A Three-Dimensional Orthogonal Protection Scheme for Solid-Phase Peptide Synthesis under Mild Conditions[1,2]

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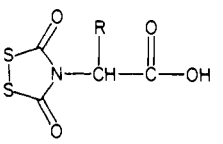
Abstract: Several N^{α}-dithiasuccinoyl (Dts) amino acids (**1**) were esterified, by use of *N,N'*-dicyclohexylcarbodiimide (DCC) and without racemization, to *tert*-butyl 4-(hydroxymethyl)-3-nitrobenzoate (**8**). The resultant handle derivatives **4** were treated with trifluoroacetic acid to yield crystalline 4-(N^{α}-Dts-aminoacyloxymethyl)-3-nitrobenzoic acids (**3**), which were quantitatively incorporated onto aminomethylcopoly(styrene-1% divinylbenzene)-resins by DCC-mediated couplings to give the starting point for stepwise solid-phase synthesis of peptides anchored as *o*-nitrobenzyl (ONb) esters. Assembly of the protected leucine-enkephalin-resin derivative Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-ONb-resin (**2**) was achieved from the appropriate Dts-amino acids by a highly efficient protocol. By carrying out, in each conceivable order, either in solution or on the solid phase, one, two, or all three of the following orthogonal treatments [(i) thiolytic removal of the Dts group; (ii) acidolytic cleavage of the *tert*-butyl ether; and (iii) photolytic cleavage at 350 nm of the ONb ester], the common resin-bound intermediate **2** became the source of four partially or fully deblocked leucine-enkephalin derivatives. These four, namely Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH, Dts-Tyr-Gly-Gly-Phe-Leu-OH, H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH, and H-Tyr-Gly-Gly-Phe-Leu-OH, were each obtained pure in good yields and were characterized by amino acid composition, HPLC, 300-MHz ^{1}H NMR, and fast atom bombardment mass spectrometry. The protected dipeptidyl sequence Prot-D-Val-L-Pro-ONb-resin was assembled with three different N^{α}-amino protecting groups and exposed to the recommended deblocking reagents. Loss of chains from the resin by diketopiperazine formation was very rapid with Prot = 9-fluorenylmethoxycarbonyl (Fmoc) and also substantial with Prot = *tert*-butoxycarbonyl (Boc), but rather negligible with Prot = Dts. Thus, these experiments demonstrate the feasibility and benefits of a mild three-dimensional orthogonal protection scheme based on Dts for N^{α}-amino protection, *tert*-butyl derivatives for side chains, and *o*-nitrobenzyl esters for anchoring.

The flexibility of stepwise schemes of solid-phase peptide synthesis[4,5] depends on the nature of the protecting groups selected, along with the corresponding methods for their removal. For example, the most commonly used strategy as optimized over the past 20 years[4-6] utilizes the N^{α}-*tert*-butoxycarbonyl (Boc) group for "temporary" protection, a series of modified benzyl alcohol and cyclohexanol derivatives for "permanent" protection of amino acid side chains, and the phenylacetamidomethyl (Pam) linkage for anchoring to the resin; deblocking of Boc is achieved with trifluoroacetic acid (TFA) whereas anhydrous liquid HF is required at the end of the synthesis to effect the remaining cleavages. Side reactions promoted by HF are well documented and have been reviewed.[5a,7] The severity of the final conditions for this scheme is a direct consequence of the fact that all classes of protecting groups including the anchoring linkage are removed by the same chemical mechanism (acidolysis), so that chemical selectivity must be attained by modulation of kinetic parameters.

With Prof. R. B. Merrifield, we have defined[5b,8,9] an *orthogonal* system as a set of completely independent classes of protecting groups, such that each class of groups can be removed in any order and in the presence of all other classes. An orthogonal protection scheme offers the prospect for use of deblocking reagents that are substantially *milder* than those used in schemes based on graduated lability to the same type of reagent, because in the orthogonal case, selectivity can be attained on the basis of differences in chemistry rather than in reaction rates.

The dithiasuccinoyl (Dts) function[8,10] (see general structure **1** for Dts-amino acids[8,11]) was conceived to meet the requirements of an orthogonally removable N^{α}-amino protecting group; it was



shown that Dts-amines resist strong acids and light but are rapidly and specifically cleaved under mild conditions by thiolysis. The

(1) A preliminary report of a portion of this work was presented by: Albericio, F. 18th European Peptide Symposium, Djuronaset, Sweden, June 10-15, 1984. See: Albericio, F.; Słomczyńska, U.; Mullen, D. G.; Zalipsky, S.; Barany, G. In "Peptides 1984"; Ragnarsson, U., Ed.; Almqvist and Wiksell: Stockholm, Sweden, 1984, pp 181-184. Financial support from the National Institutes of Health GM 28934 and Chicago Community Trust (Searle Scholars Program) is gratefully acknowledged.

