temperature. Saturated aqueous $NaHCO_3$ (0.7 mL) was added, and then the resulting precipitates were removed by filtration and washed with ethyl acetate. The filtrate was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was subjected to preparative TLC on silica gel with CH_2Cl_2 to give a 42% (6 mg) recovery of **15a** and 51% yield (7.3 mg) of **17a** (1:1 mixture of anomeric isomers) as colorless syrup: $[\alpha]^{20}_D$ +23.2° (*c* 0.293, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.156 (d, 0.5 × 3 H, *J* = 7.32 Hz, CH*Me*), 1.214 (d, 0.5 × 3 H, *J* = 6.72 Hz, CH*Me*), 2.565 (dquin, 0.5 H, *J* = 6.72 and 4.89 Hz, 2-H), 2.632 (dquin, 0.5 H, *J* = 7.32 and 3.66 Hz, 2-H), 2.775 (br d, 0.5 Hz, *J* = 6.11 Hz, OH), 3.06 (br s, 0.5 H, OH), 4.47–4.65 (m, 3 H, 4,5,5′-H), 5.323 (br s, 0.5 H, 1-H), 5.48–5.52 (m, 1 H, 3-H, 1-H), 5.605 (dd, 0.5 H, *J* = 6.11 and 3.66 Hz, 3-H), 7.37–7.64 (m, 6 H, Ar H), 7.95–8.15 (m,, 4 H, Ar H); IR ($CHCl_3$) 1720, 1270 cm^{-1}; exact mass calcd for $C_{20}H_{20}O_6$-OH 339.1232, found 339.1247.

3,5-*O*-Dibenzoyl-2-deoxy-2(*R*)-*C*-ethyl-*erythro*-D-pentofuranose (17b). This (5.8 mg, 34%, 43:57 mixture of anomeric isomers) was prepared from **15b** (17 mg, 0.046 mmol) and 1.75 M solution of DIBAL in toluene (53 μL, 0.092 mmol) in dry THF (1 mL) by a similar method as described for **17a** from **15a**. There was a 25% (4.3 mg) recovery of **15b**. **17b**: $[\alpha]^{20}_D$ +10.8° (*c* 0.195, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.017 (t, $^{43}/_{100}$ × 3 H, *J* = 7.32 Hz, CH_2CH_3), 1.036 (t, $^{57}/_{100}$ × 3 H, *J* = 7.33 Hz, CH_2CH_3), 1.56–1.80 (m, 2 H, CH_2CH_3), 2.27–2.45 (m, 1 H, 2-H), 2.6–2.9 (br s, $^{57}/_{100}$ H, OH), 2.9–3.4 (br s, $^{43}/_{100}$ H, OH), 4.454 (dt, $^{43}/_{100}$ H, *J* = 5.49 and 2.44 Hz, 4-H), 4.525 (dd, $^{57}/_{100}$ H, *J* = 11.59 and 4.88 Hz, 5-H), 4.547 (dd, $^{57}/_{100}$ H, *J* = 11.59 and 4.88 Hz, 5′-H), 4.57–4.67 (m, $^{43}/_{100}$ × 2 H, 5,5′-H), 4.57–4.64 (m, $^{57}/_{100}$ H, 4-H), 5.419 (d, $^{43}/_{100}$ H, *J* = 4.27 Hz, 1-H), 5.527 (d, $^{57}/_{100}$ H, *J* = 6.10 Hz, 3-H), 5.549 (d, $^{57}/_{100}$ H, *J* = 4.89 Hz, 1-H), 5.628 (dd, $^{43}/_{100}$ H, *J* = 6.1 and 2.44 Hz, 3-H), 7.35–7.65 (m, 6 H, Ar H), 7.95–8.10 (m, 4 H, Ar H); IR ($CHCl_3$) 1720, 1270 cm^{-1}; exact mass calcd for $C_{21}H_{22}O_6$-OH 353.1386, found 353.1381.

Registry No. **1a**, 77086-38-5; **1b**, 31469-15-5; (*E*)-**1c**, 89597-33-1; (*Z*)-**1c**, 90541-64-3; (*E*)-**1d**, 72658-09-4; (*Z*)-**1e**, 84784-64-5; (*E*)-**1f**, 58367-55-8; (*Z*)-**1f**, 58367-60-5; D-**2**, 15186-48-8; L-**2**, 22323-80-4; D-**3a**, 104578-83-8; L-**3a**, 104578-88-3; D-**3b**, 104578-84-9; L-**3b**, 104578-87-2; D-**4a**, 104578-85-0; D-**4b**, 104578-86-1; D-**5a**, 83159-90-4; D-**6a**, 34371-14-7; L-**6a**, 38996-14-4; D-**7a**, 84044-97-3; L-**7a**, 112021-04-2; D-**8a**, 81366-70-3; L-**8a**, 104578-89-4; D-**9a**, 104578-90-7; L-**9a**, 104578-92-9; D-**9b**, 104602-05-3; L-**9b**, 104578-91-8; D-**10a**, 104578-93-0; L-**10a**, 104578-94-1; D-**10b**, 111998-43-7; L-**10b**, 111998-44-8; **11a**, 111998-45-9; **11b**, 112021-05-3; **11c**, 111998-46-0; **11d**, 111998-47-1; **12a**, 111998-48-2; **12b**, 111998-49-3; **12c**, 111998-50-6; **12d**, 111998-51-7; **13a**, 111998-52-8; **13b**, 111998-53-9; **13c**, 111998-54-0; **13d**, 111998-55-1; **14a**, 111998-56-2; **14b**, 111998-57-3; **14c**, 111998-58-4; **14d**, 111998-59-5; **15a**, 98587-14-5; **15b**, 111998-60-8; **16a**, 98587-15-6; α-**17a**, 111998-61-9; β-**17a**, 111998-62-0; α-**17b**, 111998-63-1; β-**17b**, 111998-64-2; Et_3SiH, 617-86-7; Et_3SiCl, 994-30-9; CH_3CH_2COOMe, 554-12-1.

The *p*-(Methylsulfinyl)benzyl Group: A TFA-Stable Carboxyl-Protecting Group Readily Convertible to a TFA-Labile Group[1,2]

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The *p*-(methylsulfinyl)benzyl or Msib ester is recommended as a selectively cleavable carboxyl-protecting group for peptide synthesis. Peptide or amino acid esters of *p*-(methylsulfinyl)benzyl alcohol, HO-Msib (**2**), or *p*-(methylthio)benzyl alcohol, HO-Mtb (**1**), are readily prepared from the corresponding alcohols or alkyl halides. Msib esters may also be obtained from Mtb esters by oxidation with *m*-chloroperbenzoic acid. Msib esters are readily deoxygenated by excess Me_3SiCl/Ph_3P, Me_3SiCl/Me_2S, or anhydrous hydrogen chloride. Msib esters are exceedingly stable to TFA while Mtb esters solvolyze rapidly. A sample peptide synthesis demonstrates the protection of the C-terminal carboxyl group during synthesis as the Msib ester followed by Msib group removal on completion of synthesis by deoxygenation and TFA solvolysis. The stability of Mtb and Msib esters to typical acid conditions of peptide synthesis is described. The stability of Msib esters to various peptide synthesis conditions suggests that the Msib group should be quite useful as a carboxyl-protecting group in peptide synthesis.

Of the many approaches toward the development of useful peptide synthesis protecting groups, the concept of converting a stable protecting group into a labile protecting group[3] has been quite useful. This concept was behind the development of the *p*-nitrobenzyl group (reduced with zinc in acetic acid),[4] the dihydroxyborylbenzyloxycarbonyl group (oxidized with peroxide),[5] the hydroxymethylanthraquinone group (reduced by photolysis or dihydroanthraquinone),[6] the *p*-thiophenyl group (oxidized with peroxide),[7] the 5-benzisoxazolylmethyleneoxycarbonyl or Bic Group (activated with base),[8] the [2-(trifluoromethyl)-6-chromonyl]methyleneoxycarbonyl group (activated with propylamine),[9] and several groups that become base labile following oxidation.[10] The utility of each of these groups is hampered variously by the requirement of an aqueous medium (unsuitable for polystyrene-anchored solid-phase peptide synthesis),[11] the danger of Met or Trp

(1) Preliminary communication: Samanen, J.; Brandeis, E. In *Peptides: Structure and Function; Proceedings of the 9th American Peptide Symposium*; Deber, C., Kopple, K., Eds.; Pierce Chemical: Rockford, IL, 1985; pp 225–228.

(2) A number of abbreviations are used in this paper. The abbreviations for natural amino acids and nomenclature for peptide structures follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are as follows: Mtb = *p*-(methylthio)benzyl and Msib = *p*-(methylsulfinyl)benzyl, (previously abbreviated as B(S) and B(SO) esters[1]), TFA = trifluoroacetic acid, TFAA = trifluoroacetic anhydride, Fmoc = (9-fluorenylmethoxy)carbonyl, DCC = dicyclohexylcarbodiimide, MCPBA = *m*-chloroperbenzoic acid, EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, HOBt = *N*-hydroxybenzotriazole, DMF = dimethylformamide, BSA = bis(trimethylsilyl)acetamide, Ph_3P = triphenylphosphine.

(3) Kemp, D. S.; Roberts, D.; Hoyng, C.; Grattan, J.; Vellacio, F.; Reczek, J. In *Peptides: Chemistry, Structure Biology; Proceedings of the 4th American Peptide Symposium*; Walter, R., Meienhofer, J., Eds.; Ann Arbor Science: Ann Arbor, 1975; pp 295–305.

(4) Suzuki, K.; Endo, N.; Nitta, K.; Sasaki, Y. In *Peptide Chemistry 1976*; Nakajima, T., Ed.; Protein Research Foundation: Osaka, 1977; pp 45–48.

