0.6 M ;



19

minimum requirement = 1.0 M[36]) and poor overall yield for its synthesis (0.3%). With this lead case in mind, we set to accomplish four goals:

1. The design of a template, patterned after the xanthone prototype, that would exhibit at least three-fold better acyl transfer efficiency than 19.

2. The planning and execution of a short, high-yield synthesis of this material, allowing its production in large amounts (gram quantities).

3. The design of a convenient method for the preparation of O-acyl template thiols to be used in the penultimate thiol capture step.

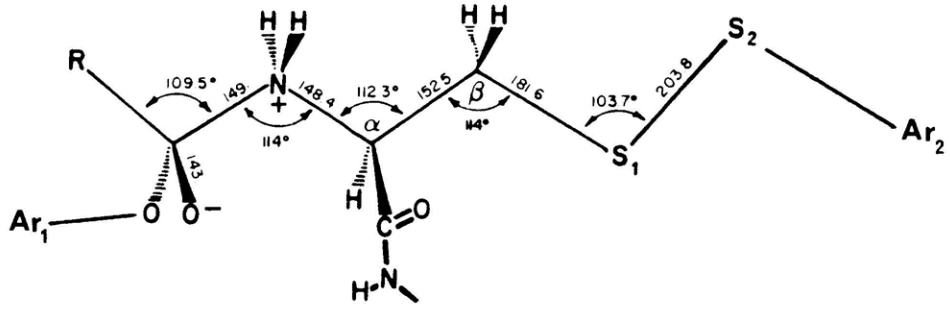
4. The in-depth investigation of the acyl transfer reaction on this template by examination of the effects of solvent polarity, O-acyl substitution, and electronic activation of the aromatic ring bearing the acyl moiety.

Structural candidates for the new template were selected by a two-fold process, involving careful inspection of Dreiding and CPK

space-filling models, as well as calculation of interatomic distances from X-ray coordinate data and assumed dihedral angles. The latter calculations were performed as follows:

First, a working model for the transition state of the acyl transfer reaction was built from available X-ray data (Figure III-1): Ar_1O and Ar_2S distances were estimated from representative aryl derivatives; OC and CN distances, as well as <OCN and <CNC_α are estimates from similar environments. All other bond distances and angles are for L-cystine.[99]

Figure III-1



Second, the geometry of this transition state model (Figure III-1) was fixed with a trans, anti orientation of the R-C and N-C_α bonds about the forming C-N linkage.[63]

Third, the $C_\beta S_1$-S_2Ar_2 dihedral angle was fixed at 90° ± 10°.[48]

Fourth, the CN-$C_\alpha C_\beta$ dihedral angle was assigned one of two values, 180° or +60 °.[28]

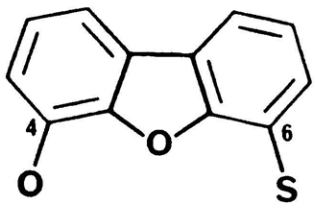
Fifth, values of ±60° or 180° were assigned to NC_α-$C_\beta S_1$ and $C_\alpha C_\beta$-S_1S_2 dihedral angles.[28]

Exclusion of conformers with large van der Waals interactions gave nine possible configurations, each characterized by an OS_2 distance. These ranges were calculated by a recursive program based on the stepwise estimation of distances across four atoms (Appendix I).

As a final exercise in this structural analysis, rigid templates were considered that would bridge each OS_2 distance with minimal steric and torsional strain. The dibenzofuranyl assembly 56a, substituted at C-4 and C-6, provided an excellent example of fit when compared with transition state 56b (Figure III-2):

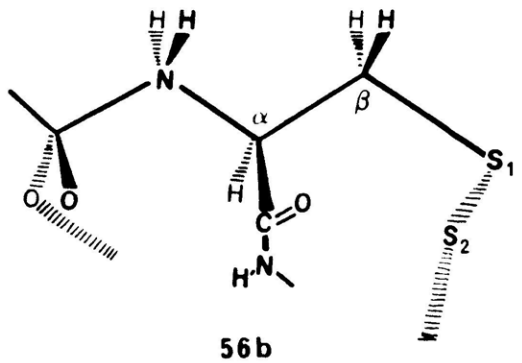
The OS distance for 56a, calculated from Banerjee's X-ray data on dibenzofuran,[100] was found to be 5.24 Angstrom, whereas the OS_2 range for the transition state model 56b, obtained by calculation as shown previously, was 5.27 Angstrom. Figure III-3 depicts this exceptionally good fitting.

Figure III-2



56a

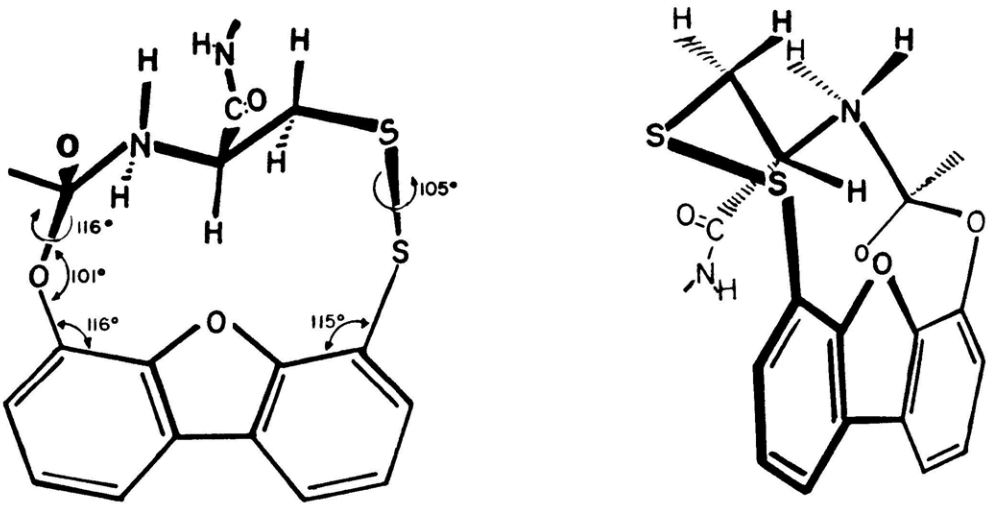
OS distance = 5.24 Å



56b

OS_2 distance = 5.27 Å

Figure III-3



Having found a good structural candidate, we proceeded to plan its synthesis.

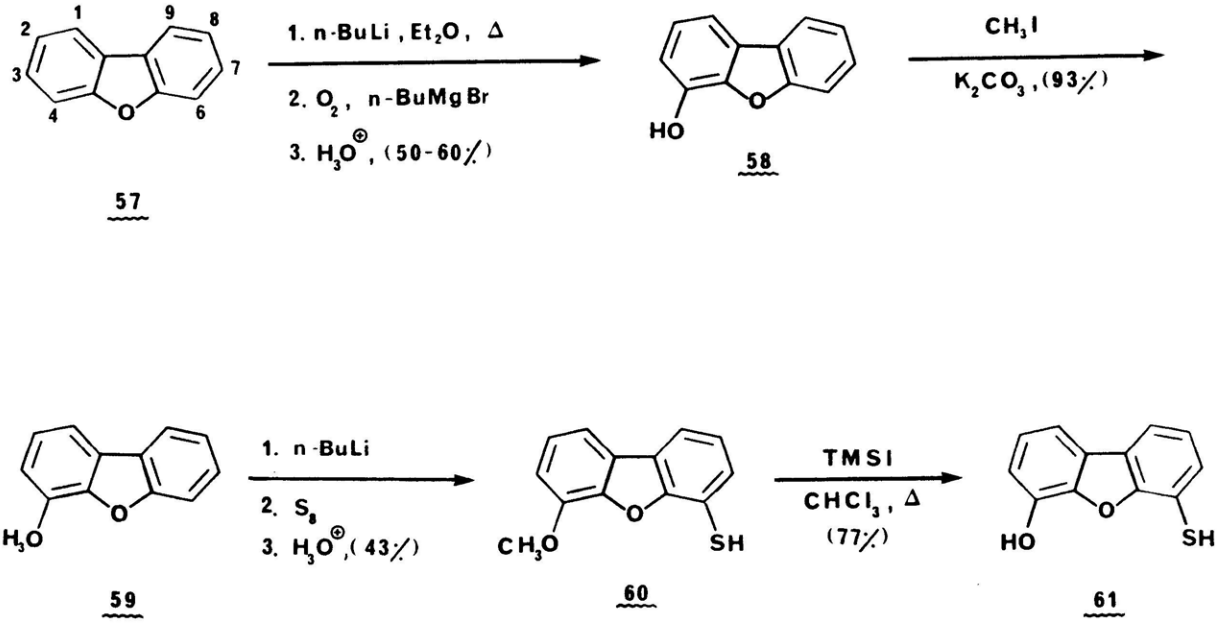
Literature search on derivatives of dibenzofuran 57 revealed that the chemistry of this ring system had been studied in much detail, especially between 1930 and 1950. This is partly because at that time it was realized that the nucleus of 57 occurs in alkaloids of the morphine group, and thus had potential chemotherapeutic utility.[101]

In contrast with the chemistry of most heterocyclic systems where ring synthesis from appropriately substituted intermediates is the method of choice, derivatives of 57 are often prepared by nuclear substitution on the readily available dibenzofuran ring. In a series of papers dating as far back as 1930 Gilman found that the otherwise inaccessible

C-4 and C-6 positions of the dibenzofuran ring can be selectively metallated by alkyllithium reagents[102,103] and mercuric salts.[104] This discovery essentially laid the foundations of metallation chemistry, which has since been elevated to high levels of sophistication.[105]

Gilman reported that 4-hydroxydibenzofuran, 58, can be obtained by a one-pot, two-step procedure in which dibenzofuran is first lithiated and then allowed to react with oxygen in the presence of n-butylmagnesium bromide.[102,105] In our hands this preparation reliably generated crude 58 in 50-60% yield. Methylation[107] of crude 58 gave ether 59 that was easily purified by vacuum distillation. Lithiation of 59 and reaction with elemental sulfur[108] produced pure 60 after recrystallization. Finally the methyl ether of the methoxy aryl thiol was cleaved with TMSI[109] in high yield to afford template 61 as a crystalline solid, stable to storage (Scheme III-1). The structure elucidation of the metallation products of 4-methoxydibenzofuran was performed by NMR and is discussed in Appendix III.

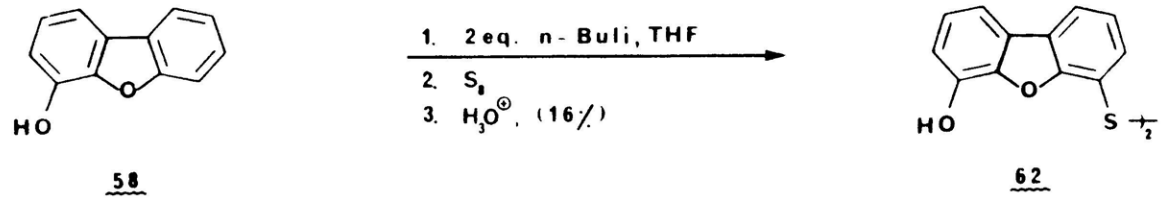
Scheme III-1



57 58 59 60 61

The yield and ease of purification of 60 were sensitive to the lithiation conditions: optimal results were obtained when tetrahydrofuran was used as the solvent, and temperatures of -28° to -32° were maintained for the lithiation step.[167] Lower yields and contamination with positional isomers were observed if higher temperatures (-20°C or over) were employed, or if diethyl ether was used at reflux as the solvent.

In one experiment the direct conversion of 58 to 61 was investigated: Treatment of 4-hydroxydibenzofuran with two equivalents of n-butyllithium, followed by quenching with sulfur, generated the symmetrical disulfide 62 in 16% yield. Presumably, the latter compound could be reduced to give 61:



58 62

In our hands, the procedure outlined in Scheme III-1 proved to be the most convenient in preparing multigram quantities of template.

Having devised a reliable and reproducible synthesis for 61, we proceeded to test its efficiency: this entailed a four-step operation:

First, the synthesis of a simple derivative of 61 patterned after the optimal sequence for prior thiol capture (see Chapter II). This assembly would serve as a working model for the measurement of the rate of the O,N-acyl migration at the transfer step.

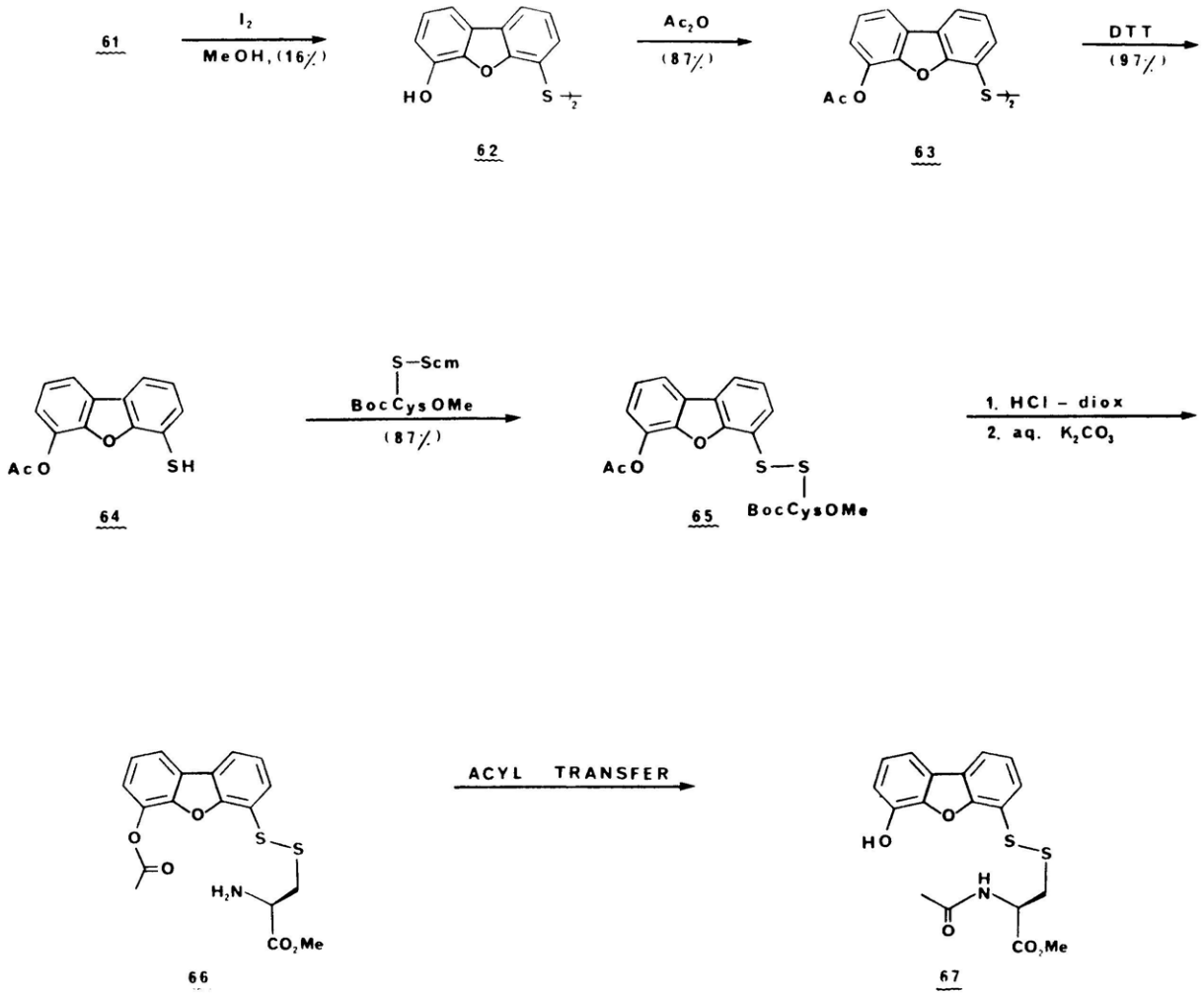
Second, the establishment of the unimolecularity of the acyl transfer reaction on the model system.

Third, the estimation of the reaction yield for the transfer step.

And fourth, the determination of the local concentration of the cysteine amine around the acetoxy group during acyl migration.

For simplicity, but also for comparative purposes with previously tested systems, we selected assembly 66 as the model compound (Scheme III-2). Its synthesis, involving the use of a symmetrical disulfide as a

Scheme III-2



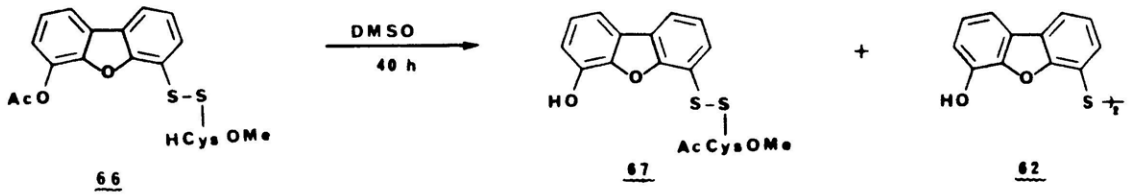
thiol protective group, and entailing the capture of an Scm-activated N-terminal cysteine derivative by the thiol functionality of the template as the key step, proceeded in high yields: Iodine oxidation[53a] of 61 generated symmetrical disulfide 62 which was acetylated to yield 63. Dithiothreitol reduction[53] of 63 afforded thiol 64 on which BocCys(Scm)-OMe was captured to give the unsymmetrical disulfide 65 in 60% overall yield (based on 61). Treatment with HCl-dioxane, followed by neutralization with aqueous potassium carbonate, afforded cysteine derivative 66, which was used immediately in the appropriate solvent for the acyl transfer step.

The rate of isomerization of 66 to 67 was measured as follows: Freshly prepared 66 was dissolved in a known volume of deuterated solvent and the O,N-acyl migration was followed by 250 MHz ^{1}H-NMR: the half-life for the reaction was taken when the integration of the appropriate acetyl methyl resonances were equivalent (Figure III-4).

When a dilute solution of cysteine amine 66 (2.9×10^{-2} M) in DMSO-d_6 was allowed to undergo this isomerization, a half-life of 2.0 h was observed; this compares favorably with the rate of acyl migration for the more highly activated ester of xanthone 19 ($t_{1/2}$ = 2.7 h).[48] A seven-fold increase in concentration of 66 (21.0×10^{-2} M) did not result in significant acceleration of the transfer rate, and a half-life of 1.6 h was measured. This result indicates that the acyl transfer observed for 66 is essentially independent of concentration and therefore the conversion of 66 to 67 proceeds by intramolecular O,N-acyl migration. Similar results were obtained when 66 was prepared by *in situ*

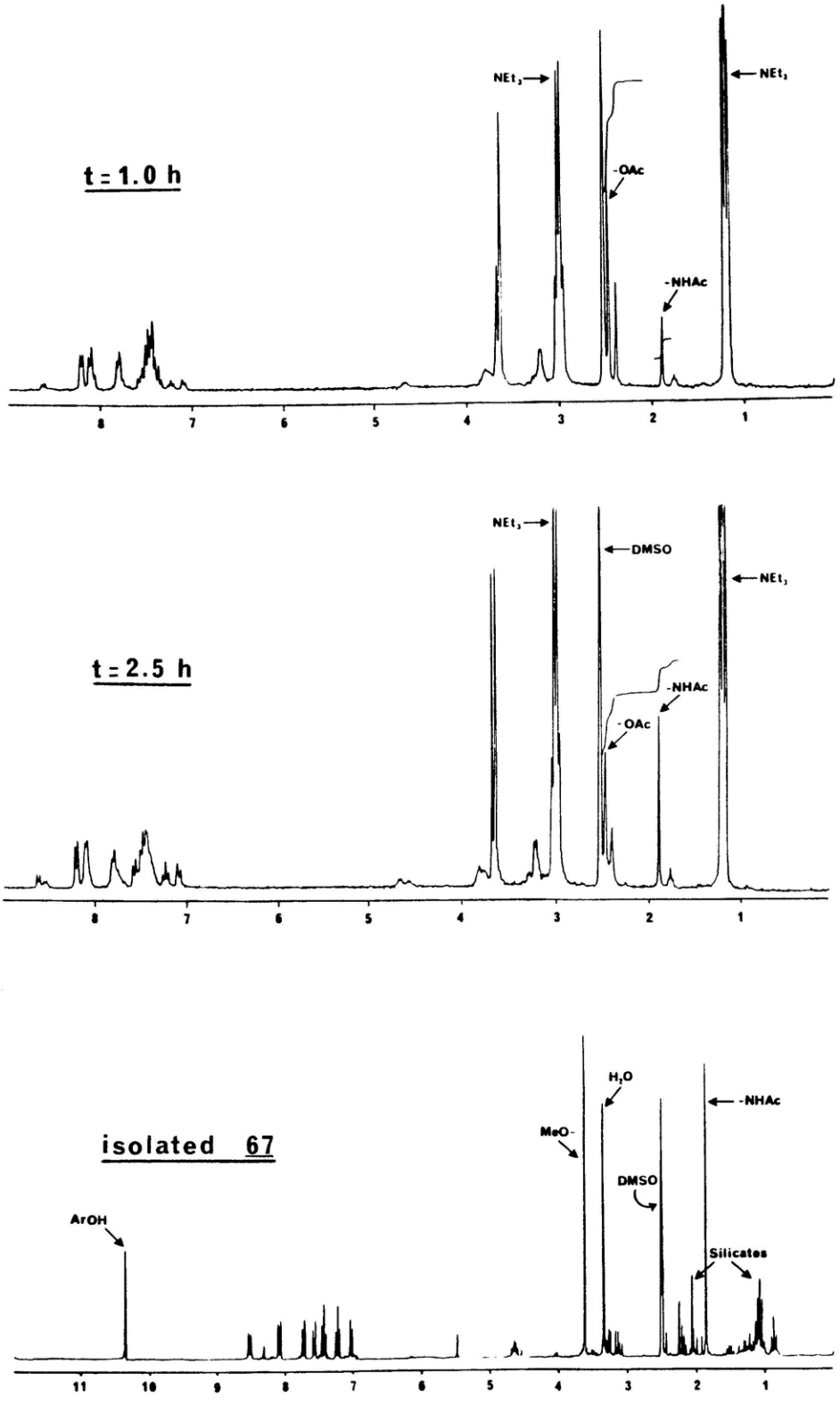
neutralization of a DMSO solution of the TFA salt of 65 with TEA; since this latter approach does not require isolation of 66, it became the method of choice for all subsequent kinetic experiments, except in the case of Z-AlaGly (see Table III-1).

The yield for 67 was estimated by NMR techniques and product isolation: At t = 40 h, the amide acetyl methyl resonance integrated for ca. nine-tenths of the total cysteine methyl ester protons, suggesting at least 90% acyl transfer; the resonance corresponding to the acetoxy methyl protons could not be detected at that point. The NMR sample was then worked up and the products purified by preparative layer chromatography: starting with 33.5 mg of crude mixture, 10.1 mg (30%) of 67 and 5.0 mg (27%) of 62 could be isolated as the only UV-active compounds:



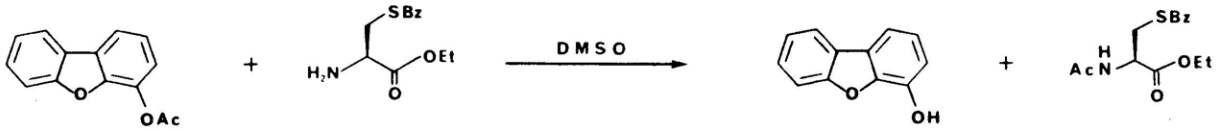
66 67 62

Figure III-4: Intramolecular acyl transfer of 66 in DMSO-d_6



This result indicates that disulfide scrambling occurs during the course of the reaction time. Since no acetylated disulfide (i.e. 63) could be detected in the product mixture, it can be assumed that disulfide interchange proceeds at a much slower rate than acyl transfer. For all practical purposes, disulfide scrambling of this nature is immaterial, since according to the scheme for amide bond formation by prior thiol capture, a reductive step is included after acyl transfer. Moreover, if these experiments were to be performed at infinite dilution, no disulfide interchange should be observed.[110]

From the reactivity of 4-acetoxydibenzofuran, 68, towards ethyl S-benzyl cysteinate in DMSO, the local concentration of cysteine amine around the acyl carbon[64] at the transfer step was determined:



68 58

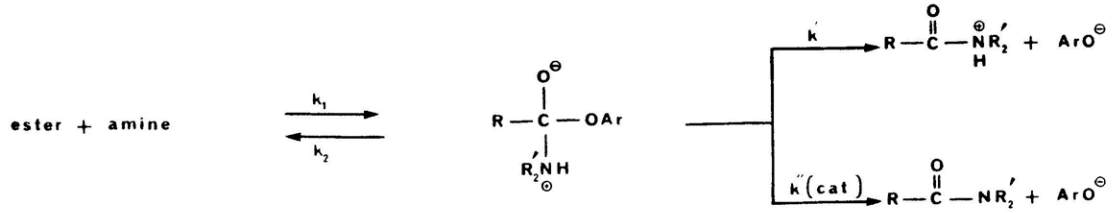
Division of the first-order rate constant for the intramolecular reaction at [66] = 2.9×10^{-2} M, by the second-order rate constant for the intermolecular aminolysis of 68, affords an effective local concentration[64] of 4.6 M. Similarly, a value of 5.7 M is obtained for the case of the more concentrated sample ([66] = 21.0×10^{-2} M). These results reflect a ten-fold improvement in efficiency over the xanthone system 19, thus demonstrating the validity of our design principles.

Having established high efficiency for the isomerization of 66 to 67, we examined the possibility of performing the reaction in solvents

other than DMSO.

We found that there is a pronounced dependence of the acyl transfer rate on the hydrogen-bond forming ability of the solvent employed for the reaction. In DMF the isomerization of 66 to 67 proceeded slowly and sluggishly, with a half-life of 30 h, in HFIP-DMSO (5:1) it gave a $t_{1/2}$ of 23 h, whereas in CH_3CN no appreciable acyl transfer was observed after 30 h. When a HMPA-DMSO (5:1) mixture was employed, the reaction rate was approximately equal to that in neat DMSO ($t_{1/2}$ = 2.2 h *vs.* 2.0 h). The 400-fold acceleration in rate observed by Watson for the catalytic effect of triphenylarsine oxide in the aminolysis of *p*-nitrophenyl acetates in chlorobenzene,[111] was not detected with our system; instead, a moderate improvement over neat DMF ($t_{1/2}$ = 23 h *vs.* 30 h) was observed when 66 was allowed to undergo acyl transfer in a 0.30 M solution of $\phi_3Ar{=}O$ in DMF.

These observations are in accordance with Menger's data on ester aminolysis in aprotic solvents,[112] and with Kemp's conclusions on the same issue:[28] Menger proposed that aminolysis of aryl esters in aprotic media proceeds by a general-base catalyzed mechanism, where the base catalyst is a second molecule of the nucleophilic amine that operates to remove the ammonium proton of the tetrahedral intermediate:[112]



For the intramolecular acyl transfer reaction, equimolar amounts of amine and aryl ester are present at high dilution. Therefore, it is unlikely that such a scheme could be realized in non-polar media (e.g. CH_3CN), and a slow rate is expected. On the other hand, solvents that enhance hydrogen-bond formation and/or proton transfer[113] (e.g. DMSO and HMPA) could serve as general base catalysts. The fact that the fastest rates for acyl migration were observed when the most polar aprotic solvents were employed is suggestive of the validity of this hypothesis.

In an attempt to explore the efficiency limitations of the dibenzofuran model, we investigated the possiblity of enhancing the rate of intramolecular acyl transfer by activating the oxygen-bearing ring with electron-withdrawing substituents:

The Hammett ρ values for the aminolysis reactions of substituted phenolic esters vary greatly with solvent and nucleophile, and are reported to span the range of 0.6-4.0, with the latter value representative of reactions in dipolar aprotic solvents.[114] Thus Menger reports a rate ratio of nearly 10^5 for reactions of phenyl and p-nitrophenyl acetates with pyrrolidine in acetonitrile.[112] Since the Hammett σ values for p-bromo and p-chloro substituents are 0.23, one estimates from the σ^- value of p-NO_2 that the chloro and bromo derivatives should be roughly an order of magnitude ($10^{0.94}$) more reactive than an unsubstituted phenyl acetate.[114]

Since a nitro-dibenzofuran was considered too labile for practical purposes, we prepared and tested the bromo and chloro derivatives shown on the next page:

The synthesis of 69 and 70 entailed relatively straight-forward manipulations and is discussed in Appendix II. Acyl transfer studies undertaken on these derivatives, demonstrated that the reaction rate was enhanced according to the literature predictions: The isomerization of 69 to 71 proceeded in DMSO with a half-life of 24 min, whereas a six-fold decrease in rate was observed when the reaction was performed in DMF ($t_{1/2}$ = 2.5 h). For 70, the acyl transfer was extremely fast; in DMSO, a half-life of 10 min was measured and no starting material could be detected after 2 h.

Aminolysis of 1-chloro-4-acetoxydibenzofuran, 73, with ethyl S-benzyl-L-cysteinate provided a second-order rate constant which was used to determine the local concentration[64] of the amine in 70 as being 4.6 M. This last result is extremely important for the evaluation of the efficiency of the dibenzofuran backbone geometry: Essentially identical estimates for ELC of the cysteine amine were obtained for both the

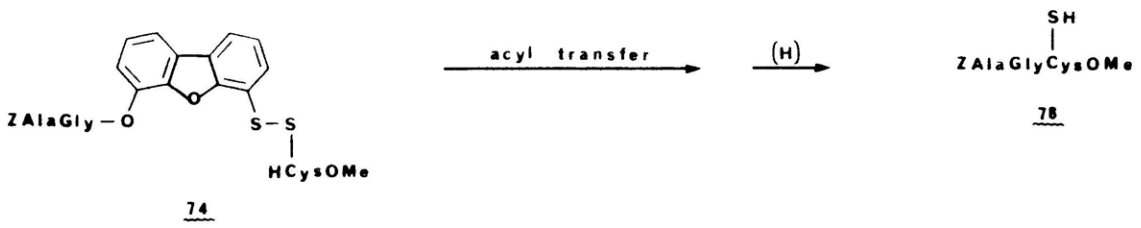
activated and the unactivated dibenzofuran template. Thus the ELC appears to be independent of the degree of activation of the phenyl ester, which suggests that it is, as we had hoped, a true measure of the efficiency of the dibenzofuran moiety in achieving a low-energy transition state for acyl transfer.

Having resolved the synthesis and efficiency issues, we directed our attention to the practical aspects of the thiol capture methodology. It was a well-timed coincidence that Bolin, in these laboratories, had at that time completed a solution-phase synthesis of the tetradecapeptide somatostatin,[115] a hormonal growth factor.[116] The thought of a somatostatin synthesis entailing amide bond formation by prior thiol capture between a dipeptide and a dodecapeptide was seriously entertained, and a study involving the preparation of a model tripeptide was undertaken:

Somatostatin:

$$\underset{1}{\text{H-Ala}}\text{-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-}\underset{14}{\text{Cys}}\text{-OH}$$

Model:



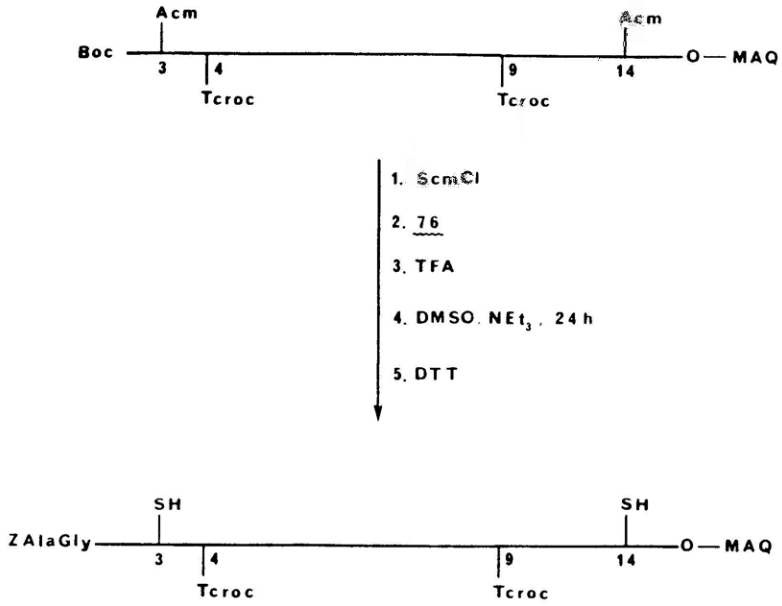
74 78

The synthesis of 74 was performed according to Scheme III-2, except that the acetylation step (62 → 63) was replaced by a DCC/HOBt-mediated coupling of N^{α}-Z-AlaGlyOH and 62. Although dicyclohexylcarbodiimide has been widely used in the preparation of active esters,[117] and is known to

be compatible with disulfides[79] the coupling proceeded in low yield (39%). The problem of acylating two phenolic functions, together with unproductive transesterification of the aryl ester with the anion of HOBt (produced during the coupling reaction), could account for this result.

In the reduction step, solubility problems were encountered with the acylated symmetrical disulfide 75, and a 73% yield of thiol 76 was obtained. Poor timing was the reason for the low product recovery in the capture step: since at that point in time we had no experience with hexafluoroisopropanol, the reaction of 76 with BocCys(Scm)OMe was performed in DMF-MeOH: most of the thiol oxidized to give symmetrical disulfides, and only 22% of the desired mixed disulfide 49 was isolated. Deprotection of cysteine 49, followed by neutralization, produced 74, which in turn provided the only positive result in this sequence: Intramolecular acyl transfer across the dibenzofuran assembly proceeded rapidly ($t_{1/2}$ = 2 h) and efficiently (91% calculated yield by NMR) and afforded the tripeptide N^{α}-Z-AlaGlyCysOMe, 78 in 76% isolated yield after dithiothreitol reduction of the crude product mixture followed by preparative layer chromatography. The acyl transfer-derived tripeptide was identical with an authentic sample of the material obtained by reduction of cystine $(Z\text{-AlaGlyCysOMe})_2$, 79.

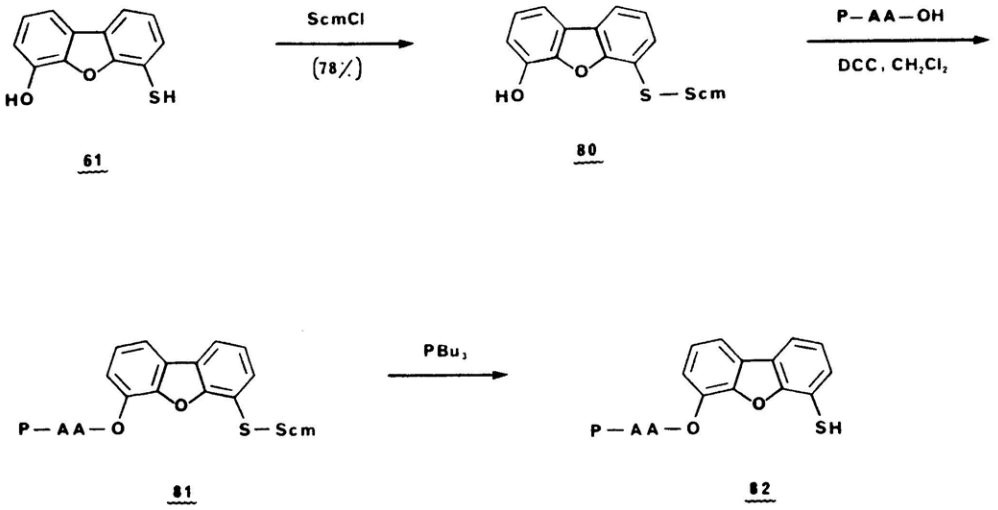
Despite these synthetic hardships, Bolin was able to synthesize somatostatin by this method: capture of dipeptide aryl thiol 76 onto an Scm-activated dodecapeptide, followed by intramolecular acyl transfer and reductive liberation of the peptide from the template, provided a material, that after purification, gave a satisfactory amino acid analysis for the desired tetradecapeptide.[123]



We reasoned that the problems encountered in the synthesis of O-acyl derivatives of template 61 resulted primarily from the inherent insolubility of symmetrical aryl disulfides in commonly used solvents. In our search for an alternative thiol protective group, we recalled that methoxycarbonylsulfenyl chloride reacts readily with thiols.[53a] Moreover, we found literature precedents that show that Scm derivatives of alkyl thiols are stable to DCC[79] and can be reduced efficiently to the corresponding mercaptans by treatment with triphenylphosphine in aqueous dioxane containing a catalytic amount of H_2SO_4.[83]

We incorporated these facts into a new synthetic strategy for preparing acetoxydibenzofuran thiols, as depicted in Scheme III-3. We found that the efficiency of the introduction of the Scm group onto 61 was dramatically dependent on the quality of the ScmCl used: Poor yields (22-40%) were obtained when the commercially available reagent (Fluka)

Scheme III-3



was employed, whereas a more laborious process involving the preparation of the reagent according to literature procedures,[118] followed by spinning-band distilllation of the crude sulfenyl chloride, afforded high yields of 80 (65-78%).

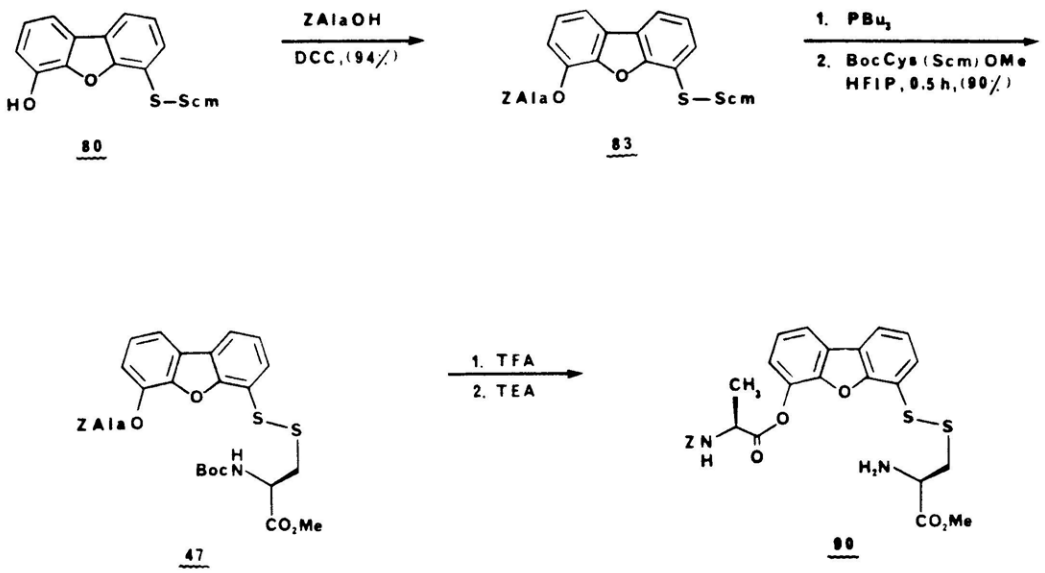
Dicyclohexylcarbodiimide-mediated coupling of a variety of N^{α}-Z, N^{α}-Boc and N^{α}-Bpoc amino acids with dibenzofuranol 80 provided active esters 81 in consistently high yield. Pure materials, obtained by recrystallization or flash chromatography[119] of the crude products, were stable compounds, and could be stored at 4°C for long periods of time (over 2 years) without decomposition. Table III-1 lists some derivatives prepared thus, and characterized.

TABLE III-1: Synthesis of peptide-Dbf-SScm derivatives

RO-Dbf-SScm	Purification[a]	Yield	mp (°C)
83: R = Z-Ala	R	94%	131
84: R = Z-Leu	C	98%	127
85: R = Boc-Ala	R or C	80%	148
85a: R = Boc-Phe	C	74%	91
86: R = Boc-Leu	R	71%	112
87: R = Bpoc-Phe	C	84%	64[b]
88: R = Bpoc-Tyr(OBu^t)	C	65%	70[b]

[a]R: recrystallization; C: chromatography. [b]Product obtained as a foam.

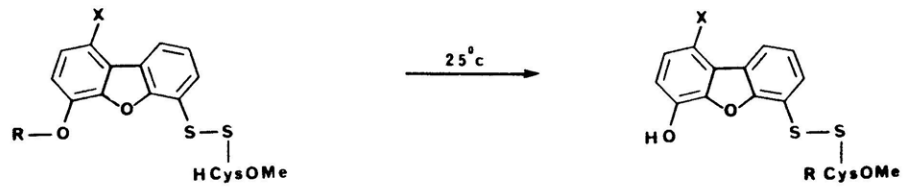
Since the use of an acid catalyst in the deprotection step is not compatible with Boc or Bpoc derivatives,[120] and in our hands, triphenylphosphine[83] and phosphine-derived insoluble resins[121] did not perform reliably, we adopted tri-n-butylphosphine as the reducing agent. In model studies, the Z-AlaOH ester of 4-hydroxydibenzofuran was found to be stable to PBu_3 in aqueous dioxane, whereas instantaneous and quantitative conversion of Scm-protected derivatives 81 to thiols 82 was observed at room temperature without the aid of an acid catalyst. Moreover, we demonstrated that 81, reduced to the mercaptan *in situ*, can undergo thiol capture with BocCys(Scm)OMe in high yields, provided that one equivalent of freshly distilled phosphine is employed for the reduction step. A synthesis depicting the merits of this new methodology is shown below:



Cysteine 90 underwent rapid O,N-acyl transfer in DMSO ($t_{1/2}$ = 3.0 h), and a 90% yield for the reaction was calculated by NMR. When the same reaction sequence was performed for the Z-LeuOH ester of 81, a half-life of 4.0 h was observed; this result, representing a rate ratio of 1.3 for Leu/Ala, compares favorably with the respective value of 0.6 observed for the corresponding intermolecular aminolysis of p-nitrophenyl esters.[63]

In an investigation of the generality of this approach, Dranginis prepared a number of O-acyl-dibenzofuran thiols and measured the rate of their intramolecular acyl transfer across the twelve-membered atom assembly of the 4-thio-6-hydroxy dibenzofuran backbone.[85] These data, along with observations made in this thesis, are summarized in Table III-2.

TABLE III-2: Intramolecular O,N-acyl transfer rates across the dibenzofuranyl assembly



91 92

R	X	SOLVENT[a]	HALF-LIFE (h)	k_{intr} (h^{-1})	ELC (M)	YIELD (calc)
Ac	H	DMSO[c]	1.6	0.43	5.7	>90%
Ac	H	DMSO[d]	2.0	0.35	4.6	>90%
Ac	H	HMPA-DMSO (5:1)	2.2	0.32	-	-
Ac	H	0.3M ϕ_3AsO in DMF	22.0	0.032	-	-
Ac	H	DMF	30.0	0.023	-	-
Ac	H	CH_3CN	-	-	-	-
Ac	Br	DMSO	0.4	1.7	-	>95%
Ac	Br	DMF	2.5	0.28	-	-
Ac	Cl	DMSO	0.2	4.1	4.6	>95%
ZAla	H	DMSO	3.0	0.23	-	-
ZLys(Z)[b]	H	DMSO	3.2	0.22	-	-
ZAsn[b]	H	DMSO	3.1	0.22	-	-
ZAsn(Mbh)[b]	H	DMSO	2.4	0.22	-	-
ZLeu	H	DMSO	4.0	0.014	-	-
ZVal[b]	H	DMSO	51.0	0.014	-	-
ZVal[b]	Br	DMSO	12.0	0.058	-	-
ZAlaGly	H	DMSO	2.0	0.35	-	91%

[a]All reactions were performed in deuterated solvents at concentrations in 91 ranging from 1×10^{-2} to 1×10^{-1} M, unless otherwise noted. [b]Experiments performed by S. Dranginis. [c][91] = 21.0×10^{-2} M. [d][91] = 2.9×10^{-2} M.

In conclusion, in this chapter we have accomplished the following goals:

1. The rational design of a new template: this was achieved in a two-step process involving first, a detailed analysis of the transition state for the intramolecular acyl transfer reaction, and second, a search for a rigid spacer to accommodate this geometry. Such a fitting was observed with the 4-thio-6-hydroxydibenzofuranyl moiety.

2. The convenient, large-scale synthesis of this template: This was accomplished by a four-step process, starting from inexpensive dibenzofuran (1 kg for $30[122]), and entailing only two isolations and no chromatography.

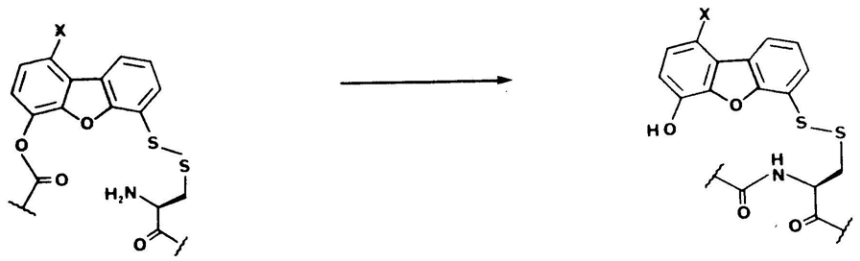
3. The efficient preparation of a variety of O-acyl template thiols by utilization of the versatility of the Scm moiety as a thiol protective group.

4. The demonstration that first intramolecular acyl transfer across the twelve-membered ring of the dibenzofuran assembly can proceed rapidly and efficiently with a half-life as low as 10 min, and second, that the process is applicable for amide bond formation between cysteine and a representative array of protected amino acid residues. What needed to be established next, is the utility of the prior thiol capture principle in the coupling of large peptide fragments. This is discussed in Chapter IV.

CHAPTER IV

APPLICATIONS OF THE PRIOR THIOL CAPTURE METHOD

In the preceding chapter we described the clean and efficient conversion of 91 to 92 by an intramolecular O,N-acyl transfer which occurs via a twelve-membered atom assembly comprised of an O-acyl dibenzofuran and a cysteine disulfide moiety. We demonstrated the generality of this amide-forming reaction with the transfer of a representative array of simple amino acid esters of dibenzofuran, and



91 92

in one experiment, Bolin successfully employed this concept in the synthesis of a dihydrosomatostatin derivative.[123] In this final chapter we propose new methodology that allows the generation of high molecular weight derivatives of 91 and addresses the question of applicability of the prior thiol capture principle to the synthesis of polypeptides.