(2) Abbreviations used: Boc, *tert*-butoxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DIEA, *N,N*-diisopropylethylamine; Dts, dithiasuccinoyl; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; MPLC, medium-pressure liquid chromatography; ONb, *o*-nitrobenzyl ester; Prot, N^{α}-amino protecting group; **R**, cross-linked polystyrene resin; TFA, trifluoroacetic acid. Amino acid symbols denote the L configuration where applicable, unless indicated otherwise.

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(4) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.

(5) Review: Barany, G.; Merrifield, R. B. In "The Peptides"; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 1-284. See especially: (a) *Ibid.*, p 76. (b) *Ibid.*, p 12. (c) *Ibid.*, pp 82-84 and 86-87. (d) *Ibid.*, pp 13-14. (e) *Ibid.*, pp 38-39.

(6) (a) Mitchell, A. R.; Kent, S. B.; Engelhard, M.; Merrifield, R. B. *J. Org. Chem.* **1978**, *43*, 2845-2852. (b) Merrifield, R. B.; Vizioli, L. D.; Boman, H. G. *Biochemistry* **1982**, *21*, 5020-5031. (c) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1983**, *105*, 6442-6455.

(7) Sakakibara, S. In "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins"; Weinstein, B., Ed.; Marcel Dekker: New York, 1971; Vol. 1, pp 51-85.

(8) Barany, G.; Merrifield, R. B. *J. Am. Chem. Soc.* **1977**, *99*, 7363-7365.

(9) For additional perspectives on the concept of orthogonality, see: (a) Kemp, D. S.; Roberts, D.; Hoyng, C.; Grattan, J.; Vellaccio, F.; Reczek, J. In "Peptides: Chemistry, Structure and Biology. Proceedings of the Fourth American Peptide Symposium"; Walter, R., Meienhofer, J., Eds.; Ann Arbor Science; Ann Arbor, MI, 1975; pp 295-305. (b) Fauchère, J. L.; Schwyzer, R. In "The Peptides"; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; Vol. 3, pp 203-252. (c) Bodanszky, M. "Principles of Peptide Synthesis"; Springer-Verlag: Berlin, 1984; pp 202-205.

(10) (a) Barany, G.; Merrifield, R. B. *Anal. Biochem.* **1979**, *95*, 160-170. (b) Barany, G.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 3084-3095.

(11) (a) Barany, G.; Zalipsky, S. In "Peptides: Structure and Function. Proceedings of the Eighth American Peptide Symposium"; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 159-162. (b) Zalipsky, S.; Albericio, F.; Słomczyńska, U.; Barany, G. *Int. J. Pept. Protein Res.*, submitted.

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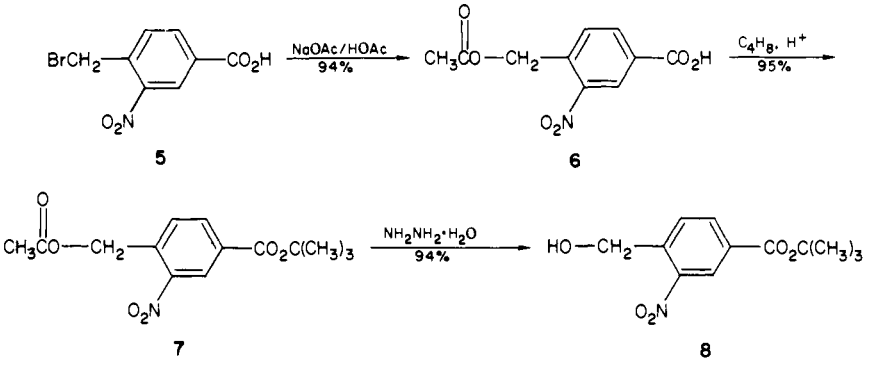
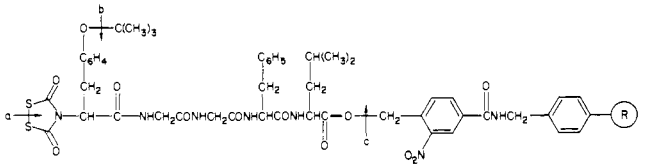
Scheme I



present paper describes the stepwise solid-phase assembly of the protected leucine-enkephalin–resin derivative **2** to demonstrate for the first time[12-14] a *three-dimensional* orthogonal protection scheme. Peptide–resin **2** was treated with thiols to remove the