(5) Kemp, D. S.; Roberts, D. C. *Tetrahedron Lett.* **1975**, 4625–4628.

(6) Kemp, D. S.; Reczck, J. *Tetrahedron Lett.* **1977**, 1031–1034.

(7) Marshall, D. L.; Liener, I. E. *J. Org. Chem.* **1970**, *35*, 867–868.

(8) Kemp, D. S.; Hoyng, C. F. *Tetrahedron Lett.* **1975**, 4625–4628.

(9) Kemp, D. S.; Hansen, G. *J. Org. Chem.* **1981**, *46*, 4971–4975.

(10) Roeske, R. W. In *The Peptides, Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1981; Vol. 3, Chapter 2, pp 122–123.

0022-3263/88/1953-0561$01.50/0

Table I. Synthesis of Mtb and Msib Derivatives[a]

starting materials	condtns	product	yield, %
Z-Phe-OH (5); HO-Mtb (1)	EDCI, HOBt, $CHCl_3$	Z-Phe-OMtb (6)	78
Z-Phe-OMtb (6)	MCPBA, $CHCl_3$, 0 °C	Z-Phe-OMsib (7)	50
Z-Phe-OH (5); HO-Msib (2)	EDCI, HOBt, $CHCl_3$	Z-Phe-OMsib (7)	100
Boc-Phe (8); HO-Msib (2)	EDCI, HOBt, $CHCl_3$	Boc-Phe-OMsib (9)	94
Boc-Met; HO-Msib (2)	EDCI, HOBt, $CHCl_3$	Boc-Met-OMsib (11)	96
Asp, Asp–Cu complex 12, HO-Mtb (1)	DMF, H_2O	(β-OMtb)Asp (13)	56
Glu, Glu–Cu complex 14, HO-Mtb (1)	DMF, H_2O	(γ-OMtb)Glu (15)	37
Ac_2O; HO-Mtb (1)	Et_3N	Ac-OMtb (16)	100
Ac-OMtb (16)	H_2O_2, AcOH	Ac-OMsib (17)	91
HO-Mtb (1)	TFA	TFA-OMtb (18)	100

[a] Temperature employed was 25 °C unless noted otherwise.

Table II. Acid Stability Studies[a]

rctn no.	starting ester	rctn condtns	anal. time	(% yield[b] of) products
1	6	glacial AcOH	3 days	5, <1; 6, >99
2	6	anhydrous TFA	30 min	5, >98; 6, <2
3	6	anhydrous HCl/dioxane	29 h	5, ≪1; 6, >99
4	7	anhydrous HCl/dioxane	30 min	6, 15; 5, 0
			4 h and 29 h	6, 100; 5, 0
		above + 10 equiv of H_2O	20 h	6, 70; 5, 30
		above + 90 equiv of H_2O	20 h	6, 50; 5, 50
5	9	anhydrous TFA	2 mo	19, 97; 20, 3.3 (by AAA)
6	9	HBr/TFA	1 h	19, 20; 20, 80
7	7	HF/anisole, 0 °C	1 h	19, >90; 20, <10

[a] Complete experimental details are available as supplementary material. Z-Phe-OH = 5, Z-Phe-OMtb = 6, Z-Phe-OMsib = 7, Boc-Phe-OMsib = 9, Phe-OMsib = 19, H-Phe-OH = 20. [b] Percent yield based on TLC.

oxidation,[1] sensitivity to visible light, lengthy reaction times, or multistep syntheses.

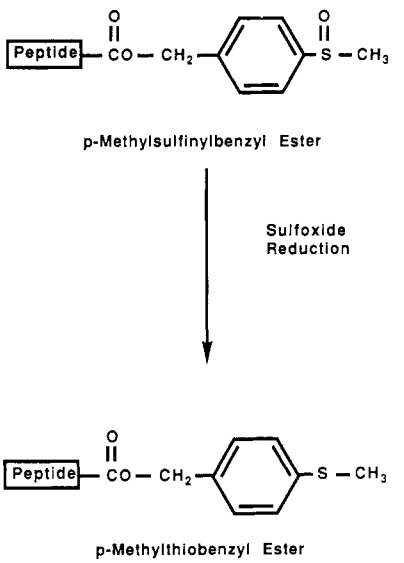
There is still a need to develop amine- and carboxyl-protecting groups that are easy to prepare, are stable to the conditions of peptide synthesis (especially DCC, Et_3N, and TFA), but are rapidly removed in high yield under mild conditions, especially while the peptide is still attached to a synthesis resin. Such protecting groups can be used for selective removal from a fully protected peptide for (a) side-chain derivatization, (b) peptide-chain elongation through the side chain, and (c) formation of a cyclic amide or ester between the peptide chain and a side chain or between side chains.

This paper describes the development of the *p*-(methylsulfinyl)benzyl or Msib group shown in Scheme I in a carboxylic acid ester.[11] Sulfoxide reduction gives a *p*-(methylthio)benzyl or Mtb group, which is cleavable in anhydrous TFA. The present paper describes the synthesis of Mtb and Msib amino acid and peptide esters, the stability of the Msib group to the conditions of peptide synthesis, the methods of reduction to the Mtb group, and the lability of the Mtb and Msib groups toward various acids.

Preparation of Msib Esters. Two reagents have been prepared for synthesis of Msib esters: *p*-(methylsulfinyl)benzyl alcohol (2) and *p*-(methylsulfinyl)benzyl chloride (4) (Scheme II). Reagent 2 is one simple step from the commercially available alcohol 1, and reagent 4 is two simple steps from alcohol 1.

These reagents can be used to form peptide or amino acid esters by standard esterification procedures (Table I). Several amino acid and peptide Msib esters have been prepared in good yields. Z-Phe-OMtb (6) and Z-Phe-OMsib (7) proved to be the best model Mtb and Msib esters for stability studies in most experiments. Msib esters may be obtained from the corresponding Mtb esters by oxidation with *m*-chloroperbenzoic acid. This route is not recommended for amino acids with oxidizable side chains

Scheme I. Structure of *p*-(Methylthio)benzyl Esters and *p*-(Methylsulfinyl)benzyl Esters



(e.g., Met, Trp, and *S*-alkylcysteine). The β and γ Mtb esters of Asp and Glu (13 and 15) have been prepared from the corresponding copper chelates (Table I).

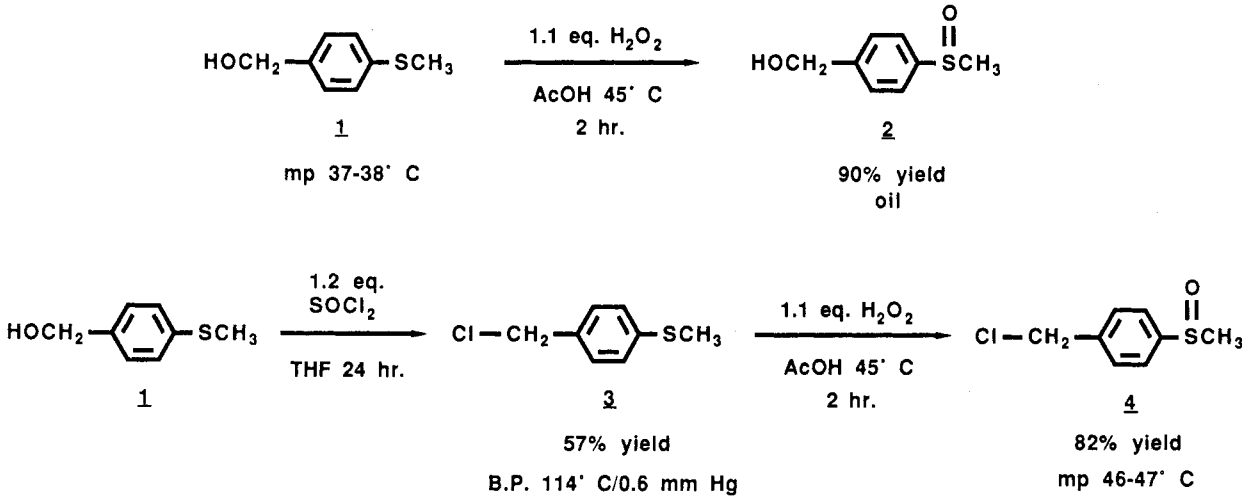
The Behavior of Mtb and Msib Esters in Typical Acid Conditions of Peptide Synthesis. A. *p*-(Methylthio)benzyl (Mtb) Esters. The *p*-(methylthio)benzyl ester 6 of Z-Phe is quite stable to glacial acetic acid (reaction 1, Table II) but solvolyzes completely in 30 min in anhydrous TFA (reaction 2). In preparative reactions the addition of Me_2S is recommended to trap the benzyl carbonium ion as the stable dimethylsulfonium salt.

Although *p*-methoxybenzyl esters are known to cleave in dilute solutions of hydrogen chloride,[12] the *p*-methylthio ester 6 was stable to anhydrous HCl in dioxane for up to 29 h (reaction 3). The σ_p^+ constant for the *p*-methoxy

(11) Stewart, J. M.; Young, J. D. In *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical: Rockford, IL, 1984; pp 1–135.

(12) Stelakatos, G. C.; Argyropoulos, N. *J. Chem. Soc. C* **1970**, 964–967.

Scheme II. Reagent Synthesis



group (–0.648)[13] is considerably larger than that of the *p*-methylthio group (–0.164).[13] Hence, solvolysis of *p*-(methylthio)benzyl esters is only observed in solvents with a very large ionizing power,[14] e.g., TFA.

As will be described in the next section, Msib esters suffer reduction in HCl/dioxane to Mtb esters.