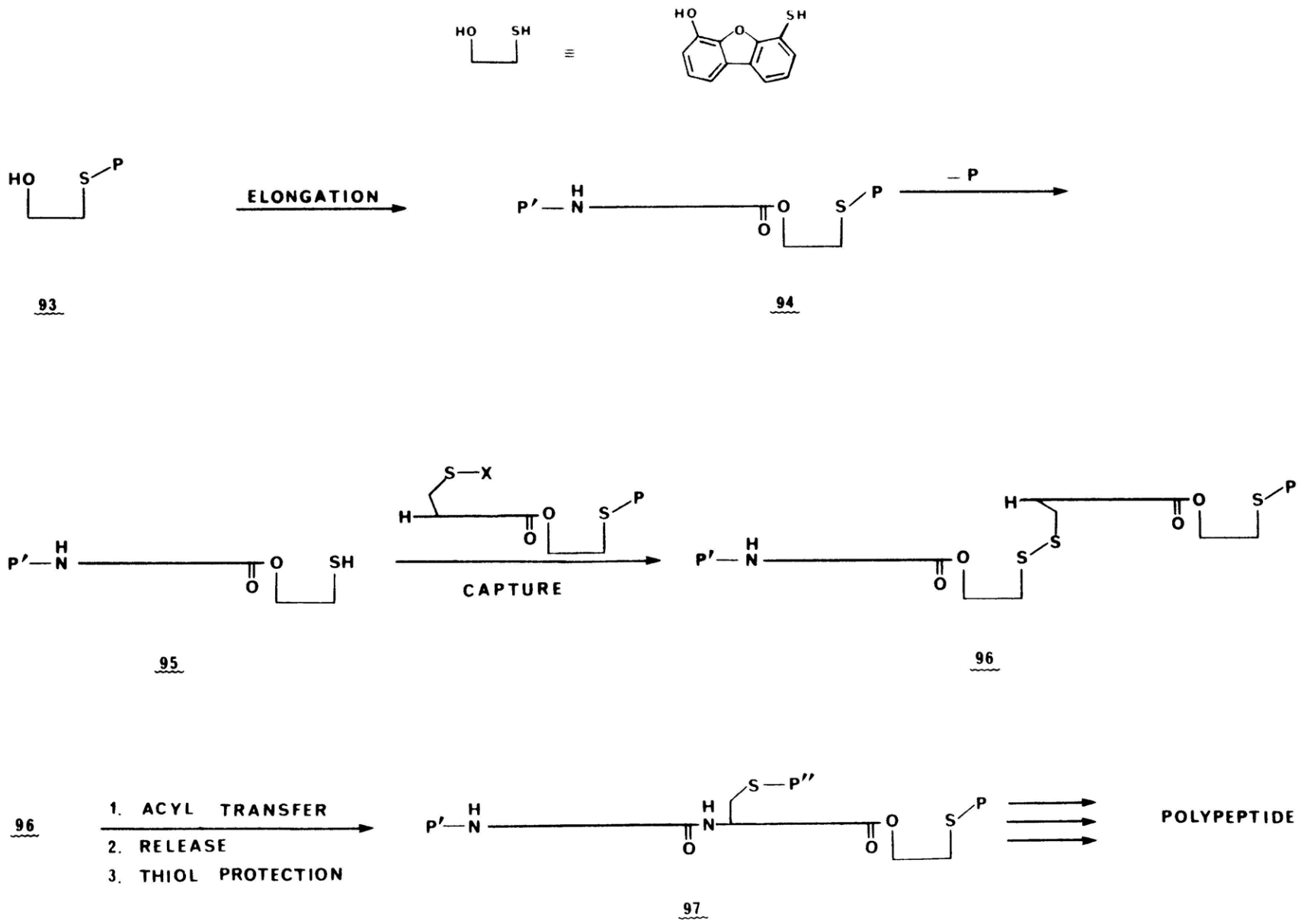
Bolin's somatostatin synthesis marked the first occasion in which the prior thiol capture principle was utilized in the generation of a medium-sized peptide. Reaction between Z-AlaGlyO-Dbf-SH and an Scm-activated dodecapeptide, linked the two fragments via an

unsymmetrical disulfide bridge, and following acyl transfer, the tetradecapeptide was isolated in good yield.[123] However, although successful, this undertaking also served to identify a number of significant limitations in the synthetic methodology employed. These problems, stemming primarily from the application of protocols designed for simple acyl transfer precursors to a complex molecule, can be traced to the inefficient generation of the appropriately functionalized assembly fragments. For example, the dipeptide aryl thiol was prepared in a mere 23% yield by a method suitable only for peptides bearing glycine at the C-terminus that involved the side-reaction prone, DCC-mediated esterification between Z-AlaGlyOH and the template.[4] Furthermore, the activation of the N-terminal cysteine peptide was inefficient: It required fourteen equivalents of ScmCl to go to completion, owing to the use of a solvent which itself consumes the reagent.[123]

Having already established the feasibility of the thiol capture and acyl transfer reactions, we sought to develop new methodology that would address these synthetic problems, and at the same time take the opportunity to formulate a general strategy for the synthesis of polypeptides by intramolecular acyl transfer. Our results are described in this Chapter.

The thiol capture principle is not intended for synthesis of small peptides. Instead, it is an elaborate, refined methodology designed to solve the one problem for which conventional methods fail, namely the reliable coupling of medium to large-sized peptides.[36] As outlined in the preceding Chapters and shown in Scheme IV-1, medium-sized fragments

Scheme IV-1

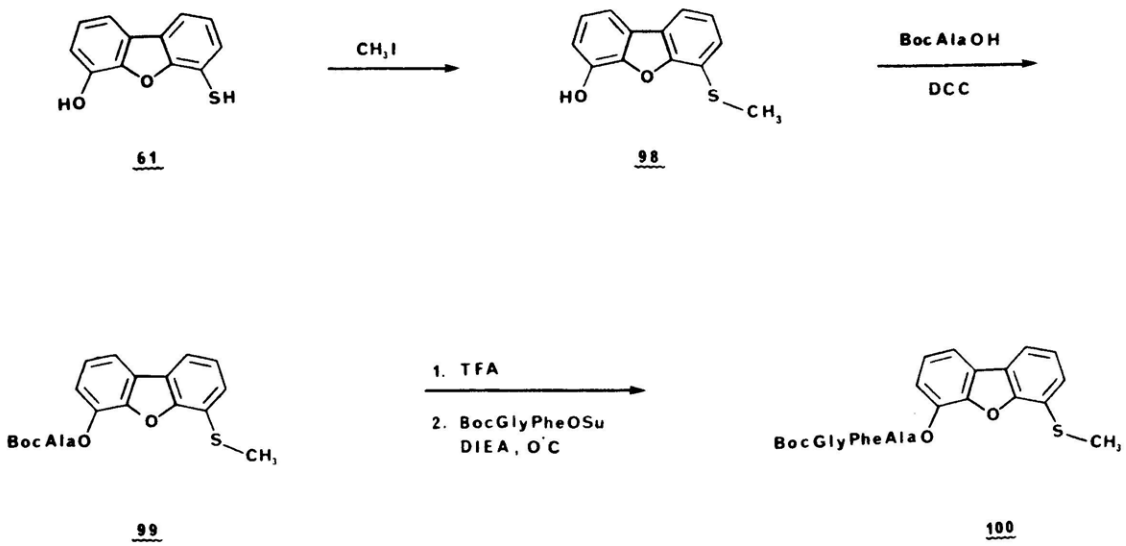

HO SH
≡
HO SH
O
HO S P
ELONGATION
93
P′ H N O O S P
– P
94
P′ H N O O SH
S X
H O O S P
CAPTURE
95
H S X O O S P
P′ H N O O S S H O O S P
96
96
1. ACYL TRANSFER
2. RELEASE
3. THIOL PROTECTION
P′ H N O H N S P″ O O S P
POLYPEPTIDE
97

bearing capture sites at their C-termini are to be linked successively in a convergent fashion to afford the polypeptide. The model experiments of those Chapters on SS bond formation and acyl transfer established the first step of feasibility; it now remained to apply these results to more challenging and complex polypeptides. However, before one could look to generalizations, a simple, versatile and reliable method had to be devised for constructing the fragments themselves.

A priori, one can envisage three possible ways of generating the desired fragments bearing dibenzofuran functions, i.e. species of type 95 (Scheme IV-1). The simplest would consist of classical or solid-phase synthesis of a fully protected polypeptide C-terminal acid, followed by esterification with dibenzofuran phenol 93. Two difficulties render this approach unacceptable: First, it is intrinsically difficult to prepare the required peptide acids, given the limitations of the experience-tested protective groups.[124] And second, none of the available amide or ester-forming reagents generate phenyl esters efficiently and without side reactions such as racemization.[125]

A second, more promising approach entails stepwise chain elongation in solution, a method employed by Schwyzer and Goodman in the preparation of p-nitrophenyl esters of C-terminal peptides.[126] An example of how this approach could be applied to the synthesis of O-acyl derivatives of S-blocked dibenzofuran is shown in Scheme IV-2, where for convenience and illustration, the thiol function of 61 is blocked with a simple methyl group. Removal of the N-terminal protection of species 99, followed by conversion of the resulting salt to the free amine immediately prior to acylation, constitutes the "backing-off" strategy.[126]

SCHEME IV-2



Thus, thioether 98 was readily acylated with BocAlaOH in the presence of DCC to generate ester 99 in 79% yield. Removal of the Boc protection followed by quenching of the resulting TFA salt with a mixture of one equivalent of diisopropylethylamine and three equivalents of activated dipeptide BocGlyPheOSu, afforded the coupled product 100 in 88% yield (HPLC).

The virtue of this protocol is that a phenol is esterified only with a simple N-blocked amino acid, a highly efficient operation compared to ester linkage formation with polypeptide acids.[127] In principle then, the "backing-off" strategy is compatible with amide bond formation between a medium to large peptide acid and an excess of a simple amino acid ester of an S-blocked template. Alternatively, a classical Bodanszky stepwise elongation approach could be employed,[128] starting with such an ester. However, both of these protocols would require a yet untested S-protective function that can be removed in the presence of a phenyl

ester and the side-chain blocking groups.[129]

Despite the several unexplored manipulations it would require, the "backing-off" approach may yet be promising; however, we were attracted to an unconventional scheme, which if realized, could offer unique versatility and efficiency. This third approach, would enable us to utilize the virtues of solid-phase peptide synthesis and involves the employment of a polymer as the protective group for the template mercaptan.

Solid phase peptide synthesis is a well-developed art that differs strikingly from conventional organic synthesis in solution, both in tactics of execution and in experimental procedure. This being the case, it seems appropriate that a digression must be made at this point to allow the discussion of the scope and limitations of the solid-phase principle.

In a solid-phase synthesis, the carboxyl terminus of an amino acid or peptide is immobilized by attachment to an insoluble polymer, which most frequently is a functionalized, 1-2% cross-linked polystyrene resin. The reactive amine terminus can then be treated with a soluble acylating agent and the resulting resin-bound amide isolated by simple filtration. Removal of the N-terminal protective group, followed by a second acylation results in linear chain elongation, free of the difficulties associated with insolubility and the need to isolate and purify intermediates.[22]

However, the virtues of the solid-phase principle are balanced by a range of special constraints. Since intermediates cannot be conveniently detached, purified and reattached to the resin, yields must be close to quantitiative, and side-reactions must be suppressed.[22] Large excesses of

acylating agents are conventionally used,[22] and repeated washes with carefully purified solvents are required to prepare the resin for the next stage of a solid-phase synthesis.

Moreover, identical functional groups bound within a resin may react at strikingly different rates. This inconsistency stems from the heterogeneity of the polymer, and solvents for solid phase synthesis are usually selected for their capacity to swell the resin (e.g. dichloromethane, DMF) rather than shrink it (e.g. ethanol, water, acetic acid), in an effort to maximize opportunities for a soluble reagent to encounter a bound functional group.[147] Practicing solid-phase peptide chemists have developed a wide variety of protocols for solvent washing cycles,[5] but it is not obvious at this stage that any one of these offers clear advantages.

In summary then, modern solid-phase synthesis, in any of its usual forms, offers rapid and convenient access to essentially clean samples of medium sized peptides (10-15 amino acid residues). In the opinion of many, although not all of its practicioners, the methodology becomes much less reliable when applied to large peptides.[20,22]

As outlined in Table IV-1, three main patterns in blocking group combinations have found extensive use. The oldest, pioneered by Merrifield, involves a benzyl ester linkage to the solid support, $\underline{N}^{\alpha}$-Boc protection and benzyl side-chain blocking.[130] Although chain elongation proceeds smoothly by repetitive acidolysis of the Boc function followed by neutralization and acylation, harsh acids such as anhydrous HF are required for the final deblocking.[143] A second combination, involving t-butyl side-chain blocking and α-amino protection by the 9-fluorenyl-

methyloxycarbonyl (Fmoc) group,[131,162] is incompatible with the phenyl ester of the template since secondary amines are used in Fmoc removal. A third choice, involving t-butyl side-chain protection and $\underline{N}^{\alpha}$ amine blocking by the Bpoc group (cleaved by 0.5% trifluoroacetic acid in dichloromethane), has been introduced and applied by Merrifield and his coworkers.[132,164]

TABLE IV-1: Strategies in solid-phase synthesis

Combination	Resin[a]	$\underline{N}^{\alpha}$-protection/deprotection	Side-Chain	Final Release
1	A	t-Boc/50% TFA-CH_2Cl_2	benzyl	HF,CF_3SO_3H
2	B	Fmoc/50% piperidine-CH_2Cl_2	t-butyl	50% TFA-CH_2Cl_2
3	B	Bpoc/0.5% TFA-CH_2Cl_2	t-butyl	50% TFA-CH_2Cl_2

[a]A: chloromethylated polystyrene; B: polystyrene benzyloxybenzyl alcohol.

Of the possibilities shown in Table IV-1, the first offers well-tested efficiency in the chain elongation process, and therefore seemed most suitable for exploratory work on our methodology. In the long run though, we envisaged the application of the third combination, which with its inherent versatility and mildness of conditions, complements best our synthetic protocol.

Although earlier workers often employed acylating agents that required hours to react to completion, most current solid phase syntheses are carried out with large excesses of the symmetrical anhydrides of $\underline{N}$-blocked amino acids.[20] These latter reactions are routinely performed in CH_2Cl_2, or mixtures of it with DMF, and result in

the clean and rapid acylation of resin-bound amines. Because of ready neighboring group participation reactions, asparagine and glutamine cannot be introduced using symmetrical anhydrides.[133] Instead, preformed active esters of N-hydroxylamine derivatives such as N-hydroxysuccinimide or N-hydroxybenzotriazole are employed.[134]

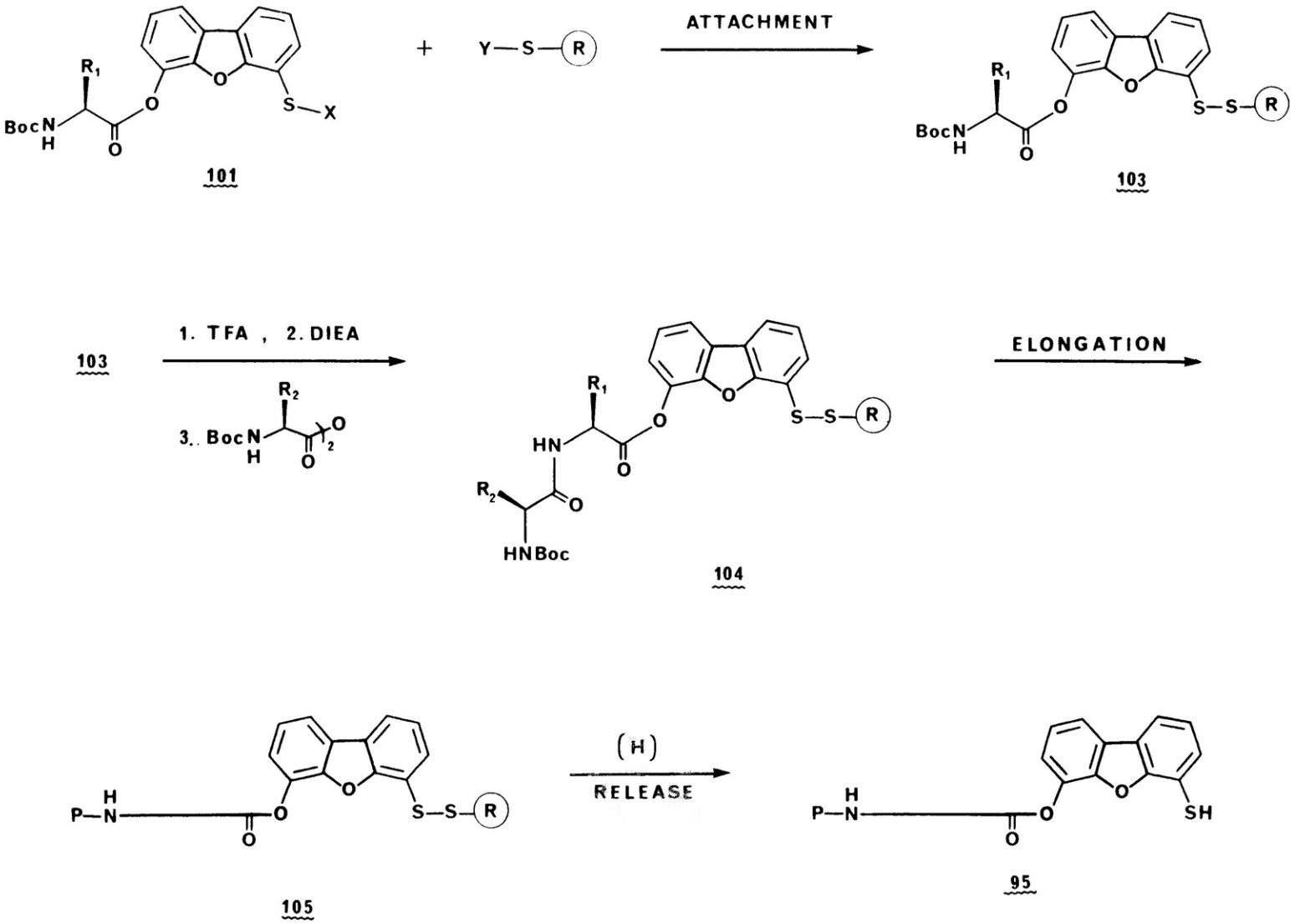
Incomplete acylation is the most frequent source of impurities during solid-phase synthesis, and a qualitative ninhydrin assay is commonly employed to check for the presence of unreacted free amino functions.[135] A price paid for the convenience of synthesis on a solid support therefore, is an inherent difficulty in characterizing the resin-bound products of chain elongation.[22] For this reason, analytical methods assume an unusual importance in this type of synthesis.

In this thesis we propose to explore the possibility of constructing a polystyrene resin that reversibly bears the dibenzofuran template, and on which the most efficient of the conventional chain elongation protocols can be applied. Of the possible linkages between the template and the solid support, disulfides offer several very attractive features as well as special constraints. These are explored in this Chapter.

The underlying principle for our synthetic protocol is outlined in Scheme IV-3. Thus, deblocking of resin-bound ester 103, followed by neutralization and acylation of the resulting free amine with an activated N-protected amino acid would generate the blocked dipeptide 104. Repetition of the coupling cycle would allow chain elongation, and reductive cleavage of the final aryl ester from the resin would provide the liberated dibenzofuran-functionalized polypeptide 95.

The applicability of this methodology, depicted in Scheme IV-3,

Scheme IV-3


ATTACHMENT
101
103
1. TFA , 2. DIEA
3.
ELONGATION
104
(H)
RELEASE
105
95

depends largely on the stability of the arene ester bond and the unsymmetrical disulfide linkage of the attachment product 103 to the repetitive acidolytic and neutralization conditions required for chain elongation on conventional solid supports.

The employment of ester linkages as attachment sites for solid-phase synthesis is not novel: Pivaille and coworkers found that simple phenolic esters of polystyrene can withstand conventional solid phase manipulations.[136] Moreover, recently, Buckle utilized the phenyl ester linkage generated between the phenolic site of tyrosine and a peptide acid as the attachment site for the growing peptide chain.[137] Finally, Arshady[138] and Ledwith[139] describe the use in solid phase synthesis of phenolic polymers based on styrene and acrylonitrile. These literature precedents, along with Kenner's successful utilization of phenyl esters as C-terminal protective groups,[40] and our own results on the applicability of the "backing-off" strategy on dibenzofuranyl esters in solution,[140] provided substantiated optimism.

Concerning the stability of disulfide linkages to the various steps of the solid-phase procedure, Lukenheimer and Zahn found that resin-bound symmetrical cystine peptides are not affected by HBr in TFA, nor by 10% (v/v) triethylamine in CH_2Cl_2 or DMF.[141] Recently, Gutte extended this observation to include unsymmetrical disulfides,[142] and in this thesis cysteine aryl disulfides of type 65 have been repeatedly shown to be inert to the acidolytic conditions required for the removal of the N^{α}-Boc group.[140]

The novel principle of unsymmetrical disulfide attachment of a peptide residue to a resin offers an array of attractive features:

Provided that the conditions employed for deprotection and acylation of the N-terminal amine of 103 do not affect the disulfide linkage, the latter bond can be conveniently cleaved at the end of the synthesis with phosphines, reagents that do not attack the commonly used protective groups nor affect the phenolic ester site.[140] This is in direct contrast with the often destructive requirement that strongly acidic reagents, such as HF, be used for the liberation of the completed polypeptide from the classical Merrifield resin.[143]

The unprecedented orthogonality between the final cleavage reagent and the peptide chain protective group offers unique synthetic versatility: For example, using Bpoc as the N^{α} amine protection and tert-butyl groups for side-chain blocking, it should be possible to release the polypeptide from the resin either before, or after the removal of the protective groups. In the event that impure products are formed at an intermediate stage of the synthesis, the peptide aryl thiol can in principle be reductively liberated from the solid support, then purified, and finally reattached to the resin for further manipulation.

In addition to the attractive synthetic features described above, the nature of the disulfide attachment site provides a unique analytical handle on the chain elongation process: Accurate (± 1%), and rapid (within 20 min) estimates of the efficiency of each coupling cycle should be obtained by a two-step process involving first phosphine reduction of a small portion of preswelled disulfide resin, followed by HPLC quantitation of the resulting peptide aryl thiol.

Finally, the multidetachable nature of 105 (Scheme IV-3) offers a choice of cleavage sites for the resin-bound aryl peptide. For the

purposes of the prior thiol capture strategy, release from the solid support should be affected at the disulfide linkage. However, if conventional formation of the amide bond is desired, hydrazinolysis of the phenolic ester 105 would allow application of the azide coupling method.[144]

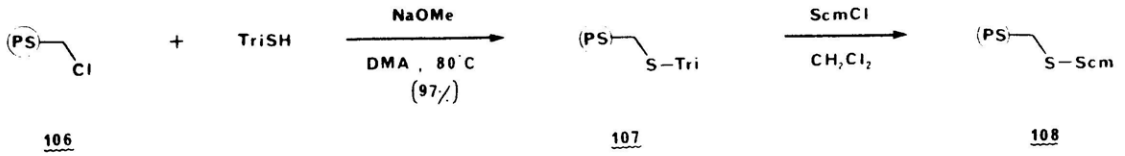
Practical realization of these attractive features requires demonstration of efficacy of the following three operations:

1. The reliable and efficeint formation of an unsymmetrical disulfide bond between the thiol functionalities of the dibenzofuran template and the resin (101 to 103).

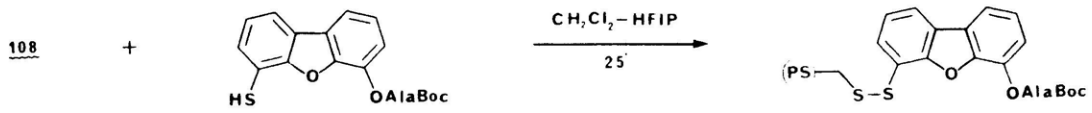
2. The application of the repetitive acidolytic, neutralization and coupling conditions required for chain elongation on a solid support in the presence of an unsymmetrical disulfide linkage and a phenolic ester bond (103 to 105).

3. The rapid and selective cleavage of the disulfide attachment at the end of the synthesis (105 to 106).

Although the utility of the Scm protocol for unsymmetrical disulfide formation in solution has been amply documented,[78-80] no examples of successful adaptation of this methodology to solid-phase synthesis could be found in the literature.[145] Our initial laboratory experience with this latter issue was equally unproductive. In an approach paralleling our successful solution-phase capture experiments described in Chapter II, we first attempted to realize unsymmetrical disulfide bond formation between the activated resin 108 and the aryl thiol 109.



106 107 108

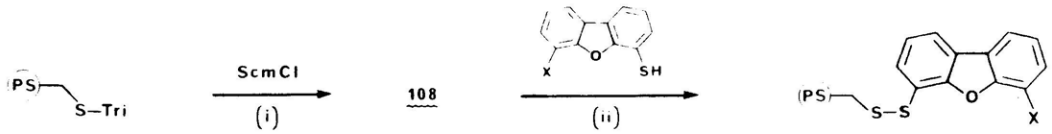


109 110

Treatment of chloromethylated polystyrene 106 (Chemalog, 1.03 mmol Cl/g) with the sodium salt of trityl mercaptan afforded thioether 107,[26] which was then converted to the Scm derivative 108 according to the procedure used by Kamber in the solution-phase synthesis of human insulin.[10] However, upon treatment of resin 108 with an excess of thiol 109 (prepared by reduction of its Scm derivative 85 with a stoichiometric amount of PBu_3), only 2-8% attachment product was obtained. This yield determination was reproducibly performed by a two-step process involving first, phosphine reduction of a pre-weighed amount of 110 suspended in a known volume of aqueous dioxane, and second, HPLC quantitation of the supernatant.

Somewhat better, but nevertheless unsatisfactory results were obtained when unacylated dibenzofuran thiols were allowed to react with 108. Table IV-2 summarizes all the attachment yields acquired by this method.

TABLE IV-2: Reaction yields for the conversion of 107 to 111.



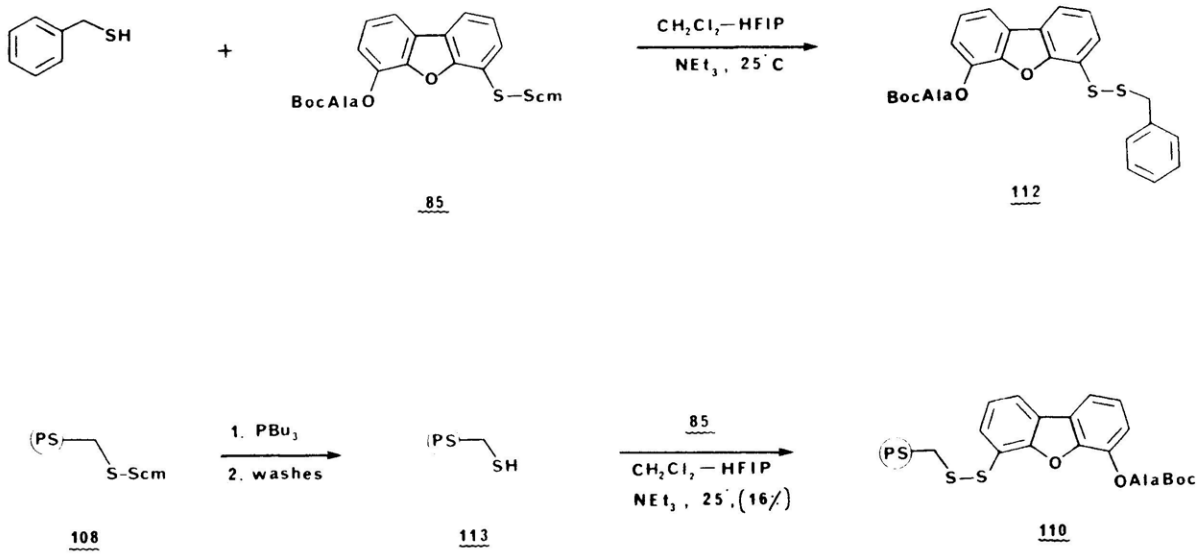
107 111

(i)	(ii)	X	Yield[a]
CH_2Cl_2, 14h, 25°C	DMF-HFIP-H_2O (10:9:1) cat NEt_3, 14h	H	5%
CH_2Cl_2, 14h, 25°C	CH_2Cl_2-HFIP (1:1) cat NEt_3, 13h	BocAlaO	2%
DMF, 16h, 25°C	DMF-HFIP-H_2O (10:9:1) cat NEt_3, 8h	H	7%
DMF, 16h, 25°C	CH_2Cl_2-HFIP (1:4) cat NEt_3, 22h	H	32%
DMF, 16h, 25°C	CH_2Cl_2-HFIP (1:1) cat NEt_3, 7h	HO	39%
DMF, 16h, 25°C	CH_2Cl_2-HFIP (1:1) 13h	BocAlaO	8%

[a]Yields determined by reductive cleavage of 111.

The discouraging nature of these results led us to investigate the reverse approach, namely the formation of the unsymmetrical disulfide linkage between the resin-bound mercaptan 113 and the Scm-activated aryl thiol 85 (Scheme IV-4). Although in model studies high yields of the mixed disulfide 112 were obtained upon treatment of benzyl mercaptan with

SCHEME IV-4

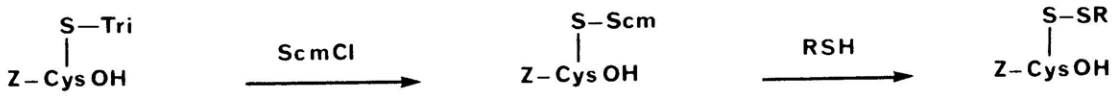


85,[78] reaction of freshly prepared thiol resin 113 with the Scm derivative 85 afforded only 16% of the attachment product 110, as evidenced by HPLC quantitation of its reduced form.

Given the poor applicability of highly promising solution-phase thiol capture systems to simple polystyrene analogs, it is reasonable to assume that the key element in the attachment process must be the nature of the solid support. However, one should not forget the requirement of solvent polarity for rapid reaction of the Scm function with thiols, and the tendency of the lipophilic polystyrene matrix to exclude the polar components of solvent mixtures. Conceivably, the S-trityl functions,

themselves highly hydrophobic, are deeply buried within the lipophilic resin interior thus rendering themselves inaccessible to the ScmCl electrophile.

Hoping that enhanced accessibility of the S-trityl function would improve the efficiency of the capture reaction, we investigated the possibility of introducing a spacer between the thioether site and the resin backbone. We chose N^{α}-carbobenzoxy, S-trityl-L-cysteine, 114 as the most promising structural candidate because in addition to the fact that it can be conveniently prepared in large quantities by known procedures,[146] once introduced, it can serve as an internal standard for the determination of attachment yields by amino acid analysis. Moreover, the chemistry of 114 has been well studied. Hiskey reports that conversion of 114 to its Scm derivative 115, followed by treatment of the latter with cysteine thiols, affords unsymmetrical disulfides in high yields.[79]

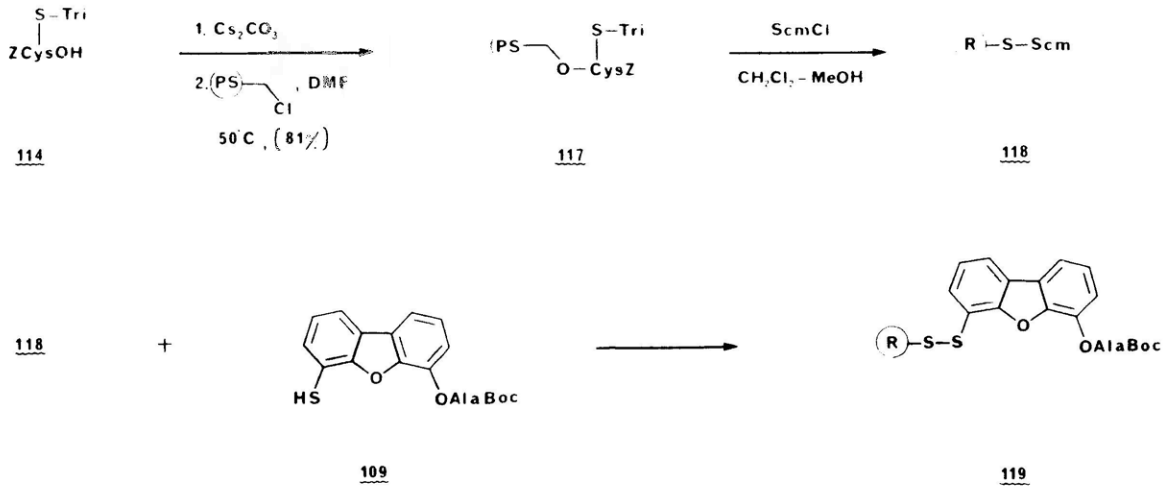


114 115 116

Initial experiments that investigated the introduction of the Z-Cys(Tri)OH spacer were encouraging: Kemp found that treatment of the Merrifield chloromethylated polystyrene (1.03 mmol Cl/g) with a stoichiometric amount of the cesium salt of 114[148] generated benzyl ester 117 in acceptable yield. In this thesis, Volhard titration[149] 117

prepared thus indicated that 81% of the total chloride content of the starting chloromethylated resin was replaced by functionalized cysteine residues (Scheme IV-5).

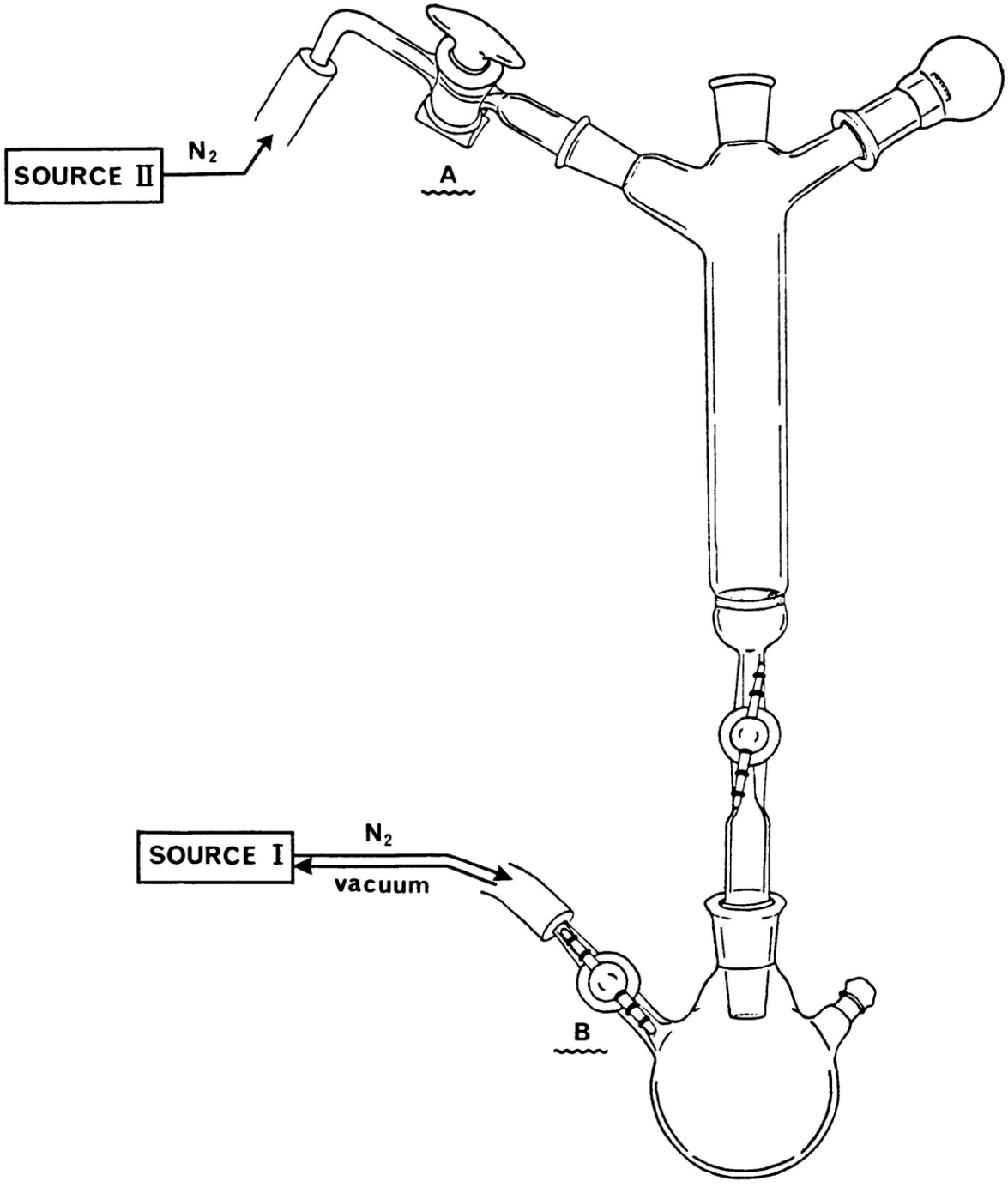
SCHEME IV-5



Conversion of 117 to 118 was undertaken in CH_2Cl_2 containing 10% MeOH and the product was washed well with dichloromethane and methanol so that traces of unreacted ScmCl would be removed. The activated resin was then placed in a fritted tube under a blanket of nitrogen and treated with aryl thiol 109. For this step a special apparatus was constructed that maintained the reaction mixture under oxygen-free nitrogen at all times, and allowed gradual evaporation of the solvent mixture (Figure IV-1). The successive reaction of 117 with ScmCl and the thiol resulted in the formation of only 32-63% of the theoretical disulfide linkages, but this level of functionalization appeared to be satisfactory for subsequent operations. Table IV-3 summarizes the various conditions employed for the attachment step (117 to 119).

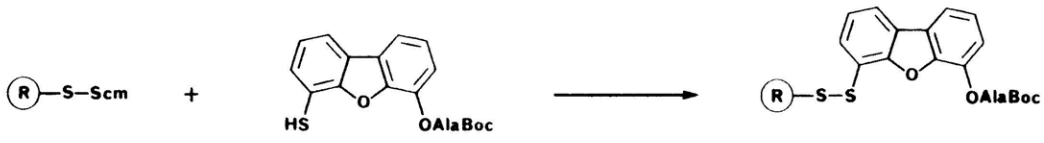
As shown in Table IV-3, in all the cases examined (with the exception of Kemp's original experiment) partial deacylation of 119

Figure IV-1



Thiol Capture Apparatus

TABLE IV-3:

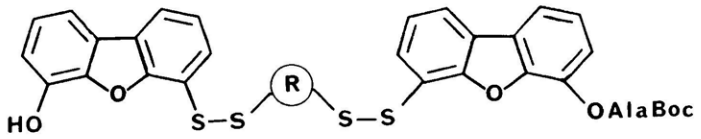


117 109 119

Run	Loading of Resin (mmol, S-Tri/g)	mmol S-Tri	mmol 109	ScmCl commercial	ScmCl fresh	Amount of Resin (mg)	Solvent[d]	Mixture Evaporated to Dryness	Total Capture Yield[e]	[109]/[61]
1[a]	0.76	0.207	0.104	?	?	272	CH_2Cl_2-aq. HFIP (4:3) cat. NEt_3	✓	61%	-
2[a]	0.76	0.236	0.173	✓		310	CH_2Cl_2-aq. HFIP (4:3) cat. NEt_3	✓	63%	3.7
3[a]	0.76	0.313	0.287	✓		412	CH_2Cl_2-aq. HFIP (3:2) cat. NEt_3	No	42%	11.5
4[a]	0.66	0.242	0.316		✓	367	CH_2Cl_2-aq. HFIP (3:2) cat. NEt_3	No	54%	6.9
5[a]	0.35	0.124	0.135		✓	356	CH_2Cl_2-aq. HFIP (3:2) cat. NEt_3	No	53%	2.6
6[a]	0.66	0.348	0.326	✓		527	CH_2Cl_2-aq. HFIP (3:2) cat. NEt_3	No	53%	11.3
7[a]	0.65[c]	0.403	0.488	✓		620	CH_2Cl_2-aq. HFIP (2:1) cat. NEt_3	No	32%	4.6
8[a]	0.65[c]	0.360	0.580		✓	554	CH_2Cl_2-aq. HFIP (2:1) cat. NEt_3	✓	34%	1.3
9[a]	0.63[c]	0.349	0.577	✓		554	CH_2Cl_2-HFIP (3:2)	✓	37%	19.0
10[b]	0.63[c]	0.348	0.580	✓		552	CH_2Cl_2-HFIP (3:2)	✓	41%	13.2

[a]The S-Tri resin was preswelled in CH_2Cl_2 and treated with ScmCl and MeOH. [b]MeOH was added at 0°C, 1h after the ScmCl treatment. [c]The resins contained 0.06 mmol Z-Gly/g; maximum loading possible:0.78mmol/g. [d]Aq. HFIP constitution: HFIP-H_2O (9:1). [e]Capture yields are based on mmol S-Tri, and were calculated by HPLC after reductive liberation of the aryl thiols.

during the attachment process afforded resins of type 120. Most likely this is attributable to simple ester hydrolysis, since deacylation was significantly suppressed when the reaction was performed under anhydrous conditions.



120

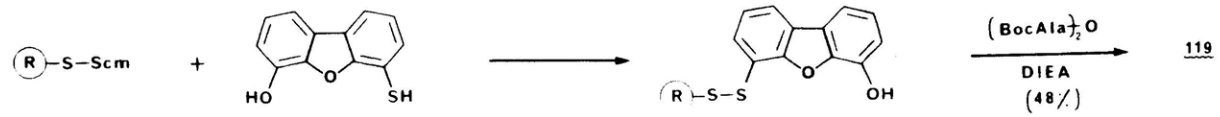
Noteworthy is also the fact that no appreciable variation in yield was observed when technical grade (Fluka; 90% pure) instead of the painstakingly spinning-band distilled, freshly prepared ScmCl reagent[118] was employed in the activation step. This result is in direct contrast with the critical dependence of yield on the purity of ScmCl in equivalent transformations performed in solution.[140]

Finally, blockage of the unreacted chloromethylated sites of 117 with Z-GlyOH prior to treatment with ScmCl did not affect the efficiency of the attachment process. It must be noted that treatment of 117 with the cesium salt of carbobenzoxyglycine[148] resulted in the displacement of only 50% of the residual chlormethylated sites, (product: 117a) presumably due to steric effects.

In one experiment, the attachment product 119 was prepared in 48% overall yield by a two-step process involving first, capture of template 61 onto the Scm-functionalized resin 118, and second, acylation with the symmetrical anhydride of BocAlaOH (Scheme IV-6). This approach greatly simplified the synthetic methodology leading to 119 since it eliminates

the requirement for the thiol protection and deprotection steps needed in the preparation of O-acyl aryl thiols of type 109.[150]

SCHEME IV-6



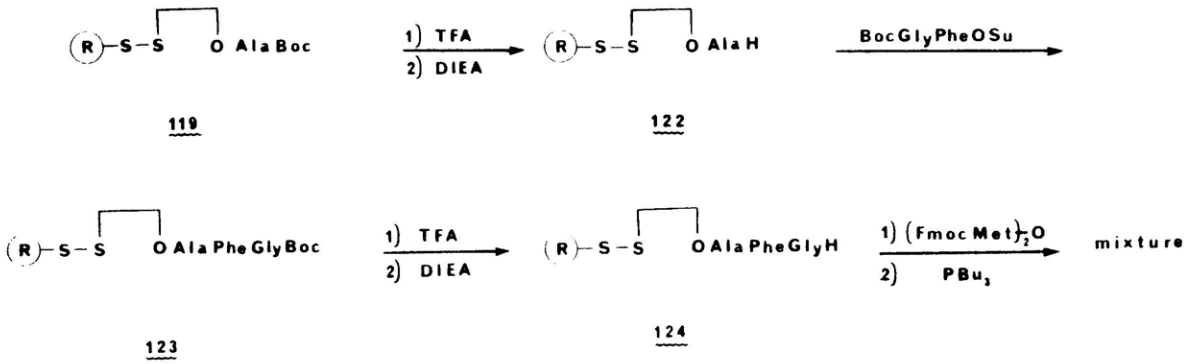
118 61 121

Having resolved the attachment problem we proceeded to investigate the possibility of preparing esters of type 94 (Scheme IV-1) using conventional solid-phase chain elongation protocols. As it was discussed earlier in this chapter, substantiated optimism for the successful outcome of this latter undertaking is derived from the literature, where examples were found in which aryl esters and unsymmetrical disulfide linkages are shown to be inert to the repetitive acidolytic and neutralization conditions required for such an operation.[40,136-142]

Chain elongation on the disulfide resin was first attempted in these laboratories by Kemp: Removal of the Boc protection of 119 with 50% TFA in CH_2Cl_2, followed by neutralization of the resulting salt with 10% diisopropylethylamine in CH_2Cl_2, generated free amine 122. Excess base was washed off with CH_2Cl_2 and the resin-bound amine was then acylated with the HOSu ester of BocGlyPheOH which was introduced in the form of a dipeptide active ester in order to avoid diketopiperazine formation. The resulting tripeptide aryl disulfide 123 was washed with CH_2Cl_2 and then subjected to a new deprotection-neutralization cycle. Acylation of the resulting amine 124 with the symmetrical anhydride of FmocMetOH, followed

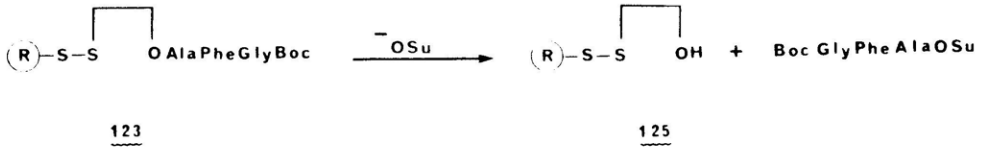
by thorough washings with CH_2Cl_2, and reductive liberation of the peptide aryl thiol from the support, afforded a mixture of four compounds (by HPLC), that were not characterized (Scheme IV-7). However, amino acid analysis of the crude product showed that in toto, the mixture contained the correct ratio of amino acids (Met: 1.05; Gly: 0.95; Phe: 0.96; Ala: 1.04).