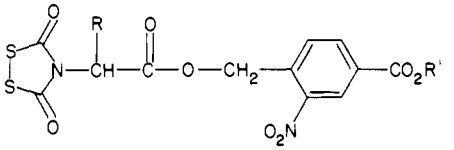


2 Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-ONb-resin

Dts group (bond a), or with TFA to break the *tert*-butyl ether (bond b) protecting the tyrosine side chain, or photolyzed at 350 nm to cleave (bond c) the *o*-nitrobenzyl (ONb) ester anchoring linkage[12a,b] and thus release a partially protected peptide from the support. Regardless of the order in which the steps were carried out, either in solution or on the solid phase, the appropriate soluble intermediates could be obtained in good yields and purities. After complete deblocking, the peptide leucine-enkephalin was obtained. We also comment here on the advantages of the described protection scheme with regard to minimization of diketopiperazine formation,[5c,15] a side reaction which in other systems leads to major losses of chains at the dipeptidyl-resin stage.

Results and Discussion

Anchoring of Dts-Amino Acids. By analogy to successful precedents in other systems,[5,16] 4-(N^{α}-dithiasuccinoylamino-acyloxymethyl)-3-nitrobenzoic acids (**3**) were targeted as the key "performed handle" derivatives for linking Dts-amino acids (**1**) via an *o*-nitrobenzyl ester onto polymeric supports. Starting with



3 R'=H
4 R'=*t*-Bu
a, R=$CH(CH_3)_2$ (Val)
b, R=$CH_2CH(CH_3)_2$ (Leu)
c, R=$CH_2C_6H_5$ (Phe)

4-(bromomethyl)-3-nitrobenzoic acid (**5**) prepared by reproducible modifications of the procedures of Rich and Gurwara,[12a] *tert*-butyl 4-(hydroxymethyl)-3-nitrobenzoate (**8**) was obtained in an overall yield of 84% for three steps (Scheme I). Subsequently, the appropriate Dts-amino acids (**1**) were coupled to the common intermediate **8** by use of *N,N'*-dicyclohexylcarbodiimide (DCC) in dichloromethane to furnish handle derivatives **4**, cleanly obtained in 40–60% yields upon silica gel chromatography to separate from unreacted **8**. The *tert*-butyl esters of **4** were readily cleaved in >90% yields by orthogonal treatment with TFA which did not affect the Dts group, and finally the resultant crystalline **3** were quantitatively incorporated onto aminomethylcopoly(styrene–1% divinylbenzene)-resins by DCC-mediated couplings in dichloromethane.

The stability of *o*-nitrobenzyl esters to strong acids is well-known,[4,12a] but the stability to thiols needed to be established to validate the proposed orthogonality. Thus, Dts-aminoacyl-resins were exposed for 24 h to β-mercaptoethanol–*N,N*-diisopropylethylamine (0.5/0.5 M) in dichloromethane, simulating over 300 cycles of Dts removal. The amino acid was entirely retained on the resin, and none was released into solution. In another experiment, a Dts-valyl-resin was taken through one solid-phase synthetic cycle of deprotection followed by coupling of Dts-glycine to create a Dts-glycyl-valyl-resin, which was then irradiated at 350 nm in trifluoroethanol–dichloromethane (7:3) under nitrogen at 30–35 °C for 9 h. The product obtained (58% cleavage yield, 97% purity), Dts-glycyl-valine, was indistinguishable from an