B. *p*-(Methylsulfinyl)benzyl (Msib) Esters. The Msib ester is exceptionally stable to trifluoroacetic acid. The *p*-(methylsulfinyl)benzyl ester **19** of phenylalanine was stable to anhydrous trifluoroacetic acid for 2 months. Only a trace of cleavage product phenylalanine (**20**) (3.3%) was detected after 2 months by amino acid analysis (reaction 5, Table II). This stability would correspond to the exposure of an Msib ester to at least 2880 half-hour TFA treatments during peptide synthesis with 0.000038% ester cleavage/min.

As with other sulfoxides,[15] the *p*-(methylsulfinyl)benzyl ester **7** suffers reduction on exposure to hydrogen chloride in anhydrous dioxane (reaction 4). Reduction is complete after 4 h. Only a trace of cleavage product Z-Phe (**5**) was detected. Addition of H_2O is followed by incomplete solvolysis to Z-Phe. Only 30% Z-Phe was observed 20 h after addition of 10 equiv of H_2O to an HCl/dioxane mixture (reaction 4).

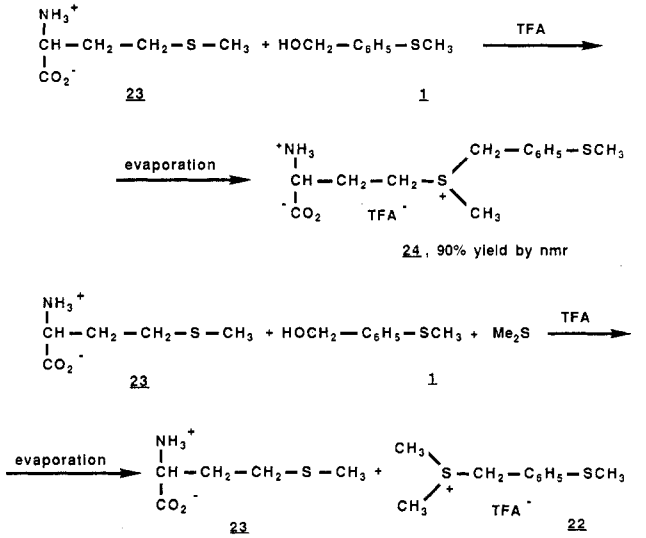
Treatment of Boc-Phe-OMsib (**9**) in a solution of trifluoroacetic acid saturated with hydrogen bromide gave a mixture of Phe-OMsib (**19**) (20%) and Phe (**20**) (80%) (reaction 6). The product mixture presumably arose from sulfoxide reduction followed by ester cleavage.

Treatment of Z-Phe-OMsib (**7**) with anhydrous hydrogen fluoride gave mostly Phe-OMsib (**19**) (>90%) and a small amount of Phe (**20**) (<10%) (reaction 7). Presumably sulfoxide reduction is much less efficient in anhydrous HF at 0 °C. This stability of *p*-(methylsulfinyl)benzyl ester to HF is comparable to the stability of a *p*-nitrobenzyl ester, which was reported to suffer 15% cleavage at 20 °C after 1 h.[16]

This demonstration of HF stability suggests that peptide esters attached to an analogous solid phase synthesis resin anchor group would be much more resistant to acidolysis than the PAM anchor group.[17] Peptides attached to PAM resin are completely cleaved in anhydrous HF.

C. Trapping the *p*-(Methylthio)benzyl Carbonium Ion with Dimethyl Sulfide (Scheme III). TFA solutions of *p*-(methylthio)benzyl alcohol (**1**) and acetate **16** form deep red to blue colors. Solutions from **1** and **16** lose color on evaporation, and *p*-(methylthio)benzyl trifluoroacetate (**18**) is recovered in quantitative yield (Scheme III). The TFA solution of *p*-(methylthio)benzyl chloride (**3**) is colorless, but on evaporation a mixture of **3** and **18** is obtained. Presumably these compounds solvolyze to the benzyl carbonium ion **20**, which during solvent removal collapses to the colorless trifluoroacetate. Addition of 5 equiv of Me_2S to such a TFA solution is followed by loss of blue color. Upon evaporation the dimethyl[*p*-(methylthio)benzyl]sulfonium salt **22** is obtained in quantitative yield.

Evaporating an equimolar TFA solution of *p*-(methylthio)benzyl alcohol and methionine gives a mixture that contains 83% (3-amino-3-carboxypropyl)methyl[*p*-(methylthio)benzyl]sulfonium trifluoroacetate (**24**). In the final



stages of evaporation, the dialkyl sulfide side chain of methionine successfully competes with TFA for trapping the benzyl carbonium ion. Repeating the same experiment with the addition of 20 equiv of Me_2S, however, gives a clean mixture of methionine (**23**) and dimethyl[*p*-(meth-

(13) Swain, C. G.; Lupton, E. C., Jr. *J. Am. Chem. Soc.* **1968**, *90*, 4328–4337.
(14) March, J. In *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 2nd ed.; McGraw-Hill: New York, 1977; p 333.
(15) Drabowicz, J.; Numata, T.; Oae, S. *Org. Prep. Proced. Int.* **1977**, *9*, 64–83.
(16) Sakakibara, S. In *Chemistry and Biochemistry of Amino Acids*; Weinstein, B., Ed.; M. Deckker: New York, 1971; Chapter 3, p 72.
(17) Mitchell, A. R.; Erickson, B. W.; Ryabtsev, M. N.; Hodges, R. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1976**, *98*, 7357–7362.

Scheme III. Trapping the *p*-(Methylthio)benzyl Carbonium Ion with Dimethyl Sulfide

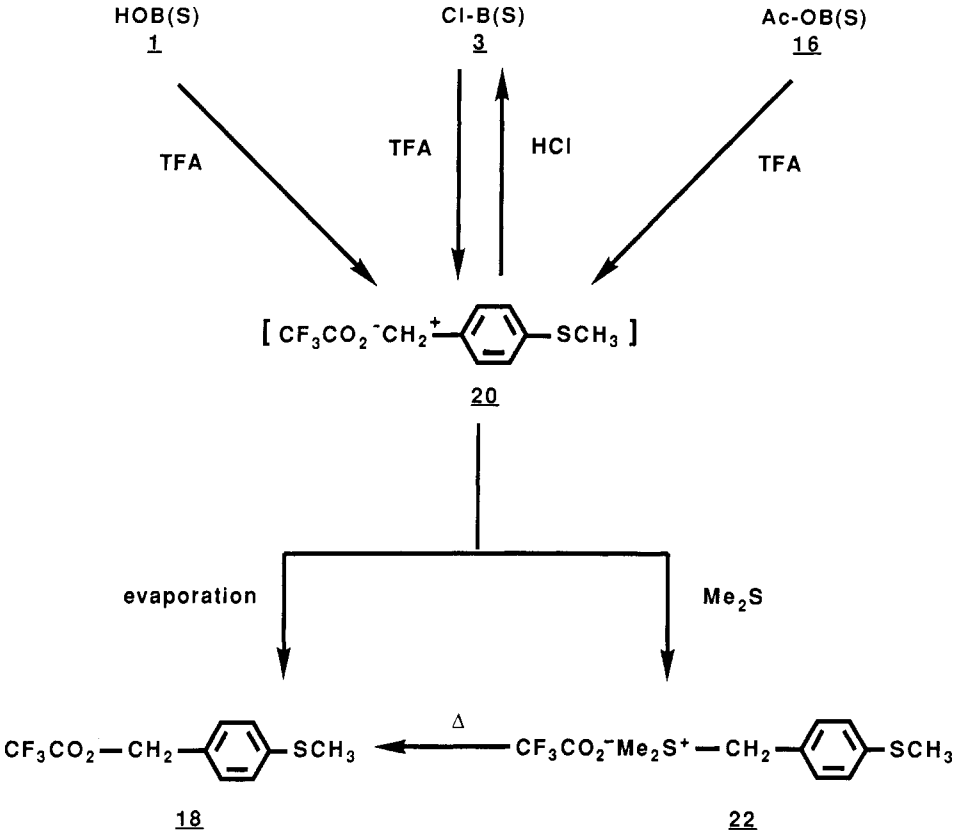


Table III. Sulfoxide Deoxygenation Studies[a]

rctn no.	starting ester	rctn condtns	anal. time	(TLC % yield of) products
1	7	10 equiv of $HSCH_2CO_2H$, pH 2.6, aqueous AcOH	22 h	**6**, 0
2	7	10 equiv of $HSCH_2CO_2H$, pH 7	20 h	**6**, 0
3	11	$CDCl_3$	32 mo	**25**, 0 (by NMR)
4	7	1.1 equiv of TFAA, 2 equiv of Me_2S, −18 °C, THF	10 min	**6**, ≫98
5	7	10 equiv of $TiCl_4$, 20 equiv of Zn, 0 °C, THF	2 h	**6**, 95; **5**, 0
			6 h	**6**, 10; **5**, 90
6	7	10 equiv of $(Me_3Si)_2S$, THF	5 h	**6**, ≫98
7	7	20 equiv of Me_3SiCl, 10 equiv of Ph_3P, THF	1 min	**6**, ≫98
8	7	10 equiv of $ZnCl_2$, 20 equiv of Me_2S, THF	24 h	**6**, 0
9	7	20 equiv of Me_3SiCl, 40 equiv of Me_2S, THF	1 h	**6**, >95; **7**, <5
10	9	10 equiv of Me_3SiCl, 20 equiv of Me_2S, 1 drop of pyridine, THF	5 h	**10**, >98
11	26	10 equiv of Me_3SiCl, 20 equiv of Me_2S, THF	2 h	**27**, 100 (isolated)

[a] Complete experimental details are available as supplementary material. Z-Phe-OMtb = **6**, Z-Phe-OMsib = **7**, Boc-Phe-OMsib = **9**, Boc-Phe-OMtb = **10**, Boc-Met-OMsib = **11**, Boc-Met(O)-OMtb = **25**, Z-Met(O)-OMe = **26**, Z-Met-OMe = **27**.

ylthio)benzyl]sulfonium trifluoroacetate (**22**). Hence in preparative reactions it is recommended that Mtb ester cleavage be performed in the presence of excess Me_2S. The dimethyl[*p*-(methylthio)benzyl]sulfonium salt **22** is soluble in acetone, ethyl acetate, dioxane, and water, but insoluble in ether, hexane, and CCl_4. Workup should avoid concentration of the TFA solution but should include either peptide precipitation from the TFA solution or dilution with water followed by peptide recovery via precipitation, extraction, gel filtration, or ion exchange. In a subsequent section, synthesis of an enkephalin derivative is described in which Mtb ester cleavage proceeded without degradation of the methionine residue.