SCHEME IV-7



Preliminary attempts to reproduce this result failed, and in all cases, extensive cleavage of the aryl ester linkage resulted in the generation of resin-bound template 125. Careful examination of the PBu_3 reduction adducts of all the synthetic intermediates of Scheme IV-7 revealed that 125 is formed under the following three circumstances:

First, when the efficent acyl exchange reagent HOSu[151] is used for C-terminal activation for BocGlyPheOH: N-Hydroxysuccinimide-induced deacylation, also observed in the context of our "backing-off" strategy experiments in solution, presumably results from nucleophilic attack of the anion of HOSu (generated during esterification) at the phenolic ester site. This constitutes a side reaction that is unique to unusually nucleophilic hydroxylamine derivatives, such as HOSu[151] or HOBt.[134]



123 125

Second, when free N-terminal amines (i.e. 122 and 124) are allowed to stand for prolonged periods of time in solvents that swell the resin, i.e. CH_2Cl_2. And third, when alcohols such as 2-propanol, are employed as solvents: Incomplete removal of such protic compounds, followed by acid and base treatments in aprotic media can cause transesterification, as noted by Pivaille[136] and Li.[152] Quantitative assessments of the magnitude of the former two effects are given in Table IV-4.

TABLE IV-4: Stability of blocked and free N-terminal disulfide resins to acylation conditions

t(h)	%123	%125	%other
Experiment 1: 123 $\xrightarrow{HOSu}$ 125[a,b,c]			
0.0	77	0	23
2.5	74	3	23
4.0	73	4	23
Experiment 2: 123 $\xrightarrow[DIEA]{HOSu}$ 125[a,b,c,d]			
0.0	77	0	23
0.3	70	9	21
1.3	54	25	26
Experiment 3: 124 $\xrightarrow{CH_2Cl_2}$ 125[a,c]			
0.0	79	1	20
0.1	51	12	37
3.3	12	50	38

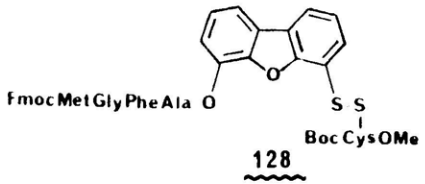
[a]Resin (20 mg, 6.1 μmol) suspended in CH_2Cl_2-THF (10:1, 11 mL). [b][HOSu]= 5×10^{-2} M. [c]Percentages determined by HPLC(D). [d][DIEA]= 5×10^{-2}M.

In the process of devising ways to overcome these problems we observed that employment of high concentrations of BocGlyPheOSu and short reaction times suppresses the deacylation reaction. Moreover, we reasoned that the problem of lability of free N-terminal amines such as 124 could in principle be overcome if the functionality could be acylated immediately after it was formed. Such an operation, can be affected if the TFA salt of the penultimate amine is allowed to react with a mixture of a hindered tertiary base and an acylating agent. Finally, we found that 2-propanol can be effectively substituted by anhydrous dioxane as the wash solvent, and thus solvent-induced transesterification would no longer be possible.

Alteration of Kemp's synthetic protocol to accommodate these observations, resulted in an exceptionally clean synthesis of the test tetrapeptide aryl thiol 128 (Figure IV-2). Thus, removal of the Boc protection of 119 (50% TFA; 1 x 5 min and 2 x 15 min), followed by repeated washings of the resulting salt with CH_2Cl_2 (5 x 2 min), afforded a resin that exhibited a positive ninhydrin test.[135] Treatment of this resin-bound amine salt with a 0.2 M solution of BocGlyPheOSu in CH_2Cl_2 containing 5% (v/v) diisopropylethylamine, generated completely acylated product 123 within 3 min (negative ninhydrin test), which was washed with CH_2Cl_2 (3 x 2 min), anhydrous dioxane (3 x 2 min), and CH_2Cl_2 (4 x 2 min). HPLC analysis of the PBu_3 reduction adduct of 123 indicated a near-quantiative yield for the acylation reaction and the regeneration of only 1-2% of phenolic sites. Following acetylation of 123, the coupling cycle was repeated, this time using the preformed symmetrical anhydride of FmocMetOH as the acylating agent. The coupling proceeded

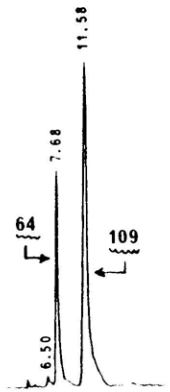
Figure IV-2

Chain Elongation: Synthesis of

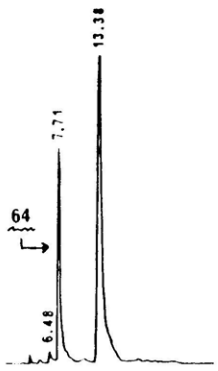


128

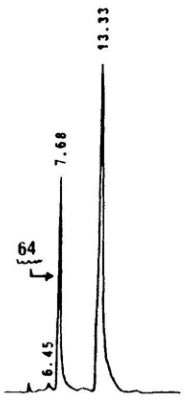
HPLC-1



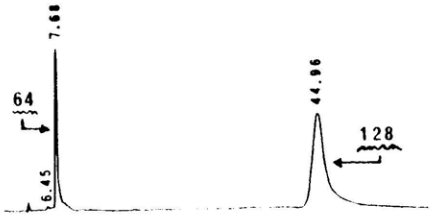
HPLC-2



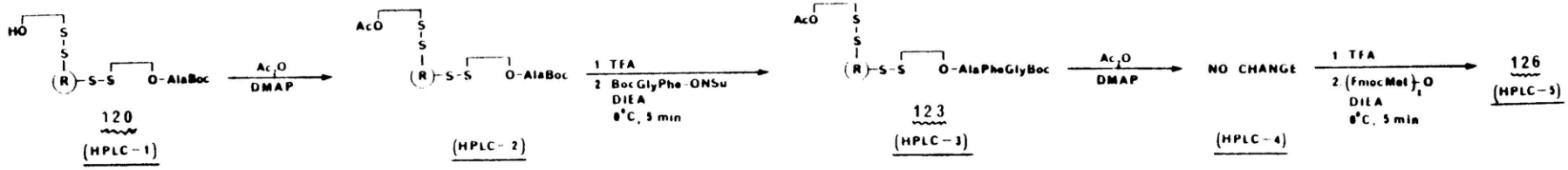
HPLC-3



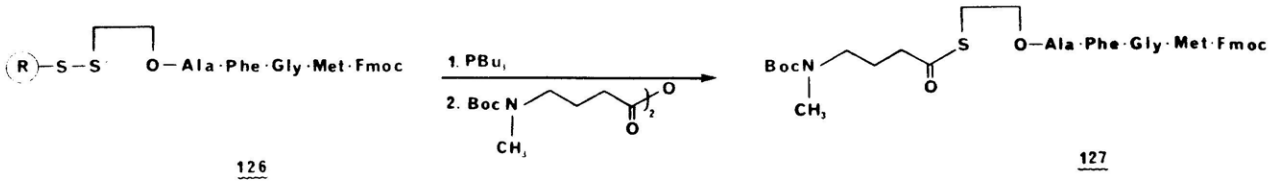
HPLC-4



HPLC-5



uneventfully and the resulting tetrapeptide aryl thiol was reductively liberated from the resin and immediately converted to thioester 127 as shown below:[129]



126 127

The thioester was homogeneous by HPLC and analyzed well for its amino acid content (Met: 0.95; Gly: 1.01; Phe: 0.98; Ala: 1.09).

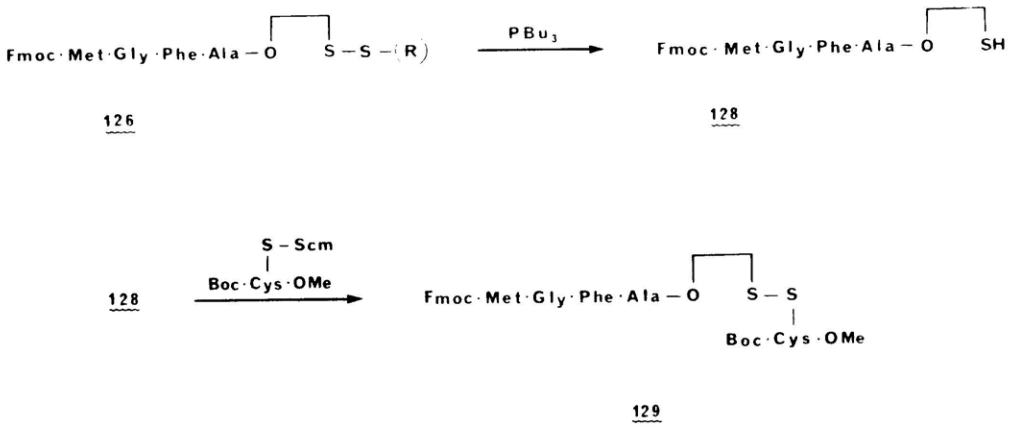
The above described protocol was refined further to circumvent the requirement that the second and third amino acids be introduced as preformed dipeptides: Thus, removal of the Boc group of 119 followed by treatment of the resulting salt with the symmetrical anhydride of BocPheOH and DIEA (according to the protocol described above) generated dipeptide 123a quantitatively. Acidolytic deprotection of 123a, followed by quenching of the amine salt product with a 0.3 M solution of $(BocGly)_2O$ in CH_2Cl_2 containing 5% DIEA (v/v) afforded 123, which was identical with the material prepared by the dipeptide condensation approach (HPLC). During the second acylation, only 2% deacylation occurred, presumably due to diketopiperazine formation.

This last result marked the successful conclusion of our efforts to establish a rapid, reliable and efficient methodology for generating template esters of C-terminal peptide acids. In summary, our protocol consists of a five-step operation: First, the penultimate phenolic template, or a simple N^{α}-blocked amino acid ester of it, is attached to the solid support by a thiol capture reaction. Second, acidolysis removes the N^{α}-protective function and generates the amine salt of the resin-bound amino acid. Third, acylation according to the "backing-off" strategy of Schwyzer and Goodman[126] affords a dipeptide. Fourth, successive repetition of the second and third steps generates the polypeptide, and finally fifth, phosphine cleavage of the disulfide attachment liberates the peptide aryl thiol. In addition, the purity of each synthetic intermediate could be rapidly and quantitatively tested by HPLC analysis of the phosphine reduction products of the disulfide resins. At this point then, what needed to be shown next was that the peptide aryl thiols prepared by this protocol can undergo thiol capture with Scm-activated N-terminal cysteine peptides, as in the successful experiments of Chapter II.

The efficacy of this latter operation was demonstrated with the exceptionally clean synthesis of the 4 + 1 transfer system 129 (Scheme IV-8). Disulfide resin 126 was placed in the Schlenck tube of the no-air apparatus (Figure IV-1), preswelled in aqueous dioxane and treated for 2 min with PBu_3. Longer reaction times were avoided so that thioester formation between the liberated resin thiol sites and the aryl ester functionality of the template would be suppressed. Filtration, followed by flash evaporation of the supernatant and trituration of the residue with

anhydrous Et_2O under a blanket of N_2 afforded aryl thiol 128, free of 61 and 64. The mercaptan was then immediately treated with BocCys(Scm)OMe, 39, in aqueous HFIP and the capture reaction, followed by HPLC, appeared to be complete within 1 h (Figure IV-3).

SCHEME IV-8

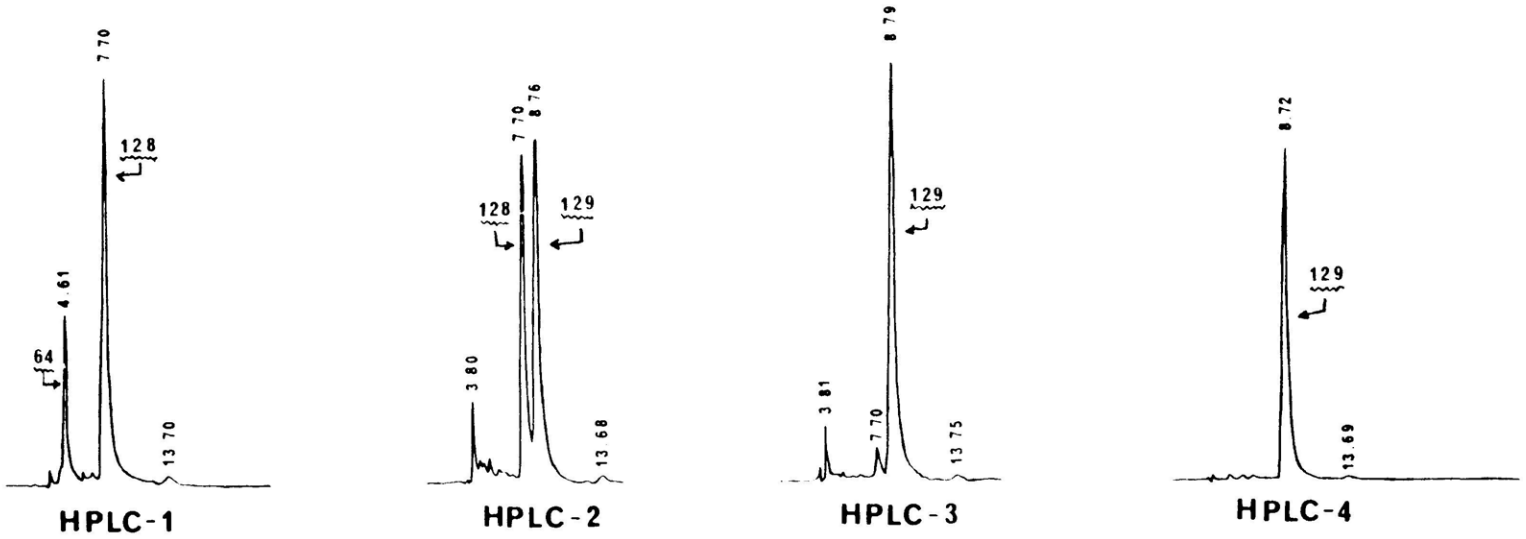


Although evaporation of the reaction mixture upon completion of the capture process resulted in partial hydrolysis of the aryl ester linkage of 129, lyophilization proceeded uneventuflly and after the final residue was triturated with Et_2O, the capture product was obtained as a sharp melting solid, homogeneous by HPLC (Figure IV-3), and containing the desired amino acids at the correct ratio. Moreover, an exceptionally clean 270 MHz ^{1}H-NMR spectrum of 129 was obtained, displaying sharp singlets for the methyl resonances corresponding to the thioether of Met and the ester of cysteine (Figure IV-4).

Having achieved two of the three goals set in this Chapter, namely the efficient preparation of O-acyl aryl thiols and the demonstration of the feasibility of thiol capture in the context of our newly developed solid-phase methodology, we proceeded to address the topic of appli-

Figure IV-3

Thiol Capture of Fmoc-Tetrapeptide Aryl Thiol 128 by BocCys(Scm)OMe, 39.



HPLC-1 : RELEASE OF THIOL FROM RESIN WITH $P(n\text{-}Bu)_3$

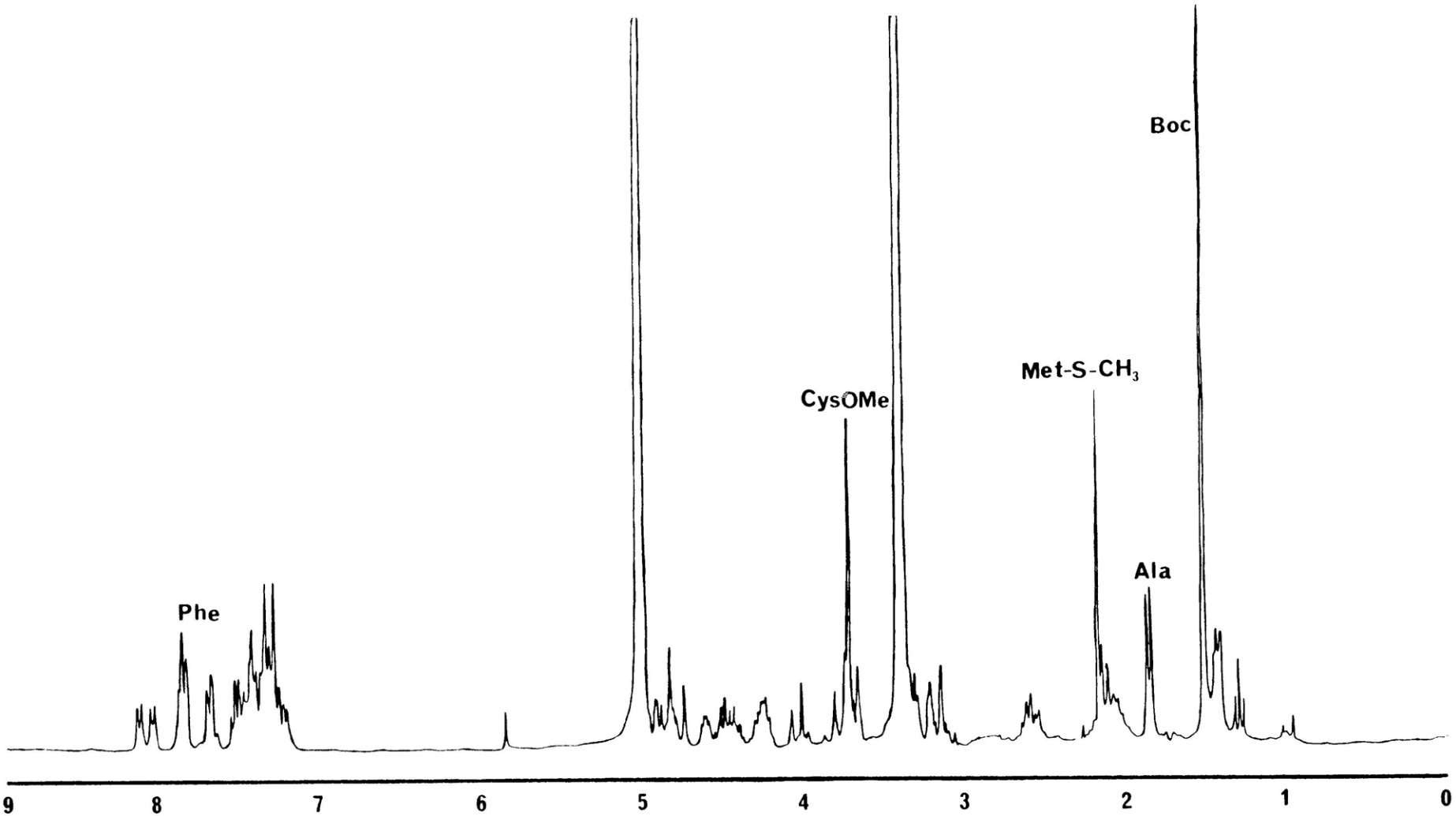
HPLC-2 : CAPTURE WITH BocCys(S-Scm)OMe ; t = 5 min

HPLC-3 : CAPTURE ; t = 1.8 h

HPLC-4 : ISOLATION OF THIOL CAPTURE PRODUCT

Figure IV-4

270 MH_z ^{1}H-NMR of 129 in CD_3OD



cability of intramolecular acyl transfer in the coupling of large peptide fragments.

As a first major effort in applying the prior thiol capture principle to a difficult synthetic problem, we selected a sequence from the peptide hormone urogastrone. First purified[153] and sequenced[154] by Gregory, this substance is believed to be derived from larger precursors in the submandibular glands and in Brunner's glands of the duodenum,[155] and can be isolated from human urine where it occurs at a concentration of ca. 30 μg/L. Urogastrone is a potent and selective inhibitor of gastric acid secretion. At doses of 0.2-0.5 μg/kg in dogs stimulated with 30 μg/ kg-h of histamine or 1 μg/kg-h of pentagastrin, it produces 60-80% inhibition of maximal rate of acid secretion. Moreover, it appears to have no effect on secretion of pancreatic fluid, saliva or bile.[156] Clearly then, this substance, along with its analogs offers very great clinical promise. As summarized by Gregory: "... large amounts of pure urogastrone ... would allow for detailed studies on the healing of ulcers of the gastrointestinal tract, but it may also allow for the realization of the potential to promote healing of epithelial wounds in other parts of the body."[154]

Urogastrone (δ-species) consists of a single chain of fifty-three amino acid residues, bridged by three disulfide linkages[154] (Scheme IV-9). Although a number of laboratories is engaged in the biosynthesis of urogastrone and the structurally related epidermal growth factor (EGF) by recombinant DNA techniques,[157] this approach cannot be used to form analogs with structure-enhancing amino acid residues at key sites. Chemical synthesis, which has greater versatility in this regard, has

been problematic. Attempts to generate urogastrone by the Yajima methodology have thus far failed, and Meienhofer has suggested that this is a molecule which defies conventional chemical methodologies.[157]

At the outset, we believed urogastrone is in fact ideally suited for synthesis by thiol capture. As shown in Schem IV-9, urogastrone contains six cysteine residues, five of which are conveniently placed for use in assembly by our methodology.

SCHEME IV-9

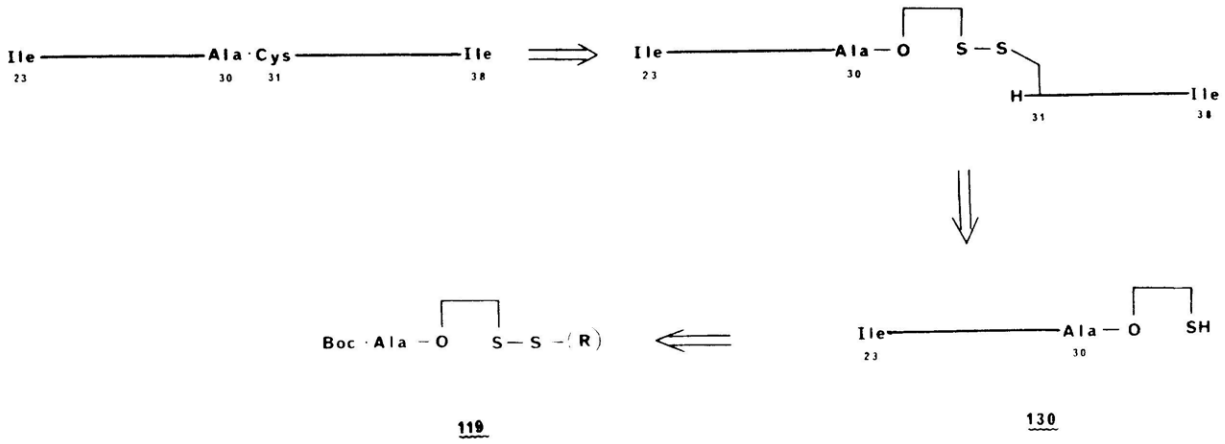
H-^{1}Asn-Ser-Asp-Ser-Glu-^{6}Cys-Pro-Leu-Ser-His-Asp-Gly-Tyr-^{14}Cys-Leu-His-Asp-Gly-Val-^{20}Cys-Met-Tyr-Ile-Glu-Ala-Leu-Asp-Lys-Tyr-Ala-^{31}Cys-Asn-^{33}Cys-Val-Val-Gly-Tyr-Ile-Gly-Glu-Arg-^{42}Cys-Gln-Tyr-Arg-Asp-Leu-Lys-Trp-Trp-Glu-Leu-^{53}Arg-OH

Disulfide bridges at ^{6}Cys-^{20}Cys, ^{14}Cys-^{31}Cys, ^{33}Cys-^{42}Cys

Given the size and complexity of our target molecule, on the one hand and on the other the severe time constraints imposed on this thesis, we reasoned that the thiol capture methodology developed thus far would be put through a thorough test by the synthesis of the ^{23}Ile-^{38}Ile central decahexapeptide portion of urogastrone. We selected this fragment primarily because we envisoned the penultimate step in its synthesis to involve intramolecular amide bond formation between N-terminal ^{31}Cys and C-terminal ^{30}Ala residues, a system that was well studied with models. Such an assembly would be constructed by thiol capture of the Scm derivative of the N-terminal ^{31}Cys octapeptide by the C-terminal ^{30}Ala octapeptide dibenzofuranyl thiol 130, which in turn would be generated according to the disulfide resin methodology described

earlier in this Chapter (Scheme IV-10).

SCHEME IV-10



119 130

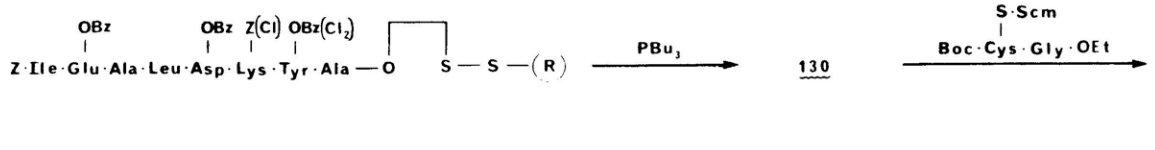
In this synthesis, we were concerned first with the applicability of our solid-phase protocol to the generation of 130, a fragment possessing four branched amino acid residues. In selecting side-chain protective groups for Tyr and Lys we recalled Merrifields' recent observation that O-2,6-dichlorobenzyl Tyr and N^{α}-2-chlrobenzyloxycarbonyl Lys are 5200 and 820 times more stable towards 50% TFA than their respective unchlorinated derivatives.[158] For Asp and Glu we chose benzyl ester blocking, since such functionalities are known to be inert to repetitive acidolysis.[159]

Chain elongation on disulfide resin 119 was undertaken according to the protocol described for the test tetrapeptide 126 and all amino acids were introduced as their N^{α}-Boc derivatives by the symmetrical anhydride method[20] (N-terminal ^{23}Ile was introduced as its N^{α}-Z derivative). At the end of each coupling cycle, HPLC analysis of the reductively liberated aryl thiol provided a quantitative assessment of the efficiency

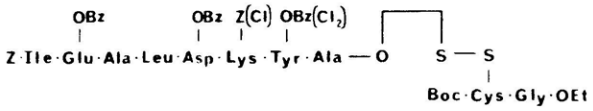
of each acylation. This latter operation appeared to be dependent on the solvent in which the resin was suspended. Erratic results were obtained when the disulfide resin was reduced with PBu_3 in aqueous dioxane, whereas employment of the same phosphine in a 1:1 CH_2Cl_2-HFIP mixture provided reproducible results. This solvent dependence presumably results from the enhanced solubilizing and swelling capacity of the latter medium, and is in accordance with Wünsch's preference of hexafluoroisopropanol as the optimal solvent for the final phosphine reduction of Somatostatin-28.[160]

Although yield assessments at the last stages of the synthesis of 130 were complicated by the increased insolubility of the peptide aryl thiols in the commonly used HPLC mobile phases, an exceptionally clean profile of the final product was obtained (Figure IV-5). This material, 130, also appeared to have the correct amino acid constitution, as evidenced by an excellent amino acid analysis of the octapeptide aryl disulfide resin 131.[16]

In one experiment, thiol 130 was liberated from the resin and captured with BocCys(Scm)GlyOEt.[161] The reaction was clean and rapid (complete within 3.4 h), and generated mixed disulfide 132 in 94% yield (HPLC).

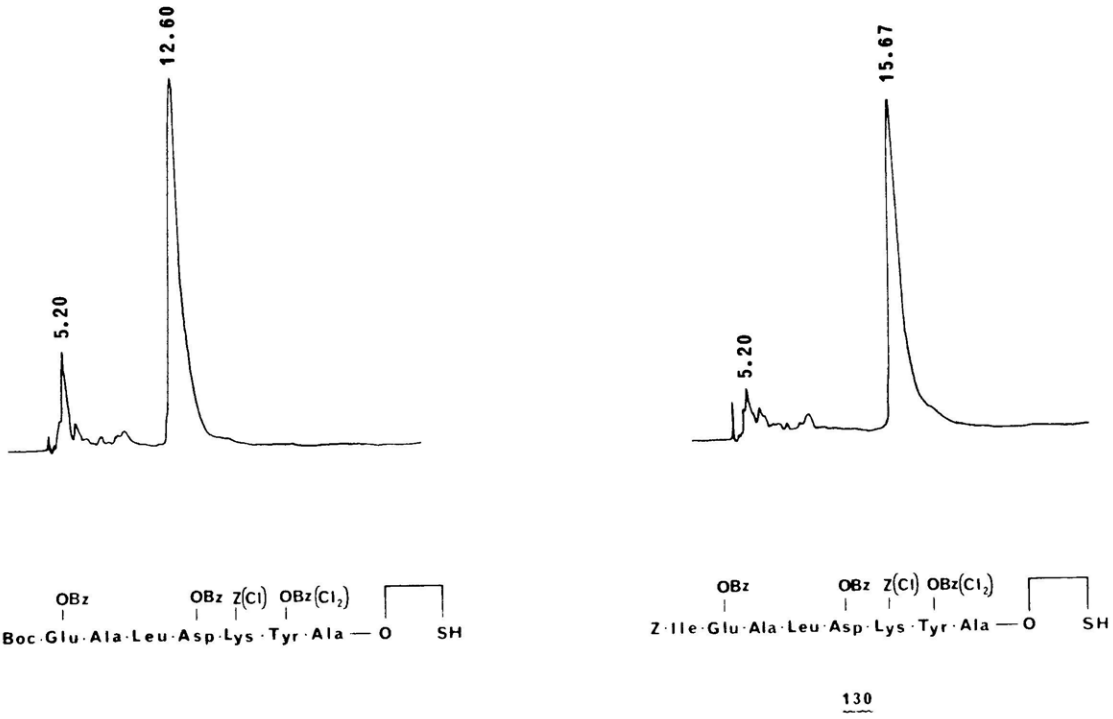


131



132

FIGURE IV-5



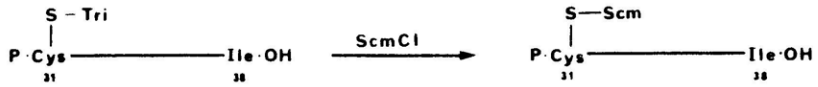
130

Having successfully prepared the left-hand-side portion of our target molecule, and established its reactivity in the thiol capture process, we focused our attention on the problem of efficient generation of the Scm-activated N-terminal cysteine octapeptide 133.

Although, in principle, 133 can be prepared from its S-Tri precursor by treatment with ScmCl, in practice, complications are expected to arise because such transformations proceed best in media that are poor solubulizing agents for peptides:[78-80] In model studies we found that while the conversion of Z-Cys(Tri)OH, 114, to its Scm derivative proceeded rapidly and efficiently in $CHCl_3$-MeOH, or neat CH_2Cl_2, sluggish reaction

and slow capture rates were observed when the same conversion was performed in DMF-MeOH.

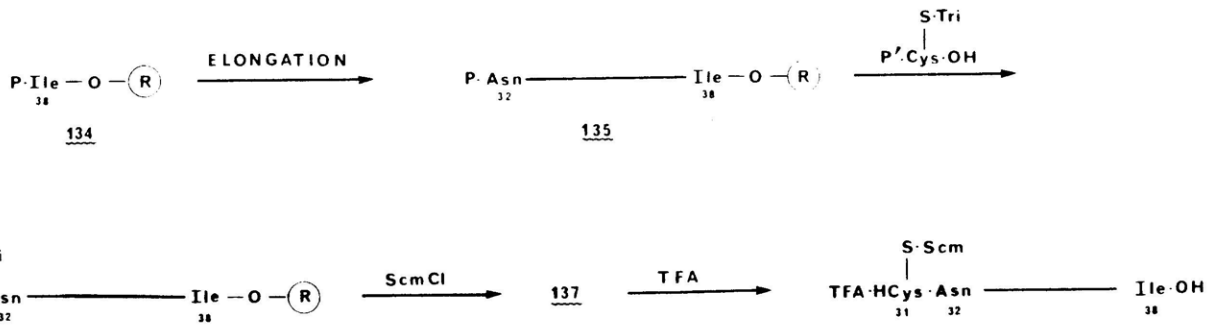
SCHEME IV-11



133

Rather than explore the feasibility of Scheme IV-11 which is compatible with solution-phase synthesis, we were attracted to a similar strategy that is best suited to a solid-phase protocol: Chain elongation on a highly acid-labile solid support using base-removable N^{α} protection would afford heptapeptide 135 which would be acylated with BocCys(Tri)OH or its N^{α}-Bpoc analog. Treatment of the resulting S-Tri octapeptide 136 with ScmCl would generate 137 which could be acidolytically liberated from the resin to afford the S-Scm salt 138 (Scheme IV-12).

SCHEME IV-12



134 135 136 138

The approach outlined in Scheme IV-12 offers three major advantageous features: First, Scm activation of 136 can be performed in CH_2Cl_2, a solvent that favors both, the transformation in solution-phase systems,[78,80] and meets the swelling requirement of most resins.[147] Although this

activation reaction is unprecedented in the context of an S-Tri octapeptide-resin complex, it has been shown earlier in this Chapter to be applicable to the Z-Cys(Tri)O-polystyrene system (attachment studies). Since in 136 the S-Tri functionality is eight amino acid residues removed from the solid support, the activation reaction with ScmCl is expected to be enhanced due to the increased accessibility of the thioether moiety. Second, N-terminal deblocking, and acidolytic liberation of the final peptide from the solid support can be performed in either one or two steps, depending on the choice of P' (Scheme IV-12). For example, if Bpoc is used as the N^{α} protection for ^{31}Cys, and the solid support is an alkoxybenzyl resin,[26] the N-terminal amino function can be removed either prior (0.5% TFA-CH_2Cl_2), or together with liberation of the peptide from the resin (50% TFA-CH_2Cl_2).[163] Such versatility would allow thiol capture and acyl transfer either on or off the solid support. And third, concurrent acidolysis of the N^{α}-terminal blocking group and cleavage of the peptide-alkoxybenzyl ester attachment site would generate the activated salt 138, which is expected to be orders of magnitude more reactive towards thiols than its N-blocked analog. This prediciton, substantiated by the model studies described in Chapter II, is rationalized on the basis of the inductive effect exerted by the ammonium salt on the Scm moiety.

Realization of these attractive features, required the selection of a convenient resin elongation protocol combination that would be compatible with our synthetic scheme. In a somatostatin synthesis designed to demonstrate chain elongation without repetitive acidolysis, Meienhofer[163] successfully employed the base-labile Fmoc function[131,162]

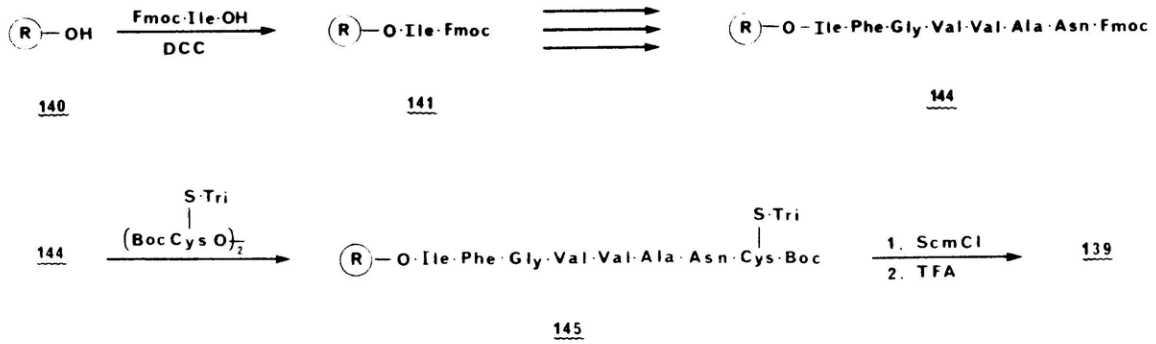
as N^{α}-amine protection. Moreover, he showed that the final N-terminal Fmoc-blocked peptide could be readily cleaved from a benzyloxybenzyl resin using 50% TFA in CH_2Cl_2 for 1 h.[163]

We incorporated these observations in the formalization of a synthetic scheme for 139, an analog of the ^{31}Cys-^{38}Ile octapeptide fragment of naturally occuring urogastrone.

139

Thus, DCC-mediated acylation of p-benzyloxybenzyl alcohol resin 140 (0.98 mmol/g) with FmocIleOH afforded the anchored product 141 in 43% yield, as determined by Meienhofer's spectrophotometric procedure.[163] The unreacted sites of 141 were deactivated with benzoyl chloride,[163] and then the product was treated first with 50% piperidine in CH_2Cl_2 and then with the preformed symmetrical anhydride of BpocPheOH to generate 142.[20] The Bpoc protection of 142 was removed with 1.5% TFA in CH_2Cl_2 containing 1% mercaptoethanol[164] and the resulting salt was treated with $(FmocGly)_2O$ and diisopropylethylamine ("backing-off" strategy). Piperidine-induced deblocking of the N^{α}-Fmoc tripeptide resin, followed by acylation with $(FmocVal)_2O$ and repetition of the coupling cycle with a second equivalent of FmocValOH and then FmocAlaOH, afforded hexapeptide 143. Introduction of FmocAsnOH on 143 was undertaken by the DCC/HOBt method, as proposed by Geiger and König,[134] so that dehydration of the β-amide functionality of Asn would be suppressed. The resulting N^{α}-Fmoc heptapeptide-resin 144

was deblocked, and then acylated with the preformed symmetrical anhydride of BocCys(Tri)OH to generate octapeptide-resin 145 that was found to contain the correct ratio of the desired amino acid residues.[16]



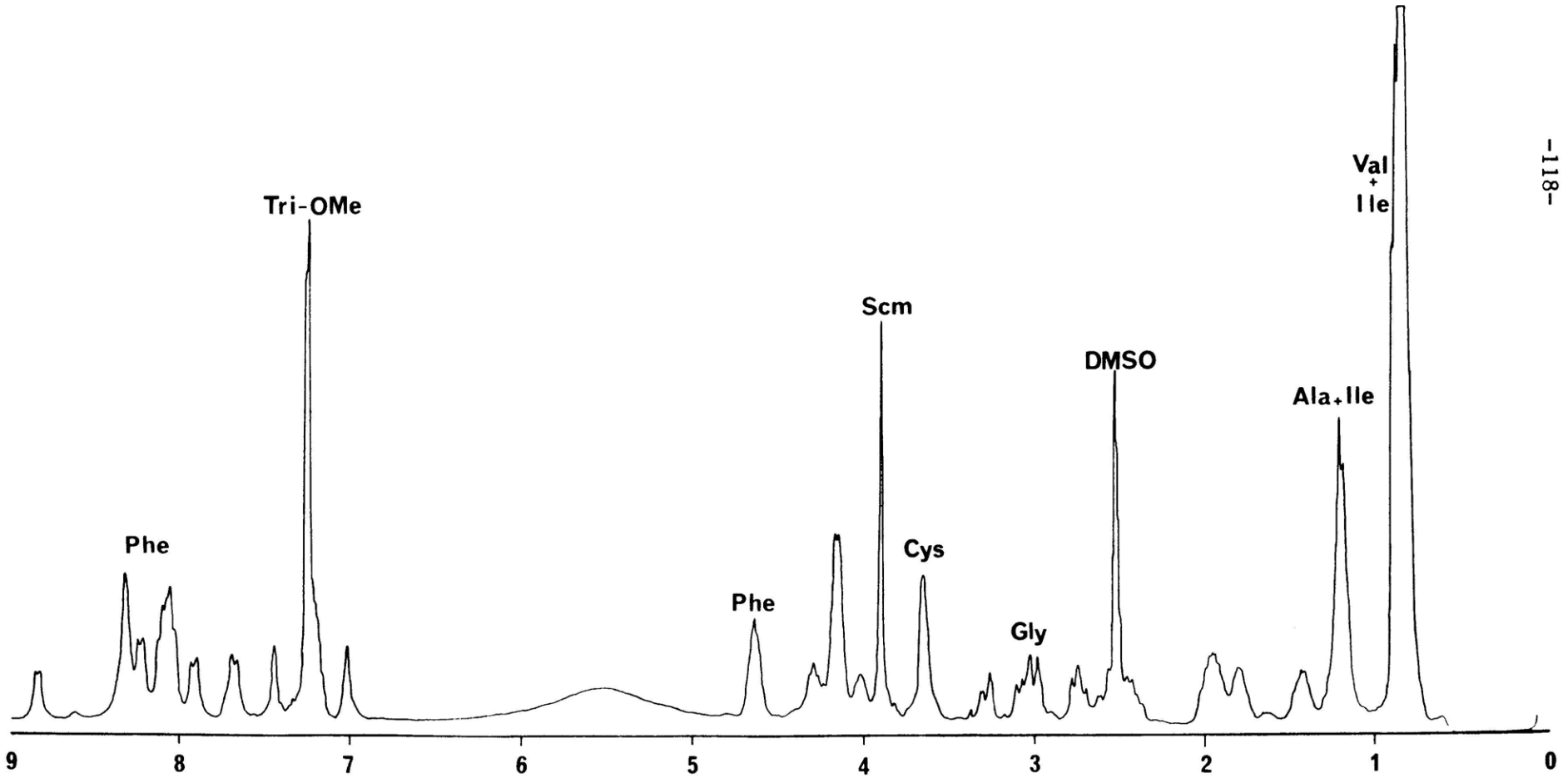
When 205 mg of this peptide-containing resin was treated first with ScmCl in CH_2Cl_2-MeOH and then with 55% TFA in CH_2Cl_2, 55 mg of the TFA salt of the Scm-functionalized octapeptide, 139, was recovered (tan powder). This material appeared to be homogeneous by HPLC and exhibited an exceptionally clean ^{1}H-NMR spectrum (Figure IV-6).

The completion of the synthesis of the octapeptide aryl disulfide resin 131 and the Scm-activated N-terminal cysteine fragment 139 set the stage for the final task in this thesis, namely the demonstration that when formed, thiol capture assembly 140 can undergo intramolecular acyl transfer to generate the target hexadecapeptide.

Our synthetic scheme for this undertaking was straightforward and consisted of operations that have been well documented in this thesis (Scheme IV-13). Thus, reduction of the disulfide bridge of 131 would afford aryl thiol 130 which would be trapped with 139 to generate mixed disulfide 140. Neutralization, followed by intramolecular acylation

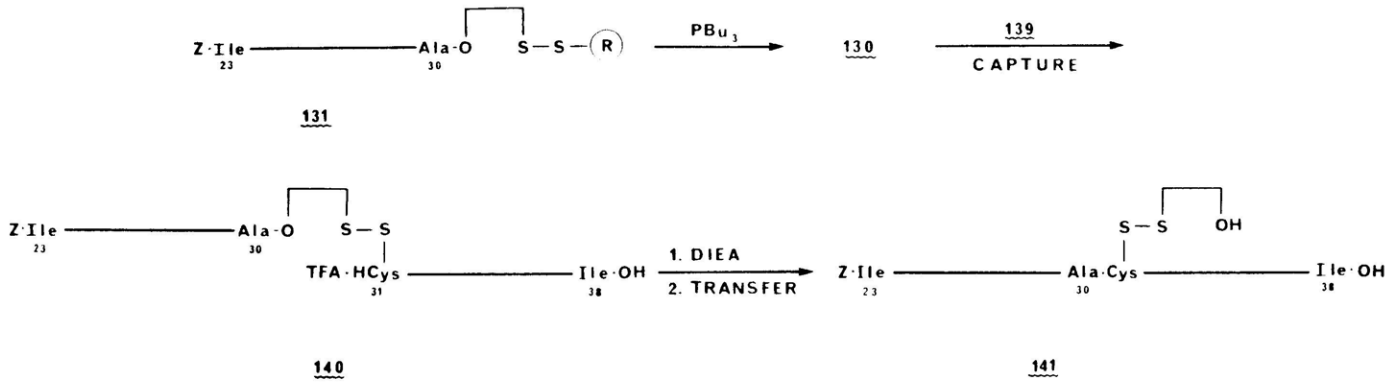
Figure IV-6

270 MHz ^{1}H NMR of 139



would yield the desired hexadecapeptide to which the template would be attached at ^{31}Cys via a disulfide bridge (141). At that point the dibenzofuranyl thio functionality can either serve as a protective group for the cysteine mercaptan or be reductively removed and the resulting thiol converted to a thioether or a thioester.