(12) Protection schemes with two independent dimensions of orthogonality have been reported from several laboratories. Combinations are listed in order: N^{α}-protection/permanent protection/anchoring linkage. For Boc/Bzl/ONb (acid/strong acid/light), see: (a) Rich, D. H.; Gurwara, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 1575–1579 and referenes cited therein. And: (b) Giralt, E.; Albericio, F.; Pedroso, E.; Granier, C.; Van Rietschoten, J. *Tetrahedron* **1982**, *38*, 1193–1201. For Fmoc/*t*-Bu/*p*-alkoxybenzyl ester (base/acid/acid), see: (c) Atherton, E.; Hübscher, W.; Sheppard, R. C.; Wooley, W. *Hoppe Seyler's Z. Physiol. Chem.* **1981**, *362*, 833–839. And (d) Chang, C. D.; Felix, A. M.; Jimenez, M. H.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1980**, *15*, 485–494. For Fmoc/*t*-Bu/dialkoxybenzyl ester (base/acid/mild acid), see: (e) Sheppard, R. C.; Williams, B. J. *J. Chem. Soc., Chem. Commun.* **1982**, 587–589. For *o*-nitrophenylsulfenyl/*t*-Bu/*p*-alkoxybenzyl ester (2-mercaptopyridine + TFA/acid/acid), see: (f) Fries, J. L.; Coy, D. H.; Huang, W. Y.; Meyers, C. A. In "Peptides: Structure and Biological Function. Proceedings of the Sixth American Peptide Symposium"; Gross, E., Meienhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, 1979; pp 499–502. For 3-nitro-2-pyridine-sulfenyl/*t*-Bu/*p*-alkoxybenzyl ester (triphenylphosphine + pyridine·HCl/acid/acid), see: (g) Wang, S. S.; Matsueda, R.; Matsueda, G. R. In "Peptide Chemistry 1981"; Shioiri, T., Ed.; Protein Research Foundation: Osaka, Japan, 1982; pp 37–40. For Dts/*t*-Bu/*p*-alkoxybenzyl ester (thiolysis/acid/acid), see: (h) ref 1.

(13) Orthogonal cleavages are also part of the fundamental logic of "multidetachable" resins: see: Tam, J. P.; Tjoeng, F. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 6117–6127.

(14) The Cambridge group has mentioned at two symposia their exploratory studies on the three-dimensional orthogonal combination Fmoc/*t*-Bu/ONb ester (base/acid/light), evidently not pursued because of base-promoted side reactions on the resin that affected overall yields. See (a) Atherton, E.; Jarvis, D.; Priestly, G. P.; Sheppard, R. C.; Williams, B. J. In "Peptides: Structure and Biological Function. Proceedings of the Sixth American Peptide Symposium"; Gross, E., Meienhofer, J., Eds.; Pierce Chemical Co.; Rockford, IL, 1979; pp 361–364. And: (b) Atherton, E.; Brown, E.; Priestly, G.; Sheppard, R. C.; Williams, B. J. In "Peptides: Synthesis-Structure-Function. Proceedings of the Seventh American Symposium"; Rich, D. H., Gross, E., Eds.; Pierce Chemical Co.; Rockford, IL, 1981; pp 163–175.

(15) (a) Gisin, B. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 3102–3106. (b) Giralt, E.; Eritja, R.; Pedroso, E. *Tetrahedron Lett.* **1981**, 3779–3782.

(16) For corresponding handles to anchor Boc-amino acids with Pam-resins, see: (a) Tam, J. P.; Kent, S. B.; Wong, T. W.; Merrifield, R. B. *Synthesis* **1979**, 955–957 and references cited therein. To anchor Boc-amino acids as ONb esters, see: (b) Hemmasi, B.; Stuber, W.; Bayer, E. *Hoppe-Seyler's Z. Physiol. Chem.* **1982**, *363*, 701–708. To anchor Fmoc-amino acids as *p*-alkoxybenzyl esters, see: (c) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1984**, *23*, 342–349. This latter paper also describes some of the methodology of peptide synthesis as practiced in this laboratory and gives experimental details and references for racemization studies.

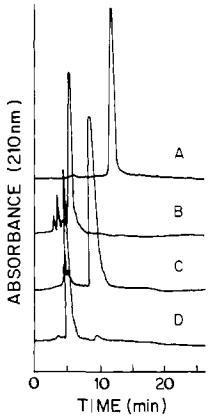


Figure 1. HPLC analyses of partially and fully deprotected leucine-enkephalin derivatives. Conditions used were as follows: C-18 column; linear gradient starting from CH_3CN–0.1% TFA in H_2O (7:13) taken over 20 min to pure CH_3CN; flow rate 1.1 mL/min. (A) Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH, purified by MPLC; (B) Dts-Tyr-Gly-Gly-Phe-Leu-OH, directly from purified protected peptide acid; (C) H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH, purified by MPLC; (D) fully deprotected H-Tyr-Gly-Gly-Phe-Leu-OH, purified by MPLC. The early peaks in chromatograms B and C are most likely residual reagents and solvents, and their amounts relative to the main peak were decreased 2- to 3-fold when absorbance was monitored at 280 nm rather than 210 nm.

authentic standard prepared[11b] by conversion of the free dipeptide to its Dts derivative, thereby demonstrating for a simple case the orthogonality of the Dts and ONb functions to the photolytic mode.