Methods of Sulfoxide Reduction (Deoxygenation). The *p*-(methylsulfinyl)benzyl ester is a relatively stable sulfoxide. Mercaptoacetic acid, commonly used to reduce methionine sulfoxide in peptides,[18] does not reduce Z-Phe-OMsib (**7**) (reactions 1, 2, Table III). An Msib ester of a peptide or amino acid containing methionine or an *S*-alkylcysteine will not be deoxygenated by such side chains, since a solution of Boc-Met-OMsib (**11**) failed to rearrange to Boc-Met(O)-OMtb (**25**) after 32 months (reaction 3).

A number of reagents were found to reduce an Msib ester at or below room temperature: TFAA/Me_2S,[20] $TiCl_4$/Zn,[21] $(Me_3Si)_2S$,[22] $Me_3SiCl/HSC_6H_4CH_3$,[23] HCl/dioxane, Me_3SiCl/Ph_3P, and Me_3SiCl/Me_2S. The reagent of choice proved to be Me_3SiCl/Ph_3P, while Me_3SiCl/Me_2S

(18) Houghton, R. A.; Li, C. H. *Anal. Biochem.* **1979**, *98*, 36–46.

(19) Castrillon, J. P. A.; Szmant, H. H. *J. Org. Chem.* **1965**, *30*, 1338–1339.

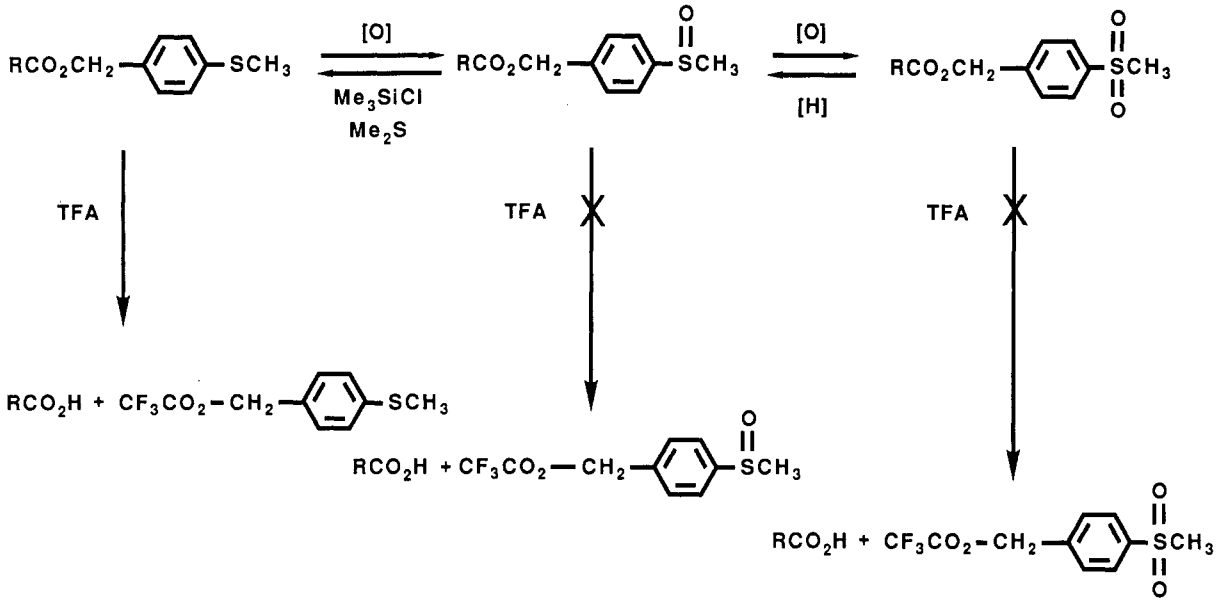
(20) Tanikaga, R.; Nakayama, K.; Tanaka, K.; Kaji, A. *Chem. Lett.* **1977**, 395–396.

(21) Drabowicz, J.; Mikolajczyk, M. *Synthesis* **1978**, 138–139.

(22) Dilanjan-Soysa, H. S.; Weber, W. P. *Tetrahedron Lett.* **1978**, 235–238.

(23) Numata, T.; Togo, H.; Oae, S. *Chem. Lett.* **1979**, 329–332.

Scheme IV. Oxidation, Reduction, and Acidolytic Pathways of p-(Methylthio, -sulfinyl, and -sulfonyl)benzyl Esters



and hydrogen chloride are quite satisfactory for many applications. Complete reduction of Z-Phe-OMsib (7) occurs in about 1 min in the presence of excesses of both Me_3SiCl and triphenylphosphine (reaction 7). Without Me_3SiCl, sulfoxide deoxygenation by phosphines typically employs elevated temperatures.[15,19] Deoxygenation of silyl sulfoxide by Ph_3P, however, proved to be quite rapid (reaction 7). Complete reduction is also achieved in the presence of excesses of both Me_3SiCl and Me_2S (reaction 9), but the reaction is more sluggish (1 h). When HCl is vigorously excluded by employment of freshly distilled Me_3SiCl and a drop of pyridine, reduction can be performed in the presence of Boc groups (reaction 10). Since TFA is employed to subsequently cleave the p-(methylsulfinyl)benzyl ester, concern for traces of HCl in the reduction mixture will usually be unnecessary. Deoxygenation by hydrogen chloride has been described in the previous section.

An added benefit from employment of the Msib group is that any oxidized methionine or S-alkylcysteine in a peptide would be reduced simultaneously during reduction of the Msib group. This was demonstrated by reduction of Z-Met(O)-OMe (26) with Me_3SiCl/Me_2S in quantitative yield (reaction 11).

Several reaction mechanisms could be advanced for sulfoxide deoxygenation by Me_3SiCl/Ph_3P or Me_3SiCl/Me_2S. On the basis of other similar reagents,[20,22-24] the deoxygenation mechanism is presumed to proceed via an O-silylated sulfoxide.

A study of optimal conditions for reduction of silylated sulfoxides (as detailed in the supplementary material) revealed that excesses of both silylation and deoxygenation reagents are important for pushing the reaction to completion in reasonable time. Chlorotrimethylsilane alone fails to effect reduction. While deoxygenation by 1.1 equiv of Me_3SiCl/1.0 equiv of Ph_3P proceeded to 90% yield in 1 h, deoxygenation was complete after just 1 min with 10 equiv of Me_3SiCl/10 equiv of Ph_3P.

Deoxygenation with $Me_3SiCl/HSC_6H_4CH_3$, previously reported by Numata et al.,[23] gave results analogous to those for Me_3SiCl/Me_2S. Since the odor of methylbenzenethiol is much stronger, dimethyl sulfide is preferred. Olah[24] has also described sulfoxide reduction with Me_3SiCl/NaI. The potential for iodination with this reagent, due to contamination with I_2, limits the utility of this reagent.

Large excesses of amines block reduction of silyl sulfoxides. No reaction was observed with hexamethyldisilazane or with 10 equiv of Me_3SiCl/20 equiv of Me_2S in the presence of 20 equiv of triethylamine. Excess bis(trimethylsilyl)acetamide in the presence of excess Me_2S did not reduce Z-Phe-OMsib. The greater stability of silylamine or silylamide over O-silyl sulfoxide is inferred by these reactions.

Since Me_3SiCl is used in excess in these deoxygenations, side chain or C-terminal carboxyl groups will be converted to trimethylsilyl (TMS) esters during the reaction. Deoxygenation reactions of Msib peptide esters containing unprotected carboxyl groups should be quenched with dilute acid or base to hydrolyze the TMS esters. A quench with alcohol could produce the corresponding alkyl ester.[25]

Stability of p-(Methylsulfinyl)benzyl Esters to Oxidation. Oxidizing agents should be avoided in peptide syntheses employing the Msib group. Oxidation of the sulfoxide to sulfone is a reaction that would prevent reduction to sulfide when needed. Sulfones are generally resistant to reduction except under harsh conditions, e.g., diisobutylaluminum hydride.[26]

Stability of p-(Methylthio)benzyl and p-(Methylsulfinyl)benzyl Esters to Oxidation. The p-(methylsulfinyl)benzyl function is quite resistant to air oxidation. Air was bubbled into a D_2O solution of p-(methylsulfinyl)benzyl alcohol (2) for 29 days. NMR analysis of the solution revealed the presence of only 1.3% sulfone. Thus Msib esters should not suffer oxidation under normal handling. Reoxidation of a p-(methylthio)benzyl ester to sulfoxide would prevent cleavage of the ester in THF. As an example, p-(methylthio)benzyl alcohol (1) is fairly resistant to oxidation. Only 21.5% of the sulfinylbenzyl alcohol 2 was obtained after air was bubbled into a solution of the thiobenzyl alcohol 1 for 73 days. A 40% yield of 2 was obtained after treatment of 1 with sodium metaperiodate for 2 h at 45 °C while a 90% yield of 2 was

(24) Olah, G. A.; Narang, S. C.; Gupta, B. G. B.; Malhorta, R. *Synthesis* **1979**, 61–62.

(25) Nakao, R.; Oka, K.; Fukumoto, T. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1267–1268.