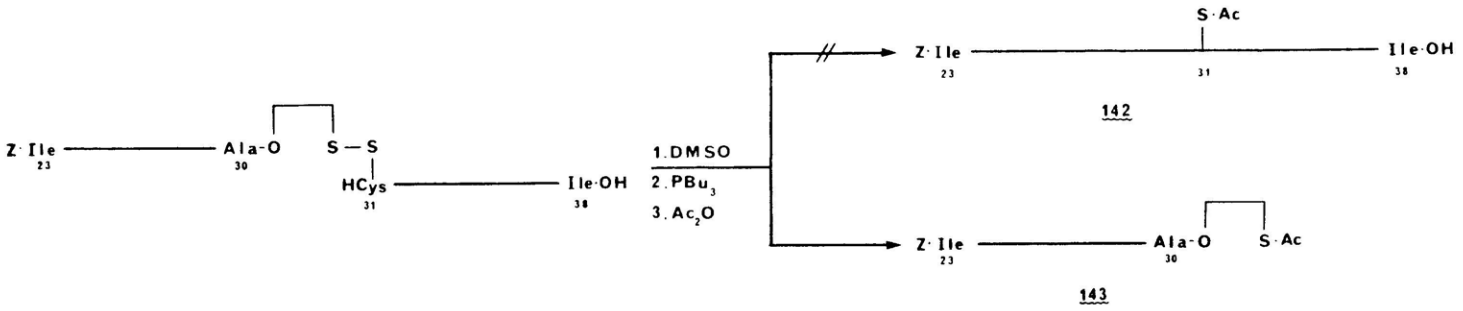
SCHEME IV-13



Deceptive preliminary results suggested that this scheme had been realized: Treatment of 131 with phosphine generated peptide aryl thiol 130 which was captured with excess 139 in aqueous HFIP. The reaction was followed by HPLC at 280 nm and appeared to be complete within 1.8 h (Figure IV-7). Although quantitative assessments of the progress of the capture process could hardly be pronounced definitive given the exceptionally poor solubility of these materials in all common HPLC mobile phases,[165] significant change in retention times (2 min) between the starting material and the product was recorded, and a change in the shape of the corresponding peaks was observed. Careful lyophilization of the reaction mixture, followed by neutralization with diisopropylethylamine, generated the free amine which was allowed to undergo acyl transfer in anhydrous DMSO. After 30 h the solvent was removed and the

crude product was treated with PBu_3 to yield a mixture of three components, the major of which (38% of total absorbance) was identified by coinjection of an authentic sample as being template 61. This latter material was removed by trituration with Et_2O, and the resulting two-component mixture was treated with Ac_2O (Figure IV-7). HPLC analysis of the product mixture indicated that the major component (61% of total) eluted faster than prior to acetylation, hence suggesting thioester formation.[166]

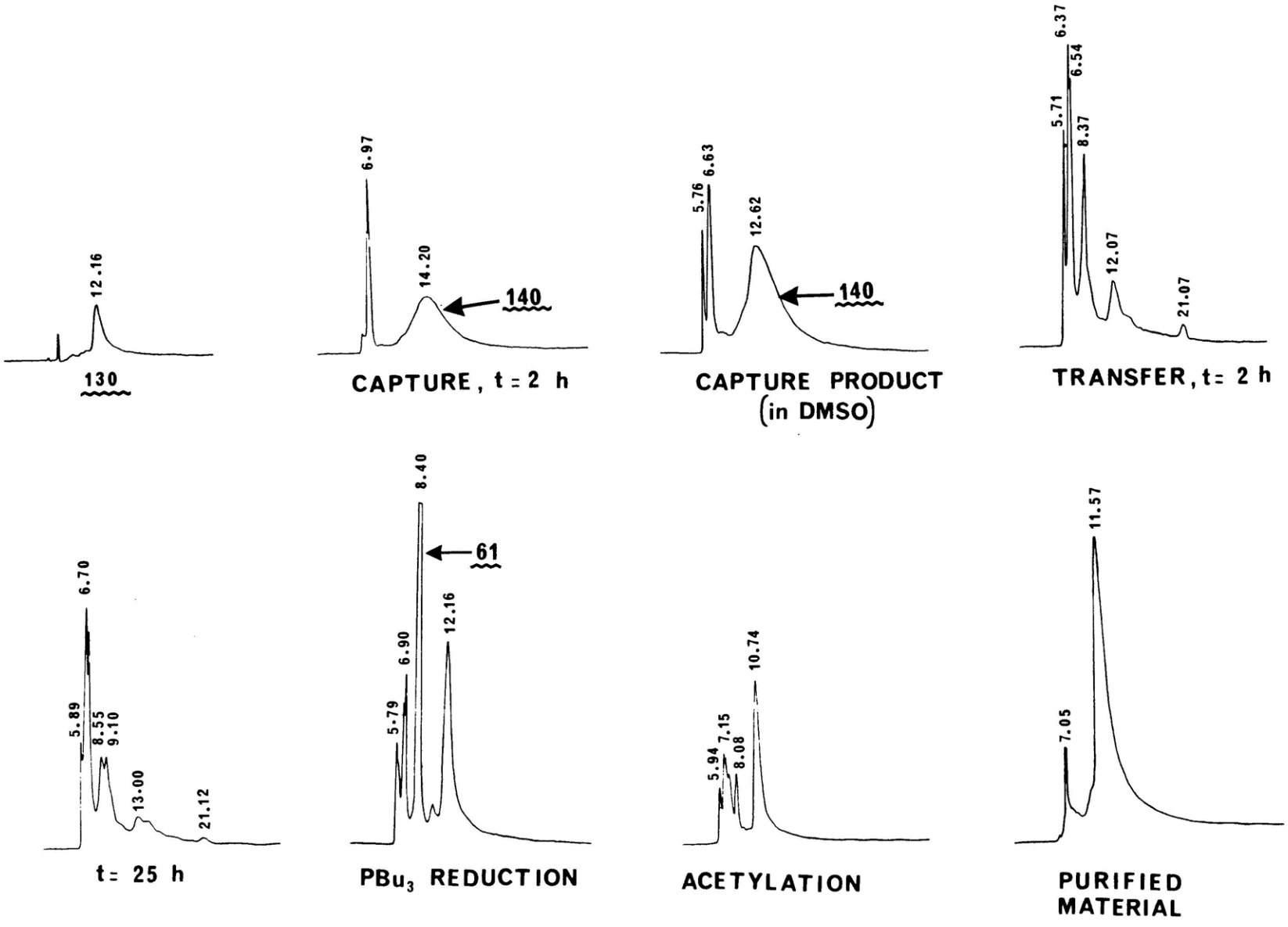
Optimistically, we concluded that the newly formed thioester was the acetylated form of the hexadecapeptide (i.e. 142). However, to our dismay, amino acid analysis of the pure form of presumed 142 (isolated and purified by preparatory HPLC) indicated that the product in question contained only the amino acids found in the octapeptide aryl thiol 130. Hence, it appears that we had generated thioester 143.



Since severe time constraints did not allow the repetition of this experiment, only some speculations can be made about the outcome of this attempted 8 + 8 coupling.

The generation of thioester must have been present either due to incomplete capture, incomplete transfer followed by disulfide reduction,

Figure IV-7


12.16
130
6.97
14.20
140
CAPTURE, t = 2 h
5.76
6.63
12.62
140
CAPTURE PRODUCT
(in DMSO)
6.37
6.54
5.71
8.37
12.07
21.07
TRANSFER, t= 2 h
6.70
5.89
8.55
9.10
13.00
21.12
t= 25 h
8.40
61
6.90
5.79
12.16
PBu3 REDUCTION
10.74
5.94
7.15
8.08
ACETYLATION
11.57
7.05
PURIFIED
MATERIAL

or both. Given the HPLC evidence provided for the capture step, the former possibility seems unlikely. As for the latter two options, only an observation can be stated: The HPLC traces depicting the progress of the acyl transfer reaction clearly show the gradual disappearance of the acyl transfer amine assembly and the appearance of products. If the HPLC data are to be rationalized in terms of template generation after phosphine reduction, then cleavage of the aryl ester bond could have occurred either at the lyophilization step following thiol capture (hydrolysis), or wishfully, during acyl transfer. Since the HPLC trace taken immediately after dissolution of the captured salt 140 in DMSO showed no absorption corresponding to the template, the former possibility must be discarded.

Unfortunately, the complex nature of this experiment does not allow the formulation of any decisive conclusions. Moreover, since all our information on the progress of this coupling is derived from HPLC data, any deductions made on this basis should be tentative, given the severe solubility problems encountered with the various synthetic intermediates. These results then, should best be viewed as part of a single, inconclusive experiment where for the first time an attempt was made to realize amide bond formation between medium-sized fragments by prior thiol capture. This being the case, we can now summarize the goals achieved in this Chapter.

First, we established new methodology, based on a novel variation of existing solid-phase protocols, that allows the rapid and efficient generation of medium to large-sized peptide esters of aryl mercaptans. The key feature of this methodology entails the employment of an

unsymmetrical disulfide attachment site, and given its exceptional versatility, this approach, or a variant thereof, may become a useful tool in routine synthetic manipulations of peptides.

Second, we devised a convenient and versatile method for the generation of Scm-activated cysteine peptides of medium to large size. Our strategy involves chain elongation on a solid support, then introduction of the Cys residue as its S-Tri derivative, ScmCl activation, and finally acidolytic liberation of the functionalized peptide from the resin.

And third, we demonstrated the feasibility of thiol capture between medium-sized fragments by a series of successful condensations (4 + 1, 8 + 2, and 8 + 8).

In conclusion, it is the belief of the author that the work presented in this thesis has advanced the prior thiol capture principle for peptide synthesis to the application stage. Further development remains for the future.

EXPERIMENTAL SECTION

PART A
Solution-phase: general methods

Apparatus:

Melting points were taken in capillary tubes using a Thomas-Hoover Unimelt apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 283-B grating infrared spectrometer. Low resolution ^{1}H-NMR spectra were taken on a Varian T-60 spectrometer. High resolution proton and carbon-13 NMR spectra were obtained on either a Bruker WM-250 or a Bruker WM-270 instrument. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (internal standard), and splitting patters are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. Low resolution mass spectra were determined on a Varian MAT-44 spectrometer. High resolution and field desorption mass spectra were courtesy of Dr. C.E. Costello (M.I.T). Ultraviolet (UV) spectra were taken on a Perkin-Elmer Model 330 UV-VIS spectrophotometer (1.0 cm path length). Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

Spinning-band distillations were undertaken using a Neslab-Faust Model 151 apparatus. Low temperature reactions were performed in an ethyleneglycol-water bath cooled to the desired temperature with a Haake Model F-423 constant temperature unit connected to a Neslab Model PBC-4 bath cooler. For the metallation reactions, unless otherwise stated, the glassware used was dried for at least one day in an oven thermostated at 125°C.

Chromatography:

Analytical thin layer chromatography (TLC) was performed on glass precoated silica gel 60 (E. Merck F-254) plates employing the following solvent systems:

(A)	Chloroform-ethyl acetate	9:1
(B)	Chloroform	100%
(C)	Chloroform-ethyl acetate	1:1
(D)	Dichloromethane	100%
(E)	Cyclohexane-ethyl acetate	7:1
(F)	Chloroform-ethanol-acetic acid	98:10:2
(G)	Cyclohexane-dichloromethane	9:1
(H)	Chloroform-acetic acid	93:7
(I)	Cyclohexane-chloroform	1:1
(J)	Chloroform-cyclohexane	4:1
(K)	Chloroform-methanol-acetic acid	50:35:15

Compounds were visualized by UV absorption (254 nm), staining with iodine vapors, phosphomolybdic acid, 1% ninhydrin in a 9:1 absolute ethanol-trifluoroacetic acid mixture (primary and secondary amines), and Ellman's reagent[168] (thiols).

Preparative layer chromatography was performed on Analtech GF 1,000 μ and GF 2,000 μ silica gel plates. Flash chromatography[119] was conducted in custom-made columns (diameter 55 mm, length = 60 cm) equipped with a N_2 inlet and were freshly packed with silica gel 60, 230-400 mesh (E. Merck).

High pressure liquid chromatography (HPLC) was performed on a Waters Associates 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 conditions shown on the following page (Table E-1). Data are reported as the retention time (rt) in minutes, followed by the relative integration given by the data module.

Solvents:

Tetrahydrofuran (THF) and p-dioxane were obtained dry and peroxide-free by distillation from the sodium benzophenone ketyl. Dimethyl formamide (DMF) was dried over molecular sieves (Linde 4A) for at least two days, then distilled from ninhydrin under high vacuum and stored in brown bottles at 4°C over molecular sieves. Dimethylsulfoxide (DMSO) was freshly distilled (76°C at 12 torr) from calcium hydride and stored over sieves. Trifluoroacetic acid (TFA) was fractionally distilled from P_2O_5, then redistilled from anhydrous L-valine to remove traces of the anhydride. Reagent grade dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), acetone and acetonitrile (CH_3CN) were dried over molecular sieves (Linde 4A); methanol over Linde 3A sieves. Unless otherwise stated, reagent grade hexafluoroisopropanol (HFIP) was purchased from Du Pont and used without further purification.

Deuterated solvents were obtained either from Aldrich, KOR, Sigma or MDS and were used without further purification.

TABLE E-1: HPLC column and solvent systems

System	Column	Mobile Phase	Gradient (curve; time)	Flow rate (mL/min)	Wavelength (nm)
A	10/25 ODS-1	68% MeOH-1% HOAc to 100% MeOH	#6; 17 min	1.00	280
B	10/25 ODS-1	75% MeOH-1% HOAc to 100% MeOH	#6; 15 min	1.00	280
C	10/25 ODS-1	80% MeOH-1% HOAc	--	1.00	280
D	5/25 ODS-1	85% MeOH-1% HOAc	--	1.00	280
E	10/25 ODS-1	70% MeOH-water	--	1.00	280
F	10/25 ODS-1	80% MeOH-water	--	1.00	280
G	10/25 ODS-3	90% MeOH-1% HOAc	--	1.00	280
H	10/25 ODS-3	90% MeOH-1% HOAc to 100 MeOH	#6; 7 min	1.00	254
I	10/25 ODS-1	100% MeOH	--	1.00	280
J	10/25 ODS-3	67% MeOH-1% HOAc	--	1.00	280
K	10/25 ODS-1	82% MeOH-1% HOAc	--	1.00	280
L	5/25 ODS-3	80% MeOH-1% HOAc	#6; 10 min	1.00	280
M	5/25 ODS-3	99% (90% CH_3CN-water)-MeOH	--	0.80	280
N	5/25 ODS-3	90% MeOH-1% HOAc	--	1.00	280
O	5/25 ODS-3	82% MeOH-1% HOAc	--	1.00	280
P	5/25 ODS-3	79% MeOH-1% HOAc	--	1.00	280
Q	5/25 ODS-3	92% MeOH-1% HOAc	--	1.00	280
R	10/25 ODS-3	75% i-PrOH-1% HOAc	--	0.50	280
S	5/25 ODS-3	95% i-PrOH-1% HOAc	--	0.50	280

Reagents:

Dibenzofuran (98% pure), iodotrimethylsilane (TMSI), deuterium chloride (DCl) in D_2O (20% solution) and dithiothreitol (DTT) were purchased from Sigma. Solutions of n-butyllithium (n-BuLi) in n-hexane were obtained from Alfa and titrated[169] prior to use.

Triethylamine (TEA) was distilled first from ninhydrin, then from sodium, and stored in sealed ampules at -20°C; tri-n-butylphosphine (PBu_3) was fractionally distilled under vacuum and stored under N_2 at 4°C; p-dimethylaminopyridine (DMAP) was recrystallized from anhydrous diethyl ether and 1-hydroxybenzotriazole (HOBt) from anhydrous acetonitrile (3x); dicyclohexylcarbodiimide was distilled under vacuum and stored in brown bottles under N_2 at -20°C.

Methoxycarbonylsulfenyl chloride (ScmCl) was prepared according to published procedures[118] and purified by spinning-band distillation (bp. 73-75°C 100 torr). N^{α}-Z-L-AlaOH was synthesized following Goodman's procedure;[170] N^{α}-Z-L-AlaGlyOH according to Naithani,[171] and N^{α}, $N^{\alpha'}$-bis-Boc-L-cystine dimethyl ester as described by Dranginis.[85] O-Bu^t, N^{α}-Bpoc-L-tyrosine (mp 70-73°C) was prepared by Dr. M. Kolovos (MIT). N^{α}-Boc-L-AlaOH, N^{α}-Boc-L-LeuOH, N^{α}-Z-L-LeuOH and N^{α}-Bpoc-L-PheOH DCHA salt were purchased from Chemalog.

Nomenclature:

Amino acids and their derivatives are abbreviated according to the notation recommended by IUPAC-IUB Commission on Biochemical Nomenclature.[172]

Dibenzofuran, 57

The commercially available material was used without further purification.

TLC: R_f 0.75 (A). ^1H NMR (250 MHz, $CDCl_3$): δ 7.17 (1H, td: J = 8,1 Hz, C_2-H), 7.29 (1H, td; J = 8,1 Hz, C_3-H), 7.42 (1H, dm, C_4-H), 7.78 (1H, dm, C_1-H). ^{13}C NMR (62.83 MHz, $CDCl_3$): δ 111.7 (d, C_4), 120.7 (d, C_1), 122.7 (d, C_2), 124.3 (s, C_{4b}), 127.2 (d, C_3), 156.3 (s, C_{4a}). Mass spectrum (70 eV) m/e (rel. intensity): 168 (M^+, 100), 139 (60), 113 (13), 34 (32), 69 (28), 63 (36), 39 (33).

4-Hydroxydibenzofuran, 58

Prepared by modification of published procedures:[102,106] To a solution of 57 (118.9 g, 0.71 mol) in anhydrous diethyl ether (1.30 L) was added dropwise (30 min) at room temperature under N_2 a solution of 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 4-necked flask equipped with a mechanical stirrer was added anhydrous diethyl ether (1.20 L). The vessel was cooled to -78°C (acetone-CO_2 bath), and the solvent was saturated with O_2 (passed through a $CaSO_4$ tower). To the cooled solvent mixture was added simultaneously and over a period of 35 min, the solution of lithiodibenzofuran in Et_2O and n-butylmagnesium bromide (1.15 mmol) in diethyl ether (freshly prepared as in Vogel[175] from n-butyl bromide (124.0 mL) and magnesium turnings (32.3 g) in anhydrous diethyl ether (600 mL)).

During the simultaneous addition, oxygen was bubbled through the reaction mixture and the flow of the gas was maintained for an additional 1 h. The mixture was then allowed to warm up slowly to room temperature and was stirred for 12 h under dry air atmosphere.

The resulting white suspension was then cooled to 0°C, cautiously acidified with 6 N HCl until both phases became clear. After separating the layers the aqueous phase was extracted with Et_2O (3x), the combined organic layers were washed with 1 N HCl and extracted with 1 N NaOH (3x). The alkaline phases were combined, acidified to pH 1 with concentrated HCl, and extracted with Et_2O (4x). The organic layers were combined, washed with water, 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 58 as white needles (55.3 g, 42%), mp 97-99°C (lit.[106] mp 98.5-99.5°C).

TLC: R_f 0.43 (A). HPLC: rt 4.50 min, 99% (D). IR (KBr): ν_{max} 3400-3010 (b), 1630, 1600, 1503, 1435 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 5.59 (1H, bs, Ar:OH), 7.01 (1H, dd; J = 8,1 Hz, C_3-H), 7.20 (1H, t; J = 8 Hz, C_2-H), 7.34 (1H, td; J = 8,1 Hz, C_8-H), 7.44 (1H, td; J = 8,1 Hz, C_7-H), 7.49 (1H, dd; J = 8,1 Hz, C_1-H), 7.58 (1H, dm, C_6-H), 7.92 (1H, dm, C_9-H). Mass spectrum (70 eV) m/e (rel. intensity): 184 (M^+, 100), 155 (24), 128 (41), 102 (29), 92 (39), 63 (46), 51 (60), 39 (26).

4-Methoxydibenzofuran, 59

To a solution of 58 (crude material, 63.08 g, 0.34 mol) in acetone (2.40 L) was added anhydrous potassium carbonate (71.7 g, 0.52 mol) at room temperature under N_2. The suspension was heated to 40°C, methyl

iodide (182.0 mL, 2.92 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 (3x), the organic layers were combined, washed with 1 N NaOH, water, brine, dried ($MgSO_4$), and evaporated. The resulting brown oil residue was distilled under reduced pressure (150°C 0.8 torr) to afford pure 59 as a colorless oil that solidified on standing (62.44 g, 93%), mp 50.5-51.0°C (lit.[176] mp 52°C).

TLC: R_f 0.58 (B), 0.69 (A). HPLC: rt 6.52 min, 100% (D). ^1H NMR (250 MHz, $CDCl_3$): δ 4.04 (3H, s, Ar:OCH_3), 6.95 (1H, d; J = 8 Hz, C_3-H), 7.24 (1H, t; J = 8 Hz, C_2-H), 7.33 (1H, t; J = 8 Hz, C_8-H), 7.44 (1H, t; J = 8 Hz, C_7-H), 7.52 (1H, d; J = 8 Hz, C_1-H), 7.62 (1H, d; J = 8 Hz, C_6-H), 7.91 (1H, d; J = 8 Hz, C_9-H). Mass spectrum (70 eV) m/e (rel. intensity): 198 (M^+, 100), 183 (41), 155 (72), 127 (49), 101 (16), 77 (24).

4-Mercaptodibenzofuran, 42

To a solution of 57 (33.6 g, 0.20 mol) in anhydrous diethyl ether placed under N_2 (400 mL) was added dropwise (20 min) at 0°C a solution of n-butyllithium (0.22 mol) in n-hexane (92.0 mL) and the mixture was heated to reflux for 20 h. The resulting yellow-green suspension was cooled to 0°C, elemental sulfur (Baker, sublimed, N.F.; 7.04 g, 0.22 mol) was added in 4 portions (15 min total), the mixture was allowed to warm up to room temperature and then was heated to reflux for 2 h. The mixture was then cooled to 0°C and cautiously acidified to pH 1 with 20%

HCl (80 mL). The aqueous phase was extracted with Et_2O and the organic layers were combined, washed with 1 N HCl, water, and extracted with 1 N NaOH (3x). The alkaline phases were combined, washed with Et_2O (2x), acidified to pH 1 with concentrated HCl and the aqueous mixture was extracted with Et_2O. The organic layers were combined, washed with 1 N HCl, water, dried ($MgSO_4$), and evaporated. The resulting dark yellow oil residue (32.79 g, 82%) was distilled under reduced pressure (160°C, 0.4 torr) to afford 42 as a light yellow-green oil that solidified on standing, (28.6 g, 71%), mp 46.0-47.5°C (lit[108] 33%, mp 50.5-52.0°C).

TLC: R_f 0.74 (A). HPLC: rt 10.17 min, 100% (L); 4.28 min, 99% (M). ^1H NMR (250 MHz, $CDCl_3$): δ 3.93 (1H, s, Ar:SH), 7.21 (1H, t; J = 8 Hz), 7.29-7.38 (2H, m), 7.45 (1H, td; J = 8,1 Hz), 7.60 (1H, dd; J = 8,1 Hz), 7.71 (1H, dd; J = 8,1 Hz), 7.91 (1H, dd; J = 8,1 Hz). Mass spectrum (70 eV) m/e (rel. intensity): 200 (M^+, 67), 199 (100), 198 (34), 170 (86), 168 (38), 167 (62), 126 (52), 85 (66). UV^{HFIP}: λ_{max} 282nm (15,500).

Anal. Calcd for $C_{12}H_8OS$: C, 71.97; H, 4.03; S, 16.01. Found: C, 71.98; H, 3.97; S, 15.97.

4-Mercapto-6-methoxydibenzofuran, 60

To a solution of 59 (17.19 g, 86.82 mmol) in tetrahydrofuran (130 mL) contained in a 3-necked flask equipped with a low temperature thermometer and cooled under N_2 to -28°C (o-xylene-liquid N_2)[177] was added dropwise (20 min) a solution of 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 condi-

tions for an additional 1 h. To the mixture was then added in one portion elemental sulfur (Baker; sublimed, N.F.; 3.52 g, 109.8 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 (3x), the organic layers were combined, washed with water and extracted with 0.5 N NaOH (3x). The alkaline phases were combined, washed with Et_2O and acidified to pH 1 with concentrated HCl. The aqueous mixture was then extracted with CH_2Cl_2, the organic layers were combined, washed with water, dried ($MgSO_4$) and evaporated to afford a white solid residue possessing a strong thiol odor (14.45 g, 72%). HPLC analysis of the crude product indicated that it was free of positional isomers and symmetrical disulfides (rt 11.64 min, 98% (A)). Recrystallization from CH_2Cl_2-pentane yielded pure 60 as odorless white shiny crystals (8.61 g, 43%), mp 101-2°C.

TLC: R_f 0.62 (A). HPLC: rt 11.70 min, 99% (A); 7.19 min, 100% (D). IR ($CDCl_3$): ν_{max} 3005, 1628, 1585, 1497, 1410 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 3.98 (3H, s, Ar:OCH_3), 4.02 (1H, s, Ar:SH), 6.90 (1H, dd; J = 8,1 Hz, C_7-H), 7.15 (1H, t; J = 8 Hz), 7.18 (1H, t; J = 8 Hz), 7.29 (1H, dd; J = 8,1 Hz, C3-H), 7.42 (1H, dd; J = 8,1 Hz, C_9-H), 7.63 (1H, dd; J = 8,1 Hz, C_1-H). Mass spectrum (70 eV) m/e (rel. 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, 61

To a solution of 60 (3.52 g, 15.3 mmol) in chloroform (23.0 mL) was added in one portion at room temperature under N_2 trimethylsilyl iodide (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 (3x). The organic layers were combined, washed successively with 10% $Na_2S_2O_3$ (2x), water, and brine, then dried ($MgSO_4$), and evaporated to afford a yellow powder (3.18 g, 96%), mp 130-5°C. Dissolution in CH_2Cl_2 followed by cooling to -20°C yielded pure 61 as a light-yellow odorless fluffy solid (2.55 g, 77%), mp 133.5-135.5°C.

TLC: R_f 0.20 (A). HPLC: rt 5.52 min, 98% (B), 5.03 min, 99% (D). IR ($CHCl_3$): ν_{max} 3670, 3600, 3020, 1600, 1500, 1410 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 3.90 (1H, bs, Ar:SH), 5.80 (1H, bs, Ar:OH), 7.04 (1H, dd; J = 8,1 Hz, C_7-H), 7.22 (1H, td; J = 8,1 Hz), 7.23 (1H, td; J = 8,1 Hz), 7.36 (1H, dd; J = 8,1 Hz, C_3-H), 7.47 (1H, dd; J = 8,1 Hz, C_9-H), 7.72 (1H, dd; J = 8,1 Hz, C_1-H). Mass spectrum (70 eV) m/e (rel. 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.

(6-hydroxy-4-dibenzofuranyl) disulfide, 62

To a solution of 61 (1.00 g, 4.63 mmol) in methanol (5.0 mL) was added dropwise (10 min) a solution of iodine (3.50 g, 13.8 mmol) in the same solvent (60 mL). Initially a colorless solution was observed, but the characteristic iodine color persisted after ca. a quarter of the volume of the iodine solution was added. The mixture was then poured into 5% ascorbic acid (200 mL) and extracted with CH_2Cl_2 (3x). The organic layers were combined, back-washed with 5% ascorbic acid, washed with water, brine, dried ($MgSO_4$), and evaporated to dryness to yield an off-white solid (975 mg, 98%), mp 203-5°C. The crude product was triturated with a 1:1 mixture of anhydrous diethyl ether and hexane to afford a white powder (802 mg, 81%), mp 206-207°C.

TLC: R_f 0.59 (C). HPLC: rt 9.57 min, 100% (D). IR ($CHCl_3$): ν_{max} 3575, 3010, 1405, 1300, 710 cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): δ 7.08 (1H, d; J = 8 Hz, C_7-H), 7.24 (1H, t; J = 8 Hz, C_8-H), 7.40 (1H, t; J = 8 Hz, C_2-H), 7.57 (1H, d; J = 8Hz), 7.80 (1H, d; J = 8 Hz), 8.02 (1H, d; J = 8 Hz, C_1-H), 9.29 (1H, S, Ar:OH). Mass spectrum (70 eV) m/e (rel. intensity): 430 (M^+, 6), 397 (2), 215 (66), 187 (35), 158 (22), 115 (100).

Anal. Calcd for $C_{24}H_{14}O_4S_2$: C, 66.96; H, 3.28; S, 14.89. Found: C, 66.79; H, 3.47; S, 15.01.

(6-Acetoxy-4-dibenzofuranyl)disulfide, 63

To a solution of 62 (62.5 mg, 0.145 mmol) in acetic anhydride (1.70 mL) was added a catalytic amount of concentrated sulfuric acid (7 µL) to cause immediate precipitation of a white solid. The resulting thick

suspension was stirred at room temperature for 4 h, was then poured into saturated $NaHCO_3$-ice and stirred for 10 min. The mixture was then extracted with CH_2Cl_2, the organic layers were combined, back-washed with 5% $NaHCO_3$ (5x), washed with water, brine, dried ($MgSO_4$) and evaporated to dryness to afford 63 as a white fluffy solid (66 mg, 89%), mp 203-5°C.

TLC: R_f 0.67 (A). ^1H NMR (250 MHz, $CDCl_3$): δ 2.43 (3H, s, Ar:OAc), 7.21-7.36 (3H, m), 7.71 (1H, dd; J = 8,1 Hz), 7.79 (1H, dd; J = 8,1 Hz), 7.85 (1H, dd; J = 8,1 Hz). Mass spectrum (70 eV) m/e (rel. intensity): 514 (M^+, 3), 472 (2), 430 (9), 215 (20), 115 (17).

[6-(N$^\alpha$-benzyloxycarbonyl-L-alaninyloxy)-4-dibenzofuranyl]disulfide.

A solution of 62 (200.0 mg, 0.465 mmol), N$^\alpha$-Boc-L-alanine (239.4 mg, 1.07 mmol) and 1-hydroxybenzotriazole (182.0 mg, 1.35 mmol) in dimethylformamide (3.5 mL) was cooled to 0°C under N_2. Dicyclohexylcarbodiimide (254 mg, 1.23 mmol) was added, the mixture was stirred first at 0°C for 2 h and then at room temperature for an additional 15 h. To the resulting suspension was added glacial acetic acid (0.20 mL), the mixture was stirred for 40 min, was then cooled to 0°C, filtered and the filtrate was evaporated to dryness. The moist solid residue was dissolved in EtOAc (125 mL), and cooled to 0°C for 1 h. The resulting suspension was filtered, the filtrate was collected and washed with 5% $KHSO_4$, 5% $NaHCO_3$ (2x), water, and brine. It was then dried ($MgSO_4$) and evaporated to yield a moist tan solid (457 mg). Precipitation from DMF-Et_2O, afforded the acylated symmetrical disulfide as a white powder (135 mg, 35%), mp 187-90°C.

TLC: R_f 0.83 (F). ^1H NMR (270 MHz, DMSO-d_6): δ 1.56 (3H, d; J = 7 Hz, Ala: methyl), 4.40-4.51 (1H, m, Ala: methine), 5.10 (2H, s, benzyl), 7.29-7.39 (7H, m), 7.45 (1H, t; J = 8 Hz), 7.75 (1H, d; J = 8 Hz), 8.06 (1H, d; J = 8 Hz), 8.12 (1H, d; J = 8 Hz), 8.18 (1H, d; J = 8 Hz). Field desorption mass spectrum: m/e 840 (M^+).

[6-N^{α}-benzyloxycarbonyl-L-alaninylglycyloxy)-4-dibenzofuranyl]disulfide, 75

Prepared from 62 and N^{α}-Z-L-alaninylglycine according to the procedure described for the preceding acylated disulfide. The crude product was precipitated from DMF-Et_2O to afford pure 75 as a white powder in 39% yield, mp 188-90°C.

TLC: R_f 0.36 (C). ^1H NMR (250 MHz, 3:1 DMSO-d_6:$CDCl_3$): δ 1.29 (3H, d; J = 7 Hz, Ala:methyl), 4.12-4.20 (1H, m, Ala:methine), 4.24-4.35 (2H, m, Gly), 5.02 (2H, m, benzyl), 7.27-7.53 (10H, m), 7.79 (1H, d; J = 8 Hz), 8.03 (1H, d; J = 8 Hz), 8.15 (1H, d; J = 8 Hz), 8.45-8.60 (1H, m).

4-(Methoxycarbonyldithio)-6-hydroxydibenzofuran, 80

To a solution of 61 (1.02 g, 4.72 mmol) in methanol (10.0 mL) was added in one portion with vigorous stirring methoxycarbonylsulfenyl chloride (0.50 mL, 5.45 mmol). Exothermicity was detected, and the clear yellow-green mixture was stirred at room temperature under N_2 for 2 h. The solvent was then evaporated, the residue was dried under high vacuum and azeotroped with CH_3CN (2x) to yield a viscous oil (1.640 g). Flash chromatography (eluent 100% CH_2Cl_2) afforded pure 80 as a colorless oil that solidified on standing (1.13 g, 78%). Trituration with a 1:1

mixture of anhydrous Et_2O and hexane afforded a white powder (1.01 g, 70%), mp 106-8°C.

TLC: R_f 0.20 (D), 0.44 (A). HPLC: rt 5.95 min, 100% (D). IR ($CHCl_3$): ν_{max} 3560 (b), 3005, 1732, 1600, 1409, 1135 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 3.90 (3H, s, Ar:SScm), 6.89 (1H, bs, Ar:OH), 7.06 (1H, dd; J = 8,1 Hz, C_7-H), 7.21 (1H, t; J = 8 Hz), 7.29 (1H, t; J = 8 Hz), 7.42 (1H, dd; J = 8,1 Hz, C_9-H), 7.68 (1H, dd; J = 8,1 Hz, C_3-H), 7.87 (1H, dd; J = 8,1 Hz, C_1-H). Mass spectrum (70 eV) m/e (rel. intensity): 306 (M^+, 17), 247 (6), 216 (19), 184 (32), 155 (23), 115 (74), 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.

4-(Methoxycarbonyldithio)-6-acetoxydibenzofuran, 35

To a solution of 80 (176 mg, 0.575 mmol) in acetic anhydride (2.50 mL) was added a catalytic amount of concentrated sulfuric acid (5 μL) and the clear mixture was stirred at room temperature under N_2 for 4 h. The solution was then poured into 5% $NaHCO_3$-ice, and extracted with CH_2Cl_2 (3x). The organic layers were combined, back-washed with 5% $NaHCO_3$, washed with water, brine, dried ($MgSO_4$), and evaporated. The oil residue (208 mg) was azeotroped with CH_3CN (2x), and dried under high vacuum to afford pure 35 as a white powder (194 mg, 97%), mp 95-6°C.

TLC: R_f 0.62 (A). IR ($CHCl_3$): ν_{max} 3022, 1769, 1738, 1430, 1408, 1180, 1142 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.50 (3H, s, Ar:OAc), 3.90 (3H, s, Ar:SScm), 7.25-7.38 (3H, m), 7.73 (1H, dd; J = 8,1 Hz), 7.79 (1H,

dd; J = 8,1 Hz), 7.91 (1H, dd; J = 8,1 Hz, C_1-H). Mass spectrum (70 eV) m/e (rel. intensity): 348 (M^+, 11), 306 (17), 262 (14), 215 (23), 158 (8), 115 (16). High resolution mass spectrum: Calcd for $C_{16}H_{12}O_5S_2$: 348.0126; Found: 348.0115.

4-(Methoxycarbonyldithio)-6-(N^{α}-tert-butyloxycarbonyl-L-alaninyloxy)-dibenzofuran, 85

To a solution of 80 (1.430 g, 4.670 mmol) in dichloromethane (15.0 mL) was added in one portion a solution of N^{α}-Boc-L-alanine (973 mg, 5.143 mmol) in the same solvent (20.0 mL) and the clear mixture was cooled to 0°C under N_2. To this solution was added in one portion with vigorous stirring dicyclohexylcarbodiimide (1.200 g, 5.83 mmol). Dicyclohexylurea (DCU) formed within 2 min, 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, the DCU was collected by filtration and washed with ice-cold CH_2Cl_2 (2x). 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 $NaHCO_3$ (2x), water, dried ($MgSO_4$), and evaporated. To the resulting white solid residue (2.91 g) was added EtOAc (50 mL) and the suspension that formed was heated briefly over a steam bath until DCU remained as the only insoluble material in the mixture. The resulting suspension was allowed to cool to room temperature, was filtered, and the residue was washed with ice-cold EtOAc. The filtrates were combined, condensed to ca. 25 mL, and the DCU that precipitated was collected. The above described process was repeated, and evaporation of the combined final filtrates afforded a white solid

residue (2.166 g, 97%). Recrystallization from EtOAc (10 mL) yielded white needles (1.327 g, 60%), mp 148-9°C. A second crop was obtained by cooling the filtrates to -20°C overnight (0.246 g, 11%), mp 146-8°C.[178]

TLC: R_f 0.20 (B), 0.57 (A). HPLC: rt 6.37 min, 99% (C); 8.70 min, 98% (D). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.49 (9H, s, Ala:Boc), 1.78 (3H, d; J = 7 Hz, Ala:methyl), 3.89 (3H, s, Ar:Scm), 4.74-4.85 (1H, m, Ala:methine), 5.27 (1H, bd; J = 8 Hz, urethane), 7.27-7.39 (3H, m), 7.76 (1H, dd; J = 8,1 Hz), 7.82 (1H, dd; J = 8,1 Hz), 7.92 (1H, dd; J = 8,1 Hz, C_1-H). Field desorption mass spectrum: m/e 476 (M^+-1).

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^{α}-tert-butyloxycarbonyl-L-phenylalaninyloxy)-dibenzofuran, 85a

Prepared from 80 and N^{α}-Boc-L-phenylalanine according to the procedure described for 85. The crude product was purified by flash chromatography (eluent 100% CH_2Cl_2) to yield pure 58a as a white crystalline solid in 74% yield, mp 91-3°C.

TLC: R_f 0.27 (D). HPLC: rt 13.15 min, 100% (D). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.45 (9H, s, Phe:Boc), 3.35-3.62 (2H, m, Phe: benzyl), 3.89 (3H, s, Ar:SScm), 4.95-5.10 (1H, m, Phe: methine), 5.18 (1H, bd; J = 8 Hz, urethane), 7.21-7.43 (8H, m), 7.45 (1H, dd; J = 8,1 Hz), 7.82 (1H, dd; J = 8,1 Hz), 7.94 (1H, dd; J = 8,1 Hz, C_1-H). Field desorption 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^{α}-tert-butyloxycarbonyl-L-leucinyloxy)-dibenzofuran, 86

Prepared from 80 and N^{α}-Boc-L-leucine according to the procedure described for 85 except that the commercially available protected amino acid monohydrate was azeotroped with CH_3CN (3x) and dried under high vacuum over P_2O_5 for 6 h prior to use. The crude product was flash chromatographed (eluent 100% CH_2Cl_2) to afford a light yellow solid in 71% yield, mp 111-113°C. Recrystallization from Et_2O-hexane yielded analytically pure 86 as white needles in 53% yield (overall), mp 112.0-112.5°C.

TLC: R_f 0.60 (A). HPLC: rt 6.50 min, 100% (F). ^1H NMR (270 MHz, $CDCl_3$): δ 1.11 (6H, m, Leu: i-Pr), 1.49 (9H, s, Leu:Boc), 1.84-1.91 (1H, m), 1.99-2.10 (2H, m, Leu: methylene), 3.89 (3H, s, Ar:Scm), 4.70-4.78 (1H, m, Leu: methine), 5.12 (1H, bd; J = 8 Hz, urethane), 7.29-7.40 (3H, m), 7.75 (1H, dd; J = 8,1 Hz), 7.81 (1H, dd; J = 8,1 Hz), 7.93 (1H, 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^{α}-benzyloxycarbonyl-L-alaninyloxy)-dibenzofuran, 83

Prepared from 80 and N^{α}-Z-L-alanine according to the procedure described for 85. The crude product was recrystallized from EtOAc-cyclohexane to afford 83 as white needles in 94% yield, mp 130-131°C.

TLC: R_f 0.83 (F), 0.57 (A). ^1H NMR (270 MHz, $CDCl_3$): δ 1.80 (3H, d; J = 7 Hz, Ala: methyl), 3.85 (3H, s, Ar:Scm), 4.80-4.91 (1H, m, Ala: methine), 5.17 (2H, s, benzyl), 5.23 (1H, bd; J = 8 Hz, urethane), 7.29-7.37 (8H, m), 7.73 (1H, dd; J = 8,1 Hz), 7.81 (1H, dd; J = 8,1 Hz), 7.91 (1H, dd; J = 8,1 Hz, C_1-H). Field desorption 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^{α}-benzyloxycarbonyl-L-leucinyloxy)-dibenzofuran, 84

Prepared from 80 and N^{α}-Z-L-leucine according to the procedure described for 85, except that the commercially available protected amino acid hydrate was azeotroped with CH_3CN (3x) and dried under high vacuum over P_2O_5 for 6 h prior to use. The crude product was purified by preparative layer chromatography (1000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford a white solid in 98% yield. Trituration with hexane gave pure 84 as white crystals (75% yield overall), mp 126-127°C.

TLC: R_f 0.64 (A). HPLC: rt 7.48 min, 100% (F). ^1H NMR (250 MHz, $CDCl_3$): δ 1.12 (6H, m, Leu: i-Pr), 1.78-2.13 (3H, m), 3.86 (3H, s, Ar: Scm), 4.78 -4.87 (1H, m, Leu: methine), 5.17 (2H, s, benzyl), 5.38 (1H, bd; J = 8 Hz, urethane), 7.30-7.38 (8H, m), 7.75 (1H, dd; J = 8,1 Hz), 7.82 (1H, dd; J = 8,1 Hz, C_1-H).

4-(Methoxycarbonyldithio)-6-(N^{α}-p-biphenylisopropyloxycarbonyl-L-phenylalaninyloxy)-dibenzofuran, 87

Prepared from 80 and N^{α}-Bpoc-L-phenylalanine according to the procedure described for 85, except that the commercially available DCHA salt of the protected amino acid was liberated according to Merrifield[27] prior to use. The crude product was purified by flash chromatography (eluent 100% CH_2Cl_2) to afford pure 87 as a white foam in 84% yield, mp 62-64°C.

TLC: R_f 0.64 (A). HPLC: rt 11.87 min, 98% (F). ^1H NMR (250 MHz, CD_2Cl_2): δ 1.72 (3H, s, Bpoc: methyl), 1.76 (3H, s, Bpoc: methyl), 3.29-3.46 (2H, Phe: methylene), 3.89 (3H, s, Ar:Scm), 4.85-5.10 (1H, m, Phe: methine), 5.44 (1H, bd; J = 8 Hz, urethane), 7.19-7.63 (17H, m), 7.78 (1H, d; J = 8 Hz), 7.88 (1H, d; J = 8 Hz), 8.01 (1H, d; J = 8 Hz, C_1-H). Field desorption 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^{α}-p-biphenyl-isopropyloxycarbonyl-L-tyrosinyloxy)-dibenzofuran, 88

Prepared from 80 and O-Bu^t-N^{α}-Bpoc-L-tyrosine according to the procedure described for 87. The crude product was purified by preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford 88 as a white foam in 65% yield, mp 67-70°C.

TLC: R_f 0.62 (A). HPLC: rt 18.46 min, 98% (F). ^1H NMR (250 MHz, CD_2Cl_2): δ 1.33 (9H, s, Tyr: O-Bu^t), 1.74 (3H, s, Bpoc: methyl), 1.78 (3H, s, Bpoc: methyl), 3.35-3.60 (2H, m, benzyl), 3.87 (3H, s, Ar:Scm),

4.78-4.95 (1H, m, Tyr: methine), 5.49 (1H, bd; J = 8 Hz, urethane), 7.01 (2H, d; J = 9 Hz), 7.18 (1H, d; J = 8 Hz), 7.28-7.63 (13H, m), 7.79 (1H, d; J = 8 Hz), 7.87 (1H, d; J = 8 Hz), 8.00 (1H, d; J = 8 Hz, C_1-H).

Field desorption 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.

4-Mercapto-6-acetoxydibenzofuran,

64 (Method A), or 38 (Method B)

Method A: To a suspension of 63 (27.7 mg, 53.8 μmol) and dithiothreitol (9.0 mg, 58.2 μmol) in a 1:1 mixture of dimethylformamide and methanol was added a catalytic amount of 1.0 N KOH (5.3 μL, 5.3 μmol) and the mixture was stirred at room temperature under N_2 for 2 h. The resulting clear solution was then poured into 0.1 N HCl-ice, and extracted with CH_2Cl_2 (3x). The organic layers were combined, washed with water, brine, dried ($MgSO_4$) and evaporated. The resulting oil residue was azeotroped with CH_3CN (2x) and dried under high vacuum to afford a tan solid (27.0 mg, 97%), mp 107-112°C. Recrystallization from CH_2Cl_2-pentane yielded large light-yellow prisms (17 mg, 62%), mp 115-116°C. See p. 61.

TLC: R_f 0.56 (A). ^1H NMR (250 MHz, $CDCl_3$): δ 2.48 (3H, s, Ar:OAc), 3.94 (1H, s, Ar:SH), 7.22-7.40 (4H, m), 7.76 (1H, dd; J = 8,1 Hz), 7.81 (1H, dd; J = 8,1 Hz).

Anal. Calcd for $C_{14}H_{10}O_3S$: C, 65.10; H, 3.90; S, 12.41. Found: C, 65.33; H, 4.05; S, 12.44.

Method B: Prepared from 35 according to the procedure described for 45 (87%). See p. 40.

4-Mercapto-6-dibenzofuranyl N^{α}-benzyloxycarbonyl-L-alaninate, 45

To a solution of 83 (92.4 mg, 0.181 mmol) in a 4:1 dioxane-water mixture (1.40 mL) was added at room temperature under N_2 tri-n-butylphosphine (46 µL, 0.185 mmol) and the solution was heated to 45°C for 0.5 h.[179] The mixture was then allowed to cool to room temperature, was lyophilized, the resulting white solid residue (117 mg) was triturated with ice-cold CH_3CN (3.5 mL), and filtered. The residue was washed with ice-cold CH_3CN (2 x 0.5 mL), and dried under high vacuum to afford white shiny crystals (47 mg, 62%), mp 158-60°C.

TLC: R_f 0.42 (A). ^1H NMR (270 MHz, $CDCl_3$): δ 1.75 (3H, d; J = 7 Hz, Ala: methyl), 4.03 (1H, s, Ar:SH), 4.76-4.90 (1H, m, Ala: methine), 5.12-5.24 (2H, m, benzyl), 5.42 (1H, bd; J = 8 Hz, urethane), 7.23-7.45 (7H, m), 7.74 (1H, dd; J = 8,1 Hz), 7.83 (1H, 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^{α}-tert-butyloxycarbonyl-L-cysteinate, 36

To a solution of N^{α},$N^{\alpha'}$-bis-Boc-L-cystine dimethyl ester (0.80 g, 1.71 mmol) and dithiothreitol (0.29 g, 1.88 mmol) in methanol (12.0 mL) was added a catalytic amount of 1.0 N KOH (165 µL), and the clear mixture was stirred at room temperature under N_2 for 2 h. The solution was then poured into ice-cold 0.1 N HCl and extracted with CH_2Cl_2 (3x). The organic layers were combined, washed with water (2x), dried ($MgSO_4$), and evaporated. The resulting clear oil residue was azeotroped with CH_3CN (2x) and dried under high vacuum to afford 36 as a clear colorless oil (745 mg, 93%).