Importantly, the overall reaction sequence for anchoring and cleavage was entirely *free* of racemization. This fact was established by preparing the dipeptide L-Ala-L-Val (attach **3a** to resin; deprotect; couple and deprotect Dts-Ala; photolyze) and examining the crude product directly by ion-exchange chromatography.[16c] The single peak detected corresponded to the expected L,L-diastereomer (sensitivity limit for L,D- or D,L-dipeptides, 0.05%).

Orthogonal Solid-Phase Synthesis of Leucine-Enkephalin. First, Dts-leucine was transformed to handle derivative **3b**, which was then anchored to the support by the methodology just described. Next, each appropriate Dts-amino acid[8,11] (**1**) of the leucine-enkephalin sequence was in turn incorporated by cycles at 25 °C involving (i) deprotection[17] with β-mercaptoethanol–*N*,*N*-diisopropylethylamine (0.5/0.5 M) in dichloromethane (2 × 2 min), (ii) CH_2Cl_2 washes (5 × 1 min), (iii) Dts-amino acid (3 equiv) in minimal CH_2Cl_2 (3 min), (iv) DCC (3 equiv) in CH_2Cl_2 (90 min), (v) CH_2Cl_2 washes (5 × 1 min), and (vi) a qualitative ninhydrin test[18] on a resin aliquot, negative in all cases. The phenolic side chain of tyrosine was blocked as the thiol- and light-stable, albeit acid-labile *tert*-butyl ether. Amino acid analysis of the protected peptide–resin **2** thus obtained revealed that all residues had been introduced in the expected integer ratios and importantly that the substitution level corrected for peptide weight was unchanged from the loading of the first amino acid. These results demonstrate the efficacy of the overall protocol, particularly the lack of chain loss from the resin.

To obtain free leucine-enkephalin, peptide–resin **2** was successively treated with thiol (Dts removal, standard conditions), TFA–CH_2Cl_2 (1:1) (*t*-Bu ether cleavage, 1 h, 25 °C), and light (cleavage from resin). The photolysis yield was 63%, and product purity judged by HPLC was >90%. Pure peptide (>99.8%, Figure 1D) was obtained in 70% recovery by reversed-phase MPLC and had the correct amino acid composition, 300-MHz ^1H NMR spectrum (Figure 2D),[19] and fast atom bombardment mass spectrum (FABMS).[20,21]

Alternatively, the protected peptide acid retaining the N^{α}-Dts group and the *t*-Bu ether could be directly released from peptide–resin **2**. The yield of this photolysis was 62%, with product purity of 92%. The protected peptide acid was readily purified by MPLC and characterized by HPLC (Figure 1A), ^1H NMR (Figure 2A), and FABMS[21] which showed a clear MH^+ quasi-molecular ion at *m/z* 730 and a loss of 2-butene at *m/z* 674.

Finally, in accordance with the orthogonality requirements, removal in solution of the two remaining protecting groups of the protected peptide acid could be cleanly carried out in two discrete steps, independent of the order. The appropriate partially protected intermediates, containing, respectively, only N^{α}-Dts or only

Table I. Retention of Chains from Protected Dipeptidyl *o*-Nitrobenzyl Ester–Resins as a Function of Deprotective Agent and Time[a]

protected–resin	reagent	time, min	chains retained, %
Boc–**9**	DIEA–CH_2Cl_2 (1:19)[b]	2	66
		5	37[c]
Fmoc–**10**	piperidine–CH_2Cl_2 (1:1)	2	25[d]
Dts–**11**	β-mercaptoethanol–DIEA (0.5/0.5 M) in CH_2Cl_2	2	97
		15	92
		120	65[e]
Dts–**11**	*N*-methylmercaptoacetamide–*N*-methylmorpholine (0.5/0.5 M) in CH_2Cl_2	15	69
Dts–**11**	*N*-methylmercaptoacetamide–HOBt (0.5/0.1 M) in *N*,*N*-dimethylformamide	2	77
		5	60[f]