(26) Gardner, J. N.; Kaiser, S.; Krabiner, A.; Cevas, H. *Can. J. Chem.* **1973**, *51*, 1419–1421.

Table IV. Stability of Amino Acids to Msib Reduction Conditions[a]

rctn no.	amino acid	condtns	anal. time	% remaining
1A	Z-Trp	20 equiv of Me_3SiCl, 40 equiv of Me_2S, THF	5 h	>95
1B	Z-Trp	above + 1 equiv of Z-Phe-OMsib after 5 h	40 min	<40
2A	Z-Trp	20 equiv of Me_3SiCl, 10 equiv of Ph_3P, THF	5 h	100
2B	Z-Trp	above + 1 equiv of Ac-OMsib after 5 h	40 min	98*[b]
			24 h	98*
3A	Z-Trp	HCl(g), dioxane	3 h	60
3B	Z-Trp	above + 1 equiv of Ac-OMsib after 3 h	2 h	0
4A	Z-Trp	HCl(g), 10 equiv of Ph_3P, dioxane	3 h	97
4B	Z-Trp	above + 1 equiv of Ac-OMsib after 3 h	2 h	95
5	Z-(NO_2)Arg	20 equiv of Me_3SiCl, 10 equiv of Ph_3P, 1 equiv of Ac-OMsib, dioxane	8 h	95
6	Z-(Tos)Arg	20 equiv of Me_3SiCl, 10 equiv of Ph_3P, 1 equiv of Ac-OMsib, dioxane	8 h	100
7	Z-Gln-OMe	20 equiv of Me_3SiCl, 10 equiv of Ph_3P, 1 equiv of Ac-OMsib, dioxane	8 h	100

[a] Complete experimental details are available as supplementary material. [b] After TFA quench.

obtained with hydrogen peroxide oxidation of 1 for 2 h at 45 °C. These reactions demonstrate that *p*-(methylthio)benzyl compounds will not suffer oxidation under normal handling. Scheme IV summarizes the oxidation, reduction, and acidolytic pathways of *p*-(methylthio, -sulfinyl, and -sulfonyl)benzyl esters.

Stability of Amino Acids to Msib Reduction. Most of the amino acids should not suffer degradation during Msib deoxygenation by any of the methods described. Choice of deoxygenation reagent is critical for tryptophan, however. Msib deoxygenation can be performed with Me_3SiCl/Ph_3P in the presence of tryptophan with only minor degradation and no color generation (Table IV, reactions 2A and 2B). Msib deoxygenation with either Me_3SiCl/Me_2S or HCl/dioxane will lead to extensive degradation of tryptophan (reactions 1A, 1B, 3A, and 3B). In both cases a chlorosulfonium ion is presumed to be the species that halogenates the indole group.[27] When triphenylphosphine is present during HCl/dioxane treatment of Z-Trp, degradation is not observed (reaction 4A), and only trace amounts of degradation products are observed when Ac-OMsib is added (reaction 4B).

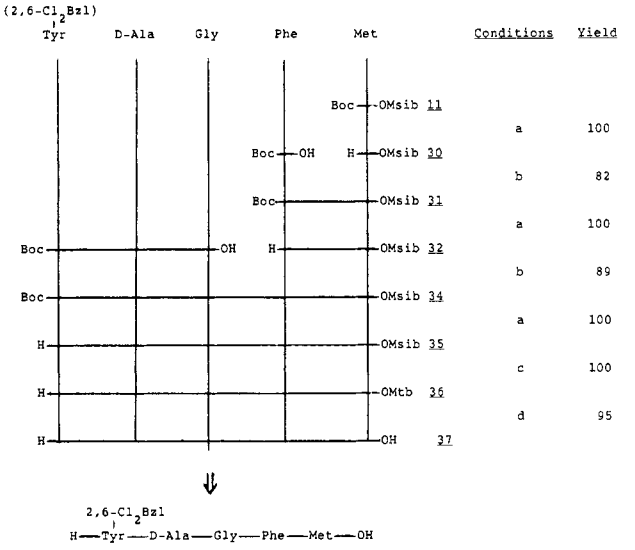
Triphenylphosphine has been employed by Mukaiyama as a component in a novel peptide decoupling reaction.[28] Under these conditions, protected amino acid side chains (even unprotected threonine and serine) have not suffered degradation. Since the effects of Me_3SiCl/Ph_3P were not known, the potentially vulnerable Cbz-protected amino acids, (nitro)arginine and methylglutaminate, were exposed to excess Me_3SiCl/Ph_3P for 8 h. Only minor degradation (5%) was detected by TLC in the reaction of Z-(NO_2)Arg (reaction 5), while Z-(Tos)Arg was completely stable for 8 h (reaction 6). Z-Gln-OMe showed no reaction after 8 h (reaction 7).

The colorless solutions obtained by Msib deoxygenation with Me_3SiCl/Ph_3P in the presence of tryptophan attest to the importance of selecting Me_3SiCl/Ph_3P as the reagent for deoxygenation of Msib peptide esters containing tryptophan. The exceedingly rapid rate of reduction should make this the reagent of choice in general.

Methionine does not suffer decomposition by either Me_3SiCl/Ph_3P, Me_3SiCl/Me_2S, or HCl/dioxane.

Stability of *p*-(Methylsulfinyl)benzyl Esters to Peptide Synthesis Conditions. The *p*-(methylsulfinyl)benzyl function has been exposed to several different sets of conditions typical to peptide synthesis. No degradation of Z-Phe-OMsib (7) by dicyclohexylcarbodiimide was observed for 1 month. The sulfoxide function in *p*-(methylsulfinyl)benzyl chloride (4) remained unchanged after exposure either for 60 days at room temperature to acetic anhydride, a model for the acylation reaction in peptide synthesis, or for 30 days to Ac_2O/Et_3N. As was previously described, the Msib ester **19** was stable to anhydrous TFA for at least 2 months. Msib esters are removed by catalytic hydrogenation albeit slowly (63% cleavage after 20 h at 33 psi of H_2). The methylsulfinyl group (with a pK_a of 32.5)[29] should be completely stable to deprotonation by organic amine bases.

Scheme V. Employment of the Msib Group in the Synthesis of a Peptide[a]



[a] (a) Anhydrous TFA, 40 min; (b) EDCI, HOBt, Et_3N, DMF; (c) Me_3SiCl, Me_2S, THF, then EtOH; (d) 50% TFA/Me_2S, then H_2O.

The Msib Ester in Peptide Synthesis. Presentation of a new protecting group is incomplete without demonstration of utility through successful peptide synthesis. In this regard we have prepared a derivative of enkephalin in high yield. An Msib ester **34** of Boc-[(2,6-Cl_2Bzl)-Tyr[1],D-Ala[2],Met[5]]enkephalin was prepared from Boc-(2,6-Cl_2Bzl)Tyr-D-Ala-Gly (**33**) and TFA·Phe-Met-OMsib (**32**) (Scheme V). As expected, treatment of **34** with anhydrous TFA gives the TFA salt of **35** in quantitative yield without disturbing the Msib group. Exposure to 20 equiv of Me_3SiCl and 40 equiv of Me_2S in anhydrous THF for 4 h gives the deoxygenated Mtb ester **36** in quantitative yield following ethanol treatment to remove silyl groups. Final treatment of the Mtb ester **36** with 50% TFA/Me_2S followed by water quench produces TFA·[(2,6-Cl_2Bzl)-Tyr[1],D-Ala[2],Met[5]]enkephalin (**37**) in 95% yield.

(27) Savage, W. E.; Fontana, A. *Int. J. Pept. Protein Res.* **1980**, *15*, 285–297.
(28) Mukaiyama, T. *Synth. Commun.* **1972**, *2*, 243–265.
(29) Bordwell, F. G.; Branca, J. C.; Johnson, C. R.; Vanier, N. R. *J. Org. Chem.* **1980**, *45*, 3884–3889.

Table V. Comparative Stabilities of Mtb and Msib Esters to Some Other Protecting Groups Typically Employed in Peptide Synthesis[a]

	CO_2Msib → CO_2H	CO_2Mtb → CO_2H	Z-NH → NH_2	Boc-NH → NH_2	Fmoc-NH → NH_2
glacial AcOH	–	–	–	–	–
TFA	–	+	–	+	–
HCl/dioxane	R	–	–	+	–
HBr/TFA	R	+	+	+	–
HF	(R)	+	+	+	–
Me_3SiCl/Me_2S[b]	R	–	–	–	–
Et_3N	–	–	–	–	(+)

[a] R = reduction to RCO_2Mtb, (R) = partial reduction, – = stable, + = cleaves, (+) = partial cleavage. The stabilities of the benzyl ester are inferred by those shown for the Z group, and the stabilities of the *tert*-butyl ester are inferred by those shown for the Boc group. [b] Chlorotrimethylsilane must be freshly distilled from pyridine to avoid *tert*-butyl group cleavage.

Summary of Stability Experiments. Anhydrous hydrogen chloride, often used for N^{α} protecting group cleavage, should only be employed when Msib deoxygenation is desired. The resulting *p*-(methylthio)benzyl esters are stable to anhydrous HCl/dioxane. Anhydrous trifluoroacetic acid has been the reagent used in recent years in place of anhydrous hydrogen chloride for N^{α} protecting group cleavage during solid-phase peptide syntheses. Msib esters are quite stable to TFA. Triphenylphosphine should be employed in Me_3SiCl or HCl deoxygenations of Msib peptides containing tryptophan residues.

The Msib group is stable to the main reagents used in solid-phase synthesis: TFA, Et_3N, DCC, and symmetrical anhydride. There are no indications that one's ability to assemble peptide sequences would be restricted by the presence of Msib groups. Groups on the peptide chain that are silylated during the sulfoxide reduction reaction are efficiently liberated with ethanol (following prior acid cleavage of trimethylsilyl esters).