TLC: R_f 0.53 (A). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.40 (1H, t; J = 9 Hz, Cys:SH), 1.45 (9H, s, Boc), 2.95-3.06 (2H, m), 3.80 (3H, s, Cys:OMe), 4.66-4.72 (1H, m, Cys: methine), 5.72 (1H, bd; J = 9 Hz, urethane).

Methyl N^{α}-tert-butyloxycarbonyl, S-methoxycarbonylsulfenyl-L-cysteinate, 39

To a solution of freshly prepared 36 (2.38 g, 10.13 mmol) in methanol (30.0 mL) was added with vigorous stirring at 0°C under N_2 methoxycarbonylsulfenyl chloride (1.00 mL, 10.91 mmol) followed by triethylamine (1.52 mL, 10.92 mmol). The yellow-green clear mixture was stirred at 0°C for 10 min and then at room temperature for an additional 50 min. The solution was then poured into ice-cold 0.5 M citric acid, extracted with CH_2Cl_2 (3x), the organic phases were combined, back-washed with 0.5 M citric acid, washed with water (2x), dried ($MgSO_4$), and evaporated to yield a white solid residue (3.09 g, 94%). Recrystallization from hexane afforded white needles (2.81 g, 85%), mp 76.5-78.0°C (lit.[180] 66%, mp 78°C).

TLC: R_f 0.44 (A). HPLC: rt 5.58 min, 100% (L). ^{1}H NMR (250 MHz, acetone-d_6): δ 1.42 (9H, s, Boc), 3.21-3.40 (2H, m, methylene), 3.72 (3H, s, Cys:OMe), 3.89 (3H, s, Cys:Scm), 4.51-4.63 (1H, m, Cys: methine), 6.47 (1H, bd; J = 8 Hz, urethane). Mass spectrum (70 eV) m/e (rel. intensity): 325 (M^+, 0.1), 224 (2), 193 (3), 178 (15) 165 (14), 57 (100). UV^{HFIP} λ_{max}: 270 nm (ε 823).

Anal. Calcd for $C_{11}H_{19}O_6NS_2$: C, 40.60; H, 5.88; N, 4.30. Found: C, 40.79; H, 6.00; N, 4.25.

Methyl N^{α}-tert-butyloxycarbonyl-S-(6-acetoxy-4-dibenzofuranylthio)-L-cysteinate, 40 (Method A) or 65 (Method B)

Method A: To a solution of 35 (0.52 g, 1.49 mmol) in a 1:1 chloroform-methanol mixture (2.10 mL) was added dropwise (8 min) at room temperature under N_2 a solution of freshly prepared 36 (365 mg, 1.55 mmol) in the same solvent (3.2 mL) and the clear mixture was stirred under these conditions for 0.5 h. The solvent was then evaporated, the resulting yellow oil residue was dissolved in EtOAc, and the solution was washed with water, dried ($MgSO_4$), and evaporated to dryness. The resulting oil residue (802 mg) was purified by flash chromatography (eluent 9:1 CH_2Cl_2-EtOAc) to afford the unsymmetrical disulfide 40 as a light-yellow oil that crystallized on standing (235 mg, 32%), mp 63.5-64.5°C.

TLC: R_f 0.52 (A). IR ($CHCl_3$): ν_{max} 2987, 1748, 1705, 1487, 1175, 1156 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.43 (9H, s, Boc), 2.48 (3H, s, Ar-OAc), 3.29-3.48 (2H, m, Cys: methylene), 3.70 (3H, s, Cys:OMe), 3.75 (3H, s, Ar:Scm), 4.62-4.72 (1H, m, Cys: methine), 5.47 (1H, bd; J = 8 Hz, urethane), 7.26 (1H, dd; J = 8,1 Hz, C_7-H), 7.35 (2H, tm, C_2-H and C_8-H), 7.70 (1H, dd; J = 8,1 Hz), 7.81 (1H, dd; J = 8,1 Hz), 7.89 (1H, dd; J = 8,1 Hz, C_1-H). Mass spectrum (70 eV) m/e (rel. intensity): 435 (M^+-56, 2), 375 (14), 258 (19), 216 (100), 178 (17), 146 (20). High resolution mass spectrum: Calcd for $C_{23}H_{25}O_7NS_2$: 491.1073; Found: 491.1081.

Anal. Calcd for $C_{23}H_{25}O_7NS_2$: C, 56.20; H, 5.13; N, 2.85; S, 13.04. Found: C, 55.99; H, 5.31; N, 2.77; S, 13.20.

Method B: To a solution of 64 (22.5 mg, 87.1 μmol) in methanol (0.50 mL) was added a solution of 39 (28.3 mg, 87.1 μmol) in a 1:1 chloroform-

methanol mixture (1.0 mL). The clear mixture was then treated with a catalytic amount of triethylamine (2 μL, 14 μmol) and was stirred at room temperature under N_2 for 0.5 h. The solution was then evaporated to dryness and the resulting oil residue was dissolved in CH_2Cl_2. The solution was washed with ice-cold 0.1 N HCl, water, brine, was dried ($MgSO_4$) and evaporated. The residue was purified by preparative layer chromatography (1,000 μ; eluent: 9:1 $CHCl_3$-EtOAc) to afford a clear oil that solidified on standing (37.4 mg, 87%), mp 63-64°C. The product was shown to be identical with 40 by mixed mp, TLC and ^1H NMR.

Methyl N^{α}-tert-butyloxycarbonyl-S-[6-(N^{α}-benzyloxycarbonyl-L-alaninyloxy)-4-dibenzofuranylthio]-L-cysteinate, 47

To a suspension of 83 (40.8 mg, 79.8 μmol) in a 4:1 dioxane-water mixture (2.00 mL) was added in one portion at room temperature under N_2 tri-n-butylphosphine (20 μL, 80.3 μmol) and the mixture was warmed to 40°C where it was stirred under N_2 for 0.5 h.[179] The resulting clear solution was then lyophilized, and the white solid residue (50.8 mg) was dissolved in a 14:1 hexafluoroisopropanol-chloroform mixture (1.50 mL) and added in one portion to a solution of Cys-SScm 39 (26.1 mg, 80.3 μmol) in hexafluoroisopropanol (0.70 mL). The clear mixture was stirred at room temperature under N_2 for 0.5 h, the solvent was then evaporated and the residue dried under high vacuum to yield a white solid (82.6 mg). Preparative layer chromatography (1,000 μ; eluent 9:1 chloroform-EtOAc) afforded pure 47 as a white solid (46.7 mg, 90%), mp 133-135°C. An analytical sample was obtained by recrystallization from EtOAc, mp 133.5-134.5°C.

TLC: R_f 0.47 (A). ^1H NMR (270 MHz, $CDCl_3$): δ 1.40 (9H, s, Boc), 1.80 (3H, d; J = 7 Hz, Ala: methyl), 3.29-3.44 (2H, m, Cys: methylene), 3.67 (3H, s, Cys:OMe), 4.63-4.75 (1H, m, Cys: methine), 4.78-4.90 (1H, m, Ala: methine), 5.18 (2H, m, benzyl), 5.42 (1H, bd; J = 8 Hz, Cys: urethane), 6.01 (1H, bd; J = 8 Hz, Ala: urethane), 7.28-7.42 (8H, m), 7.70 (1H, dd; J = 8,1 Hz), 7.83 (1H, dd; J = 8,1 Hz), 7.92 (1H, dd; J = 8,1 Hz, C_1-H).

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.

4-(Methoxycarbonyldithio)-dibenzofuran

Prepared from 42 and methoxycarbonylsulfenyl chloride according to the procedure described for 80. The crude oil product was azeotroped with CH_3CN (3x) and dried under high vacuum to afford the Scm material as a light-yellow solid in 97% yield, mp 66-67°C.

TLC: R_f 0.70 (A). ^1H NMR (60 MHz, $CDCl_3$): δ 3.86 (3H, s, Ar:Scm), 7.10-7.92 (7H, m).

Methyl N^{α}-tert-butyloxycarbonyl, S-(4-dibenzofuranylthio)-L-cysteinate, 43

Prepared from 42 and 39 according to the procedure described for 65 except that the reaction was performed in the absence of base in a 3:2 acetonitrile-hexafluoroisopropanol solvent mixture. The crude product was purified by preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford 43 as a pale oil in 87% yield.

Also prepared from 4-(Methoxycarbonyldithio)-dibenzofuran according to the two-step procedure developed for 47: the crude material was purified as described above to afford a light-yellow oil 83% overall) that was identical with 43 by TLC, HPLC and ^{1}H NMR.

TLC: R_f 0.60 (A). HPLC: rt 11.95 min, 100% (L). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.43 (9H, s, Cys:Boc), 3.21-3.43 (2H, m, Cys:methylene), 3.73 (3H, s, Cys:OMe), 4.65-4.76 (1H, m, Cys:methine), 5.43 (1H, bd; J = 8 Hz, urethane), 7.30 (1H, dd; J = 8,1 Hz), 7.37 (1H, td; J = 8,1 Hz), 7.48 (1H, td; J = 8,1 Hz), 7.63 (1H, dd; J = 8,1 Hz), 7.93 (1H, dd; J = 8,1 Hz). High resolution mass spectrum: Calcd for $C_{21}H_{23}O_5NS_2$: 433.1018; found: 433.1020. UV^{HFIP}: λ_{max} 283 nm (ε 15,700), 238 nm (ε 24,000), 209 nm (ε 38,600).

Methyl N^{α}-tert-butyloxycarbonyl-S-(6-hydroxy-4-dibenzofuranylthio)-L-cysteinate, 46

Prepared from 61 and 39 according to the procedure described for 65 except that methanol was used as the solvent and the reaction was allowed to proceed for 1 h. The crude material was purified by preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford 46 as a yellow oil in 87% yield.

TLC: R_f 0.41 (A). IR ($CHCl_3$): ν_{max} 3420, 3320 (b), 3000, 2972, 1738, 1700, 1493, 1180 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.46 (9H, s, Cys:Boc), 3.13-3.24 (1H, m, Cys:methylene), 3.77 (3H, s, Cys:OMe), 3.86-3.97 (1H, m, Cys:methylene), 4.84-4.95 (1H, m, Cys:methine), 5.76 (1H, bd; J = 8 Hz, urethane), 7.11 (1H, dd; J = 8,1 Hz, C_7-H), 7.27 (1H, td; J = 8,1 Hz), 7.31 (1H, td; J = 8,1 Hz), 7.47 (1H, dd; J = 8,1 Hz), 7.66 (1H, dd; J = 8,1 Hz), 7.95 (1H, dd; J = 8,1 Hz, C_1-H), 8.32 (1H, bs,

Ar:OH). High resolution mass spectrum: Calcd for $C_{21}H_{23}O_6NS_2$: 449.0967; found: 449.0974.

Methyl N^{α}-tert-butyloxycarbonyl-S-[6-(N^{α}-benzyloxycarbonyl-L-alaninylglycyloxy)4-dibenzofuranylthio]-L-cysteinate, 49

The symmetrical disulfide 75 was cleaved with dithiothreitol according to the procedure described for 64 to give the free thiol 48 as a white crystalline solid in 73% yield, mp 134-136.5°C. This material was used in the next step without further purification.

To a solution of 39 (62 mg, 0.19 mmol) in a 2:1 methanol-dimethylformamide mixture (1.0 mL) was added dropwise (5 min) a solution of freshly prepared 48 in the same solvent (2.5 mL). The mixture was then treated with triethylamine (3 μL, 20 μmol) and stirred at room temperature under N_2 for 1.5 h. The solution was then poured into 5% $KHSO_4$-ice (100 mL), and the white solid that precipitated was collected by filtration, washed with water (2x) and dried under high vacuum over P_2O_5 to afford a white powder (106 mg, 78%), mp 65-85°C. The crude product was purified by preparative layer chromatography (2,000 μ; eluent 1:1 $CHCl_3$-EtOAc) to afford a moist solid. Trituration with diethyl ether-petroleum ether yielded a pale powder (30 mg, 22%), mp 153-155°C.

TLC: R_f 0.40 (C). ^1H NMR (250 MHz, $CDCl_3$): δ 1.42 (9H, s, Boc), 1.45 (3H, d; J = 7 Hz, Ala:methyl), 3.33-3.50 (2H, m, Ala:methylene), 3.71 (3H, s, Cys:OMe), 4.35-4.53 (2H, m, Gly:methylene), 4.56 (1H, bd; J = 8 Hz, amide), 4.62-4.74 (1H, m, Ala:methine), 5.03-5.17 (2H, m, benzyl), 5.58 (1H, bd, urethane), 5.75 (1H, bd, urethane), 7.29-7.41 (8H,

m), 7.70 (1H, dd; J = 8,1 Hz), 7.85 (1H, dd; J = 8,1 Hz), 7.95 (1H, dd; J = 8,1 Hz, C_1-H). Field desorption mass spectrum: m/e 712 (M^+).

Trifluoroacetic acid salt of methyl S-methoxycarbonylsulfenyl-L-cysteinate, 50

To a solution of 39 (69.4 mg, 213 μmol) in trifluoroacetic acid (0.40 mL) was added at 0°C under N_2 anisole (2.2 μL, 20 μmol) and the solution was stirred under these conditions for 1 h. The mixture was then evaporated to dryness under high vacuum and the colorless oil residue azeotroped with toluene (3x). This new residue was dried under high vacuum to afford 50 as a gum (67 mg, 100%) that was immediately used in the capture step.

^{1}H NMR (250 MHz, $CDCl_3$): δ 3.47 (2H, bs, Cys:methylene), 3.88 (3H, s, Cys:OMe), 3.95 (3H, s, Cys:Scm), 4.17-4.36 (1H, m, Cys:methine), 8.33 (3H, bs, ammonium salt).

Trifluoroacetic acid salt of methyl S-[6-(N$^{\alpha}$-benzyloxycarbonyl-L-alaninyloxy)-4-dibenzofuranylthio]-L-cysteinate, 53

Method A: A solution of 47 (27.9 mg, 42.7 μmol) in trifluoroacetic acid (0.60 mL) was stirred at 0°C under N_2 for 1 h. The resulting light-red clear mixture was then evaporated, the oil residue azeotroped with toluene (3x), and dried under high vacuum. The final product was triturated with a 1:1 diethyl ether-petroleum ether mixture to afford 53 as a tan solid (23 mg, 81%), mp 85-88°C.

^{1}H NMR (270 MHz, DMSO-d_6): δ 1.57 (3H, d; J = 7 Hz, Ala:methyl), 3.15-3.49 (2H, m, Cys:methylene), 3.67 (3H, s, Cys:OMe), 4.45 (2H, m, Cys

and Ala methines), 5.07 (2H, s, benzyl), 7.14-7.56 (8H, m), 7.78 (1H, d; J = 8 Hz), 8.18 (2H, m), 8.22 (1H, d; J = 8 Hz), 8.68 (3H, bs, ammonium salt). Field desorption mass spectrum: m/e 555 (M^+-TFA).

Method B: To a solution of freshly prepared cysteine trifluoroacetate 50 (60.30 mol) in hexafluoroisopropanol (2.20 mL) was added in one portion at 0°C a solution of 45 (24.3 mg, 57.65 mol) in the same solvent. The resulting pale solution was stirred first at 0°C for 7 min, then at room temperature for 25 min and the clear mixture was evaporated to dryness. The residue was dried under high vacuum and triturated with petroleum ether to afford a pale powder (37.1 mg, 96%), mp 84-86°C (dec).

This compound was identical with authentic 53 (Method A) by mixed mp and ^{1}H NMR.

Trifluoroacetic acid salt of (4-dibenzofuranylthio)-L-cysteinate, 51

Prepared from 42 and 50 according to the procedure described for 53 (Method B) except that the reaction was performed in a 5:1 hexafluoroisopropanol-chloroform solvent mixture. The crude product was triturated with anhydrous diethyl ether to afford 51 as a white powder in 81% yield, mp 147-149°C (dec).

Also prepared by trifluoroacetic acid treatment of 43, according to the procedure developed for 53 (Method A). Trituration of the crude product with anhydrous diethyl ether afforded a white powder (72%, mp 148-151°C) that was identical with 51 by mixed mp, HPLC and ^{1}H NMR.

HPLC: rt 6.03, 98% (M). ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.36 (2H, m, Cys:methylene; obscured by H_2O impurity in DMSO-d_6), 3.52 (3H, s, Cys:OMe), 3.78 (1H, t; J = 6 Hz, Cys:methine), 7.45 (2H, m), 7.62 (1H, t; J = 8 Hz), 7.76-7.86 (2H, m), 8.18-8.27 (2H, m), 8.59 (3H, bs, ammonium salt).

Trifluoracetic acid salt of methyl S-(6-acetoxy-4-dibenzofuranylthio)-L-cysteinate, 52

Prepared from 38 and 50 according to the procedure described for 53 (Method B). The crude product was triturated with anhydrous diethyl ether to afford 52 as a white powder in 82% yield, mp 143-145°C (dec).

Also prepared from 40 according to the procedure for 53 (Method A). Purification of the crude product as described above yielded a tan powder (58% yield, mp 140-142°C) that was identical with 52 by ^{1}H NMR and showed no depression by mixed mp.

IR (KBr): ν_{max} 3450 (b), 2970 (b), 1750, 1403, 1180 cm^{-1}. ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.47 (3H, s, Ar:OAc), 3.30-3.54 (2H, m, Cys:methylene), 3.77 (3H, s, Cys:OMe), 4.53 (1H, t; J = 6 Hz, Cys:methine), 7.38-7.58 (3H, m), 7.82 (1H, dd; J = 8,1 Hz), 8.11 (1H, dd; J = 8,1 Hz), 8.24 (1H, dd; J = 8,1 Hz), 8.68 (3H, bs, ammonium salt). High resolution mass spectrum: Calcd for $C_{18}H_{17}O_5NS_2$ (M^+-TFA): 391.0548; found: 391.0563.

(4-Dibenzofuranyl)disulfide, 54

A solution of 42 (308 mg, 1.54 mmol) in anhydrous dimethylsulfoxide (1.20 mL) was heated at 85°C for 14 h[181] and was then allowed to cool to room temperature. The resulting white suspension was poured into ice-cold water (150 mL) the white precipitate that formed was collected by filtration, washed with water (4x), and dried under high vacuum at 50°C over P_2O_5 to afford 54 as a light-yellow solid (257 mg, 84%), mp 138-141°C. Recyrstallization from CH_2Cl_2-pentane afforded a light yellow solid, mp 143.5-145°C.

TLC: R_f 0.77 (A). HPLC: rt 17.05 min, 98% (L); 15.69 min, 100% (M). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.23-7.51 (3H, m), 7.68 (1H, d; J = 8 Hz), 7.83-7.94 (2H, m). Mass spectrum (70 eV) m/e (rel. intensity): 398 (M^+, 42), 365 (10), 334 (17), 199 (100), 171 (91), 126 (90).

Rate determination for the thiol capture reaction which occurs on treatment of 42 with 39

Within two days before a kinetic run, 4-mercaptodibenzofuran, 42, was distilled and methyl S-(Scm)-N^{α}-Boc-L-cysteinate, 39, was recrystallized (hexane) to constant mp. (78°C). Triethylamine (TEA) and diisopropylethylamine (DIEA) were distilled first from ninhydrin, then from sodium and stored in sealed ampules at -20°C; 2,6-di-tert-butyl, 4-methylpyridine was obtained courtesy of Professor G. Büchi (M.I.T.) and distilled prior to use. Hexafluoroisopropanol (HFIP) was fractionally distilled; reagent grade acetonitrile (CH_3CN) was dried over molecular sieves (Linde 4A) for at least 2 d prior to use; water was freshly distilled (Corning AG-1B constant flow apparatus).

Cysteine 39 was dissolved in HFIP or aqueous HFIP; thiol 42 and the amine bases in CH_3CN. Reactions were conducted at 4.3×10^{-4} M to 8.6×10^{-4} M in thiol, and zero to 17.5×10^{-3} M in amine at ambient temperature (25°C). In all but one case pseudo-first-order conditions were employed with cysteine being in 21-fold excess of the thiol.

To initiate a run, volumes of freshly prepared solutions of the reagents were transferred by syringe (Hamilton) into a borosilicate microvial (Wheaton; capacity: 0.30 mL) equipped with a magnetic flea bar and a teflon-coated screw cap in the following order: First 39 (200 μL), second 42 (20 μL), and last the appropriate base (if any). The reaction time was measured with a digital timer (Precision Scientific Co., cat. #69235) and zero time was taken when the last reagent was added.

The reaction was followed by HPLC (using column-eluent system L; monitored by injecting 10.0 μL aliquots of the reaction mixture at regular intervals). The retention times of the peaks of interest were predetermined by injection of authentic samples of the appropriate compounds under the same conditions and are as follows: 39 (5.58 min), 42 (10.17 min), 43 (11.95 min), 54 (17.05 min).

The peaks of interest were integrated and the sum of the integrals for 42, 43 and 54 gave the value for A_o at any given time. The relative concentration of thiol 42 (A_t) was obtained in every case by dividing the integral for 42 by A_o (obtained as described above). The reaction was followed for at least 3 half-lifes and the infinity point was taken at 6 half-lifes as the relative concentration of 54 in the reaction mixture (calculated as for 42). Pseudo-first-order rate constants were derived

for each run from the slope of the line obtained for a plot of $\ell n(A_o-A_\infty)/\ell n(A_t-A_\infty)$ versus time; second-order rate constants were calculated from the equation:

$$k_2 = \frac{k_\psi}{[\underline{39}]}$$

Rate determination of the thiol capture reaction which occurs on treatment of 42 with 50

The kinetics of this thiol capture were performed as described above except that (a) the run employed equal concentrations of 42 and freshly prepared 50 (4.54×10^{-4} M) in a 91:9 HFIP-CH_3CN solvent mixture and (b) it was monitored under HPLC condition M.

Methyl N^{α}-acetyl, S-(6-hydroxy-4-dibenzofuranylthio)-L-cysteinate, 67

A solution of 65 (231 mg, 0.470 mmol) in dioxane saturated with dry HCl gas (15 mL) was stirred at room temperature under N_2 for 20 min. The mixture was then lyophilized, and the residue dried under high vacuum. The resulting white solid product was triturated with anhydrous diethyl ether to afford the amine salt as a white powder (178 mg, 89%), mp 177-180°C (dec).

A portion of this material (93.0 mg, 0.217 mmol) was suspended in water (3.0 mL), two drops of 50% potassium carbonate in water was added, and the alkaline mixture extracted with $CHCl_3$ (3x). The organic layers were combined, dried ($MgSO_4$) and evaporated to give 66 as a clear oil (33.5 mg, 39%).

The free amine obtained thus was dissolved in DMSO-d_6 (0.40 mL) and the clear mixture was allowed to stand at room temperature for 20 h. The solvent was then evaporated under high vacuum and the resulting oil residue was purified by preparative layer chromatography (1,000 μ; eluent 1:1 $CHCl_3$-EtOAc) to afford 67 as a clear oil (10.1 mg, 30% based on 66) and symmetrical disulfide 62 as an oil that solidified on standing (5.0 mg, 27% based on 66), mp 204-107°C.

Unsymmetrical disulfide 67 was independently synthesized in 12% yield by allowing 80 to react with freshly prepared methyl N^{α}-Ac-L-cysteinate under the conditions described for 40 and was found to be identical with the acyl transfer product by TLC, IR and ^{1}H NMR. Similarly, symmetrical disulfide 62 was identical with an authentic sample of the material prepared as described on p. 136 by TLC and ^{1}H NMR.

For the amine salt: IR (KBr): ν_{max} 3450 (b), 2930-2800 (b), 1750, 1403, 1180 cm^{-1}. ^{1}H NMR (250 MHz, CD_3OD): δ 2.45 (3H, s, Ar:OAc), 3.32-3.52 (2H, m, Cys: methylene), 3.80 (3H, s, Cys:OMe), 4.51-4.60 (1H, m, Cys: methine), 7.32 (1H, dd; J = 8,1 Hz, C_7-H), 7.42 (1H, td; J = 8,1 Hz), 7.45 (1H, td; J = 8,1 Hz), 7.77 (1H, dd; J = 8,1 Hz), 7.96 (1H, dd; J = 8,1 Hz), 8.09 (1H, dd; J = 8,1 Hz, C_1-H).

High resolution mass spectrum: Calcd for $CGGH_{17}O_5NS_2$ (M^+-HCl): 391.0548; found: 391.0563.

For 67: TLC: R_f 0.29 (C). IR ($CHCl_3$): ν_{max} 3420, 2992, 1739, 1665, 1405, 1180 cm^{-1}. ^{1}H NMR (50 MHz): δ 1.87 (3H, s, Cys:Ac), 3.06-3.32 (2H, m, Cys: methylene), 3.63 (3H, s, Cys:OMe), 4.59 -4.71 (1H, m, Cys: methine), 7.02 (1H, dd; J = 8,1 Hz, C_7-H), 7.22 (1H, td; J = 8,1 Hz, C_8-H), 7.43 (1H, td; J = 8,1 Hz, C_2-H), 7.57 (1H, dd; J = 8,1 Hz),

7.74 (1H, dd; J = 8,1 Hz), 8.06 (1H, dd; J = 8,1 Hz, C_1-H), 8.53 (1H, d; J = 8 Hz, amide). High resolution mass spectrum: Calcd for $C_{18}H_{17}O_5NS_2$: 391.0548; found: 391.0549.

N^{α}, $N^{\alpha'}$-bis-(Benzyloxycarbonyl-L-alaninyl glyclyl)-L-cystine dimethyl ester, 79

To a solution of L-cystine dimethyl ester dihydrochloride (194 mg, 0.570 mmol) in dimethylformamide (4.80 mL) was added triethylamine, and the mixture was cooled to 0°C under N_2. To the resulting fine suspension was added N^{α}-Z-L-alaninylglycine[171] (339 mg 1.21 mmol) followed by 1-hydroxybenzotriazole (280 mg, 1.83 mmol); the mixture was stirred at 0°C (15 min), dicyclohexylcarbodiimide (311 mg, 1.51 mmol) was added and the fine suspension was stirred first at 0°C for 15 min and then at room temperature for 20 h. The resulting white suspension was then cooled to 0°C, 50% aqueous acetic acid (0.15 mL) was added dropwise, the mixture was stirred for 10 min, filtered and the residue was washed with ice-cold DMF (2 x 1 mL). The filtrates were combined, evaporated to dryness under high vacuum and the oil residue was dissolved in EtOAc (5.0 mL) and cooled to 0°C. The DCU that precipitated was collected by filtration, washed with ice-cold EtOAc (2 x 1 mL) and the filtrates were combined, washed with 5% $KHSO_4$, 5% $NaHCO_3$, water (2x), brine, dried ($MgSO_4$), and evaporated to yield a pale solid (453 mg, 100%), mp 109-113.5°C. Recrystallization from EtOAc afforded pure 79 as white crystals (257 mg, 57%), mp 123-125°C.

TLC: R_f 0.89 (K). ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.22 (3H, d; J = 7 Hz, Ala: methyl), 2.88-3.00 (1H, m, Cys: methylene), 3.08-3.20 (1H, m,

Cys: methylene), 3.65 (3H, s, Cys:OMe), 3.76 (2H, d; J = 5 Hz, Gly: methylene), 3.99-4.14 (1H, m, Ala: methine) 4.54-4.65 (1H, m, Cys: methine), 5.01 (2H, m, benzyl), 7.33 (5H, bs, phenyl), 7.44 (1H, d; J = 7 Hz, Ala: urethane), 8.17 (1H, m, AlaGly amide), 8.33 (1H, d; J = 7 Hz, GlyCys amide). Mass spectrum (70 eV) m/e (rel. intensity): 397 (M^+-monomer, 0.2), 363 (02.), 306 (0.2), 263 (1), 228 (1), 178 (2), 134 (8), 91 (100).

Methyl (N^{α}-benzyloxycarbonyl-L-alaninylglycyl)-L-cysteinate, 78

The Boc group of 49 was quantitatively removed with HCl-dioxane according to the procedure described for 65, and the resulting hydrochloride salt was neutralized with aqueous potassium carbonate as for 66 in 84% overall yield.

A solution of amine 74 prepared thus (8.0 mg, 13.1 μmol) in DMSO-d_6 (0.40 ml) was allowed to stand at room temperature for 40 h. The solvent was then evaporated under high vacuum, and the oil residue was dissolved in methanol (0.60 mL). Dithiothreitol (2.2 mg, 14.4 μmol) and 1.0 N KOH (1.0 μL, 1.0 μmol) was added and the solution was stirred at room temperature under N_2 for 2 h. The mixture was then poured into ice-cold 0.1 N HCl and extracted with CH_2Cl_2 (3x). The organic phases were combined, washed with water, brine, dried ($MgSO_4$), and evaporated. The resulting moist solid residue (8.50 mg) was purified by preparative layer chromatography (1,000 μ, one-half plate; eluent 98:10:2 $CHCl_3$-MeOH-HOAc) to afford pure 78 as a white solid (3.9 mg, 76% from 74), mp 104-107°C.

Tripeptide 78 was independently prepared by dithiothreitol reduction of 79 in 76% yield, mp 106-108°C and was identical with the acyl transfer product by mixed mp, TLC and ^1H NMR.

For 78: TLC: R_f 0.38 (F). ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.22 (3H, d; J = 7 Hz, Ala: methyl), 2.54 (1H, t; J = 9 Hz, Cys:SH), 2.75-2.86 (2H, m, Cys: methylene), 3.65 (3H, s, Cys:OMe), 3.76 (2H, m, Gly: methylene), 4.01-4.12 (1H, m, Cys: methine), 4.45-4.57 (1H, m, Gly: methine), 4.95-5.09 (2H, m, benzyl), 7.36 (5H, bs, phenyl), 7.57 (1H, d; J = 7 Hz, urethane), 8.15 (1H, d; J = 7 Hz), 8.24 (1H, m). Mass spectrum (70 eV) m/e (rel. intensity): 397 (M^+, 0.3), 306 (0.2), 263 (0.6), 236 (0.4), 159 (5), 134 (7), 91 (100).

Rate determinations for the intramolecular acyl transfer reaction

Trifluoroacetate salts of the protected capture materials were freshly prepared by treating the appropriate Boc derivatives with anhydrous trifluoroacetic acid at 0°C for 1 h then evaporating under high vacuum, azeotroping the residue with toluene (3x) and triturating the final product with anhydrous diethyl ether. Commercially available deuterated solvents were used without further purification; triethylamine (TEA) was distilled as described in General Methods.

The reaction was followed by 250 MHz ^{1}H NMR at ambient temperature (25°C). Equimolar amounts of the TFA salts and triethylamine were employed at concentrations ranging from 3.3×10^{-2} M to 21×10^{-3} M. To initiate a run, a sample of the TFA salt was weighed in an NMR tube, then dissolved in a measured volume of deuterated solvent and the appropriate amount of TEA was added. The tube was shaken to afford complete dissolution 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 measured as follows:

a. In the case of the acetoxy derivatives, when the integration of th appropriate acetyl methyl resonances was equivalent.

b. In the case of the benzyloxycarbonyl-protected derivatives, when the integration of C_7-H (δ 7.0) was equivalent to one-quarter of the integration of the benzylic protons of the Z group (δ 4.9-5.1).

Reaction yields were calculated at t = 40 h according to the equation:

$$\% \text{ yield} = \frac{2 \times (\text{Integr. of } C_7\text{-H})}{(\text{Integr. of bzl protons})}$$

and intramolecular acyl transfer rates according to the integrated form of the pseudo-first-order expression:

$$k_{intr.} = \frac{\ell n\ 2}{t_{1/2}}$$

Table III-2 summarizes (p. 75) the kinetic data obtained thus.

4-Acetoxydibenzofuran, 68

Prepared from 58 according to the procedure described for 63 in 99% yield, mp 98.5-99.5°C. Recrystallization from absolute ethanol afforded pure 68 as long white needles (76% overall), mp 99-100°C.

TLC: R_f 0.70 (H). IR (KBr): ν_{max} 1758, 1452, 1189, 749 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.43 (3H, s, Ar:OAc), 7.19 (1H, dd; J = 8,1 Hz, C_3-H), 7.29 (1H, t; J = 8 Hz, C_2-H), 7.31 (1H, td; J = 8,1 Hz), 7.46 (1H, td; J = 8,1 Hz), 7.56 (1H, d; J = 8 Hz, C_6-H), 7.77 (1H, dd; J = 8,1 Hz, C_1-H), 7.89 (1H, dm; C_9-H). Mass spectrum (70 eV) m/e (rel. intensity): 226 (M^+, 6), 184 (76), 155 (13), 127 (29), 102 (12), 43

(100). Anal. Calcd for $C_{14}H_{10}O_3$: C, 74.33; H, 4.46. Found: C, 74.23; H, 4.50.

Rate determination for the intermolecular aminolysis of 68 with ethyl S-benzyl-L-cysteinate

Ethyl S-benzyl-L-cysteine hydrochloride was liberated from its salt by treatment with 10% aqueous sodium carbonate, and the free amine was extracted with CH_2Cl_2 (3x). The combined organic phases were backwashed with brine, evaporated to dryness and the residue distilled under vacuum (Kugelrohr, 62°C/2 torr) to afford a clear oil that was used immediately. 4-Acetoxydibenzofuran, 68, was recrystallized (absolute ethanol) to constant mp (99-100°C). Commercially available deuterated solvents were used without further purification.

The reaction was followed by 250 MHz ^1H NMR at ambient temperature (25°C) and employed 68 and the cysteine amine at 1.97×10^{-1} M and 1.00 M respectively. To initiate a run, a sample of 68 was weighed in an NMR tube, was then dissolved in a measured volume of deuterated solvent and the appropriate amount of cysteine was introduced by syringe (Hamilton) into the solution (time zero).

The relative amounts of starting material and product were measured by integration of the appropriate acetyl methyl resonances. Second-order rate constants were calculated according to the expression shown below[212] and are reported as averages of two data points. Effective local

$$k_2 t = \frac{1}{B_o - A_o} \ln\left(\frac{A_o B}{B_o A}\right)$$

concentrations (E.L.C.) were tabulated using Kirby's[64] expression:

$$E.L.C. = \frac{k_{intr}}{k_2}$$

Thus:

$$k_2 = 7.58 \times 10^{-2}\ \underline{M}^{-1}h^{-1}$$

Table III-2 summarizes (p. 75) the data obtained in this fashion.

Methyl 6-hydroxy-4-dibenzofuranylthioether, 98

A solution of 61 (423 mg, 1.96 mmol) in methanol (5.0 mL) was treated with methyl iodide (134 μL, 2.15 mmol), then triethylamine (272 μL, 1.95 mmol) and stirred at room temperature under N_2 for 45 min. The solvent was then evaporated, the oil residue was redissolved in CH_2Cl_2, and washed with 0.5 N HCl and water. The solution was then dried ($MgSO_4$), and evaporated to dryness to give a light brown solid (430 mg, 95%). Purification by flash chromatography (eluent: 100% CH_2Cl_2) afforded pure 98 as a light brown powder (342 mg, 76%), mp 115-116°C.

TLC: R_f 0.48 (A). HPLC: rt 5.28 min, 99% (D). ^1H NMR (250 MHz, $CDCl_3$): δ 2.63 (3H, s, Ar:SCH_3), 5.65 (1H, s, Ar:OH), 7.04 (1H, d; J = 8 Hz, C_7-H), 7.21-7.39 (3H, m), 7.50 (1H, d; J = 8 Hz), 7.77 (1H, dd; J = 8,1 Hz). High resolution mass spectrum: Calcd for $C_{13}H_{10}O_2S$: 230.0402; found: 230.0422.

Methyl 6-(N^{α}-tert-butyloxycarbonyl-L-alaninyloxy)-4-dibenzofuranylthioether, 99

Prepared by acylation of 98 with N^{α}-Boc-AlaOH according to the procedure described for 85. The crude product was recrystallized from CH_2Cl_2-pentane to afford pure 99 as a white solid (79%), mp 96-97°C.

HPLC: rt 7.55 min, 98% (D). ^1H NMR (250 MHz, $CDCl_3$): δ 1.49 (9H, s, Ala:Boc), 1.74 (3H, d; J = 7 Hz, Ala:CH_3), 2.61 (3H, s, Ar:SCH_3), 4.67- 4.80 (1H, m, Ala:methine), 5.09-5.18 (1H, bs, Ala:urethane), 7.27-7.41 (4H, m), 7.77 (1H, dd; J = 8,1 Hz), 7.32 (1H, dd; J = 8,1 Hz).

Anal. Calcd for $C_{21}H_{23}O_5NS$: C, 62.82; H, 5.77; N, 3.49; found: C, 64.03; H, 6.73; N, 3.50.

Methyl 6-(N^{α}-tert-butyloxycarbonyl-glycyl-L-phenylalanyl-L-alaninyloxy)-4-dibenzofuranylthioether, 100

Synthesis of BocGlyPhe-OSu: To a solution of N-hydroxysuccinimide (125 mg, 1.09 mmol) in tetrahydrofuran (5.0 mL) was added at 0°C under N_2 N^{α}-BocGlyPheOH (350 mg, 1.08 mmol). The clear solution was stirred under these conditions for 3 min and then treated with dicyclohexylcarbodiimide (210 mg, 1.02 mmol). The resulting white suspension was stirred at 0°C for 20 min and then at room temperature for 1 h. The DCU that formed was collected by filtration, washed with ice-cold THF (2 x 1 mL) and the combined filtrates were evaporated to dryness to afford a white foam. The activated dipeptide obtained thus was redissolved in dichloromethane (2.2 mL) and used immediately in the acylation step.

Deprotection of 99: To thioether 99 (145 mg, 0.361 mmol) anisole (60 μL, 0.552 mmol) was added, the mixture was cooled to 0°C under N_2 and treated with trifluoroacetic acid (2.0 mL). The resulting light-yellow solution was stirred under these conditions for 45 min. The solvent was then evaporated under high vacuum and the residue was azeotroped with toluene (2 x 10 mL). The white solid residue was then triturated with a 1:1 mixture of anhydrous diethyl ether and petroleum ether (10 mL), was filtered, and the residue was collected and dried under high vacuum to afford the TFA salt of deblocked 99 as a white powder (137 mg, 91%).

Acylation: To a suspension of this amine salt in dichloromethane (2.0 mL) was added at 0°C under N_2 the solution of BocGlyPheOSu in the same solvent and the mixture was stirred under these conditions for 2 min. The suspension was then treated with diisopropylethylamine (59 μL, 0.339 mmol), the suspension cleared instantaneously, and the resulting solution was stirred at 0°C for 5 min and then at room temperature for 13 h. HPLC analysis (system D) at this time indicated the presence of 61 (12%) and 100 (88%).

The solution was then diluted with dichloromethane and was washed with sodium citrate buffer (0.5 M in citric acid, pH = 3.5), 5% $NaHCO_3$ and water. It was then dried ($MgSO_4$), evaporated, and the resiude was triturated with anhydrous diethyl ether to afford a white solid (155 mg, 71% based on the TFA salt), mp 157-159°C. Recrystallization from EtOAc yielded an analytical sample (116 mg, 58%), mp 162.5-163.5°C.

HPLC: rt 7.86 min, 98% (D). ^1H NMR (250 MHz, $CDCl_3$): δ 1.47 (9H, s, Gly:Boc), 1.73 (3H, d; J = 7 Hz, Ala:CH_3), 2.65 (3H, s, Ar:SCH_3), 3.05-3.27 (2H, m, Gly:methylene), 3.80 (2H, d, J = 6 Hz, Ala:methylene),

4.70-4.95 (2H, m), 6.52 (1H, bs), 6.72 (1H, bd; J = 8 Hz), 7.17-7.42 (1H, m), 7.78 (1H, dd; J = 8,1 Hz), 7.82 (1H, dd; J = 8,1 Hz).

Anal. Calcd for $C_{32}H_{35}O_7N_3S$: C, 63.45; H, 5.82; N, 6.94; found: C, 63.64; H, 6.03; N, 7.20.

PART B

Solid Phase: General Methods

Apparatus

An Ace-Burlitch inert atmosphere system (Ace Glass cat #7818-10) connected to a vacuum pump and a N_2 tank provided a means of facile interchange between high vacuum and nitrogen (oxygen, moisture-free). To this was connected a Schlenk filter tube (Ace Cat. #7791-06) and the apparatus (Figure IV-1; p. 96) was employed thus in the anchoring and thiol capture steps.

Solid phase reaction vessels were custom-made (R. DiGiacomo, M.I.T.) according to Merrifield's prototype.[185] The vessel was clamped onto a Con-Torque mechanical stirrer (Eberbach Co.) which in turn was connected to an electronic timer (GRA Lab, Model 171) and was rotated 360° at ca. 11 rpm (Figure E-1). Filtration of the reaction mixture was performed by removing the vessel's teflon-coated screw-cap, attaching a screw-on nitrogen inlet adaptor (Ace Cat. #10924-200) and forcing the solvent through a fine porosity (4-8 μ) glass frit with a stream of N_2 (Figure E-2).

The low-temperature equipment employed in the solid phase experiments is as described in General Methods of the solution-phase section.

Quantitation Methods

The ninhydrin test[135] was employed as a qualitative test for the completion of the amine acylation reactions.

High pressure liquid chromatography (HPLC) was used for quantitation of the cysteine resins as described on p. 178. The hardware employed are as described on p. 127 and a table of column-eluent systems can be found on p. 128.

Figure E-1:
Rotation Mode

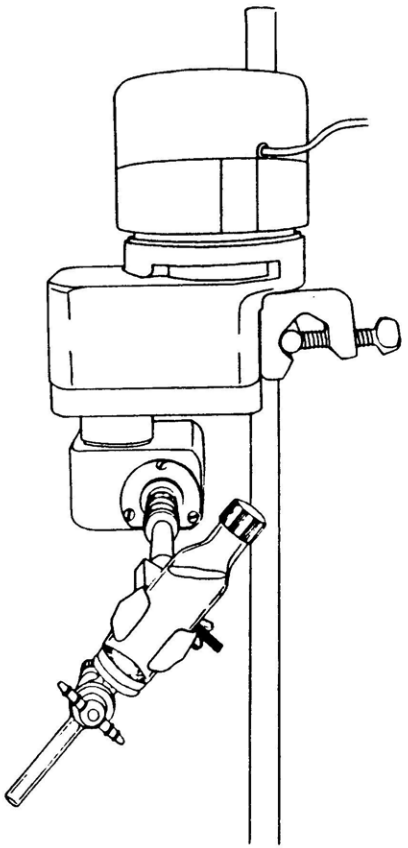
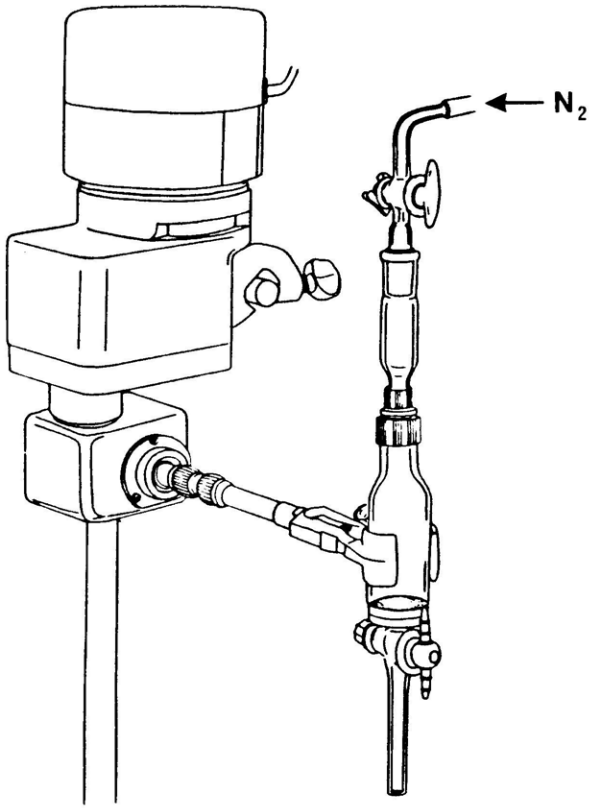


Figure E-2:
Filtration Mode



Free and resin-bound peptides were examined by amino acid analysis conducted on a Glenco Model MM-60 micro-column analyzer. Prior to analysis peptides were hydrolyzed 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 M HCl-glacial acetic acid-phenol mixture[17] (resin-bound peptides).

Reagents and Solvents

N^{α}-Z-glycine was purchased from Aldrich; N^{α}-Z-isoleucine from Sigma; N^{α}-Boc-glycyl-L-phenylalanine was prepared by C.F. Hoyng, M.I.T.; all other amino acid derivatives, chlormethylated polystyrene resin (2% crosslinked, Cat. #57-8620-00), p-benzyloxybenzyl alcohol resin (Cat. #57-8620-80) were obtained from Chemalog and used without purification.

Methoxycarbonylsulfenyl chloride (ScmCl) was either purchased from Fluka or was freshly prepared;[118] benzyloxycarbonyl chloride (Z-Cl) was obtained from Aldrich. Tri-n-butylphosphine (PBu_3) was distilled *in vacuo* and used fresh; pyridine was dried over calcium hydride, then fractionally distilled; piperidine was distilled over potassium hydroxide (2x) and was stored in sealed ampules at -20°C; diisopropylethylamine (DIEA) and triethylamine (TEA) were distilled first from ninhydrin, then from sodium and stored at -20°C; trifluoroacetic acid (TFA) was fractionally distilled from phosphorus pentoxide, then redistilled from anhydrous L-valine. Dicyclohexylcarbodiimide (DCC) was vacuum distilled under N_2 and stored at -20°C; N-hydroxysuccinimide (HOSu) and 1-hydroxybenzotriazole (HOBt) were recrystallized from ethyl acetate and anhydrous acetonitrile respectively.

Dichloromethane was fractionally distilled from P_2O_5, stored over molecular sieves (Linde 4A) and passed through a column of basic aluminum

oxide (Woelm, W-200; ICN Nutritional Biochemicals) immediately prior to use; reagent grade methanol and 2-propanol (i-PrOH) were dried over molecular sieves (Linde 3A); dimethyl formamide (DMF), dioxane and tetrahydrofuran (THF) were purified as described on p. 127.