[a] See Scheme II for structures of protected dipeptidyl–resins. Resin samples were withdrawn and washed at the indicated times, hydrolyzed in 12 N HCl–HOAc (1:1), 12 h, 130 °C, and the ratio (Val + Pro)/(Leu = "internal reference" amino acid) was determined to give the levels of chains retained on the resin. For Boc (**9**) and Fmoc (**10**), diketopiperazine formation **12** was demonstrated directly by HPLC analysis of the filtrates; amounts were consistent with the values from hydrolysis. [b] Trace diketopiperazine **12** (≤1%) detected in filtrate after Boc group was removed with TFA–CH_2Cl_2 (3:7) (2 × 5 min). [c] Chain loss continued, at a somewhat slower rate (compared to initial half-time = 3.4 min). After 3 h, only 6% of chains were retained and there was no free N^{α}-amino group by a ninhydrin test (ref 18). [d] Within experimental error, no further chain loss occurred beyond 5-min reaction time. The final observed level, 17% after 3 h, may reflect an irreversible side reaction in which the N^{α}-amino group of the dipeptide is blocked (ninhydrin test, ref 18, was negative). [e] The peptide–resin at this point was challenged with freshly prepared thiolysis reagent, and chain loss continued over 1–2 h at a rate comparable to that observed previously as reported in the table. A second portion was exposed to piperidine–CH_2Cl_2 (1:1) for 5 min, leading to a final level of 34% of chains retained. Lastly, a portion was photolyzed (88% cleavage = 8% of chains retained). These experiments all suggest that at this stage of exposure to thiolysis reagent, the integrity of the *o*-nitrobenzyl ester anchoring linkage is unaffected. [f] For reasons that were not pursued, but may relate to the life-time of the thiolysis reagent, little further cleavage occurred at time points from 15 min to 3 h (level of chains retained was 43–50%).

(17) An equally successful synthesis was carried out involving deprotection with *N*-methylmercaptoacetamide–*N*-methylmorpholine (0.5/0.5 M) in dichloromethane (2 × 2 min); except *N*-methylmercaptoacetamide–HOBt (0.5/0.1 M) in *N*,*N*-dimethylformamide (2 × 2 min) was used to deprotect Dts-Phe-Leu-ONb–resin.

(18) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.

(19) Assignments of the ^1H NMR spectra were carried out by (a) comparison to the conclusions for methionine-enkephalin reached by: Bleich, H. B.; Cutnell, J. D.; Day, A. R.; Freer, R. J.; Glasel, J. A.; McKelvy, J. F. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2589–2593. (b) According to guidelines of: Wüthrich, K. "NMR in Biological Research: Peptides and Proteins"; North-Holland/American Elsevier: New York, 1976. (c) Comparison to spectra of Dts-Tyr(*t*-Bu)-OH and Dts-Tyr-OH in ref 11b. And: (d) Inspection. In the Supplementary Material, Table III summarizes chemical shift and coupling constant information, and Figures 3–6 provide expanded views of the spectra.

(20) Gross, M. L.; Chess, E. K.; Lyon, P. A.; Crow, F. W. *Int. J. Mass Spectrum. Ion Phys.* **1982**, *42*, 243–254. These authors are from the Midwest Center for Mass Spectrometry at the University of Nebraska, an NSF Regional Instrumention Facility (CHE 8211164).

(21) The full FABMS spectra on all peptides of this study are contained with the Supplementary Material (Figures 7–10).