Table V summarizes the comparative stabilities of *p*-(alkylsulfinyl)benzyl esters and *p*-(alkylthio)benzyl esters with some of the standard protecting groups used in peptide synthesis. Benzyl, Z, and Fmoc groups, used as semipermanent protecting groups, should be stable to conditions for reduction of the *p*-(alkylsulfinyl)benzyl group and stable to the conditions for cleavage of the *p*-(alkylthio)benzyl group. Final catalytic hydrogenation would remove the benzyl and Z groups from the cleaved peptide.

We envision the Msib ester to be particularly useful as a semipermanent carboxyl-protecting group[30] for peptide syntheses by fragment condensation, for Asp or Glu side chain derivatization or protection,[31] and synthesis of cyclic amides or esters. The Mtb ester constitutes a convenient alternative to the *tert*-butyl or *p*-methoxybenzyl ester[12] owing to its selective stability in anhydrous HCl/dioxane. An Msib-like anchor group on solid phase synthesis resin would be a logical development of this work.

Experimental Section

p-(Methylthio)benzaldehyde, *p*-(methylthio)benzyl alcohol, and other reagents were obtained from Aldrich Chemical Co. Amino acids and protected amino acids were obtained from Protein Research Foundation of Bachem Chemicals Inc. Infrared spectra were determined on a Beckman IR4260 spectrometer. NMR spectra were determined on a Varian EM390 spectrometer. Elemental analyses were performed by Galbraith Laboratories Inc. Chemical ionization (CI) mass spectra were obtained on a Finnigan Model 3600 mass spectrometer employing ammonia as the ionizing beam. FAB mass spectra were obtained on a VG ZAB-1F-HF mass spectrometer with a standard FAB source employing a glycerol matrix. Amino acid analysis was performed by initial hydrolysis in 6 N HCl containing 0.2% (v/v) mercaptoethanol for 48 h followed by analysis on a Beckman 6300 amino acid analyzer. Melting points were determined by using a Büchi melting point apparatus and are uncorrected. Thin-layer chromatography was performed with Brinkmann/Machery-Nagel Sil G-25 silica gel TLC plates. TLC identity of products was established by coelution with genuine compounds and visualization with iodine vapor or ninhydrin reagent. In stability determinations where yields were estimated by TLC, approximate quantitation of yields (±3%) was obtained by serial dilution of starting or product compounds. The TLC solvent systems and the mobilities of the compounds described in this paper are listed in a table in the supplementary material.

4-(Methylsulfinyl)benzyl Alcohol (HO-Msib, 2). Aqueous H_2O_2 (7.97 mL of a 30% solution, 77 mmol) was added dropwise with stirring to a solution of HO-Mtb (1, 11.80 g, 77 mmol) in glacial HOAc (150 mL) maintained below 20 °C by chilling in an ice/water bath. After 2 h, TLC showed no presence of starting sulfide. The reaction mixture was diluted with water (500 mL) and washed with Et_2O (2 × 75 mL). The ether layers were combined and lyophilized to give the oil **2** (11.72 g, 90%), a portion of which was distilled bulb to bulb (170 °C, 0.2 mmHg) to give an analytical sample: TLC, sys A R_f 0.07, sys B R_f 0.27; ^{1}H NMR ($CDCl_3$) δ 7.50 (s, 4 H), 4.68 (s, 2 H), 3.70 (s, 1 H), 2.68 (s, 3 H). Anal. Calcd for $C_8H_{10}SO_2 \cdot H_2O$: C, 54.98; H, 6.06; S, 18.35. Found: C, 54.78; H, 6.01; S, 18.48.

4-(Methylthio)benzyl Chloride (Cl-Mtb, 3). A solution of $SOCl_2$ (11.4 mL, 155 mmol) in freshly distilled THF (500 mL) was added dropwise at 25 °C to a stirred solution of HO-Mtb (1, 20.0 g, 130 mmol). After overnight stirring, aqueous saturated NaCl solution (500 mL) was added and the organic layer was evaporated in vacuo to an orange residue (20.0 g, 89%). The residue was distilled in vacuo (114 °C, 0.6 mmHg) to a colorless oil **3** (12.7 g, 57%): TLC, sys A R_f 0.97, sys B R_f 0.98; IR (Nujol) 1600, 1470, 1380 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.34 (m, 4 H), 4.68 (s, 2 H), 2.47 (s, 3 H). Anal. Calcd for C_8H_9ClS: C, 55.64; H, 5.25; Cl, 20.54; S, 18.57. Found: C, 55.71; H, 5.33; Cl, 20.65; S, 18.65.

4-(Methylsulfinyl)benzyl Chloride (Cl-Msib, 4). Aqueous hydrogen peroxide (8.4 mL of a 30% solution, 81.2 mmol) was added dropwise to a solution of 4-(methylthio)benzyl chloride (3, 12.7 g, 73.8 mmol) in glacial HOAc (200 mL) stirred at 50 °C. The solution was stirred for 1 h until TLC showed the disappearance of starting sulfide. After cooling to 25 °C, Me_2S (1 mL) was added to remove excess peroxide. The solution was diluted with deionized water to 4000 mL and neutralized by addition of solid sodium carbonate. The mixture was washed with an equal volume of EtOAc. The EtOAc layer was removed and rotary evaporated to a colorless oil **4** (11.5 g, 82% yield, 95% pure by TLC). The oil was sufficiently pure to be used as a reagent. On standing in a refrigerator, the oil crystallized to a white solid. A portion of the solid was recrystallized from EtOAc/Et_2O/hexane (1:5:1) to white needles: TLC, sys A R_f 0.51, sys B R_f 0.68; mp 46–47 °C; ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 4 H), 4.60 (s, 2 H), 2.70 (s, 3 H). Anal. Calcd for C_8H_9ClOS: C, 50.92; H, 4.81; Cl, 18.79. Found: C, 50.84; H, 4.89; Cl, 18.60.

Z-Phe-OMtb (6). A solution of Z-Phe-OH (299 mg, 1 mmol), HO-Mtb (616 mg, 4 mmol), EDCI (211 mg, 1.1 mmol), and HOBt (168 mg, 1.1 mmol) in $CHCl_3$ (20 mL) was stirred at 25 °C for

(30) Fauchere, J.-L.; Schwyzer, R. In *The Peptides, Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1981; Vol. 3, Chapter 5, pp 203–205.

(31) Yang, C. C.; Merrifield, R. B. *J. Org. Chem.* **1976**, *41*, 1032–1041.

2 days. The solution was washed with 1 N HCl (2 × 100 mL), 10% Na_2CO_3 (2 × 100 mL), and H_2O (50 mL). The aqueous solutions were washed with fresh $CHCl_3$ (50 mL). The $CHCl_3$ layers were combined, dried over anhydrous Na_2SO_4, and rotary evaporated to an oil, which was allowed to crystallize in 50% hexane/Et_2O to give a hard white powder **6** (339 mg, 78%): TLC, sys A R_f 0.48, sys B R_f 0.99; mp 62–65 °C; ^1H NMR ($CDCl_3$) δ 7.40–6.85 (m, 14 H), 5.14 (s, 2 H), 5.07 (s, 2 H), 4.68 (m, 1 H), 3.14 (s, 1 H), 3.03 (s, 1 H), 2.46 (s, 3 H); NH_3 CI mass spectrum $(M{\cdot}NH_3)^+$ 453. Anal. Calcd for $C_{25}H_{25}NSO_4$: C, 68.94; H, 5.79; N, 3.22; S, 7.36. Found: C, 68.76; H, 5.87; N, 3.19; S, 7.43.

Z-Phe-OMsib (7) from Z-Phe-OMtb (6). A solution of MCPBA (0.308 g, 1.79 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a solution of Z-Phe-OMtb (0.710 g, 1.63 mmol) in CH_2Cl_2 (50 mL) at 0 °C. After 30 min, the solution was allowed to warm to 25 °C. After 2 h, H_2O was added (50 mL) and CH_2Cl_2 was partially removed (to ≃10 mL) by rotary evaporation. The mixture was washed with Et_2O (50 mL). The ether solution was then washed with 10% Na_2CO_3 (30 mL) and H_2O (10 mL), dried over anhydrous Na_2SO_4, and rotary evaporated to an oil. TLC (sys A) indicated the presence of starting sulfide. The oil was eluted through a column of silica gel (100 g, 40–140 mesh, wet packed in $CHCl_3$) with a mixture of $CHCl_3$/AcOH, 95:5, with collection of fractions containing Z-Phe-OMsib as analyzed by TLC (sys A). Evaporation of the pooled fractions gave a colorless oil **7** (0.37 g, 50%): TLC, sys A R_f 0.87, sys B R_f 0.75; ^1H NMR ($CDCl_3$) δ 7.60 (d, 2 H, J = 8 Hz), 7.36 (d, 2 H, J = 8 Hz), 7.3–6.85 (m, 10 H), 5.13 and 5.06 (2 s, 2 H), 4.6 (t, 1 H, J = 6 Hz), 3.09 (d, 2 H, J = 6 Hz), 2.68 (s, 3 H); NH_3 CI mass spectrum $(M{\cdot}NH_4)^+$ 469. Anal. Calcd for $C_{25}H_{25}NSO_5$: C, 66.50; H, 5.58; S, 7.10. Found: C, 66.40; H, 5.66; S, 7.20.

Z-Phe-OMsib (7) from Z-Phe + HO-Msib (2). A solution of Z-Phe-OH (748 mg, 2.5 mmol), HO-Msib (2.125 g, 12.5 mmol), EDCI (719 mg, 3.75 mmol), and HOBt (383 mg, 2.5 mmol) in $CHCl_3$ (30 mL) was stirred at 25 °C for 4 days. The solution was washed with 1 N HCl (2 × 100 mL), 10% Na_2CO_3 (2 × 100 mL), and H_2O (15 mL). The aqueous solutions were washed with fresh $CHCl_3$ (20 mL). The $CHCl_3$ solutions were combined, dried over anhydrous Na_2SO_4, and rotary evaporated to a yellow oil **7** (1.11 g, 98%): TLC, sys B 95% pure.