S-Trityl, N^{α}-benzyloxycarbonyl-L-cysteine diethylammonium salt, 114

To a suspension of S-trityl-L-cysteine (6.51 g, 17.9 mmol) in a 2:1 dioxane-water mixture (60 mL) was added 4 N NaOH (3.8 mL, 15.2 mmol) followed by potassium bicarbonate (16.3 g, 163 mmol). The mixture was then cooled to 0°C, benzyloxycarbonyl chloride (2.75 mL, 19.3 mmol) was added dropwise with vigorous stirring and the heterogeneous mixture was allowed to warm up slowly to room temperature (4 h). Most of the dioxane was then removed under high vacuum, the resulting aqueous suspension was diluted with water, Et_2O was added, and the mixture was shaken in a separatory funnel. Of the three phases that formed, the top one was discarded. To the remaining two, Et_2O was added and the process was repeated twice. The final aqueous solution was acidified to pH 3.5 with solid citric acid, and extracted with CH_2Cl_2 (3x). The organic phases were combined, washed with sodium citrate buffer (0.5 M in citric acid, pH = 3.5), water (2x), dried ($MgSO_4$), evaporated, and the residue was azeotroped with CH_3CN (2x).

The resulting light-yellow foam was dissolved in EtOAc (20 mL) with mild heat, was cooled to 0°C and treated with diethylamine (1.90 mL, 18.4 mmol). The white solid that formed was collected by filtration, washed with cyclohexane and dried under high vacuum to afford 114 as white needles (7.92 g, 78%), mp 156-160°C. Two recrystallizations from

acetone afforded pure 114 as long white needles (4.02 g, 39%), mp 166-168°C (lit.[146] mp 168°C).

HPLC: rt 4.81 min, 100% (C). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.17 (3H, t; J = 8 Hz, ethyl), 2.52-2.78 (4H, m), 4.12-4.23 (1H, m, methine), 5.08 (2H, s, benzyl), 5.68 (1H, bd; J = 8 Hz, urethane), 7.10-7.29 (10H, m, trityl), 7.31-7.44 (8H, m, trityl), 10.23 (2H, bs, salt).

S-Trityl, N^{α}-benzyloxycarbonyl-L-cysteine benzyl ester resin, 117

Liberation of the salt: A solution of 114 (1.85 g, 3.24 mmol) in dichloromethane (60 mL) was washed with sodium citrate buffer, 0.5 M in citric acid, pH = 3.5 (3 x 60 mL) which was back-washed with dichloromethane (1 x 40 mL). The organic layers were then combined, dried ($MgSO_4$), evaporated and the residue was dried under high vacuum to yield the free acid as a white foam (1.60 g, 99%).

Formation of the cesium salt: The free acid (3.13 mmol) was dissolved in a 3:1 absolute ethanol-water mixture (20 mL) and the solution was brought to pH 7.6 with aqueous cesium carbonate (process monitored by pH paper). The resulting clear mixture was then evaporated to dryness under high vacuum and the residue was azeotroped with dimethylformamide (3 x 15 mL) to afford the cesium salt as a light-yellow viscous oil.

Benzyl ester formation with the resin: The cesium salt obtained as described above was dissolved in dimethylformamide (26 mL) with mild heat. It was then added to chloromethylated polystyrene (3.00 g, 309 mmol) that had been quantitated for chloride content by Volhard titration[149] and the resulting heterogeneous mixture was stirred at 50°C under N_2 for 18 h. The suspension was then filtered through a glass fritted funnel and the residue was washed with

1.	3 x 25 mL	5:1	DMF-water
2.	3 x 25 mL	100%	DMF
3.	2 x 25 mL	5:1	DMF-water
4.	3 x 25 mL	100%	absolute EtOH
5.	3 x 25 mL	100%	DMF
6.	3 x 25 mL	100%	absolute EtOH
7.	4 x 25 mL	100%	CH_2Cl_2

The final residue was collected and dried under high vacuum overnight to afford 117 as a white solid (3.90 g). The reaction yield was estimated by Volhard titration for residual chloride (see below) and was found to be 81%.

Quantitation: The displaceable chloride content of both starting material and product resins was assayed by Volhard titration[149] according to the following procedure:

A suspension of 159.8 mg of the commercially available chloromethylated polystyrene (starting material) in pyridine (4.0 mL) was heated in a 10 mL round-bottomed flask at 100°C for 2 h. The contents of the flask were then quantitatively transferred to a 125 mL Erlenmeyer flask (washed by soaking in cleaning solution for 24 h, then rinsed with distilled water (5x) and dried in an oven thermostated at 125°C) and the 10 mL flask was washed with 50% aqueous acetic acid (3 x 10 mL). The washes were combined, cautiously treated with concentrated nitric acid (5 mL) and standard 0.100 N aqueous silver nitrate (5.00 mL) was added (pipette). The resulting suspension was then vigorously stirred for 3 min at room temperature and then treated with water (15 mL), toluene (5 mL) and ferric alum (saturated solution of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in water) (3 drops). The resulting heterogeneous

mixture was then vigoroulsy stirred, and titrated with standard 0.050 N aqueous ammonium thiocyanate until the first permanent tinge of a red-brown color was observed (end point).

Found: starting material: 1.03 mmol Cl/g resin

1.04 mmol Cl/g resin

product (on 455 mg resin): 0.15 mmol Cl/g resin

Yield calculation: Cl_o: 3.00 (g) x 1.03 (mmol Cl/g) = 3.09 mmol Cl

Cl_f: 3.90 (g) x 0.15 (mmol Cl/g) = 0.58 mmol Cl

$$\text{\% yield} = 100 - \frac{0.58}{3.09} = 81\% \text{ of displaceable chloride replaced by Z-Cys(Tri)}O^{-} \text{ residues}$$

$$\text{Loading of resin} = \frac{3.09 - 0.58}{3.90} = 0.64 \text{ mmol Cys/g resin}$$

(S-Trityl, N^{α}-benzyloxycarbonyl-L-cysteine benzyl ester), (N^{α}-benzyloxycarbonyl)glycine benzyl ester)-resin, 117a

Prepared from 117 (3.37 g, 2.16 mmol Cys) and N^{α}-benzyloxycarbonyl-glycine (300 mg, 1.43 mmol) according to the procedure described for 117 except that the "liberation of salt" part was omitted. Drying the final product under high vacuum overnight afforded 117a as a white solid (3.43 g).

Volhard titration: 0.07 mmol Cl/ g resin

Loading of resin: 0.63 mmol Cys/ g resin

0.08 mmol Gly, g resin

0.07 mmol Cl/g resin

[S-[6-(N^{α}-tert-butyloxycarbonyl-L-alaninyloxy)-4-dibenzofuranylthio)-N^{α}-benzyloxycarbonyl-L-cysteine benzyl ester], [N^{α}-benzylcarbonyl-glycine benzyl ester]-resin, 119

A. Activation of the S-trityl resin: Cysteine resin 117a (552 mg, 0.348 mmol Cys) was weighed in an oven-dried 10 mL round-bottomed flask equipped with a flea stirring bar, was suspended in dichloromethane (5.0 mL) and sealed with a rubber septum equipped with a balloon filled with N_2. The vessel was then immersed in an ethylene glycol-water bath electtronically thermostated at -18°C. To the chilled mixture was added in one portion methoxycarbonylsulfenyl chloride (Fluka; 0.45 mL, 4.98 mmol) and the suspension was allowed to warm up slowly to 0°C with stirring (1 h). The resulting yellow-green mixture was treated with methanol (0.45 mL) and the suspension was allowed to warm up slowly to room temperature (2.5 h). The mixture was then filtered through a glass fritted funnel, washed with

1. 4 x 10 mL CH_2Cl_2
2. 3 x 10 mL 1:1 CH_2Cl_2-MeOH
3. 5 x 10 mL CH_2Cl_2

and the resulting white solid was quantitatively transferred to the Schlenk tube of the no-air apparatus (Figure IV-1) where it was immediately used in the anchoring step (Part C).

B. 4-Mercapto, 6-dibenzofuranyl N^{α}-tert-butyloxycarbonyl-L-alaninate, 109: A solution of 85 (277 mg, 0.580 mmol) in a 9:1 dioxane-water mixture (5.00 mL) was prepared in a 50 mL round-bottomed flask equipped with a stopcock linked to the N_2-vacuum line, a side arm with a small serum cap, and a 14/20 joint leading to the Schlenk tube

(Figure IV-1; stopcock A closed). The solution was degassed and saturated with N_2 by a few quick cycles of evacuation and N_2 introduction. Tri-n-butylphosphine (147 µL, 0.590 mmol) was introduced by syringe through the serum cap and the clear mixture was stirred at room temperature under positive pressure of N_2 (Source I) for 30 min. The solvent was then evaporated, the oil residue was dried under high vacuum for 10 min to remove most of the residual water. Thiol 109 prepared thus was used immediately in the next step (Part C).

HPLC: rt (N): 5.13 min, 98%: BocAlaO-Dbf-SH, 109; 9.64 min, 2%: symmetrical disulfide of 109.

C. Anchoring: The Scm-functionalized cysteine resin contained in the Schlenk tube (Part A) was suspended in dichloromethane (4.0 mL). Stopcock A (Figure IV-1) was opened, the solvent was forced into the 50 mL flask with a stream of N_2 (nitrogen source II) and thiol 109 (Part B) was dissolved in this solvent with stirring. The N_2 flow from source II was interrupted, the resin was resuspended in dichloromethane (4.0 mL) and agitated with a stream of N_2 originating from source I (upwards flow of the gas through the fritted plate). To the suspension stirred thus was introduced by syringe the solution of 109 in dichloromethane, and the 50 mL flask was washed with hexafluoroisopropanol (5 x 1 mL) and 1:1 hexafluoroisopropanol-dichloromethane (2 x 1 mL). The washings were added to the mixture by syringe and the suspension was agitated with a medium flow of N_2 (source I) until all the solvent had evaporated (48 h). The resin was then washed with

1. 3 x 2 min 10 mL CH_2Cl_2
2. 3 x 2 min 10 mL dioxane
3. 4 x 2 min 10 mL CH_2Cl_2

the filtrates were combined, analyzed (Part D), and the resin was quantitatively transferred to an 10 mL round-bottomed flask where it was dried under high vacuum overnight to afford 119 as a white solid (555 mg).

D. Quantitation:

(a) Standard 109: To a solution of 85 (recrystallized to constant mp (147.0-148.0°C), 19.0 mg, 39.79 μmol) in a 9:1 dioxane-water mixture (3 mL) was added tri-n-butylphosphine (50 μL, 200 μmol) and the mixture was diluted to the mark in a 10 mL volumetric flask with 10% aqueous dioxane. A 15.0 μL aliquot of this mixture was quantitated by HPLC to give the standard molar integration (in absorption units per mmol thiol) for 109.

HPLC: rt (D): 6.84 min, 100%, 187585 au (Avg. of two injections; ± 2%).

Molar integration at λ = 280 nm :

$$1.88 \times 10^6 \times \frac{10.0}{0.015} \times \frac{1}{39.79} = 3.125 \times 10^6 \text{ au/mmol 109}$$

(b) Filtrates: The washes of the resin (Part C) were combined, diluted to the mark in a 100 mL volumetric flask with dichloromethane, and a 20.0 μL aliquot was analyzed by HPLC under the same conditions as standard 109.

HPLC: rt (D): 6.90 min, 92%, 209300 au: 109

20.86 min, 4%, 9804 au

22.35 min, 4%, 8042 au

Total filtrates: $0.227 \times 10^6 \times \frac{100.0}{0.020} \times \frac{1}{3.125 \times 10^6} \times 1000 = 363$ μmol

(c) Resin: To a suspension of dried 119 (38.1 mg) in a 9:1 dioxane-water mixture contained in a 5 mL round-bottomed flask equipped with a flea stirring bar was added tri-n-butylphosphine (50 μL, 200 μmol). The mixture was then stirred at room temperature for 2 min and a 15.0 μL aliquot of the supernatant was analyzed by HPLC under the same conditions as the standard.

HPLC: rt (D): 5.00 min, 7%, 29109: 61

6.85 min, 93%, 412248: 109

Total resin: $0.441 \times 10^6 \times \frac{1.050}{0.015} \times \frac{1}{3.125 \times 10^6} \times \frac{555}{38.1} \times 1000 = 144$ μmol

Loading of resin: $\frac{144 \times 0.93}{555} = 0.24$ mmol 109/g resin

(d) Yields:

Total anchoring yield (based on Cys): 144/348 = 41%

Total anchoring yield (based on 109): 144/580 = 25%

Total filtrates (based on 109): 363/580 = 63%

Alternative synthesis of 119

A. [S-(6-Hydroxy-4-dibenzofuranylthio)-N^{α}-benzyloxycarbonyl-L-cysteine benzyl ester], [N^{α}-benzyloxycarbonyl-glycine benzyl ester]-resin, 121

The S-trityl-cysteine resin 117a (602 mg, 0.379 mmol Cys) was allowed to react with methoxycarbonylsulfenyl chloride according to the procedure described for 119 (Part A) and the resulting Scm-functionalized resin was treated with 4-mercapto-6-hydroxydibenzofuran, 61, (151 mg, 0.699 mmol) as described for 119 (Part C) except that a 24:10:1:1 dichloromethane-hexafluoroisopropanol-dioxane-water mixture was employed.

Complete evaporation of the solvent could not be achieved due to precipitation of 61 upon concentration of the reaction mixture to ca. one-quarter of the original volume. The suspension was agitated with a medium flow of N_2 for 48 h, the resin was filtered, washed according to the protocol described for 119 and dried under high vacuum to afford 121 as a tan solid (555 mg).

B. Acylation of 121

To a solution of N^{α}-tert-butyloxycarbonyl-L-alanine (703 mg, 3.71 mmol) in dichloromethane (6.0 mL) was added at 0°C under N_2 dicyclohexylcarbodiimide (370 mg, 1.80 mmol) and the mixture was stirred under these conditions for 45 min. The DCU that formed was collected by filtration, washed with ice-cold dichloromethane (2 x 1.2 mL) and dried (375 mg, 93%). The filtrates were combined, cooled to 0°C, diisopropylethylamine (0.20 mL, 1.15 mmol) was added and the resulting yellow clear mixture was used immediately in the acylation step.

Incorporation of N^{α}-Boc-L-alanine on resin 121 (495 mg) involved the following operations (performed in a 15 mL solid phase reaction vessel shown in Figure E-1).

1.	4 x 2 min	CH_2Cl_2 (10 mL)
2.	1 x 10 min	acylation
3.	2 x 2 min	CH_2Cl_2 (10 mL)
4.	1 x 10 min	repeat step 2 with freshly prepared acylating agent
5.	3 x 2 min	CH_2Cl_2 (10 mL)
6.	3 x 2 min	dioxane (10 mL)
7.	6 x 2 min	CH_2Cl_2 (10 mL)

The final product was collected and dried under high vacuum to afford 119 as a light-brown solid (571 mg). Quantitation of this resin was performed as described for 119 (Part D) and showed that it contained 61 (3%), 109 (78%) 4-mercapto-6-methoxydibenzofuran (impurity in 61: 13%), other (6%). The anchoring yield (117a to 119) was estimated by comparing the total integration of the PBu_3 cleavage products with that of standard 109 and was found to be 48%.

S-[(6-N^{α}-tert-butyloxycarbonylglycyl-L-phenylalanyl-L-alaninyloxy)-4-dibenzofuranylthio]-cysteine-resin, 123

Method A: Synthesis of the acylating agent: The HOSu ester of N^{α}-Boc-glycyl-L-phenylalanine was prepared as follows: To a solution of N-hydroxysuccinimide (217 mg, 1.88 mmol) in tetrahydrofuran (5.5 mL) was added N^{α}-Boc-glycyl-L-phenylalanine and the clear mixture was stirred under these conditions for 5 min. Dicyclohexylcarbodiimide (388 mg, 1.88 mmol) was then added, the mixture was stirred at 0°C for 5 min and then at room temperature for 30 min. The DCU that formed was collected by filtration, washed with ice-cold tetrahydrofuran (2 x 1 mL) and the solid residue was dried (381 mg, 90%). The filtrates were combined, evaporated to dryness, the resulting white foam was redissolved in dichloromethane (9.5 mL), treated with diisopropylethylamine (0.50 mL, 2.86 mmol) and the solution was used immediately in the acylation step (#5).

Solid phase synthesis: Resin 119 (133 mg, 24.3 μmol Ala) was prepared as previously described and analyzed by the following general method (consistently used for quantitation of disulfide resins).

To a suspension of cysteine resin (2-5 mg) in a 9:1 dioxane-water

mixture (0.1-0.2 mL) contained in a 5 mL round bottomed flask equipped with a flea stirring bar was added tri-n-butylphosphine (25 μL, 100 μmol) and the mixture was stirred at room temperature under nitrogen for 3 min. An aliquot of the supernatant was then injected in the HPLC, the recorded absorbances were integrated, and the relative abundance of each eluting compound was calculated by dividing the area of the given peak by the total. The identity of 61, 64 and 109 in the reaction mixtures was established by coinjection with authentic compounds.

For 119: HPLC (D): rt 5.03 min, 24%: 61.
6.91 min, 76%: 109.

A portion of the resin (537 mg) contained in the reaction vessel shown in Figure E-1 was acetylated according to the following protocol:

1. 3 x 2 min CH_2Cl_2 (10 mL)
2. 1 x 20 min 1.3 M Ac_2O in CH_2Cl_2 (12.5 mL)
3. 1 x 25 min addition of DMAP (20 mg, 0.163 mmol)
4. 10 x 2 min CH_2Cl_2 (10 mL).

Quantitation:

HPLC (D): rt 5.55 min, 23%: 4-mercapto-6-acetoxydibenzofuran, 64
6.85 min, 77%: 109.

Incorporation of N^{α}-Boc-glycyl-L-phenylalanine HOSu ester on the acetylated resin involved the following operations:

1. 2 x 2 min CH_2Cl_2 (10 mL)
2. 1 x 5 min 50% TFA-CH_2Cl_2 (10 mL)
3. 1 x 30 min 50% TFA-CH_2Cl_2 (10 mL)
4. 5 x 2 min CH_2Cl_2 (10 mL)
5. 1 x 5 min acylation: 0° to 25°C
6. 5 x 2 min CH_2Cl_2 (10 mL)

7. 3 x 2 min dioxane (10 mL)

8. 5 x 2 min CH_2Cl_2 (10 mL)

A postive ninhydrin test[135] was obtained after step 4, whereas the completion of the acylation was indicated by a negative test noted 3 min after the initiation of step 5.

Quantitation:

HPLC (O): rt 5.66 min, 5%: 61

6.49 min, 21%: 64

9.57 min, 74%: BocGlyPheO-Dbf-SH

HPLC (P): rt 6.51 min, 5%: 61

7.78 min, 20%: 64

13.53 min, 75%: BocGlyPheO-Dbf-SH

Method B: Synthesis of the acylating agents: To a solution of N^{α}-Boc-L-phenylalanine (796 mg, 3.00 mmol) in dichloromethane (5.0 mL) was added at 0°C dicyclohexylcarbodiimide (300 mg, 1.46 mmol) and the mixture was stirred at 0°C for 45 min. The DCU that formed was collected by filtration, washed with ice-cold dichloromethane (2 x 2.0 mL) and dried (302 mg, 92%). The filtrates were combined, cooled to 0°C, diisopropylethylamine was added (0.50 mL, 2.86 mmol) and the solution was used immediately in the acylation step.

The symmetrical anhydride of N^{α}-Boc-glycine was prepared in a similar fashion except that 5.00 mmol of the amino acid and 2.43 mmol dicyclohexylcarbodiimide in the same volume of solvent were reacted to afford DCU in 93% yield. The filtrates were collected, combined, cooled to 0°C and disopropylethylamine was added as described above.

Solid phase synthesis: Resin 119 (105 mg, 25.2 μmol Ala) was

acetylated as in Method A except that steps 2 and 3 were substituted with:

2A : 1 x 10 min 0.76 M Ac_2O in CH_2Cl_2 (9.2 mL)

3A : 1 x 5 min addition of DIEA (0.20 mL, 1.15 mmol)

Quantitation:

HPLC (P): 6.54, 1%: 61

7.78, 3%: 64

11.74, 94%: 109

Incorporation of the N^{α}-Boc amino acids on the acetylated resin involved the following operations:

1. 3 x 2 min CH_2Cl_2 (10 mL)
2. 1 x 5 min 50% TFA-CH_2Cl_2 (10 mL)
3. 2 x 15 min 50% TFA-CH_2Cl_2 (10 mL)
4. 5 x 2 min CH_2Cl_2 (10 mL)
5. 1 x 10 min acylation: 0° to 25°C
6. 3 x 2 min CH_2Cl_2 (10 mL)
7. 3 x 2 min dioxane (10 mL)
8. 4 x 2 min CH_2Cl_2 (10 mL)

Positive ninhydrin tests were obtained after step 4 and negative (within 3 min) after step 5.

Quantitation:

Amino acid added	Product distribution (HPLC, system P)
N^{α}-Boc-L-PheOH	7.75 min, 3%: 64
	16.27 min, 97%: BocPheAlaO-DBf-SH
N^{α}-Boc-GlyOH	6.52 min, 2%: 61
	7.77 min, 2%: 64
	13.51 min, 96%: BocGlyPheAlaO-Dbf-SH

S-[6-(N^{α}-9-Fluorenylmethyloxycarbonyl-L-methionyl-glycyl-L-phenylalanyl-L-alaninyloxy)-4-dibenzofuranylthio]-cysteine-resin, 126

Resin 123 (Method A) was acetylated according to the procedure described for 119 (Method A) and was analyzed:

HPLC (P): rt 6.45 min, 2%: 61
7.68 min, 27%: 64
13.33 min, 71%: BocGlyPheAlaO-Dbf-SH

The symmetrical anhydride of N^{α}-9-fluorenylmethyloxycarbonyl-L-methionine was prepared as described for N^{α}-Boc-L-PheOH, diisopropylethylamine (0.50 mL, 2.86 mmol) was added at 0°C and incorporation of the amino acid on the resin employed the protocol used for the synthesis of 123 (Method A). A positive ninhydrin test was obtained after step 4 and the completion of the acylation was indicated by a negative test 3 min after the initiation of step 5. The final product was dried under high vacuum overnight to afford 126 as a white solid (142 mg).

Quantitation:

HPLC (P): rt 6.45 min, 1%: 61
7.68 min, 24%: 64
44.91 min, 75%: FmocMetGlyPheAlaO-Dbf-SH, 128

HPLC (N): rt 4.35 min, 1%: 61
4.61 min, 21%: 64
7.60 min, 77%: 128
12.50 min, 1% other

Methyl S[6-(N^{α}-9-fluorenylmethyloxycarbonyl-L-methionyl-glycyl-L-phenylalanyl-L-alaninyloxy)-4-dibenzofuranylthio]-N^{α}-tert-butyloxycarbonyl-L-cysteine, 129

Resin 126 (73 mg) contained in the Schlenk tube of the no-air apparatus (Figure IV-1) was suspended in a 9:1 dioxane:water mixture (3.0 mL) and agitated with a medium stream of N_2 originating from source I. The mixture was treated with tri-n-butylphosphine (80 µL, 0.321 mmol), was stirred for 2 min and the supernatant was forced into the 50 mL round bottomed flask with a stream of N_2 (source II). The resulting off-white resin was washed with dioxane (3 x 2 min, 10 mL each), and the combined filtrates were evaporated to dryness under high vacuum with the aid of a warm water bath placed underneath the 50 mL flask. To the moist solid residue anhydrous diethyl ether (7 mL) was added and the resulting suspension was vigorously stirred for 1 min. The solvent was evaporated, the process was repeated once and the final residue was treated with anhydrous Et_2O (7.0 mL). The mixture was cooled to 0°C, then vigorously stirred for 2 min and the supernatant was removed under a blanket of N_2 (source I) with a Pasteur pipette possessing a fine tip. Trituration with Et_2O in the fashion described above was repeated twice and the final product was dried under high vacuum (2 min).

The resulting white powder was dissolved in hexafluoroisopropanol (0.50 mL) at room temperature under N_2, a solution of methyl S-Scm, N^{α}-Boc-L-cysteinate, 39, (13 mg, 40 µmol) in the same solvent (0.50 mL) was added, the walls of the flask were washed with hexafluoroisopropanol (4 x 0.50 mL) and the clear mixture was stirred at room temperature under N_2 for 1.6 h. The solution was then lyophilized and to the moist solid

residue anhydrous diethyl ether (10 mL) was added. The resulting suspension was cooled to 0°C, stirred for 5 min and filtered. The collected residue was then washed with ice-cold Et_2O (2x) and dried under high vacuum to afford 129 as a light-yellow powder (4.3 mg) mp 163.5-166°C (dec).

HPLC: rt 8.72, 92% (N). ^1H NMR (270 MHz, CD_3OD): Figure IV-4, p. 108. Field desorption mass spectrum: m/e 1078 (M^+).

Amino acid analysis: Met, 1.02 (1); Gly, 1.08 (1); Phe, 0.99 (1); Ala, 0.98 (1).

S-[6-(N^{α}-Z-Ile-Glu(OBzl)-Ala-Leu-Asp(OBzl)-Lys(2-Cl-Z)-Tyr(2,6-Cl_2-Bzl)-alaninyloxy)-4-dibenzofuranylthio]-cysteine-resin, 131

Synthesis of the acylating agents: N^{α}-Boc-L-Tyr(2,6-Cl_2-Bzl)OH, N^{α}-Boc-L-(2-Cl-Z)OH, N^{α}-Boc-L-Asp(OBzl)OH, N^{α}-Boc-L-AlaOH, N^{α}-Boc-L-Glu(OBzl)OH and N^{α}-Z-L-IleOH were dried under high vacuum for at least 5 h prior to use; N^{α}-Boc-L-LeuOH·H_2O was azeotroped with anhydrous acetonitrile (3x) prior to drying. All of the above amino acid derivatives (except for Ile) were purchased from Chemalog and were not checked for purity.[186,187]

To a solution of the protected amino acid (5.00 mmol) in dichloromethane (8.0 mL) was added at 0°C dicyclohexycarbodiimide (2.43 mmol) and the mixture was stirred at 0°C for 45 min. The resulting white suspension was then filtered, the DCU collected was washed with ice-cold dichloromethane (1.0 mL), the combined filtrates were cooled to 0°C, diisopropyethylamine (0.50 mL, 2.86 mmol) was added and the solution was

used immediately in the acylation step.

Solid phase synthesis: Incorporation of the amino acids onto resin 119 (prepared and acetylated as previously described; 1.00 g, 0.25 mmol Ala)[161] employed symmetrical anhydrides and was performed according to the following protocol:

1. 3 x 2 min CH_2Cl_2 (10 mL)
2. 1 x 5 min 50% TFA-CH_2Cl_2 (10 mL)
3. 2 x 15 min 50% TFA-CH_2Cl_2
4. 5 x 2 min CH_2Cl_2 (10 mL)
5. 1 x 10 min acylation: 0° to 25°C
6. 3 x 2 min CH_2Cl_2 (10 mL)
7. 3 x 2 min dioxane (10 mL)
8. 4 x 2 min CH_2Cl_2 (10 mL)

The synthesis was qualitatively monitored by the ninhydrin test: positive tests were obtained after step 4, whereas the completion of the acylation was indicated in all cases by negative tests 5 min after the initiation of the reaction. The nature of the peptide-template-thiols was quantitatively established after each cycle by the method described on p. 181 except that the solvent employed for the PBu_3 reductions was a 1:1 mixture of dichloromethane and hexafluoroisopropanol.

	Amino acid added	Major products (1-4:(N), 5-8:(Q)
1.	None	4.70 min, 7%: 64 5.15 min, 89%:109
2.	Boc-L-Tyr(2,6-Cl_2-Bzl)OH	4.74 min, 14%:64 8.13 min, 84%
3.	Boc-L-Lys(2-Cl-Z)OH	4.70 min, 10%:64 10.75 min, 88%
4.	Boc-L-Asp(OBzl)OH	4.67 min, 10%:64 10.75 min, 88%

5.	Boc-L-LeuOH	4.38 min, 11%: 64 10.82 min, 88%
6.	Boc-L-AlaOH	4.38 min, 11%: 64 9.88 min, 86%
7.	Boc-L-Glu(OBzl)OH	4.38 min, 11%: 64 12.78 min, 84%
8.	Z-L-IleOH	4.35 min, 22%: 64 15.61 min, 73%: 130

The apparent insolubility of the analytical sample in the aqueous methanolic HPLC mobile phase prompted a repeat quantitation of the same sample with aqueous isopropanol as the HPLC eluent (HPLC system R):

9.24 min, 14%: 64

12.18 min, 84%: 130

The final resin was dried under high vacuum overnight to afford 131 as a light-yellow solid (1.120 g).

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).

Ethyl S-[6-(N^{α}-Z-Ile-Glu(OBzl)-Ala-Leu-Asp(OBzl)-Lys(2-Cl-Z)-Tyr(2,6-Cl_2-Bzl)-alaninyloxy)-4-dibenzofuranylthio]-N^{α}-tert-butyoxycarbonyl-L-cysteinylglycinate, 132

A portion of resin 131 (45 mg) contained in a 15 mL solid phase reaction vessel equipped with a teflon coated screw-cap was treated with:

1. 3 x 2 min CH_2Cl_2 (10 mL)
2. 1 x 15 min 1:1 CH_2Cl_2-HFIP (8 mL) containing PBu_3 (60 µL)
3. 1 x 2 min CH_2Cl_2 (4 mL)
4. 1 x 2 min HFIP (4 mL)

The filtrates of operations 2 through 4 were combined under N_2 and

lyophilized. The white solid residue was then triturated at 0°C with anhydrous diethyl ether (6 mL) and the resulting suspension was filtered under N_2 through a glass fritted funnel. The white powder was collected, washed with ice-cold Et_2O (2 x 1 mL), quickly transferred to a 5 mL round bottomed flask and dissolved under N_2 in hexafluoroisopropanol (0.40 mL).

HPLC (Q): rt 15.11 min, 97%: 130

This clear mixture was treated with S-(Scm), N^{α}-Boc-L-cysteinylglycyl ethyl ester[161] (50 mg, 0.126 mmol) in hexafluoroisopropanol (0.40 mL). The solution was stirred at room temperature under N_2 for 1.4 h, water (0.05 mL) was added and the clear mixture was stirred under the same conditions for 2 h.

The mixture was then lyophilized and the moist solid residue was triturated with anhydrous Et_2O to afford a white powder (5.6 mg).

HPLC (Q): 14.15 min, 94%: 132

24.82 min, 6%.

A portion of this material (1.7 mg) was purified by HPLC (Whatman Partisil 5/25 ODS-3 reverse phase column; eluent: 92% MeOH-1% HOAc) to afford a white solid product (1.2 mg).

HPLC (Q): 14.95 min, 99%: 132.

Amino acid analysis: Ala, 2.17 (2); Tyr, 1.05 (1); Lys, 1.02 (1); Asp, 1.12 (1); Leu, 1.26 (1); Glu, 0.95 (1), Ile, 0.91 (1), Gly, 1.58.

N^{α}-9-Fluorenylmethyloxycarbonyl-L-isoleucine p-benzyloxybenzyl ester resin, 141

p-Benzyloxybenzyl alcohol resin[26] (2.20 g, 2.16 mmol) contained in a 40 mL solid phase reaction vessel was washed with dichloromethane (3 x

2 min; 30 mL each) and suspended in a 4:1 dichloromethane-dimethylformamide mixture (30 mL). N^{α}-9-fluorenylmethyloxycarbonyl-L-isoleucine (1.64 g, 4.65 mmol) was added and the mixture was cooled to 5°C (cold-room). Dicyclohexylcarbodiimide (0.95 g, 4.61 mmol) and dimethylaminopyridine (550 mg, 4.50 mmol) were added and the suspension was shaken at 5°C for 45 min and at room temperature for 5 h. The mixture was then cooled to 5°C, treated with benzoyl chloride (1.00 mL, 8.61 mmol) and pyridine (0.95 mL, 11.75 mmol), was shaken at 5°C for 30 min then at room temperature for 1 h. It was then filtered and washed with:

1. 2 x 2 min CH_2Cl_2 (25 mL)
2. 2 x 2 min DMF (25 mL)
3. 2 x 2 min i-PrOH (25 mL)

The washing sequence was repeated twice, the final product was washed with CH_2Cl_2 (4 x 2 min; 25 mL each) and dried under high vacuum over P_2O_5 overnight to afford a tan solid (2.428 g). Quantitation according to Meienhofer's procedure[163] gave:

At 267 nm: 0.375 mmol Ile/g resin

301 nm: 0.385 mmol Ile/g resin

Average: 0.38 mmol Ile/g resin

$$\text{Anchoring yield} = \frac{0.38 \times 2.43}{2.16} = 43\%$$

S-Trityl, N^{α}-tert-butyloxycarbonyl-L-cysteinyl-L-asparaginyl-L-alanyl-L-valinyl-L-valinyl-glycyl-L-phenylalanyl-L-isoleucine p-benzyloxybenzyl ester resin, 145

Synthesis of acylating agents: N^{α}-Bpoc-L-PheOH DCHA salt, N^{α}-Fmoc-GlyOH, N^{α}-Fmoc-L-ValOH, N^{α}-Fmoc-L-AlaOH, N^{α}-Fmoc-L-AsnOH and S-trityl,

N^{α}-Boc-L-CysOH were dried under high vacuum for at least 5 h prior to use. All of the above amino acid derivatives were purchased from Chemalog and were not checked for purity.[186,187]

Symmetrical anhydrides of protected Gly, Val, Ala and Cys were freshly prepared from the appropriate amino acid derivatives (4.17 mmol) and dicyclohexylcarbodiimide (1.94 mmol) in a 3:1 dichloromethane-dimethylformamide mixture (6.0 mL) at 0°C (45 min); N^{α}-Bpoc-L-PheOH was liberated from its salt according to Merrifield's procedure[27] and allowed to react with dicyclohexylcarbodiimide in dichloromethane using the same amounts of reagents as above; Fmoc-L-AsnOH was incorporated on the resin by DCC/ HOBt coupling[134] (2.11 mmol each) in a 1:1 dichloromethane-dimethylformamide mixture (10 mL).

Solid phase synthesis: Resin 141 (1.00 g, 0.38 mmol Ile) was contained in a 15 mL solid phase vessel and reacted with the anhydride of BpocPheOH as shown in Protocol I:[163]

Protocol I:

1.	3 x 2 min	CH_2Cl_2 (10 mL)
2.	1 x 5 min	freshly prepared 50% piperidine-CH_2Cl_2 (10 mL)
3.	2 x 25 min	50% piperidine-CH_2Cl_2 (10 mL)
4.	2 x 2 min	CH_2Cl_2 (10 mL)
5.	2 x 2 min	DMF (10 mL)
6.	2 x 5 min	2:1 dioxane-water (10 mL)
7.	3 x 2 min	DMF (10 mL)
8.	3 x 2 min	CH_2Cl_2 (10 mL)
9.	1 x 15 min	acylation
10.	1 x 15 min	addition of 0.5 M DIEA in CH_2Cl_2 (1.0 mL)
11.	1 x 2 min	CH_2Cl_2 (10 mL)

12. 1 x 2 min DMF (10 mL)

13. 1 x 2 min i-PrOH (10 mL)

14. Repeat steps 11 through 13

15. Repeat steps 11 through 13

16. 3 x 2 min CH_2Cl_2 (10 mL)

A positive ninhydrin test was obtained after step 8 whereas the completion of the acylation was indicated by a negative test performed 10 min after the initiation of step 9.

The resin obtained thus was treated according to Protocol II:[164]

Protocol II

1. 3 x 2 min CH_2Cl_2 (10 mL)

2. 1 x 5 min 1.5% TFA and 1.0% mercaptoethanol in CH_2Cl_2 (10 mL)

3. 1 x 25 min 1.5% TFA and 1.0% mercaptoethanol in CH_2Cl_2 (10 mL)

4. 5 x 2 min CH_2Cl_2 (10 mL)

5. 1 x 40 min acylation with anhydride of FmocGlyOH plus DIEA (0.50 mL)

A positive ninhydrin test was obtained at this point. The mixture was filtered, a solution of FmocGlyOH (297 mg, 1.00 mmol) in a 3:1 CH_2Cl_2-DMF mixture was added and the suspension was then treated wtih dicyclohexylcarbodiimide (200 mg, 0.97 mmol):

6. 1 x 15 min acylation: (-) ninhydrin test at 15 min

7. 1 x 2 min CH_2Cl_2 (10 mL)

8. 1 x 2 min DMF (10 mL)

9. 1 x 2 min i-PrOH (10 mL)

10. Repeat steps 7 through 9

11. Repeat steps 7 through 9

12. 4 x 2 min CH_2Cl_2 (10 mL)

The resin obtained thus was acylated with the remaining activated amino acid derivatives according to Protocol I except that for FmocAsnOH steps 9 and 10 were substituted with 1 x 2.2 h.

The homogeneity of the peptide prior to incorporation of the cysteine derivative was tested by treating a small portion of the resin (20 mg) with 55% TFA in CH_2Cl_2 (1 x 50 min), washing with CH_2Cl_2 and 55% TFA in CH_2Cl_2, evaporating the combined filtrates and HPLC analysis of the white solid residue (4.5 mg).

HPLC: rt 6.20 min, 100% (S)

For 145: Amino acid analysis: Ile, 1.13 (1); Phe, 0.97 (1); Gly, 0.99 (1); Val, 2.13 (2); Ala, 1.04 (1); Asp, 0.84 (1).[188]

Trifluoroacetic acid salt of S-(methoxycarbonylsulfenyl)-L-cysteinyl-L-asparaginyl-L-alanyl-L-valinyl-L-valinyl-glycyl-L-phenylalanyl-L-isoleucine, 139

Resin 145 (205 mg) contained in an oven-dried 10 mL round bottomed flask equipped with a flea stirring bar was suspended in dichloromethane (4.0 mL) then sealed with a rubber septum under positive pressure of N_2 (balloon) and cooled to -13°C (ethylene glycol-water thermostated bath). To the mixture was added in one portion methoxycarbonylsulfenyl chloride (freshly prepared;[118] 0.40 mL, 4.07 mmol) followed by methanol (0.40 mL) and the suspension was allowed to warm up slowly to room temperature (2.5 h). The reaction mixture was then quantitatively transferred to a 15 mL solid phase reaction vessel where it was shaken for 5 min at room temperature. The suspension was filtered and the resin was washed with:

1. 3 x 2 min CH_2Cl_2 (10 mL)
2. 3 x 2 min 1:1 CH_2Cl_2-MeOH (10 mL)
3. 5 x 2 min CH_2Cl_2 (10 mL)

The resulting off-white solid was then treated with 55% trifluoroacetic acid-dichloromethane containing anisole (20 μL) (1 x 60 min; 10 mL) and washed with 50% TFA-CH_2Cl_2 (1 x 5 min; 10 mL) and 20% TFA-CH_2Cl_2 (2 x 5 min; 10 mL). The filtrates were combined, evaporated to dryness, the resulting light-yellow oil residue was triturated with 3:1 Et_2O-petroleum ether (8 mL) and the suspension that formed was filtered. The residue was washed with Et_2O (2 x 1 mL), petroleum ether (2 x 1 mL), again with Et_2O (2 x 1 mL), and was dried under high vacuum to afford 139 as a white powder (55 mg), mp 198°C (dec).

HPLC: rt 6.91, 99% (R). ^1H NMR (270 MHz, DMSO-d_6): Figure IV-6, p. 118.

Attempted synthesis of Z-Ile-Glu(OBzl)-Ala-Leu-Asp(OBzl)-Lys(2-Cl-Z)-Tyr(2,6-Cl_2-Bzl)-Ala-Cys(Ac)-Asn-Ala-Val-Val-Gly-Phe-IleOH, 142

Resin 131 (222 mg) contained in the fritted tube of the no-air apparatus (Figure IV-1) was suspended in a 4:3 dichloromethane-hexafluroisopropanol mixture (7.0 mL) and agitated with a medium stream of N_2 originating from source I. To the mixture was added tri-n-butylphosphine (100 μL, 0.401 mmol), the suspension was stirred for 30 min and the supernatant was forced into the 50 mL round-bottomed flask with a stream of N_2 (source II). The resulting light-yellow resin was then washed with:

1. 1 x 2 min HFIP (3 mL)
2. 1 x 2 min CH_2Cl_2 (3 mL)
3. 1 x 2 min HFIP (3 mL)
4. Repeat steps 2 and 3
5. Repeat steps 2 and 3

and the combined filtrates were evaporated to dryness under high vacuum. To the white solid residue anhydrous diethyl ether (7 mL) was added, the mixture was cooled to 0°C, vigorously stirred for 2 min and then the supernatant was removed by a syringe equipped with a 20 gauge needle under a blanket of N_2 (source I). The process was repeated twice, the final product was dried under high vacuum (5 min) and the resulting white solid residue contained in the 50 mL flask was dissolved in hexafluroisopropanol (2.0 mL) under N_2 (source I).

HPLC (R): rt 9.07 min, 2%: 64
10.90 min, 6%
12.16 min, 92%.

The solution of the thiol was then treated with 139 (37 mg, 36.1 μmol) in hexafluoroisopropanol (1.50 mL), water was added (0.30 mL) and the resulting clear mixture was stirred at room temperature under N_2 for 1.8 h. The solution was then lyophilized and dried under high vacuum overnight.

HPLc (R): rt 6.95 min, 19%
14.20 min, broad, 81%.

To the capture product contained in the 50 mL round-bottomed flask was added freshly distilled dimethylsulfoxide (2.0 mL), the resulting clear mixture was treated with diisopropylethylamine (10 μL, 57.4 μmol) and the solution was stirred at room temperature under N_2 for 30 h. The

reaction mixture was then lyophilized and the resulting white solid residue was dissolved in hexafluoroisopropanol (3.0 mL). The solution was then treated with tri-n-butylphosphine[160] (50 μL, 201 μmol) and was stirred at room temperature under N_2 for 45 min.

HPLC (R): rt 5.79 min, 27%
8.40 min, 38%: 61
12.16 min, 27%.

The solvent was then removed by lyophilization and the residue was triturated with anhydrous diethyl ether under a blanket of N_2 provided by source I. The supernatant was removed by syringe as described previously, the process was repeated twice and the final product was dried under high vacuum (5 min).

To the crude product mixture contained in the 50 mL flask and set under N_2 (source I) was added in one portion a premixed solution of acetic anhydride (0.10 mL, 1.06 mmol) and diisopropylethylamine (25 μL, 143 μmol) in 50% dichloromethane-dimethylformamide (4.0 mL). The residue dissolved completely, the resulting light-yellow clear mixture was stirred at room temperature under N_2 for 15 min and was then lyophilized. The residue was triturated with anhydrous diethyl ether, the resulting suspension was filtered, the residue was washed with Et_2O (5 x 1 mL) and dried under high vacuum to afford a tan solid (48 mg).

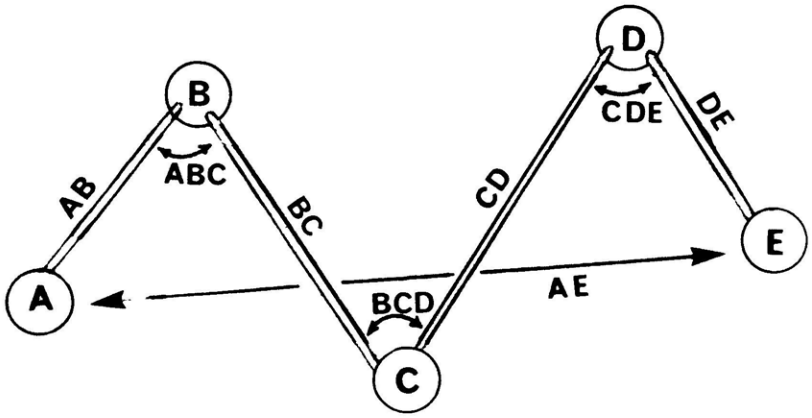
HPLC (R): rt 6.05 min, 5%
6.98 min, 34%
11.17 min, 61%

APPENDIX I

INTERATOMIC DISTANCE CALCULATION

In Chapter III we outlined the selection rules employed in our search for a rigid template that would accommodate the transition state of an intramolecular O,N-acyl transfer reaction on disulfide systems. From that discussion, it is clear that promising structural candidates should satisfy the critical requirement of fit in oxygen-sulfur interatomic distance defined by the O and S functions of the optimal transition state for the amide-forming process. In this part we describe the method used in calculating these distances.