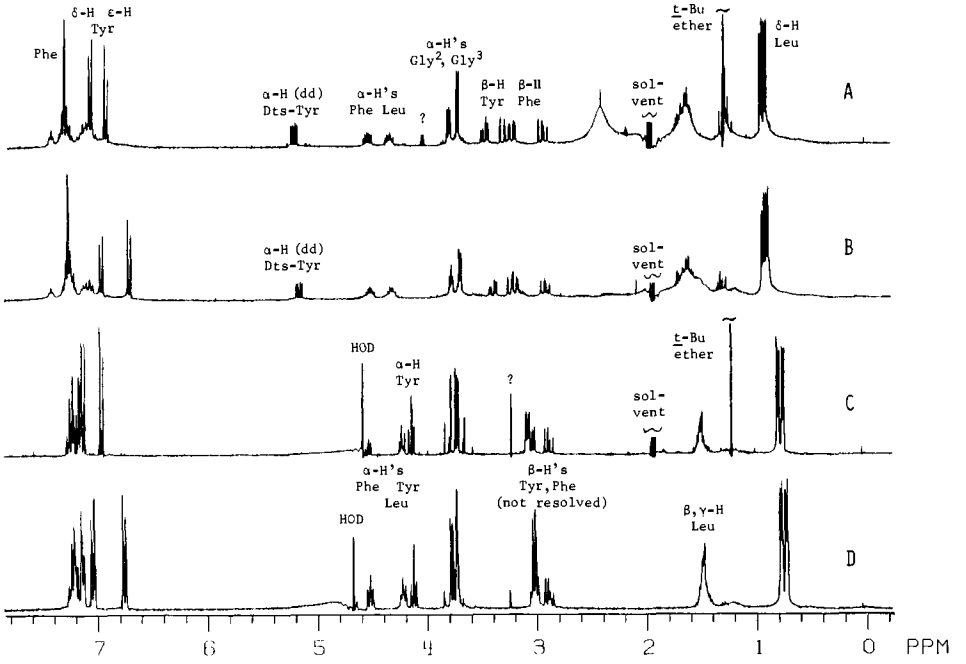


Figure 2. ^{1}H NMR spectra observed at 300 MHz and 25 °C on partially and fully deprotected leucine-enkephalin derivatives (see ref 19). Samples were ca. 1 mg in 0.3 mL of solvent, in 5-mm tubes, as follows: (A) Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH in CD_3CN; (B) Dts-Tyr-Gly-Gly-Phe-Leu-OH in CD_3CN; (C) H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH in D_2O–CD_3CN (10:1); (D) H-Tyr-Gly-Gly-Phe-Leu-OH in D_2O. Solvent resonances were suppressed with a presaturation pulse, and the spectra were resolution enhanced with a Lorentzian-to-Gaussian window. Peaks are referred to CD_2HCN = 1.930 ppm, or to external 10% sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate in D_2O. In spectra A and C, the sharp singlets due to the *tert*-butyl ether are off-scale. Peaks marked with "?" were not identified but are highly unlikely to be due to peptide material.

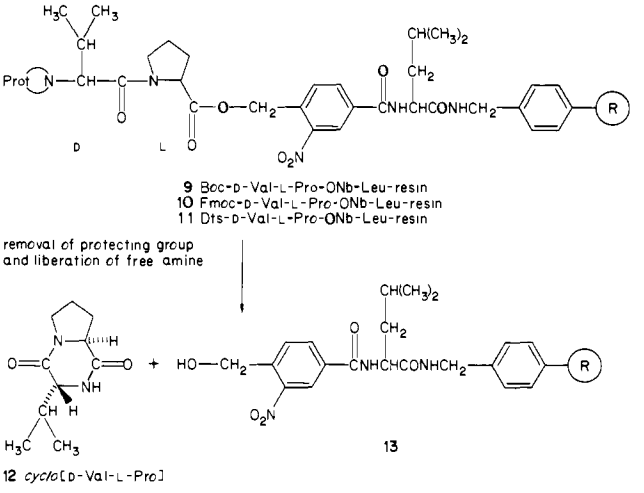
O-tert-butyl protection, were each obtained[22] in excellent purity (Figure 1B, C), and the fully deblocked leucine-enkephalin samples were indistinguishable from that obtained by carrying out both of the required deprotection steps on peptide–resin **2** prior to photolytic cleavage.

Studies on Diketopiperazine Formation. Because an important motivation for development of the dithiasuccinoyl function relates to the mild conditions for its removal, we sought a stringent system to test the comparative merits of several N^{α}-amino protecting groups. The dipeptidyl sequence[15a,b] D-Val-L-Pro is unusually prone to an acid- and base-catalyzed cyclization process which gives rise to diketopiperazine **12**, even more so with an activated *o*-nitrobenzyl alcohol leaving group as **13** (Scheme II). The three protected dipeptidyl–resins **9–11** with an "internal reference"[23] amino acid were prepared and exposed to the appropriate deblocking reagents for various lengths of time (Table I).