Boc-Phe-OMsib (9). EDCI (0.359 g, 1.9 mmol) was added to a solution of Boc-Phe (**8**, 0.332 g, 1.25 mmol), HO-Msib (1.063 g, 6.25 mmol), and HOBt (0.191 g, 1.25 mmol) in $CHCl_3$ (20 mL) with stirring at 0 °C. The mixture was allowed to warm to 25 °C overnight. The workup employed for **2** was performed on the reaction mixture to give a colorless oil **10** (0.49 g, 94%): TLC, sys A R_f 0.35, sys B R_f 0.75, sys B >98% pure; ^1H NMR ($CDCl_3$) δ 7.63 (d, 2 H, J = 8 Hz), 7.37 (d, 2 H, J = 8 Hz), 7.20 (s, 5 H), 5.14 (m, 4 H), 4.6 (m, 1 H), 3.08 (d, 2 H, J = 6 Hz), 2.70 (s, 3 H), 1.41 (s, 9 H); NH_3 CI mass spectrum $(M{\cdot}NH_4)^+$ 435.

Boc-Met-OMsib (11). By the same procedure and on the same scale as for **9**, Boc-Met-OH was converted to the colorless oil **11** (0.48 g, 96%): TLC sys A R_f 0.40, sys B R_f 0.73, sys B >98% pure; ^1H NMR ($CDCl_3$) δ 7.73 (d, 2 H, J = 8 Hz), 7.63 (d, 2 H, J = 8 Hz), 5.47 (d, 1 H, J = 6 Hz), 5.23 and 5.17 (2 s, 2 H), 4.45 (m, 1 H), 2.72 (s, 3 H), 2.5 (d, 2 H, J = 6 Hz), 2.12 (m, 2 H), 2.05 (s, 3 H), 1.43 (s, 9 H); NH_3 CI mass spectrum $(M{\cdot}NH_4)^+$ 419.

β-Aspartic Acid *p*-(Methylthio)benzyl Ester (13). Tetramethylguanidine (5.5 mL, 44.18 mmol) was added slowly to a stirred mixture of aspartic acid (2.95 g, 22.09 mmol) and the copper(II) complex of aspartic acid, $Cu(Asp)_2Cu{\cdot}4H_2O$[32] (5.87 g, 4.69 mmol), in DMF (3 mL) and water (200 μL). After 2 h more DMF (1 mL) and *p*-(methylthio)benzyl alcohol (7.8 g, 45 mmol) were added to the blue mixture. Following the workup in ref 31, a crude solid was obtained, which was triturated in $CHCl_3$ to give a white granular powder (4.7 g, 55.8%): mp 232–234 °C (recrystallized from AcOH/H_2O); ^1H NMR (AcOH-d_4) δ 7.24 (s, 4 H), 5.14 (s, 2 H), 4.30 (m, 1 H), 3.17 (m, 2 H), 2.44 (s, 3 H). Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.62; N, 5.20; S, 11.89. Found: C, 53.58; H, 5.80; N, 5.04; S, 11.80.

γ-Glutamic Acid *p*-(Methylthio)benzyl Ester (15). Following the procedure for ester **13**, we converted $Cu(Glu)_2Cu{\cdot}H_2O$[32] to a crude solid, which was recrystallized in 25% aqueous HOAc: 6.3 g (37%); mp 188–190 °C (recrystallized from AcOH/H_2O); ^1H NMR (AcOH-d_4) δ 7.24 (s, 4 H), 5.10 (s, 2 H), 4.14 (m, 1 H), 3.10–2.45 (m, 4 H), 2.45 (s, 3 H). Anal. Calcd for $C_{13}H_{17}NO_4S$: C, 55.11; H, 6.05; N, 4.94; S, 11.32. Found: C, 55.10; H, 6.10; N, 5.02; S, 11.50.

4-(Methylthio)benzyl Acetate (16). HO-Mtb (**1**, 7.53 g, 48.8 mmol) was dissolved in a mixture of Ac_2O (45.8 mL, 488 mmol) and Et_3N (68.6 mL, 488 mmol) and stirred overnight. Et_2O (40 mL) was added, and the solution was washed with 1 N HCl (2 × 150 mL), 10% aqueous Na_2CO_3 (2 × 150 mL), and water (50 mL). The aqueous solutions were backwashed with Et_2O (40 mL). The ether solutions were combined, dried over anhydrous sodium sulfate, and rotary evaporated to an oil. Pure acetate **16** was obtained by fractional vacuum distillation of the oil (6.32 g, 70%): bp 120 °C (1 mmHg); ^1H NMR ($CDCl_3$) δ 7.32 (s, 4 H), 5.10 (s, 2 H), 2.48 (s, 3 H), 2.09 (s, 3 H). Anal. Calcd for $C_{10}H_{12}SO_2$: C, 61.19; H, 6.17; S, 16.34. Found: C, 61.27; H, 6.19; S, 16.47.

4-(Methylsulfinyl)benzyl Acetate (17). Aqueous H_2O_2 (3.6 mL of a 30% solution, 34.7 mmol) was added dropwise to a solution of 4-(methylthio)benzyl acetate (**16**, 6.2 g, 31.6 mmol) in glacial HOAc (100 mL) and stirred at 45 °C. The solution was stirred for 1 h until TLC showed the disappearance of starting sulfide. The solution was allowed to cool to 25 °C. Dimethyl sulfide (1 mL) was added to remove excess peroxide. The solution was diluted with deionized water (to 2 L) and lyophilized to a white solid **17** (6.1 g, 91% yield): mp 48–51 °C; recrystallized from Et_2O, mp 49–50.5 °C; ^1H NMR ($CDCl_3$) δ 7.66 (d, 2 H, J = 8 Hz), 7.54 (d, 2 H, J = 8 Hz), 5.15 (s, 2 H), 2.73 (s, 3 H), 2.12 (s, 3 H). Anal. Calcd for $C_{10}H_{12}SO_3$: C, 56.58; H, 5.70; S, 15.11. Found: C, 56.59; H, 5.76; S, 15.30.

TFA-OMtb (18) from HO-Mtb (1), Ac-OMtb (16), and Cl-Mtb (3). Anhydrous TFA (0.5 mL) was added to a solution of HO-Mtb (**1**, 150 mg, 0.97 mmol) in $CDCl_3$ (1 mL). After 5 min, the blue solution was rotary evaporated to a colorless oil **18** (>98% pure by ^1H NMR). The oil decomposed on distillation under vacuum: ^1H NMR ($CDCl_3$) δ 7.32 (s, 4 H), 5.34 (s, 2 H), 2.48 (s, 3 H).

Under the same conditions, Ac-OMtb (**16**, 100 mg, 0.51 mmol) gave a 96% yield of **18**, containing 4% Ac-OMtb (**16**) by ^1H NMR [^1H NMR ($CDCl_3$) δ 7.32 (s, 4 H), 5.34 and 5.10 (2 s, ratio 96:4, 2 H), 2.48 (s, 3 H)], and Cl-Mtb (**3**) (98 mg, 0.567 mmol) gave a 98.5% yield of a mixture containing 47% **18** and 53% **3** by ^1H NMR [^1H NMR ($CDCl_3$) δ 7.33 (s, 4 H), 5.34 and 4.58 (2 s, ratio 8:9, 2 H), 2.48 (s, 3 H)].

TFA-OMtb from Distillation of [*p*-(Methylthio)benzyl]dimethylsulfonium Salt 22. Me_2S (1.84 mL, 25 mmol) was added to a solution of HO-Mtb (**1**, 0.72 g, 5 mmol) in anhydrous TFA (5 mL). After 5 min, the solution was rotary evaporated to an oil, 100% **22** by NMR: ^1H NMR ($CDCl_3$) δ 7.32 (s, 4 H), 4.57 (s, 2 H), 2.78 (s, 6 H), 2.48 (s, 3 H). The oil was triturated with CCl_4 (3 × 10 mL), placed under vacuum for 30 min, and then distilled in vacuo (bp 41 °C (1.1 mm)), yielding pure TFA-OMtb (**18**, 584 mg, 47% yield). Anal. Calcd for $C_{10}H_{10}SO_2F_3$: C, 47.80; H, 4.01; S, 12.76. Found: C, 48.20; H, 4.19; S, 12.85.

Exposure of Methoinine to HO-Mtb in TFA. Methionine (74.6 mg, 0.5 mmol) and HO-Mtb (**1**, 77.1 mg, 0.5 mmol) were dissolved in anhydrous TFA (0.5 mL). After 5 min, CCl_4 (5 mL) was added to the green solution and the solution was rotary evaporated to a yellow oil containing 17% methionine and 83% (3-amino-3-carboxypropyl)methyl[*p*-(methylthio)benzyl]sulfonium salt **24** by ^1H NMR and TLC: TLC, sys E R_f 0.55 methionine, R_f 0.40 **24**; ^1H NMR ($CDCl_3$, acetone-d_6) δ 2.80 and 2.08 (2 s, 3 H), 2.49 (s, 3 H). If methionine was dissolved in a 50% TFA/Me_2S solution and treated in the same manner, a colorless oil was obtained containing a 1:1 mixture of methionine (>99% pure by TLC sys F) and the dimethyl[*p*-(methylthio)benzyl]sulfonium salt **22**: ^1H NMR ($CDCl_3$) δ 7.38 (s, 4 H), 4.53 (s, 2 H), 4.40 (m, 1 H), 2.79 (s, 6 H), 2.70 (m, 2 H), 2.50 (s, 3 H), 2.33 (m, 2 H), 2.10 (s, 3 H).