Assuming that A,B,C,D,E are neighboring atoms possessing known bond lengths, bond angles, and dihedral angles, the distance between



atoms A and E can be calculated by the recursive program shown in Scheme AI-1. This is based on the following five-step operation:[197]

I. Law of cosines

$$AC = \sqrt{AB^2 + BC^2 - 2AB \cdot BC \cdot \cos\angle ABC}$$

II. Law of sines

$$\angle ACB = \arcsin\left[AB\left[\frac{\sin\angle ABC}{AC}\right]\right]$$

III. Inverse law of of cosines

$$\angle ADB = \arccos\left[\frac{AB^2 - AD^2 - BD^2}{2\ AD \cdot BD}\right]$$

IV. Angle from dihedral angle

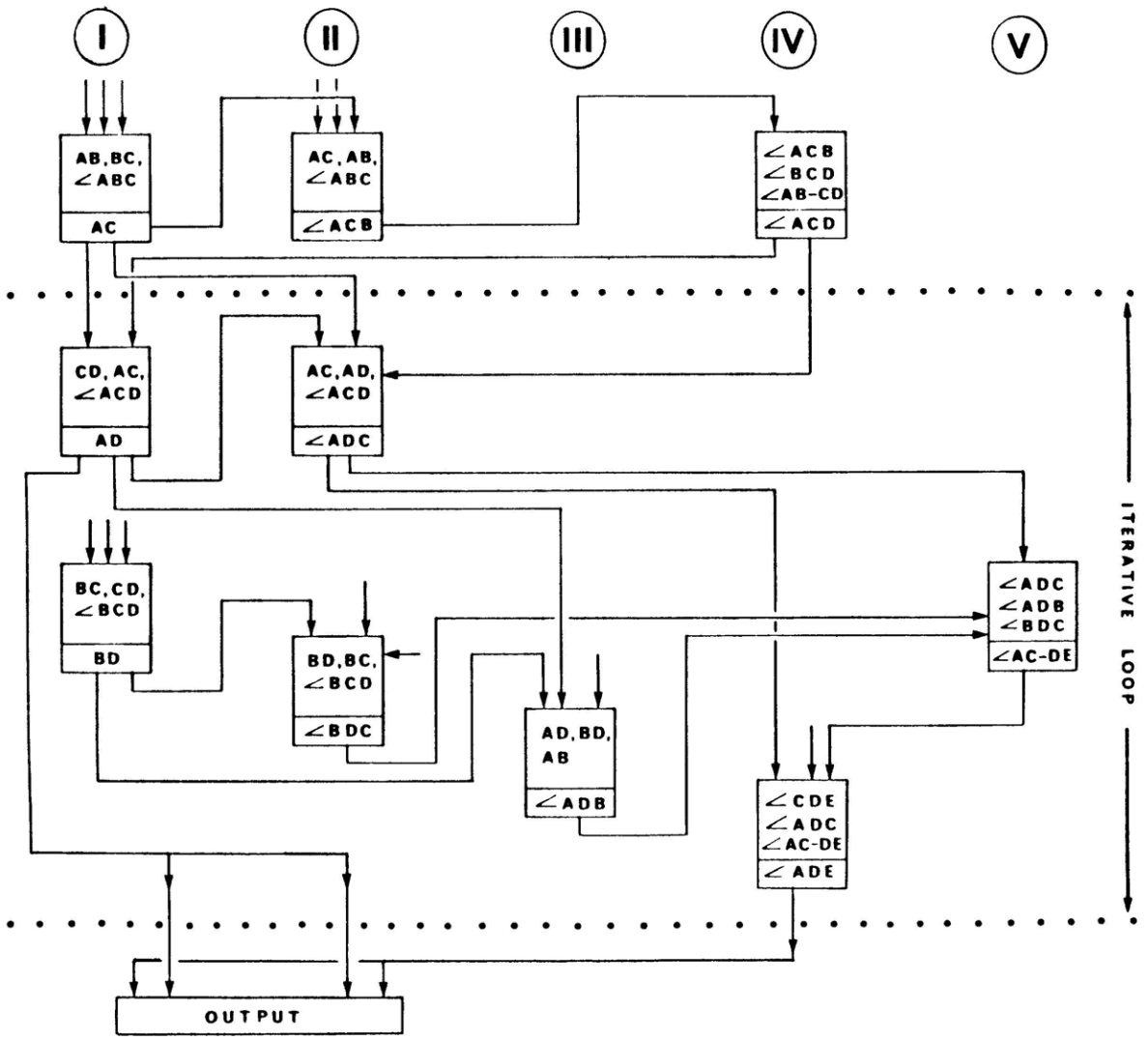
$$\angle ACD = \arccos[\cos\angle ACB \cdot \cos\angle BCD + \sin\angle ACB \cdot \sin\angle BCD \cdot \cos\angle AB,CD]$$

V. Dihedral angle from angle

$$AC,DE = \arccos\left[\frac{\cos\angle ADB - (\cos\angle ADC \cdot \cos\angle BDC)}{\sin\angle ADC \cdot \sin\angle BDC}\right]$$

Given the iterative loop incorporated in this computer program,[198] interatomic distances between A and any atom X can be calculated, provided that bond lengths, bond angles and dihedral angles between consecutive atoms are known. Thus, the OS distance across the 7-membered assemblies of 4-thio-6-hydroxydibenzofuran and the corresponding optimal acyl transfer transition state were found to be 5.24 and 5.27 Angstrom respectively.[140]

Scheme AI-1


I
II
III
IV
V
AB, BC, ∠ABC
AC
AC, AB, ∠ABC
∠ACB
∠ACB
∠BCD
∠AB-CD
∠ACD
CD, AC, ∠ACD
AD
AC, AD, ∠ACD
∠ADC
BC, CD, ∠BCD
BD
BD, BC, ∠BCD
∠BDC
AD, BD, AB
∠ADB
∠ADC
∠ADB
∠BDC
∠AC-DE
∠CDE
∠ADC
∠AC-DE
∠ADE
ITERATIVE LOOP
OUTPUT

APPENDIX II:

SYNTHESIS OF BROMO AND CHLORO DERIVATIVES OF THE DIBENZOFURANYL TEMPLATE

In the acyl transfer chapter we showed that introduction of electron-withdrawing substituents para to the acetoxy function of the dibenzofuran template enhances the rate of the amide-forming process. In this part, we describe the synthesis of these systems.

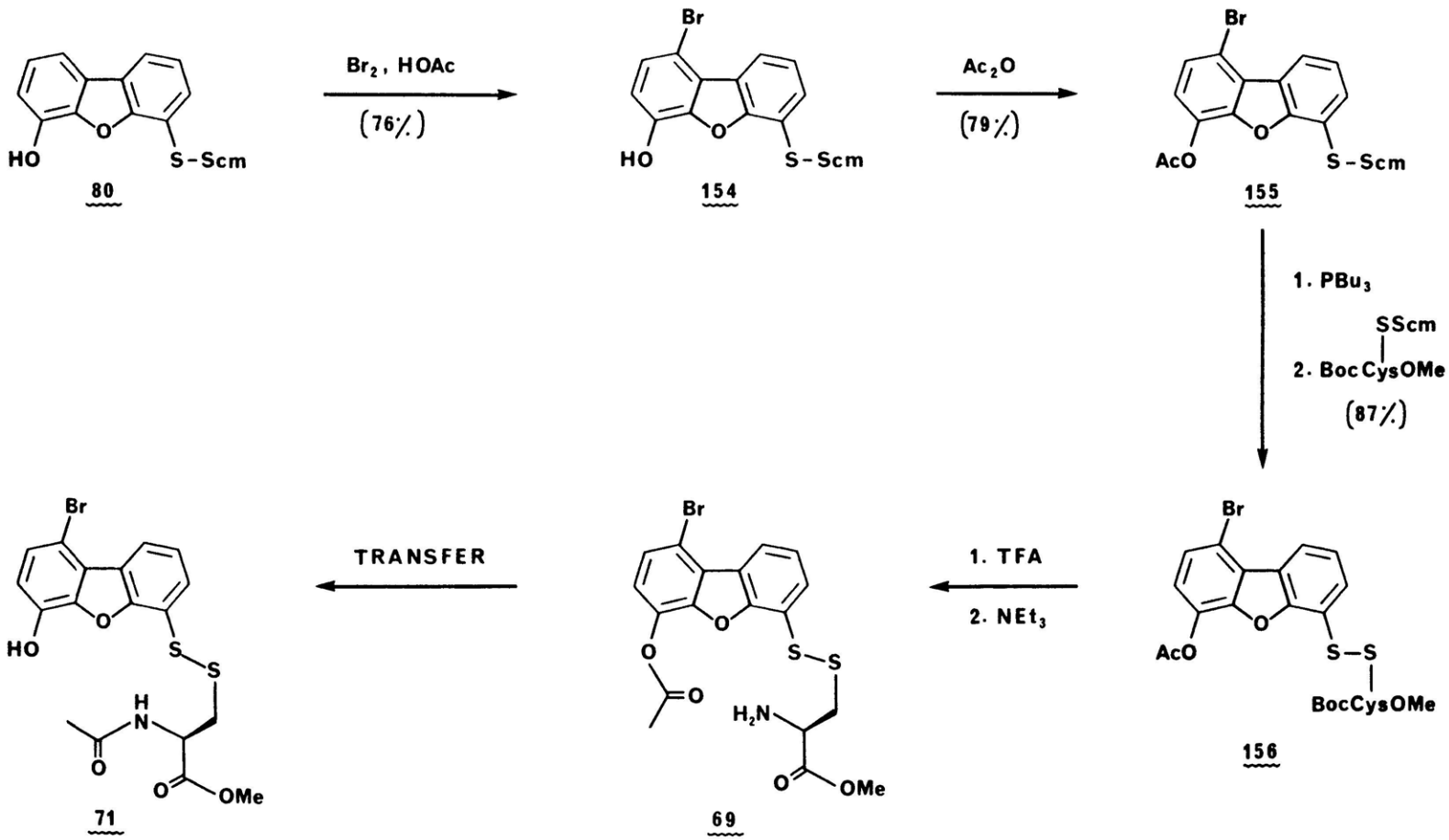
For dibenzofurans, the directing effect of a phenolic group positioned at C-4 towards electrophilic aromatic substitution reactions is much greater than that of the heterocyclic oxygen. Thus, Gilman reports that treatment of 4,6-dihydroxydibenzofuran with a solution of Br_2 in glacial acetic acid generates the 1,9-dibromo derivative in quantitative yield.[199]

In this thesis, bromination of S-blocked template 80 under Gilman's conditions afforded a mixture of para and ortho substitution products in the ratio of 76:8.[200] Structure elucidation was performed by ^{1}H-NMR and was based on the pronounced anisotropic effect exerted on C_9-H (presumably due to plane deformation induced by the spacially neighboring halogen atom). Hence, for 154, $\delta(C_9\text{-H}) = 8.41$, whereas for 80, $\delta(C_9\text{-H}) = 7.87$. The Scm-protected phenolic template 154 obtained thus was converted to the free amine 69 according to procedures established for unactivated systems, and was then allowed to undergo acyl transfer to afford N-acetyl cysteine 71 (Scheme AII-1).

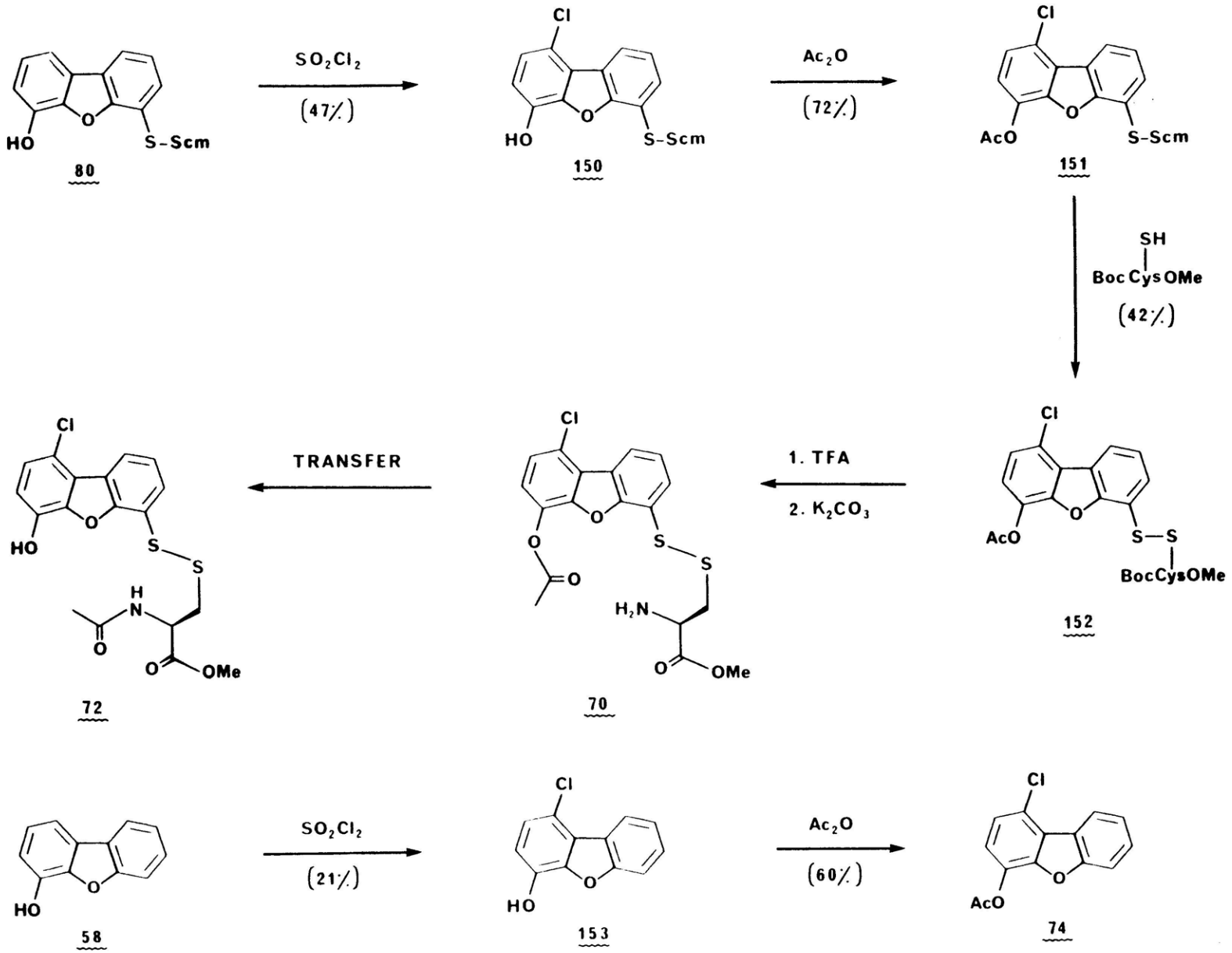
Gilman also reports that 1-chloro-6-hydroxydibenzofuran, 153, can be generated by treatment of phenol 58 with SO_2Cl_2 under conditions favoring radical reactions.[193] We applied this approach to the synthesis of acyl transfer assembly 70 (Scheme AII-2). Thus, reaction of S-Scm template 80

with SO_2Cl_2 afforded mono-chloro dibenzofuran 150 in 47% yield, which was carried through four well-precedented steps to generate 70.[140] The position of the chloro substituent was established by ^{1}H-NMR as described for the bromo analog, and the rate constants measured for the acyl transfer reaction (70 to 72) are reported in Table III-2, Chapter III.

Scheme AII-1


Br
HO
S-Scm
80
Br_2, HOAc
(76%)
154
Ac_2O
(79%)
AcO
155
1. PBu_3
SScm
2. BocCysOMe
(87%)
156
1. TFA
2. NEt_3
69
TRANSFER
71
OMe
BocCysOMe

Scheme AII-2


SO2Cl2
(47%)
Ac2O
(72%)
SH
BocCysOMe
(42%)
1. TFA
2. K2CO3
TRANSFER
SO2Cl2
(21%)
Ac2O
(60%)
80
150
151
152
70
72
58
153
74

EXPERIMENTAL SECTION

General Methods: Same as described in Part A of Experimental Section.

1-Bromo-4-hydroxy-6-(methoxycarbonyldithio)-dibenzofuran, 154

To a solution of 80 (34.4 mg, 0.112 mmol) in glacial acetic acid (1.00 mL) was added dropwise (2 min) a 0.50 M solution of bromine in acetic acid (224 μL, 0.112 mmol) and the mixture was stirred at room temperature for 30 min. The solution was then poured into water, the product was extracted with $CHCl_3$ (3x), the organic phases were combined, back-washed with water, washed with brine, dried ($MgSO_4$), and evaporated to dryness. The resulting light-yellow viscous oil was purified by preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford pure 154 as a white foam (32.6 mg, 76%).[183]

TLC: R_f 0.40 (A). ^1H NMR (270 MHz, $CDCl_3$): δ 3.92 (3H, s, Ar:Scm), 6.65 (1H, bs, Ar:OH), 6.93 (1H, d; J = 8.3 Hz, C_3-H), 7.31 (1H, d; J = 8.8 Hz, C_2-H), 7.34 (1H, t; J = 7.8 Hz, C_8-H), 7.75 (1H, d; J = 7.8 Hz, C_7-H), 8.41 (1H, d; J = 7.8 Hz, C_9-H). High resolution mass spectrum: Calcd for $C_{14}H_9O_4S_2{}^{79}Br$: 383.9126; found: 383.9122. Calcd for $C_{14}H_9O_4S_2{}^{81}Br$: 385.9105; found: 385.9124.

1-Bromo-4-acetoxy-6-(methoxycarbonyldithio)-dibenzofuran, 155

The hydroxy derivative 154 was acetylated according to the procedure described for 35 to afford a quantitative yield of crude product. Trituration with petroleum ether afforded pure 155 as a fluffy white solid (79% overall), mp 136-138°C.

TLC: R_f 0.66 (A). ^1H NMR (270 MHz, $CDCl_3$): δ 2.50 (3H, s, Ar-OAc), 3.91 (3H, s, Ar:Scm), 7.15 (1H, d; J = 8.8 Hz, C_2-H), 7.41 (1H, t; J = 7.8 Hz, C_8-H), 7.50 (1H, d; J = 8.3 Hz, C_3-H), 7.80 (1H, d; J = 7.8 Hz, C_7-H), 8.49 (1H, d; J = 7.8 Hz, C_9-H). High resolution mass spectrum: Calcd for $C_{16}H_{11}O_5S_2{}^{79}Br$: 425.9231; found: 425.9245. Calcd for $C_{16}H_{11}O_5S_2{}^{81}Br$: 427.9211; found: 427.9224.

Methyl N^{α}-tert-butyloxycarbonyl-S-(1-bromo-4-acetoxy-6-dibenzofuranylthio)-L-cysteinate, 156

Prepared from 155 and 39 according to the procedure described for 47 (p. 149). The crude material was purified by preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford pure 156 as an oil in 87% overall yield.

TLC: R_f 0.47 (A). ^1H NMR (270 MHz, $CDCl_3$): δ 1.43 (9H, s, Cys:-Boc), 2.48 (3H, s, Ar:OAc), 3.27-2.45 (2H, m, Cys: methylene), 3.75 (3H, s, Cys:OMe), 4.62-4.72 (1H, m, Cys: methine), 5.44 (1H, bd; J = 7.8 Hz, urethane), 7.14 (1H, d; J = 8.3 Hz, C_3-H), 7.42 (1H, t; J = 7.8 Hz, C_8-H), 7.50 (1H, d; J = 8.8 Hz, C_2-H), 7.76 (1H, dd; J = 7.8, 1.5 Hz, C_7-H), 8.45 (1H, dd; J = 7.8, 1.5 Hz, C_9-H). Field desorption mass spectrum: 569 (M^+: ^{79}Br), 571 (M^+: ^{81}Br).

Methyl N^{α}-acetyl-S-(1-bromo-4-hydroxy-6-dibenzofuranylthio)-L-cysteinate, 71

Cysteine derivative 156 was deprotected in 93% yield with trifluoroacetic acid, and the resulting salt was neutralized in situ with triethylamine. Free amine 69 was allowed to undergo intramolecular acyl transfer as described for 66. Purification of the crude product by preparative layer chromatography (1,000 μ; 1:1 $CHCl_3$-EtOAc) afforded pure 71 as a tan solid in 34% yield (156 to 71).

TLC: R_f 0.29 (C). IR ($CHCl_3$): ν_{max} 3920, 2924, 1746, 1650, 1400, 1244, 1187, 896 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 2.30 (3H, s, Cys:Ac), 3.05 (1H, dd; J = 15,3 Hz, Cys:methylene), 3.83 (3H, s, Cys:OMe), 3.90 (1H, dd; J = 15,3 Hz, Cys:methylene), 4.03-4.12 (1H, m, Cys:methine), 6.87 (1H, bd; J = 8 Hz, urethane), 7.00 (1H, d; J = 8,3 Hz, C_3-H), 7.34-7.46 (2H, m), 7.73 (1H, d; J = 7.8 Hz, C_7-H), 8.55 (1H, d; J = 7.8 Hz, C_9-H), 10.28 (1H, bs, Ar-OH). Field desorption mass spectrum: 469 (M^+: ^{79}Br), 471 (M^+: ^{81}Br).

Rate determination for the intramolecular acyl transfer of amine 69

The kinetics of the acyl transfer of the bromodibenzofuran template were performed as for the unactivated dibenzofuran analogs.

Solvent	Concentration (M)	$t_{1/2}$ (h)	k_{intram} (h^{-1})
DMSO-d_6	8.27×10^{-2}	0.4	1.71
DMF-d_7	5.53×10^{-2}	2.5	0.28

1-Chloro-4-hydroxy-6-(methoxycarbonyldithio)-dibenzofuran, 150

To a solution of 80 (84 mg, 0.27 mmol) in chloroform (2.00 mL) was added sulfuryl chloride (24 μL, 0.30 mmol) and the solution was stirred under N_2 first at room temperature (2 h) and then at reflux (1.5 h). The mixture was then evaporated to dryness and the oil residue purified by preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford pure 150 as a clear oil (43 mg, 47%).

TLC: R_f 0.46 (A). IR ($CDCl_3$): ν_{max} 3550 (b), 2945, 1733, 1396, 1137, 872, 679, 635. ^1H NMR (250 MHz, $CDCl_3$): δ 3.91 (3H, s, Ar:Scm), 6.71 (1H, bs, Ar:OH), 6.97 (1H, d; J = 8.4 Hz, C_3-H), 7.14 (1H, d; J = 8.4 Hz, C_2-H), 7.36 (1H, t; J = 7.7 Hz, C_8-H), 7.71 (1H, dd; J = 7.7, 1.1 Hz, C_7-H), 8.26 (1H, dd; J = 7.7, 1.1 Hz, C_9-H). Mass spectrum (70 ev) m/e (rel. intensity): 342 (M^++2, 2), 340 (M^+, 5), 248 (11), 246 (44), 189 (14), 158 (32), 126 (33), 59 (100). High resolution mass spectrum: Calcd for $C_{14}H_9O_4S_2Cl$: 339.9631; found: 339.9638.

1-Chloro-4-acetoxy-6-(methoxycarbonyldithio)-dibenzofuran, 151

The hydroxy derivative 150 was acetylated according to the procedure described for 35 to afford 92% of crude product. Trituration with ice-cold CH_3CN afforded pure 151 as a white solid (72% overall), mp 124.0-126.5°C.

TLC: R_f 0.69 (A). IR ($CDCl_3$): ν_{max} 2958, 1679, 1732, 1177, 1140, 855 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.50 (3H, s, Ar:OAc), 3.91 (3H, s, Ar:Scm), 7.19 (1H, d, J = 8.5 Hz, C_3-H), 7.33 (1H, d; J = 8.5 Hz, C_2-H), 7.39 (1H, t; J = 7.7 Hz, C_8-H), 7.80 (1H, dd; J = 7.7, 1.1 Hz, C_7-H),

8.35 (1H, dd; J = 7.7, 1.1 Hz, C_9-H). Mass spectrum (70 ev) m/e (rel. intensity): 384 (M^++2, 0.3), 382 (M^+, 0.5), 342 (2), 340 (4), 298 (2), 296 (5), 249 (17), 232 (10), 59 (26), 43 (100). High resolution mass spectrum (70 eV) m/e (rel. intensity): Calcd for $C_{16}H_{11}O_5S_2Cl$: 381.9736; found: 381.9712.

Methyl N^{α}-tert-butyloxycarbonyl-S-(1-chloro-4-acetoxy-6-dibenzofuranylthio)-L-cysteinate, 152

Prepared from 151 and 36 according to the procedure described for 40. The crude product was purified by preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) to afford pure 152 as a pale oil in 42% yield.

TLC: R_f 0.56 (A). IR ($CDCl_3$): ν_{max} 2978, 1740, 1710, 1489, 1278, 877, 684, 639 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.43 (9H, s, Cys:Boc), 2.50 (3H, s, Ar:OAc), 3.32-3.46 (2H, m, Cys:methylene), 3.77 (3H, s, Cys:OMe), 5.62-4.73 (1H, m, Cys:methine), 5.45 (1H, bd; J = 8 Hz, Cys:urethane), 7.20 (1H, d; J = 8.5 Hz, C_3-H), 7.33 (1H, d; J = 8.5 Hz, C_2-H), 7.34 (1H, t; J = 7.7 Hz, C_8-H), 7.77 (1H, dd; J = 7.7, 1.1 Hz, C_7-H), 8.34 (1H, dd; J = 7.7, 1.1 Hz, C_9-H). Mass spectrum (70 eV) m/e (rel. intensity): 526 (M^+, none), 469 (0.1), 252 (6), 250 (16), 178 (9), 146 (11), 102 (15), 57 (100). High resolution mass spectrum: Calcd for $C_{23}H_{24}O_7NS_2Cl$: 525.0683; found: 525.0663.

Methyl N^{α}-acetyl-S-(1-chloro-4-hydroxy-6-dibenzofuranylthio)-L-cysteinate, 72

The N^{α}-Boc protected cysteine 152 was converted to the free amine 70 according to the procedure described for 66 which in turn was allowed to undergo intramolecular acyl transfer as for 67. Purification of the crude product by preparative layer chromatography (1,000 μ; eluent 1:1 $CHCl_3$-EtOAc) afforded pure 72 as a light-brown solid in 42% yield (70 to 72) mp 103-106°C.

For 72: TLC: R_f 0.31 (C). IR ($CHCl_3$): ν_{max} 3920, 3005, 1738, 1645, 1292, 710, 659 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 2.30 (3H, s, Cys:Ac), 3.05 (1H, dd; J = 15,2 Hz, Cys:methylene), 3.83 (3H, s, Cys:OMe), 3.90 (1H, dd; J = 15,2 Hz, Cys:methylene), 5.04-5.12 (1H, m, Cys:methine), 6.88 (1H, bd, urethane), 7.03 (1H, d; J = 8.5 Hz, C_3-H), 7.22 (1H, d; J = 8.5 Hz, C_2-H), 7.38 (1H, t; J = 7.7 Hz, C_8-H), 7.71 (1H, dd; J = 7.7, 1.1 Hz, C_7-H), 8.42 (1H, dd; J = 7.7, 1.1 Hz, C_9-H), 10.25 (1H, s, Ar:OH). Mass spectrum (70 eV) m/e (rel. intensity): 425 (M^+, 0.4), 281 (0.4), 259 (3), 250 (8), 176 (22), 144 (32), 84 (17), 43 (100). High resolution mass spectrum: Calcd for $C_{18}H_{16}O_5NS_2Cl$: 425.0158; found: 425.0172.

Rate determination for the intramolecular acyl transfer reaction of 70

The kinetics of the acyl transfer of the chlorodibenzofuran derivative 70 were performed as described for the bromo analog, except that the free amine of cysteine was isolated prior to acyl transfer instead of

being generated in situ during the reaction.

Solvent	Concentration (M)	$t_{1/2}$ (h)	k_{intram} (h^{-1})
DMSO-d_6	5.27×10^{-2}	0.17	4.1

1-Chloro-4-hydroxydibenzofuran, 153

Prepared from 58 according to literature procedures[184] in 21% yield, mp 151-152°C (lit. 23%, mp 150-151°C).

TLC: R_f 0.49 (A). IR ($CDCl_3$): ν_{max} 3562, 2950, 1498, 1444, 1407, 1196, 875, 858, 685, 638 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 5.55 (1H, bs, Ar:OH), 6.95 (1H, d; J = 8.5 Hz, C_3-H), 7.17 (1H, d; J = 8.5 Hz, C_2-H), 7.39 (1H, td; J = 7.7, 1.1 Hz), 7.51 (1H, td; J = 7.7, 1.1 Hz), 7.58 (1H, d; J = 7.7 Hz, C_6-H), 8.35 (1H, dd; J = 7.7, 1.1 Hz, C_9-H). Mass spectrum (70 eV) m/e (rel. intensity): 220 (M^++2, 30), 218 (M^+, 100), 191 (2), 189 (6), 128 (4), 126 (45), 63 (51).

1-Chloro-4-acetoxydibenzofuran, 74

The hydroxy derivative 153 was acetylated according to the procedure described for 35 to afford 90% of a white powder, mp 122-127°C. Recrystallization from CH_2Cl_2-pentane afforded pure 74 as white needles (60% overall), mp 126-128°C.

TLC: R_f 0.69 (A). IR ($CHCl_3$): ν_{max} 3008, 1769, 1447, 1173, 704, 658 cm^{-1}. ^1H NMR (250 MHz, DMSO-d_6): δ 2.46 (3H, s, Ar:OAc), 7.42 (1H, d; J = 8.8 Hz), C_3-H), 7.52 (1H, d; J = 8.8 Hz, C_2-H), 7.53 (1H, t; J = 7.1 Hz), 7.66 (1H, t; J = 7.1 Hz), 7.83 (1H, d; J = 7.1 Hz, C_6-H), 8.34

(1H, d; J = 7.1 Hz, C_9-H). Mass spectrum (70 eV) m/e (rel. intensity): 263 (M^++2, 3), 261 (M^+, 6), 221 (35), 219 (100), 190 (7), 156 (11), 127 (29), 75 (51), 43 (49).

Anal. Calcd for $C_{14}H_9O_3Cl$: C, 64.51; H, 3.48; Cl, 13.60; found: C, 64.70; H, 3.52; Cl, 13.73.

Rate dermination for the intermolecular aminolysis of 74 with ethyl S-benzyl-L-cysteinate

The kinetics of the bimolecular aminolysis of the chlorodibenzofuran derivative 74 were performed as described for the bromo analog using 74 and ethyl S-benzyl-L-cysteinate at 1.97 x 10^{-1} M and 1.00 M, respectively. Calculation of the reaction rates and E.L.C.'s was undertaken as described for the unactivated dibenzofuran analogs.

$$k_2 = 9.18 \times 10^{-1}\ M^{-1}h^{-1}$$

E.L.C. = 4.5 M

APPENDIX III

THIOL CAPTURE VIA A MERCAPTIDE

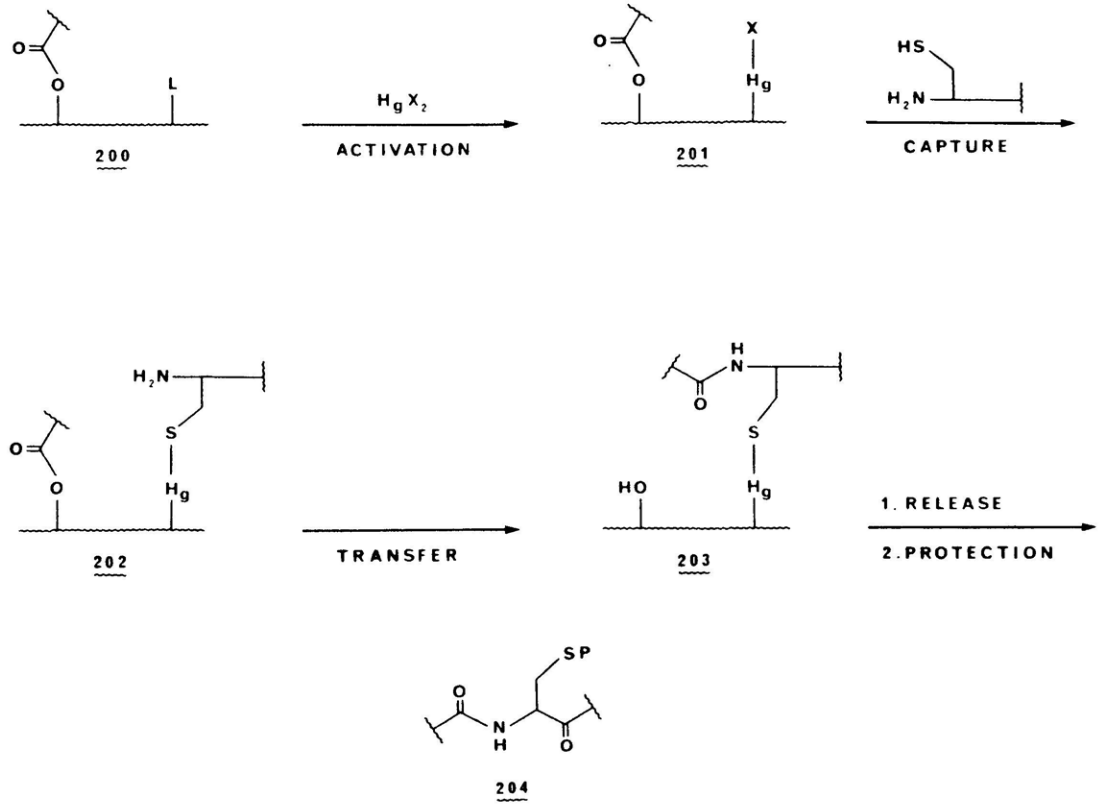
In the main part of this thesis we outlined a general scheme for amide bond formation by prior thiol capture. In summary, large peptide fragments bearing reactive amine and acyl functionalities are linked by a process of greater intrinsic affinity than the weak and unreliable interaction of a peptide amine with an electrophilic center. The resulting assembly can then undergo intramolecular O,N-acyl transfer to generate the amide bond under conditions that are independent of concentration.[36] In the preceeding chapters we utilized the methoxycarbonylsulfenyl (Scm) protocol, successfully employed in syntheses of insulin precursors,[80] to develop a strategy in which peptide segments are joined together efficiently and at very low concentrations via an unsymmetrical disulfide bridge immediately prior to acyl transfer. In this part we will use the reaction of thiols with organomercury derivatives which with its speed, reversibility and high affinity, constitutes the natural alternative to the disulfide capture process.

Aryl mercury bonds are known to be unstable to the acidic conditions often employed in many operations of peptide synthesis.[51] Therefore, in a practical capture strategy, it would be best for the mercuric capture site to be introduced at the C-terminus of the peptide fragment at the step immediately preceding thiol capture. Such a transformation would most likely involve mercury-induced transmetallation, and potential substrates for it would be derivatives of boron and silicon. This prediction stems from known chemistry of these metals: Phenylboronic acids are reported to be compatible with the operations of peptide

synthesis,[201] and can be quantitatively converted to phenylmercuric halides under mild and aqueous conditions.[202] Moreover, Benkeser observed that treatment of aryl-trimethylsilyl moieties with mercuric acetate in glacial HOAc results in the efficient generation of the respective aryl-mercuroacetate analogs.[203] Finally, in this thesis we found that aryl silanes such as 207 (Scheme AIII-2) are unreactive towards 1.0 M DCl in aqueous MeOH for 1 h at 25 °C, and 1.0 M NaOD at reflux in the same solvent for 40 min.

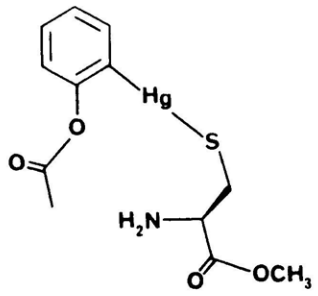
A possible formulation of these observations into a synthetic strategy is shown in Scheme AIII-1: Acylation of the template, followed by activation of the latent functionality L ($B(OH)_2$ or $SiMe_3$) of 200 would generate mercuric salt 201, on which an N-terminal cysteine peptide

SCHEME AIII-1

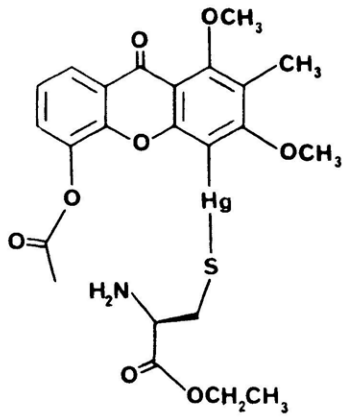


would be captured. Acyl transfer, followed by cleavage of the Hg-S linkage would afford the desired coupled product which would then be immediately acylated or alkylated at the newly formed thiol functionality of cysteine.

Because of the extended linearity of the C-Hg-S system, thiol capture via a mercaptide results in a very different geometry than in the disulfide case. Examination of Dreiding models revealed that the assemblies that best accommodated intramolecular acyl transfer in the latter cases, also appeared to be well-fitted for the mercaptide systems. Thus, on stereomodels, xanthone 21 accommodates the presumed acyl transfer transition state better than the disubstituted phenyl framework 20.



20



21

Experiments by Kerkman and Leung, confirmed this prediction:[48] Thus, although 21 underwent acyl transfer in DMSO with a half-time of 3 h and an effective local concentration of 0.6 M, 20 remained unreactive over a period of 50 h. In this thesis, the case of xanthone 21 served a dual purpose: On one hand it established the feasibility of intramolecular

acyl transfer with mercaptide systems and hence became our lead structure. But on the other hand, it helped identify serious synthetic drawbacks, the most important of which was demercuration, which was concurrent with the intramolecular acylation process. A side reaction of this sort, presumably attributable to the electron-rich character of the mercury-bearing aromatic ring, constituted a genuine twist of irony, since the substitution of the xantone system with electron-donating functionalities was specifically designed to enhance the selectivity and rate of the mercuration reaction.[65]

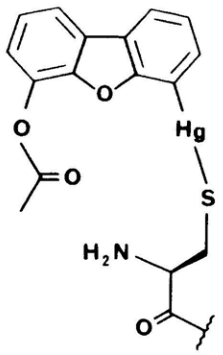
With these considerations in mind, we set out to accomplish the following three goals:

1. Design structural variants of 21 that would accommodate the acyl transfer transition state on Dreiding stereomodels, and for which demercuration would be disfavored.

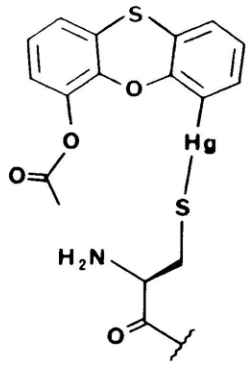
2. Efficiently generate these templates from readily available starting materials and verify the position of the mercuric capture site.

3. Test the capability of these assemblies to undergo intramolecular O,N-acyl transfer.

Application of the selection rules described in Chapter III, modified to accommodate the 180° angle requirement for the C-Hg-S system, directed us to two promising diphenyl assemblies: dibenzofuran 205 and phenoxythiin 206. Since we were uncertain of the electron-donating capacity of the heteroatoms on the aromatic rings, the demercuration reaction could only be tested experimentally.



205



206

As reported by Gilman, 4-acetoxymercurydibenzofuran is readily available by electrophilic mercuration in which dibenzofuran is heated to a melt with $Hg(OAc)_2$.[104] Although this transformation was successfully reproduced in this thesis, numerous attempts to perform this reaction on 4-acetoxydibenzofuran failed.

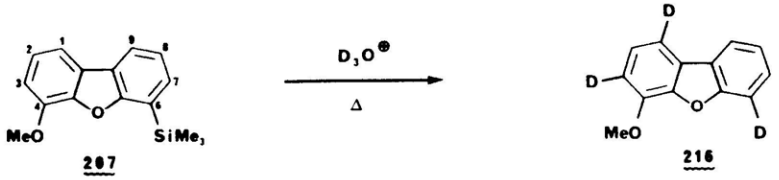
As an alternative approach to 205 we considered the silicon to mercury transmetallation reaction discussed earlier. We reasoned that such a conversion, if performed on 209, would afford the desired template 210 on which cysteine would be captured.

This synthetic approach was realized as follows (Scheme AIII-2): Metallation of 4-methoxydibenzofuran, 59, with n-BuLi generated the lithium salt at C-6, which was quenched with trimethylsilylchloride to afford 207 as a crystalline solid in 42% yield. Demethylation of 207 with TMSI[109] or BBr_3[204] was accompanied by concurrent cleavage of the C-Si linkage, but partial selectivity was accomplished by the use of a BBr_3-$NaHCO_3$ mixture. Given the poor efficiency of the latter operation

(12% yield), we investigated an alternative route for phenol 208 that would not require a methyl ether deprotection step. Thus, treatment of 4-hydroxydibenzofuran 58 with two equivalents of n-BuLi in the presence of the lithium ion chelating agent TMEDA (THF, -12 °C), followed by quenching with TMSCl and mild acid hydrolysis, afforded 208 by a one-pot procedure in 36% yield (Scheme AIII-2). It must be noted that the employment of TMEDA was essential since in its absence the same reaction produced only traces of the silane.

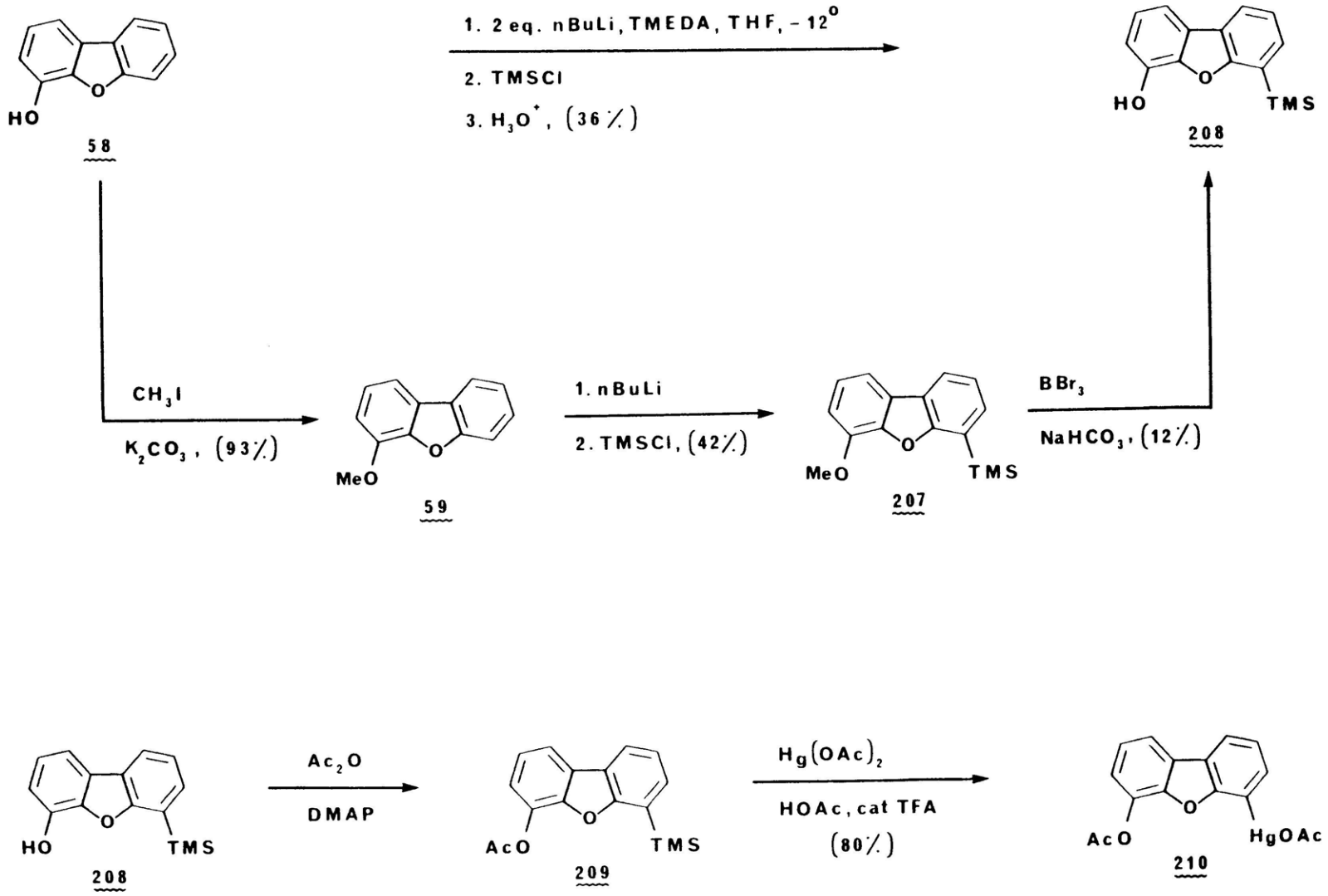
The remainder of the synthesis was uneventful. Acylation of 208 with Ac_2O and 4-dimethylaminopyridine generated 209 which upon treatment with mercuric acetate in glacial HOAc containing a catalytic amount of TFA afforded template 210 as a shiny crystalline solid in 75% overall yield (from 208).

Structure elucidation, which also constitutes a proof for the selectivity of all metallations performed on 4-methoxydibenzofuran, was undertaken with methoxy aryl silane 207, and was based on proton NMR spectroscopy. It entailed a two-step process: First, the assignement of chemical shifts to the aromatic protons of unsubstituted dibenzofuran and 4-methoxydibenzofuran, 59. And second, the examination of the ^{1}H-NMR spectrum of the tri-deuterated 4-methoxydibenzofuran 216, generated by D_3O^+-induced acidolysis of the C-Si linkage of 207.[205]



207 216

Scheme AIII-2



The proton NMR profile of dibenzofuran consists of two triplets and two doublets, sufficiently dispersed at 250 MHz to allow differentiation. Despite effors by Black,[206] no conclusive assignment of these resonances has been reported in the literature.

We achieved the unequivocal assignment of the proton absorptions of dibenzofuran by the examination of the 250 MHz spectra of 2-deuterodibenzofuran 213, and 4-deuterodibenzofuran 214, prepared from their respective known bromo derivatives 211 and 212 by metallation followed by quenching with D_3O^+. Table AIII-1 summarizes our results:

TABLE AIII-1: Proton NMR resonance assignment for dibenzofuran

δ	Dibenzofuran	2-Deuterodibenzo-furan	4-Deuterodibenzo-furan	Assignment
7.17	2H, td[a]	1H, t	2H, t	C_2-H
7.29	2H, td	2H, tm[c]	2H, tm	C_3-H
7.42	2H, dm[b]	2H, d	1H, d	C_4-H
7.78	2H, dm	2H, dm	2H, dd	C_1-H

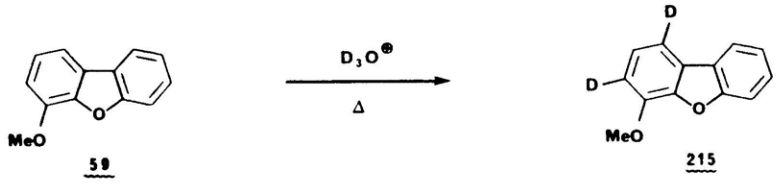
[a]td = triplet of doublets. [b]doublet of multiplets. [c]triplet of multiplets.

From Table AIII-1, it can be seen that the inherent difference in chemical shifts between C_1-H and C_3-H is ca. 0.5 ppm. We employed this observation together with the assumption that the protons on the two

aromatic rings of a monosubstituted dibenzofuran would behave independently, in the assignment of the chemical shifts observed with 4-methoxydibenzofuran, 59.