Treatment of Z-Met(O)-OMe[33] (26) with 10 equiv of Me_3SiCl and 20 equiv of Me_2S. MCPBA (318 mg, 1.85 mmol) was added to a solution of Z-Met-OMe[33] (**27**, 500 mg, 1.68 mmol) in CH_2Cl_2 (10 mL) with stirring at 0 °C. The solution was allowed

(32) van Heeswijk, W. A. R.; Eenink, M. J. D.; Feijen, J. *Synthesis* **1982**, 744–747.

(33) Iselin, B. M. *Helv. Chim. Acta* **1961**, *44*, 61–78.

to warm to 25 °C. After $2^1/_2$ h, the CH_2Cl_2 solution was washed with 10 mL of H_2O. The H_2O layer was backwashed with 10 mL of fresh CH_2Cl_2. The CH_2Cl_2 layers were combined, dried over solid Na_2SO_4, and rotary evaporated to an oil. The oil was applied to a column of silica gel (40–140 mesh, 80 g in 25 × 500 mm column) wet packed in $CHCl_3$. The soil was eluted in a mixture of $CHCl_3$/AcOH, 99:5 (5 mL/fraction). Fractions 116–181 were pooled, washed with 100 mL of H_2O, dried over anhydrous Na_2SO_4, and rotary evaporated to an oil **26** (300 mg, 57%): TLC, sys B R_f 0.11; ^{1}H NMR ($CDCl_3$) δ 7.43 (s, 5 H), 6.13 (d, 1 H, J = 7 Hz), 5.07 (s, 2 H), 4.43 (m, 1 H), 3.72 (s, 3 H), 2.73 (t, 2 H, J = 7 Hz), 2.52 (s, 3 H), 2.20 (m, 2 H).

Me_3SiCl (116 mg, 1.07 mmol) and Me_2S (133 mg, 2.14 mmol) were added to a solution of **26** (33.5 mg, 0.107 mmol) in anhydrous THF (1 mL) under argon atmosphere. After 2 h, the mixture was poured into water (80 mL). The aqueous mixture was washed with Et_2O (40 mL). The ether layer was dried over anhydrous Na_2SO_4 and rotary evaporated to an oil (100%). NMR of the oil revealed complete reduction to Z-Met-OMe **27**: ^{1}H NMR ($CDCl_3$) δ 7.27 (s, 5 H), 6.48 (d, 1 H, J = 7 Hz), 5.05 (s, 2 H), 4.40 (q, 1 H, J = 7 Hz), 3.53 (s, 3 H), 2.43 (m, 2 H), 2.0 (s, 3 H), 1.95 (m, 2 H).

Boc-Phe-Met-OMsib (31). A solution of Boc-Met-OMsib (**11**, 480 mg, 1.2 mmol) in anhydrous TFA (7 mL) was stirred for 1 h. CCl_4 (20 mL) was added, and the mixture was rotary evaporated to an oil **30** (100 % yield). The product was employed without purification.

Et_3N (152 μL, 1.1 mmol) was added dropwise to a solution of Boc-Phe (**8**) (292 mg, 1.1), the TFA salt **30** (1.2 mmol), EDCI (211 mg, 1.1 mmol), and HOBt (168 mg, 1.1 mmol) in $CHCl_3$ (10 mL) chilled at 0 °C on an ice water bath. The solution was allowed to warm to 25 °C overnight with stirring. The solution was washed with 1 N HCl (2 × 100 mL), 10% aqueous Na_2CO_3 (3 × 100 mL), and H_2O (50 mL). The aqueous solutions were backwashed with fresh $CHCl_3$ (10 mL). The $CHCl_3$ solutions were combined, dried over anhydrous Na_2SO_4, and rotary evaporated to a colorless oil **31** (495 mg, 82%): TLC, sys B R_f 0.05, >95% pure; ^{1}H NMR ($CDCl_3$) δ 7.75 (d, 2 H, J = 8 Hz), 7.65 (d, 2 H, J = 8 Hz), 7.27 (s, 5 H), 6.17 (d, 1 H), 5.30 (s, 2 H), 4.77 (m, 1 H), 4.48 (m, 1 H), 2.98 (m, 2 H), 2.69 (s, 3 H), 2.58 (t, 2 H), 2.03 (m + s, 5 H), 1.34 (s, 9 H); FAB mass spectrum $(M{\cdot}H)^+$ 549.

Boc-(2,6-Cl_2Bzl)Tyr-D-Ala-Gly-Phe-Met-OMsib (34). A solution of Boc-Phe-Met-OMsib (**31**, 156 mg, 0.267 mmol) in anhydrous TFA (3 mL) was stirred for 40 min. CCl_4 (10 mL) was added, and the mixture was rotary evaporated to a colorless glass **32** (100% yield). The product was employed without purification.

Et_3N (37 μL, 0.27 mmol) was added dropwise to a solution of Boc-(2,6hCl$_2$Bzl)Tyr-D-Ala-Gly (**33**, 167 mg, 0.293 mmol),[34] EDCI (77 mg, 0.40 mmol), and HOBt (61 mg, 0.40 mmol) in DMF (2.5 mL) chilled at 0 °C in an ice water bath. The solution was allowed to warm to room temperature overnight with stirring. The solution was rotary evaporated. The residue was partitioned between EtOAc (20 mL) and 1 N HCl (100 mL). The EtOAc layer was washed with 10% aqueous Na_2CO_3 (2 × 100 mL) and H_2O (200 mL). The aqueous solutions were backwashed with fresh EtOAc (20 mL). The EtOAc solutions were combined and rotary evaporated until a white precipitate formed. The precipitate was filtered and dried to a white powder **34** (240 mg, 89%): TLC, sys B R_f 0.47, >95% pure; ^{1}H NMR ($CDCl_3$) δ 7.69 (d, 2 H, J = 8 Hz), 7.60 (d, 2 H, J = 8 Hz), 7.38 (m, 3 H), 7.25 (s, 5 H), 7.03 (dd, 4 H), 5.80 (d, 1 H), 5.28 (s, 2 H), 3.9–5.2 (m, 6 H), 3.08 (m, 4 H), 2.68 (s, 3 H), 2.48 (t, 2 H), 2.02 (m + s, 5 H), 1.37 (s, 9 H), 1.26 (d, 3 H); FAB mas spectrum $(M{\cdot}H)^+$ 998.

TFA·(2,6-Cl_2Bzl)Tyr-D-Ala-Gly-Phe-Met-OMsib (35). A solution of **34** (39.7 mg, 0.04 mmol) in anhydrous TFA (3 mL) was stirred for 40 min. CCl_4 (10 mL) was added, and the solution was rotary evaporated to a colorless glass **35** (100% yield): TLC, sys E R_f 0.73, >95% pure; ^{1}H NMR ($CDCl_3$) revealed the absence of a Boc group and presence of the methionine methyl (2.00 ppm, s, 3 H) and the Msib methyl group (2.76 ppm, s, 3 H). The product was used without purification.

TFA·(2,6-Cl_2Bzl)Tyr-D-Ala-Gly-Phe-Met-OMtb (36). Me_3SiCl (101 μL, 0.80 mmol) was added to a solution of the Msib ester **35** (0.04 mmol) and Me_2S (117 μL, 1.6 mmol) in freshly distilled anhydrous THF (1 mL) with stirring under an argon atmosphere. A white precipitate that initially formed slowly dissolved with stirring over 30 min. After 4 h, EtOH (5 mL) was added and the solution stirred for 10 min. After rotary evaporation, the resulting residue was dissolved in ethanol (5 mL) and rotary evaporated after stirring for 10 min. The resulting residue was dissolved in EtOH (5 mL) and CCl_4 (5 mL) and rotary evaporated until a white precipitate formed. The precipitate was triturated with fresh CCl_4. The precipitate was dried to a white powder **36** (42.5 mg, >100% yield): TLC, sys E R_f 0.81, >95% pure. ^{1}H NMR ($CDCl_3$) revealed the absence of the Msib methyl group and presence of the Mtb methyl group (2.43 ppm, s) and the methionine methyl group (2.00 ppm, s). The product was used without purification.

TFA·(2,6-Cl_2Bzl)Tyr-D-Ala-Gly-Phe-Met-OH (37). A solution of 50% Me_2S/TFA (4 mL) was added to a solution of Mtb ester **36** (0.04 mmol) in $CHCl_3$ (0.5 mL) containing Me_2S (0.5 mL) with stirring. After 1 h, $CHCl_3$ (4 mL) was added and the solution was washed with H_2O (40 mL). The H_2O layer was backwashed with $CHCl_3$ (2 × 5 mL). The $CHCl_3$ solutions were combined. CH_3OH (10 mL) and CCl_4 (10 mL) were added, and the mixture was rotary evaporated to a white powder **37** (95% yield): TLC, sys E R_f 0.75, >95% pure. ^{1}H NMR ($CDCl_3$) revealed the absence of the Mtb methyl group and the presence of the methionine methyl group (2.00 ppm), as well as the following signals: 7.32, 7.24, and 7.02 (3 m, 12 H), 5.22 (s, 2 H), 3.5–4.75 (3 m, 6 H), 3.06 (m, 4 H), 2.39 (m, 2 H), 2.00 (m + s, 5 H), 1.14 (m, 3 H); FAB mass spectrum $(M{\cdot}H)^+$ 746; amino acid analysis Tyr (0.97), Ala (1.03), Gly (1.10), Phe (1.11), Met (0.78).

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Supplementary Material Available: Complete experimental details for the acid stability studies (Table II), sulfoxide deoxygenation studies (Table III), amino acid stability studies, and studies of Msib stability to various conditions (14 pages). Ordering information is given on any current masthead page.

(34) Moon, M. W. *Chem. Abstr.* **1982**, *96*, p7084w. U.S. Patent 4 251 438, Feb 17, 1981.