The assignment of C_6-H, C_7-H, C_8-H and C_9-H of 59 was easily accomplished on the basis of Table AIII-1. Of the remaining resonances, as expected, two were doublets and one was a triplet. The assignment of the latter was straightforward and was made to C_2-H. Verification of these deductions and identification of the remaining two resonances was achieved by examination of the partially deuterated methoxydibenzofuran 215, the product of a deuterium exchange reaction performed on 59.

The methoxy functionality of 59 should allow the rapid exchange of the protons ortho and para to that moiety. Moreover, the resonances of these two protons should maintain their inherent $\Delta\delta$ of 0.5 ppm. This was indeed observed, and thus the absorptions at δ 6.95 and δ 7.52 were assigned to C_3-H and C_1-H, respectively. Table AIII-2 summarizes these assignments.



59 215

The complete assignment of the proton resonances of undeuterated 59 provided the answer to the question of position of substitution of the

TABLE AIII-2: Proton NMR resonance assignment for 4-methoxydibenzofuran

δ	59	215	216	Assignment
6.95	1H, d	0.16H, d	-	C_3-H
7.24	1H, t	1H, bs	1H, s	C_2-H
7.33	1H, t	1H, t	1H, t	C_8-H
7.44	1H, t	1H, t	1H, dt	C_7-H
7.52	1H, d	0.32H, d	-	C_1-H
7.61	1H, d	1H, d	-	C_6-H
7.91	1H, d	1H, d	1H, dd	C_9-H

trimethylsilyl substituent of 207: Hydrolysis of this silane with D_3O^+ generated the trideuterated material 216, the ^{1}H-NMR spectrum of which showed disilylation and the absence of C_1-H, C_3-H (methoxy-induced deuterium exchange) and C_6-H (Table AIII-2). Since the deuteration reactions 59 to 215 and 207 to 216 were performed under identical conditions, the possibility of C_6-D formation due to simple hydrogen to deuterium conversion is unlikely. Therefore it must be concluded that deuterium introduction at C_6 resulted from the hydrolysis of the C-Si linkage at that carbon.

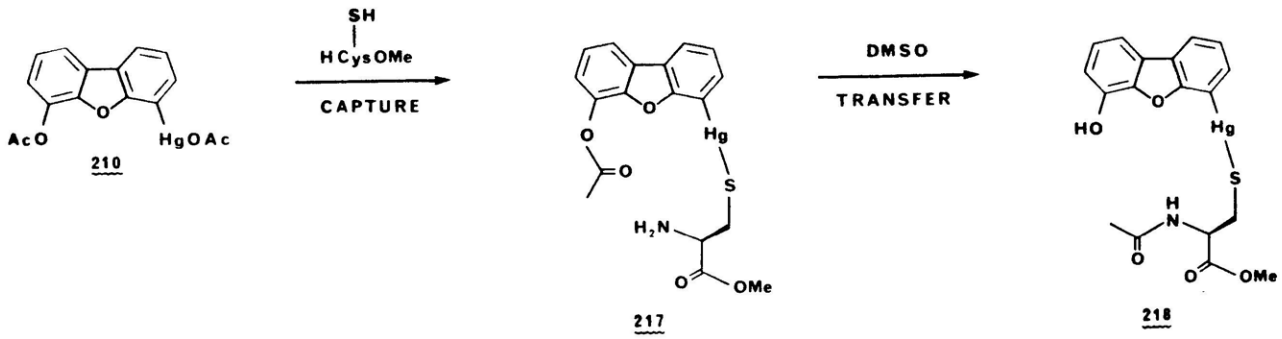
Having completed the structure elucidation of the dibenzofuranyl template, we proceeded to investigate its capability to accommodate

intramolecular O,N-acyl transfer. The demonstration of feasibility of such a reaction would also constitute independent verifcation of the structural assignment, since examination of Dreiding stereomodels revealed that the linearity of the C-Hg-S assembly prohibits intramolecular acyl migration unless the mercury function of 217 is attached at C_6.

As noted in Scheme AIII-1, the generation of the acyl transfer assembly requires the prior thiol capture of an N-terminal cysteine moiety by the Hg trapping site of the template. Since in model studies we found that the acidolytic conditions required for the deblocking of the N -Boc derivative of 217 also result in the cleavage of its C-Hg linkage,[207] we performed the thiol capture reaction by treating template 210 with unblocked methyl cysteinate (Scheme AIII-3).

Thus, treatment of a solution of 210 in DMSO with the hydrochloride salt of methyl cysteinate in the presence of DMAP resulted in rapid thiol capture, as evidenced by a negative carbazone test[189] obtained 2 min into the reaction. The capture product 217 was then allowed to undergo acyl

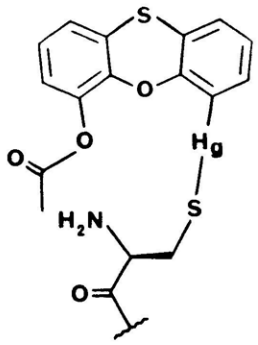
SCHEME AIII-3



transfer in the same pot ([217] = 3.1 x 10^{-} M) and the progress of the reaction was followed by proton NMR.

The intramolecular acylation of amine 217 was found to proceed moderately fast with a half-time of 12 h, which corresponds to an effective local concentration of 0.51 M. When 6.0 mg of 210 was allowed to undergo the capture and transfer operations, 5.2 mg (58%) of acyl migration product 218 was isolated. It must be noted that the demercuration reaction observed with Kerkman's and Leung's systems was not observed with 218, and this latter material was found to be stable in DMSO for at least six days.

The dibenzofuranyl system 217, although encouraging in that it served to establish the feasibility of clean intramolecular acyl transfer, nevertheless did not meet our efficiency requirement of ELC 1.0 M. For this reason we tested a structural variant of 217, the phenoxythiin assembly 206.



206

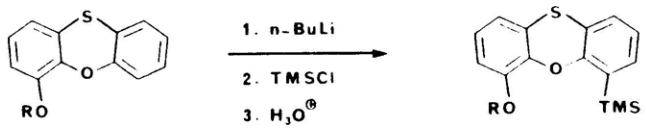
The differences in structural features between the phenoxythiin and dibenzofuran frameworks stem primarily from the presence of the sulfur spacer in the former assembly. Hosoya reports that in phenoxythiin, the

C-S bond is longer by the C-O linkage by 0.35 Å (1.75 Å *vs* 1.40 Å), which results in a large diplanar angle (138° vs *ca.* 1° in dibenzofuran) and an inwardly skewed arrangement of the C_4-H and C_6-H bonds.[208]

On Dreiding stereomodels, 206 appeared to accommodate the transition state for intramolecular acylation reaction better than the equivalent dibenzofuranyl assembly 205. However, the low activation energy butterfly flapping motion of phenoxythiin in solution, noted by Davies and Swain,[209] presented us with a novel structural parameter, namely the non-rigidity of the template framework. Since this new structural feature could not be evaluated *a priori*, we prepared 206 and investigated the rate and efficiency of its intramolecular acylation.

The synthesis of 206 was short and was based on the protocol outlined in Scheme AIII-4. Thus, hydroxylation of commercially available phenoxythiin 219, according to Gilman's procedure[193] afforded phenol 220 which was then treated with two equivalents of n-BuLi in the presence of TMEDA. This dilithiated product was then quenched with trimethylsilyl-chloride to afford a silyl ether which was readily hydrolyzed to generate silane 221 in 90% yield (Scheme AIII-4). The exceptional selectivity and high efficiency of this dimetallation reaction stemmed from a lenghty search for optimal conditions, a summary of which is shown in Table AIII-3:

TABLE AIII-3[a]

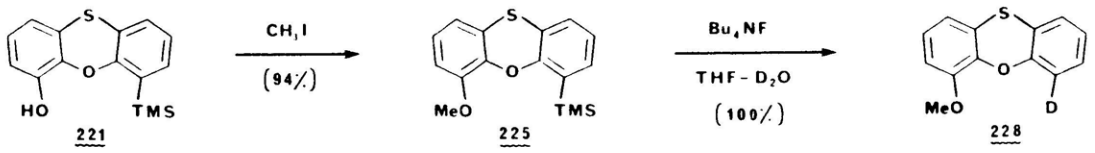


R	Starting Material	eq.'s n-BuLi	TMEDA	°C	%SM	%P	%other
CH_3-	229	1.0	-	-78	100	0	0
CH_3-	229	1.0	-	-30	88	8	4
CH_3-	229	1.0	-	-10	21	67	12
CH_3-	229	2.0	-	-10	2	20	78
t-$BuMe_2Si$-	226	1.0	-	-12		100% 220	
CH_3OCH_2-	227	1.0		-13	9	33	52
H-	220	2.0	-	-14	100	0	0
H-	220	2.0		-16	10	90	0

[a]All reactions were performed in THF; yields were estimated by HPLC.

The phenolic acyl silane 221 was then acetylated, and the resulting acetoxyphenoxythiin 222 was treated first with mercuric trifluoroacetate in anhydrous TFA and then with aqueous NaBr to afford template 223 in 84% overall yield. The employment of rather harsh conditions for the mercuration reaction was necessitated by the inertness of silane 222 towards $Hg(OAc)_2$ and was based on H.C. Brown's observation that mercuration in TFA proceeds 69 x 10 faster than in glacial HOAc.

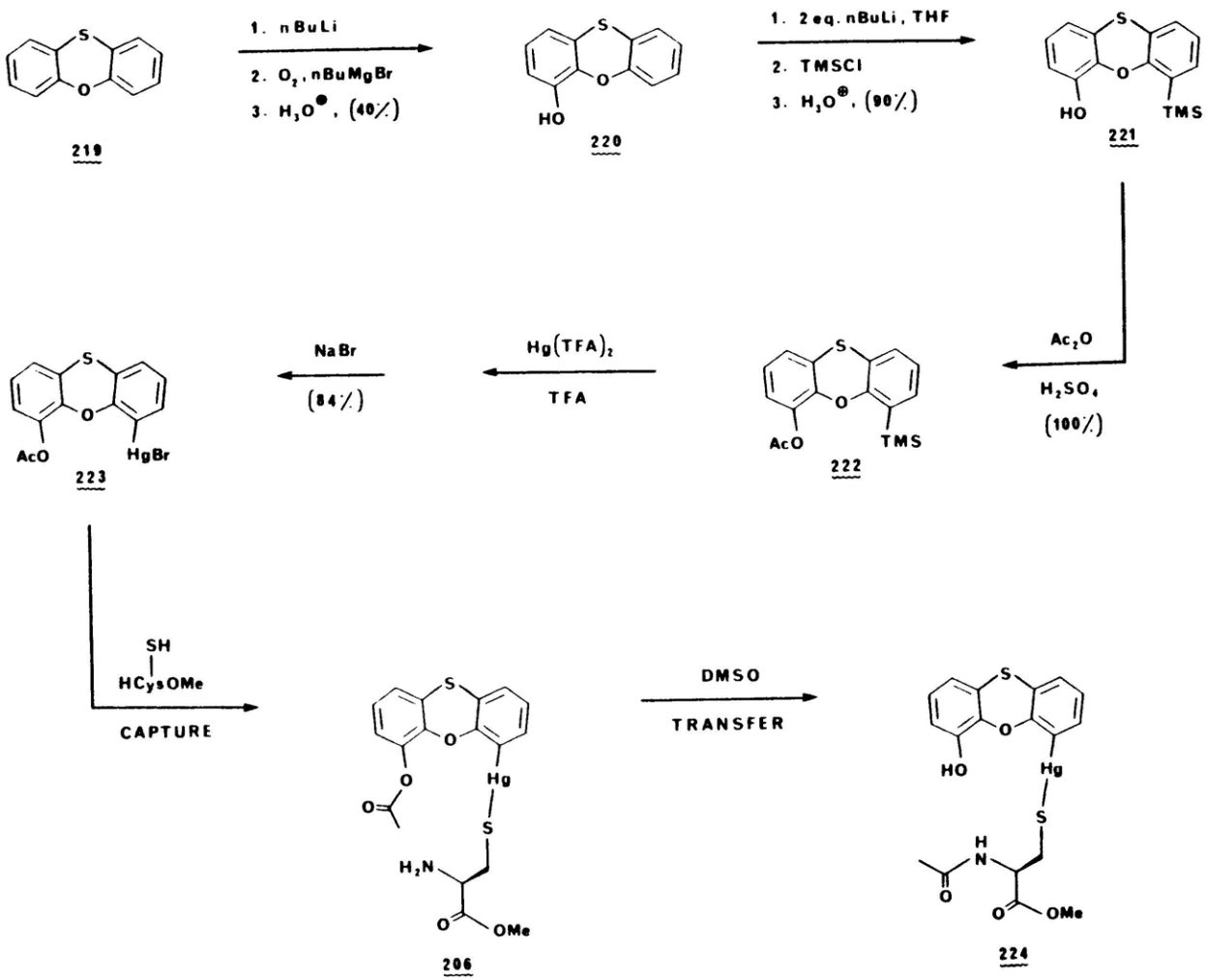
Verification of the substitution pattern of the phenoxythiin template was performed on the methoxy aryl silane 225, and was based on ^{13}C-NMR. Methylation[107] of 221 generated 225 which was then quantitatively desilylated with Bu_4NF[211] in THF containing D_2O.



Examination of the ^{13}C-NMR spectra of phenoxythiin, 4-methoxyphenoxythiin and the deuterated material 228, revealed that the absorption at δ 117.5 present in the spectra of the former two, was reduced to a low-intensity triplet in 228. Since Isbrandt[192] assigned this resonance to the absorption of C_4 or C_6, we concluded that the TMS function of 225 is attached to C_6.

Having completed the synthesis and structure elucidation of the phenoxythiin template, we proceeded to test its capability to induce intramolecular acylation. Thus, treatment of the aryl mercuric bromide 223 with methyl cysteinate generated acyl transfer system 206 (Scheme AIII-4). The reaction was rapid (over within 2 min) and the capture product was allowed to undergo acyl migration in DMSO without purification. Rate determination was undertaken by ^1H-NMR and a half-time of 12 h was observed for the reaction. Noteworthy is the fact that no demercuration of product 224 was observed for over 60 h.[48]

Scheme AIII-4



From the preceding discussion, it is clear that the rate constant obtained for the intramolecular O,N-acylation of the phenoxythiin template is almost identical with that of dibenzofuran. Moreover, our results are in close agreement with Kerkman's, where a $t_{1/2}$ of 8 h was observed with the xanthone framework 21. Although this situation may be a direct consequence of the unfavorable inflexibility of the long C-Hg lingkage, simple modifications in structure and methodology may allow the mercaptide protocol to be of practical use: Introduction of electron withdrawing substituents at the para position of the ring bearing the acetoxy functionality has been shown to result in ten-fold acceleration in rate of intramolecular acylation, without causing any undesired side-reactions (Chapter III). Moreover, addition of a temporary mercury ligand such as Me_2S may induce the bending of the linear C-Hg linkage, as is observed with tetracoordinate complexes of mercury. Finally, the fact that only three acyl transfer systems have been examined, is not preclusive that no others will perform better. We may have witnessed a case where the end points of an acyl transfer efficiency plot have been defined, and the best candidate, perhaps a simple hydrid of the known examples, remains elusive.

In summary then, in this part we accomplished the following: First, we demonstrated the applicability of a prior thiol capture scheme in which the key step consists of trapping an N-terminal cysteine moiety as its mercaptide by an organomercury template. And second, we established the feasibility of intramolecular O,N-acyl transfer across the twelve-membered atom assembly defined by the dibenzofuran and phenoxythiin frameworks 205 and 206.

APPENDIX III

EXPERIMENTAL SECTION

General Methods

L-Cysteine methyl ester hydrochloride was purchased from Fluka; boron tribromide (BBr_3) in CH_2Cl_2 (1.0 M solution) and tetrabutylammonium fluoride ($n\text{-}Bu_4NF$) in THF (1.0 M solution) from Aldrich; deuterium chloride (DCl) in D_2O (20% solution) from Sigma; t-butyldimethylsilyl chloride from Petrarch Systems. Solutions of n-butyllithium (n-BuLi) in n-hexane were obtained from Alfa and titrated[169] prior to use.

4-Hydroxydibenzofuran, 58, was recrystallized (hexane) prior to use; tetramethylethylenediamine (TMEDA) and dimethoxymethane (DMM) were dried by refluxing with sodium, then fractionally distilled and stored over molecular sieves (Linde 4A) at 4°C; trimethylsilyl chloride (TMSCl) and dimethyl sulfoxide (DMSO) were distilled from CaH_2 and used immediately; commercially available (Aldrich) mercuric trifluoroacetate was dried over P_2O_5 under high vacuum for 24h and stored in a vacuum dessicator over P_2O_5 at -20°C; trifluoroacetic acid (TFA), N,N- dimethylformamide (DMF) and tetrahydrofuran (THF) were purified as described previously; reagent grade dichloromethane was dried over molecular sieves (Linde 4A) for at least 2d and used without further purification.

For the metallation reactions the glassware used was dried for at least one day in an oven thermostated at 125°C and the reagents employed were introduced by syringe into the reaction vessel through rubber septa while positive pressure of N_2 was maintained via a balloon filled with the gas.

For the kinetics experiments commercially available deuterated solvents were used without further purification.

All instrumentation and chromatographic equipment is as described in General Method (pp. 126-128). Tables of thin layer chromatography (TLC) solvent systems and high pressure liquid chromatography (HPLC) conditions can be found on pp. 126 and 128, respectively.

4-Trimethylsilyl-6-hydroxydibenzofuran, 208

Method A: To a solution of 4-hydroxydibenzofuran, 58, (87.7 mg, 0.467 mmol) in tetrahydrofuran (3.0 mL) placed under N_2 was added tetramethylethylenediamine (0.20 mL, 1.32 mmol) at -12°C (thermostated ethylene glycol-water bath). The clear colorless mixture was stirred under these conditions for 5 min and then a solution of n-butyllithium (0.99 mmol) in n-hexane (0.45 mL) was added dropwise over a period of 5 min. After 30 min a purple color developed and the clear mixture was stirred at -12°C for 12 h. The solution was then quenched with trimethylsilyl chloride (0.19 mL, 1.35 mmol) and allowed to warm up slowly to room temperature (4 h). The resulting dark-yellow clear mixture was then poured into 5% $NaHCO_3$, extracted with CH_2Cl_2 (3x), the organic layers were combined, back-washed with 5% $NaHCO_3$, dried ($MgSO_4$), and evaporated. The dark-yellow oil residue (113.1 mg) was dissolved in MeOH (4.0 mL), 1 N HCl (50 µL) was added and the clear solution was stirred at room temperature for 5 min. The mixture was then poured into 5% $NaHCO_3$, extracted with CH_2Cl_2 the organic phases were combined,

back-washed with 5% $NaHCO_3$ and washed with water. HPLC analysis (E) indicated the presence of 58 (rt 5.70 min, 62%) and 208 (rt 11.70 min, 38%). The combined organic phases were then washed with ice-cold 1 N NaOH (2x), water (2x), dried ($MgSO_4$), and evaporated to dryness. The residue was azeotroped with CH_3CN (2x) and dried under high vacuum to afford pure 208 as a light-yellow oil (43.1 mg, 36%).

TLC: R_f 0.58 (A); 0.25 (E). HPLC: rt 11.70 min, 100% (E); 5.49 min, 98% (F). ^1H NMR (270 MHz, $CDCl_3$): δ 0.48 (9H, s, Ar:TMS), 5.32 (1H, s, Ar:OH), 7.04 (1H, d; J = 8 Hz, C_7-H), 7.24 (1H, t; J = 8 Hz, C_8-H), 7.33 (1H, t; J = 8 Hz, C_2-H), 7.53 (1H, d; J = 8 Hz, C_9-H), 7.55 (1H, d; J = 8 Hz, C_3-H), 7.96 (1H, d; J = 8 Hz, C_1-H). High resolution mass spectrum: Calcd for $C_{15}H_{16}O_2Si$: 256.0920; Found: 256.0922.

Method B. To a solution of 4-methoxydibenzofuran, 59, (2.00 g, 10.10 mmol) in anhydrous diethyl ether (50 mL) placed under N_2 was added in one portion at room temperature a solution of n-butyllithium (10.34 mmol) in n-hexane (4.70 mL) and the mixture was heated to reflux for 10 h. The resulting suspension was cooled to -78°C, quenched with trimethylsilyl chloride (1.35 mL, 10.64 mmol) and allowed to warm up slowly to room temperature (6 h). To the mixture water (50 mL) was added, the layers were separated and the aqueous phase was extracted with Et_2O (3x). The organic layers were combined, washed with water (2x), dried ($MgSO_4$), evaporated, and the residue was dried under high vacuum to afford a yellow oil (2.52 g, 92%). Flash chromatography (eluent 9:1 cyclohexane-CH_2Cl_2) yielded pure 4-trimethylsilyl-6-methoxydibenzofuran, 207, as an oil that solidified on standing (1.146 g, 42%), mp 59.5-61.5°C.

TLC: R_f 0.22 (G), 0.63 (B). ^1H NMR (250 MHz, $CDCl_3$): δ 0.46 (9H, s), 4.08 (3H, s), 6.97 (1H, dd; J = 8,1 Hz), 7.23 (1H, t; J = 8 Hz), 7.32 (1H, t; J = 8 Hz), 7.50-7.55 (2H, m), 7.93 (1H, dd; J = 8,1 Hz). High resolution mass spectrum: Calcd for $C_{16}H_{18}O_2Si$: 270.1076; Found: 270.1059.

To a solution of 207 (1.129 g, 4.18 mmol) in dichloromethane (3.0 mL) was added sodium bicarbonate (1.750 g, 20.8 mmol) and the mixture was cooled to -78°C under N_2. To the resulting suspension, a solution of boron tribromide (4.30 mmol) in dichloromethane (4.30 mL) was added dropwise (4 min) with vigorous stirring and then the mixture was allowed to warm up slowly to room temperature (16 h). The suspension was then cooled to 0°C, and quenched with saturated $NaHCO_3$ solution (10 mL). The layers were separated, the aqueous phase was extracted with CH_2Cl_2 (3x), the organic layers were combined, washed with water, brine, dried ($MgSO_4$), and evaporated to give a light-green solid residue (751 mg). Flash chromatography (eluent 100% CH_2Cl_2) afforded the desired product 208 as an oil (125 mg, 12%), and 4-hydroxydibenzofuran, 58, (615 mg, 80%).

Both compounds were identical with authentic samples of 208 and 58 respectively by TLC, ^1H NMR and mixed mp (for 58).

Dibenzofuran, 57

The commercially available material was used without further purification.

TLC: R_f 0.75 (1). ^1H NMR (250 MHz, $CDCl_3$): δ 7.17 (1H, td: J = 8,1 Hz, C_2-H), 7.29 (1H, td; J = 8,1 Hz, C_3-H), 7.42 (1H, dm, C_4-H), 7.78

(1H, dm, C_1-H). ^{13}C NMR (62.83 MHz, $CDCl_3$): δ 111.7 (d, C_4), 120.7 (d, C_1), 122.7 (d, C_2), 124.3 (s, C_{4b}), 127.2 (d, C_3), 156.3 (s, C_{4a}). Mass spectrum (70 eV) m/e (rel. intensity): 168 (M^+, 100), 139 (60), 113 (13), 34 (32), 69 (28), 63 (36), 39 (33).

2-Bromodibenzofuran, 211

Prepared from 57 according to published procedures[173] in 76% yield, mp 106-7°C (lit. 107°C).

TLC: R_f 0.48 (H). ^1H NMR (60 MHz, $CDCl_3$): δ 7.20-7.58 (5H, m), 7.80-8.05 (2H, m). Mass spectrum (70 eV) m/e (rel. intensity): 248 (M^+, 9), 246 (M^+-2, 9), 168 (8), 167 (6), 139 (33), 85 (42), 57 (66), 55 (66).

4-Bromodibenzofuran, 212

Prepared from 57 according to published procedures[174] in 56% yield, mp 69-71°C (lit. 70-71°C).

TLC: R_f 0.81 (A). ^1H NMR (60 MHz, $CDCl_3$): δ 7.03-7.75 (5H, m), 7.80-7.98 (2H, m). Mass spectrum (70 eV) m/e (rel. intensity): 248 (M^+, 34), 246 (M^+-2, 35), 167 (5), 139 (100), 85 (5), 69 (29), 63 (26), 57 (2).

2-Deuterodibenzofuran, 213

To a solution of 211 (0.80 g, 3.22 mmol) in anhydrous diethyl ether (20 mL) a solution of n-butyllithium (3.54 mmol) in n-hexane (1.42 mL) was added in one portion at room temperature under N_2, and the mixture was heated to reflux for 0.5 h. The resulting suspension was then cooled

to 0°C and quenched with a 20% solution of deuterium chloride in deuterium oxide (10 mL, 54 mmol). The layers were separated and the aqueous phase was extracted with Et_2O. The organic layers were combined, washed with water, dried ($MgSO_4$) and evaporated to yield 213 as a white solid (0.53 g, 97%), mp 75-77°C. Recrystallization from methanol afforded colorless plates (0.48 g, 88%), mp 78-79°C.

TLC: R_f 0.75 (A). ^1H NMR (250 MHz, $CDCl_3$): δ 7.17 (1H, t; J = 8 Hz), C_8-H), 7.29 (2H, tm, C_3-H and C_7-H), 7.42 (2H, d; J = 8 Hz, C_4-H and C_6-H), 7.78 (2H, dm, C_1-H and C_9-H). ^{13}C NMR (62.83 MHz, $CDCl_3$): δ 111.7 (d, C_4 and C_6), 120.6 (d, C_1), 120.7 (d, C_9), 122.5 (t, C_2), 122.7 (d, C_8), 124.3 (s, C_{4b} and C_{6b}), 127.1 (d, C_3), 127.2 (d, C_7), 156.3 (s, C_{4a} and C_{6a}). Mass spectrum (70 eV) m/e (rel. intensity): 169 (M^+, 100), 140 (41), 114 (6), 63 (11), 39 (13).

Anal. Calcd for $C_{12}H_7DO$: C, 85.21; H, 4.14. Found: C, 84.94; H, 4.30.

4-Deuterodibenzofuran, 214

The conversion of 212 to 214 employed the same procedure as for 213 except that the organomagnesium complex of 212 was formed[175] prior to the DCl/D_2O treatment. After work-up, the product was isolated in 92% yield, mp 81-82°C.

TLC: R_f 0.75 (A). ^1H NMR (250 MHz, $CDCl_3$): δ 7.17 (2H, t; J = 8 Hz, C_2-H and C_8-H), 7.29 (2H, tm, C_3-H and C_7-H), 7.42 (1H, d; J = 8 Hz, C_6-H), 7.78 (2H, dd; J = 8,1 Hz, C_1-H and C_9-H). ^{13}C NMR (62.83 MHz, $CDCl_3$): δ 111.6 (t, C_4), 111.7 (d, C_6), 120.7 (d, C_1 and C_9), 122.7 (d, C_2 and C_8), 124.3 (s, C_{4b} and C_{6b}), 127.1 (d, C_3), 127.2 (d, C_7),

156.2 (s, C_{4a}), 156.3 (s, C_{6a}). Mass spectrum (70 eV) m/e (rel. intensity): 169 (M^+, 100), 140 (50), 114 (10), 85 (16), 70 (22), 63 (24).

Anal. Calcd for $C_{12}H_7DO$: C, 85.21: H, 4.14. Found: C, 85.03; H, 4.38.

Structure Elucidation of 4-trimethylsilyl-6-methoxydibenzofuran, 207:

1,3,6-trideutero-4-methoxydibenzofuran, 216.

To a solution of 207 (7.1 mg, 26.3 μmol) in methanol-d_1 (0.60 mL) was added a 35% solution of deuterium chloride in deuterium oxide (0.30 mL) and the mixture was heated to reflux for 15 h. The solution was then poured into water, extracted with CH_2Cl_2 (3x), the organic phases were combined, washed with water (2x), dried ($MgSO_4$) and evaporated. The residue was further dried under high vacuum to afford an oil (6.1 mg). Preparative layer chromatography (1,000 μ; eluent 1:1 $CHCl_3$-cyclohexane) yielded pure 216 as an oil (3.8 mg, 72%).

TLC: R_f 0.30 (I), 0.57 (B). ^1H NMR (250 MHz, $CDCl_3$): δ 4.06 (3H, s, Ar:OCH_3), 7.25 (1H, s, C_2-H), 7.34 (1H, t; J = 8 Hz, C_8-H), 7.44 (1H, bt, C_7-H), 7.92 (1H, dd; J = 8,1 Hz, C_1-H). High resolution mass spectrum: Calcd for $C_{13}H_7D_3O_2$: 201.0869; Found: 201.0860.

Reaction of 4-methoxydibenzofuran with DCl in D_2O.

4-Methoxydibenzofuran, 59, was subjected to the same conditions as described above to yield partially deuterated 1,3-dideutero-4-methoxydibenzofuran, 215.

For 59: ^1H NMR (250 MHz, $CDCl_3$): δ 4.04 (3H, s, Ar:OCH_3), 6.95 (1H, d; J = 8 Hz, C_3-H), 7.24 (1H, t; J = 8 Hz, C_2-H), 7.33 (1H, t; J = 8 Hz,

C_8-H), 7.44 (1H, t; J = 8 Hz, C_7-H), 7.52 (1H, d; J = 8 Hz, C_1-H), 7.62 (1H, d; J = 8 Hz, C_6-H), 7.91 (1H, d; J = 8 Hz, C_9-H).

For 215: TLC: R_f 0.58 (B). ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.03 (3H, s, Ar:OCH_3), 6.95 (0.16H, d; J = 8 Hz, C_3-H), 7.24 (1H, bs, C_2-H), 7.33 (1H, t, J = 8 Hz, C_8-H), 7.44 (1H, t, J = 8 Hz, C_7-H), 7.52 (0.32 H, d; J = 8 Hz, C_1-H), 7.61 (1H, d; J = 8 Hz, C_6-H), 7.91 (1H, d; J = 8 Hz, C_9-H).

4-Trimethylsilyl-6-acetoxydibenzofuran, 209

To a solution of 208 (121.2 mg, 0.473 mmol) in acetic anhydride (0.60 mL) was added a catalytic amount of dimethylaminopyridine (2.2 mg, 18.0 μmol) and the clear solution was stirred at room temperature under N_2 for 3h. The mixture was then poured into 0.5 M citric acid and extracted with CH_2Cl_2 (3x). The organic phases were combined, washed with 5% $NaHCO_3$, water, dried ($MgSO_4$), and evaporated to dryness. The residue was azeotroped with CH_3CN (2x) and dried under high vacuum to afford 209 as a yellow viscous oil (133 mg, 94%).

TLC: R_f 0.75 (A), 0.58 (D). ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.40 (9H, s, Ar:TMS), 2.42 (3H, s, Ar:OAc), 7.16 (1H, d; J = 8 Hz, C_7-H), 7.28 (1H, t; J = 8 Hz, C_8-H), 7.33 (1H, t; J = 8 Hz, C_2-H), 7.51 (1H, d; J = 8 Hz, C_3-H), 7.78 (1H, d; J = 8 Hz, C_9-H), 7.93 (1H, d; J = 8 Hz, C_1-H). High resolution mass spectrum: Calcd for $C_{17}H_{18}O_3Si$: 298.1025; Found: 298.1021.

4-Acetoxymercury-6-acetoxydibenzofuran, 210

To a solution of 209 (59.3 mg, 0.199 mmol) in glacial acetic acid (0.30 mL) was added a solution of mercuric acetate (63.0 mg, 0.198 mmol) in the same solvent (0.75 mL). The mixture was stirred for 2 min, trifluoroacetic acid (0.11 mL) was added and the solution was stirred at room temperature for 1h. The resulting white suspension was filtered into ice-cold water, the residue was collected, washed with acetic acid (2 x 2 mL) and dried to constant weight under high vacuum to afford pure 210 as a white shiny powder (29 mg, 30%), mp 190-2°C. The filtrates were combined, cooled to 0°C and the white solid that precipitated was collected by filtration, washed with water (3 x 5 mL) and dried under high vacuum to yield a second crop (48 mg, 50%), mp 188-9°C.

HPLC: rt 13.00 min, 98% (G). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.60 (3H, s, Hg:OAc), 2.51 (3H, s, Ar:OAc), 7.20 (1H, d; J = 8 Hz), 7.33-7.39 (3H, m), 7.80 (1H, d; J = 8 Hz), 7.89 (1H, d; J = 8 Hz).

Anal. Calcd for $C_{16}H_{12}O_5Hg$: C, 39.63; H, 2.49; Hg, 41.37. Found: C, 39.43; H, 2.29; Hg, 41.46.

Methyl N-acetoxy-S-(6-hydroxy-4-dibenzofuranmercury)-L-cysteinate, 218

To a solution of the mercuric acetate 210 (6.0 mg, 12.4 μmol) and dimethylaminopyridine (3.0 mg, 24.5 μmol) in DMSO-d_6 (0.40 mL) was added L-cysteine methyl ester hydrochloride (2.1 mg, 12.2 μmol). A negative diphenylcarbazone test[189] was obtained within 2 min and the clear mixture was allowed to stand at room temperature for 6 d. During this time no precipitation of elemental mercury was observed. The solution was then poured into ice-cold 0.5 M citric acid, extracted with CH_2Cl_2 (3x), the

organic phases were combined, washed with water (2x), dried ($MgSO_4$), and evaporated to dryness to yield a white solid (11 mg). Preparative layer chromatography (1,000 μ; eluent 9:1 $CHCl_3$-EtOAc) afforded pure 218 as a white solid (5.2 mg, 58%), mp 176-8°C (dec).

TLC: R_f 0.09 (A). IR ($CDCl_3$): ν_{max} 3400, 1723, 1652, 1245 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.93 (3H, s, Cys:Ac), 3.69 (3H, s, Cys:OMe), 3.63 (1H, dd; J = 13,4 Hz, Cys: methylene), 3.96 (1H, dd; J = 13,4 Hz, Cys: methylene), 4.96-5.03 (1H, m, Cys: methine), 7.06 (1H, bs, Cys: amide), 7.07 (1H, d; J = 8 Hz, C_7-H), 7.24 (1H, t; J = 8 Hz), 7.38-7.50 (3H, m), 7.93 (1H, d; J = 8 Hz, C_1-H), 9.25 (1H, bs, Ar:OH). Field desorption mass spectrum: m/e 561 (M^+: ^{200}Hg).

Rate determination for the intramolecular acyl transfer which occurs on treatment of 210 with methyl cysteinate

Kinetics of the acyl transfer reaction were performed at ambient temperature (25°C) and followed by 250 MHz ^1H NMR at 3.1 x 10^{-2} M in each 210 and L-cysteine methyl ester hydrochloride and 6.2 x 10^{-2} M in dimethylaminopyridine. The half-time for the reaction was taken when the integration of the appropriate acetyl methyl resonances was equivalent. Effective local concentrations (E.L.C.) were calculated as described earlier.[64,140]

Solvent	$t_{1/2}$ (h)	$k_{intram.}$ (h^{-1})	E.L.C. (M)
DMSO-d_6	11.8	5.842 x 10^{-2}	0.51

Phenoxythiin, 219

Prepared from diphenyl ether and elemental sulfur according to published procedures[190,191] in 78% yield, mp 55.0-55.5°C (lit. 55-7°C).

TLC: R_f 0.78 (A). ^{1}H NMR (250 MHz, $CDCl_3$): δ 6.89-7.03 (4H, m), 7.06-7.12 (4H, m). ^{13}C NMR (62.83 MHz, $CDCl_3$): δ 117.6 (d, C_4),[192] 119.9 (s, C_{4a}), 124.3 (d), 126.6 (d), 127.5 (d, C_3), 151.9 (s, C_{1a}).[192] Mass spectrum (70 eV) m/e (rel. intensity): 200 (M^+, 100), 171 (39), 168 (41), 139 (17), 127 (13).

4-Hydroxyphenoxythiin, 220

Prepared from 219 according to published procedures[193] in 40% yield, mp 95-6°C (lit. mp 98°C).

TLC: R_f 0.52 (A), 0.23 (J). HPLC: rt 4.65 min, 100% (F). ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.83 (1H, bs Ar:OH), 6.61 (1H, dd; J = 8,2 Hz, C_3-H), 6.79 (1H, dd; J = 8,2 Hz), 6.88 (1H, t; J = 8 Hz), 6.97-7.02 (2H, m), 7.07-7.12 (2H, m). ^{13}C NMR (67.93 MHz, $CDCl_3$): δ 114.3 (d), 117.5 (d), 117.9 (d), 120.3 (s), 124.7 (d), 124.9 (d), 127.0 (d), 127.6 (d), 139.0 (s), 145.1 (s), 151.4 (s), 157.3 (s). Mass spectrum (70 eV) m/e (rel. intensity): 216 (M^+, 100), 188 (33), 185 (20), 155 (15), 115 (54), 108 (22).

4-tert-Butyldimethylsilyloxyphenoxythiin, 226

Prepared from 4-hydroxyphenoxythiin, 220, and t-butyldimethylsilyl-chloride according to published procedures.[194,195] The crude product was purified by distillation under reduced pressure (Kugelrohr, 90°C/0.8 torr) to afford pure 226 as a colorless viscous oil in 92% yield.

TLC: R_f 0.65 (J). HPLC: rt 10.15 min, 100% (K). ^1H NMR (250 MHz, $CDCl_3$), δ 0.29 (6H, s, dimethyl), 1.12 (9H, s, t-butyl), 6.73-6.78 (2H, m), 6.90 (1H, t; J = 8 Hz), 7.02=7.21 (4H, m).

4-Hydroxyphenoxythiin methoxymethyl ether, 227

Prepared from 220 and dimethoxymethane according to published procedures.[196] The crude product was purifed by vacuum distillation (Kugelrohr, 95°C/0.45 torr) to afford pure 227 as a clear oil in 38% yield.

TLC: R_f 0.34 (J). HPLC: rt 5.32 min, 99% (K). ^1H NMR (250 MHz, $CDCl_3$): δ 3.51 (3H, s), 5.21 (2H, s), 6.83 (1H, dd; J = 8,1 Hz), 6.92-7.11 (3H, m), 7.15-7.22 (3H, m).

4-Trimethylsilyl-6-hydroxyphenoxythiin, 221

For the metallation reaction the same procedure as for 208 (Method A) was used to afford 221 as a viscous light-yellow oil in 87% yield.

TLC: R_f 0.31 (E), 0.71 (D). HPLC: rt 6.53 min, 99% (F). ^1H NMR (250 MHz, $CDCl_3$): δ 0.50 (9H, s), 5.53 (1H, bs), 6.69 (1H, dd; J = 8,1 Hz), 6.86 (1H, dd; J = 8,1 Hz), 6.97 (1H, t; J = 8 Hz), 7.09 (1H, t; J = 8 Hz), 7.19 (1H, dd; J = 8,1 Hz), 7.29 (1H, dd; J = 8,1 Hz). Mass spectrum (70 eV) m/e (rel. intensity): 288 (M^+, 100), 257 (79), 242 (48), 136 (47), 75 (65).

4-Methoxyphenoxythiin, 229

Prepared from 220 following the same procedure as for 59. Kugelrohr distillation (88°C at 0.45 torr) of the crude product afforded pure 229 as a clear viscous oil in 94% yield.

TLC: R_f 0.30 (I), 0.66 (A). HPLC: rt 11.28 min, 96% (J). ^1H NMR (250 MHz, $CDCl_3$): δ 3.86 (3H, s, Ar:OCH_3), 6.68 (1H, dd; J = 8,1 Hz), 6.72 (1H, dd, J = 8,1 Hz), 6.89-7.03 (3H, m), 6.06-7.12 (2H, m). ^{13}C NMR (67.93 MHz, $CDCl_3$): δ 56.2 (Ar:OCH_3), 111.0 (d), 117.8 (d, C_6), 118.6 (d), 120.3 (s), 121.3 (s), 124.1 (d), 124.5 (d), 126.6 (d), 127.6 (d), 141.6 (s), 149.0 (s), 152.2 (s).

4-Trimethylsilyl-6-methoxyphenoxythiin, 225

Method A: For the metallation reaction the same procedure as for 208 (Method A) was used except that the base wash was omitted in the work-up. The crude product was triturated with ice-cold acetonitrile to yield pure 225 as a white crystalline solid in 49% yield, mp 98-9°C.

TLC: R_f 0.35 (G), 0.50 (I). HPLC: rt 8.43 min, 100% (C). ^1H NMR (250 MHz, $CDCl_3$): δ 0.42 (9H, s, TMS), 3.87 (3H, s, Ar:OCH_3)), 6.69 (1H, dd; J = 8,1 Hz), 6.75 (1H, dd; J = 8,1 Hz), 6.96 (1H, t; J = 8 Hz), 7.01 (1H, t; J = 8 Hz), 7.12 (1H, dd; J = 8,1 Hz), 7.26 (1H, dd; J = 8,1 Hz). Mass spectrum (70 eV) m/e (rel. intensity): 302 (M^+, 100), 287 (87), 244 (27), 144 (60), 136 (30).

Anal. Calcd for $C_{16}H_{18}O_{22}SSi$: C, 63.54; H, 6.00; S, 10.60; Si, 9.29. Found: C, 63.66; H, 5.97: S, 10.45; Si, 9.09.

Method B: 4-Trimethylsilyl-6-hydroxyphenoxythiin, 221 was methylated according to the procedure described for 59 to afford 225 as a white solid in 93% yield, mp 97-9°C. The methoxy compound prepared thus was shown to be identical with the metallation product (Method A) by mixed mp, HPLC, TLC, and ^{1}H NMR.

Structure Elucidation of 225:

Synthesis of 6-deutero-4-methoxyphenoxythiin, 228

To a solution of 4-trimethylsilyl-6-methoxyphenoxythiin, 225, (54.4 mg, 0.180 mmol) in tetrahydrofuran (2.00 mL) placed under N_2 was added at room temperature deuterium oxide (10 μL, 0.451 mmol) and tetrabutylammonium fluoride (0.66 mmol) in tetrahydrofuran (0.66 mL). The solution was stirred for 0.5 h, tetrabutylammonium fluoride (0.18 mmol) in THF (0.18 mL) was added and the mixture was stirred for 1 h. The solution was then quenched with D_2O, extracted with CH_2Cl_2 (3x), the organic layers were combined, washed with water (2x), dried ($MgSO_4$), and evaporated to yield 228 as an oil (39 mg, 94%).

TLC: R_f 0.30 (I). HPLC: rt 11.30 min, 98% (J). ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.89 (3H, s), 6.69 (1H, dd, J = 8,1 Hz), 6.73 (1H, dd, J = 8 Hz), 6.90-7.04 (2H, m), 6.07-7.11 (2H, m). ^{13}C NMR (67.93 MHz, $CDCl_3$): δ 56.3 (Ar:OCH_3), 111.1 (d), 117.5 (t, C_6), 118.7 (d), 120.4 (s), 121.4 (s), 124.0 (d), 124.5 (d), 126.6 (d), 127.5 (d), 141.8 (s), 149.2 (s), 152.3 (s). Mass spectrum (70 eV) m/e (rel. intensity): 231 (M^+, 100), 218 (24), 190 (44), 118 (38), 92 (8).

4-Trimethylsilyl-6-acetoxyphenoxythiin, 222

For the acylation reaction of 221 the same procedure as for 209, was used to afford 222 as a white solid in 100% yield, mp 112-3°C.

TLC: R_f 0.63 (D). HPLC: rt 7.20 min, 98% (F). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.40 (9H, s), 2.37 (3H, s), 6.90-7.05 (4H, m), 7.09 (1H, dd; J = 8,2 Hz), 7.22 (1H, dd; J = 7,2 Hz). Mass spectrum (70 eV) m/e (rel. intensity): 330 (M^+, 16), 228 (34), 257 (40), 242 (22), 227 (7).

4-Bromomercury-6-acetoxyphenoxythiin, 223

To a light-pink solution of 222 (33.0 mg, 0.10 mmol) in trifluoroacetic acid (3.0 mL) placed under N_2 was added in one portion at room temperature a freshly prepared solution of mercuric trifluoroacetate (42.3 mg, 0.10 mmol) in the same solvent (3.0 mL). Within 1 min the clear mixture first turned yellow and then became colorless. The solution was stirred under these conditions for 10 min and then the solvent was removed under reduced pressure. The resulting white solid residue was dissolved in acetone (2.1 mL), a solution of sodium bromide (21.0 mg, 0.20 mmol) in water (0.50 mL) was added and the mixture was stirred for 5 min. The precipitate that formed was collected by filtration, washed with water (2x), and dried under high vacuum over P_2O_5 to afford pure 223 as a white powder (38 mg, 71%), mp 194-5°C. The filtrates were extracted with CH_2Cl_2 (3x), the organic phases were combined, washed with water, dried ($MgSO_4$), and evaporated. The resulting light-yellow moist solid residue (23 mg) was recrystallized from CH_2Cl_2-pentane to yield white needles as a second crop (7 mg, 13%), mp 193-5°C.

TLC: R_f 0.43 (D). HPLC: rt 3.47 min, 100% (I). ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.53 (3H, s), 6.90 (1H, dd; J = 7,2 Hz), 6.95-7.04 (2H, m), 7.11-7.14 (3H, m). Field desorption mass spectrum: m/e 538 (M^+).

Anal. Calcd for $C_{14}H_9O_3BrHg$: C, 31.27; H, 1.69; Hg, 37.30. Found: C, 31.12; H, 1.80; Hg, 36.96.

Rate determination for the intramolecular acyl transfer which occurs on treatment of 223 with methyl cysteinate

Kinetics of the acyl transfer reaction were performed at ambient temperature (25°C) and followed by 270 MHz ^{1}H NMR at 9.8×10^{-2} M in each 223 and L-cysteine methyl ester hydrochloride, and 19.6×10^{-2} M in dimethylaminopyridine. The half-time for the reaction was measured when the integration of the appropriate acetyl methyl resonances was equivalent. After 5 half-times no precipitation of elemental mercury was observed.

Solvent	$t_{1/2}$ (h)	$k_{intram.}$ (h^{-1})
DMSO-d_6	12	5.78×10^{-2}

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