Results with Boc (resin **9**) are in accord with earlier ones in the literature;[15b] formation of **12** was established by direct methods as well as by the decreased substitution on the recovered resin. This side reaction is expressed as soon as the free amine is liberated from the initial amine hydrochloride or trifluoroacetate by neutralization with tertiary amines. Several modifications in deprotection/coupling protocols have been proposed[5c,15a,24] to keep diketopiperazine formation at manageable levels in solid-phase syntheses with Boc. In contrast, our results with Fmoc (resin **10**) show that loss of chains accompanying removal of this protecting group by the standard conditions of piperidine–dichloromethane (1:1) is so rapid that the compatibility of Fmoc with ONb can be seriously questioned.[14b]

Gratifyingly, diketopiperazine formation with Dts (**11**) was slow even in this model system which is designed to exaggerate the effects. The best results were obtained with the β-mercaptoethanol–DIEA–CH_2Cl_2 combination cited for the peptide synthesis results already discussed; here, loss of chains was negligible over the time span required to effect quantitative removal of the Dts group. Two other combinations were tested involving the more reactive thiol *N*-methylmercaptoacetamide with corresponding tempering of the base (use of *N*-methylmorpholine) or adjustment of solvent (the highly polar *N,N*-dimethylformamide, in the presence of 1-hydroxybenzotriazole to scavenge any base). In these instances, conditions known[1,10,17] to rapidly remove the Dts group were also found to promote diketopiperazine formation, although still not to the extent of the Fmoc or even Boc systems.

Scheme II



Conclusions

This paper has demonstrated the feasibility and benefits of a mild three-dimensional orthogonal protection scheme based on the dithiasuccinoyl function for N^{α}-amino protection, *tert*-butyl derivatives for side-chain protection, and an *o*-nitrobenzyl ester anchoring linkage. This methodology can be used to prepare protected peptide segments which are necessary intermediates for

(22) These partially protected intermediates were also obtained with equal results by carrying out one orthogonal deblocking step on peptide–resin **2** followed by photolytic cleavage of the *o*-nitrobenzyl ester anchoring linkage.

(23) (a) Atherton, E.; Clive, D. L.; Sheppard, R. C. *J. Am. Chem. Soc.* **1975**, *97*, 6584–6585. (b) Matsueda, G. R.; Haber, E. *Anal. Biochem.* **1980**, *104*, 215–227.

(24) (a) Suzuki, K.; Nitta, K.; Endo, N. *Chem. Pharm. Bull.* **1975**, *23*, 222–224. (b) Rothe, M.; Rott, H.; Mazañek, J. In "Peptides 1976"; Loffet, A., Ed.; Editions de l'Universitē de Bruxelles: Belgium, 1976; pp 309–318. (c) Chang, C. D.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1978**, *11*, 246–249.

solid-phase segment condensation strategies[5d] for the synthesis of large peptides and proteins. An encouraging aspect of the particular combination of protecting groups proposed here is the favorable solubility properties conferred on the partially blocked intermediates.[25]

Experimental Section

Some of the materials and methods used in this study have been detailed previously.[10b,16c,26] ^{1}H NMR spectra of organic molecules were observed with a Varian HFT 80 spectrometer whereas a Nicolet NT-300 instrument was used to study peptides. Pure N^{α}-Dts-amino acids (**1**), generally stored at 5 °C as dicyclohexylammonium (DCHA) salts, were prepared by a new method described elsewhere.[1,11] Solvent systems[27a] for TLC on silica gel GF (250 μm) were CA, $CHCl_3$–HOAc (19:1), and BPAW, *n*-butyl alcohol–pyridine–HOAc–H_2O (15:10:3:12). Solid-phase peptide synthesis was carried out by using a mechanical shaker[4,5e] and silanized screw-cap tube reaction vessels[5e,15a] each with a Teflon-lined cap, sintered glass frit, and stopcock. Aminomethylcopoly(styrene–1% divinylbenzene)–resin was from Peptides International (Louisville, KY) and had a substitution level of 0.62 mmol/g. Dichloromethane was distilled from anhydrous sodium carbonate, and benzene was dried and distilled from sodium and stored over 4-Å sieves. *N,N*-Dimethylformamide was stored over 4-Å molecular sieves plus Amberlyst 15 (H-form) and was purged by moderate bubbling through of nitrogen for 2 days, following which treatment the solvent gave a negative 1-fluoro-2,4-dinitrobenzene test[27b] for 2 weeks. All other solvents and chemicals were reagent grade and used without further purification.

Analytical HPLC was carried out on a Beckman-Altex Model 334 System, consisting of a 421 CRT controller, two 112 pumps, a 165 detector set at 210 nm, a Hewlett-Packard 3390A reporting integrator, and an Alltech Econosphere C-18 column (4.6 × 250 mm). Preparative MPLC (2–15 μmol scale, injected in a volume of 1 mL of eluent) was achieved on Merck Lobar Size A RP-8 columns, using an FMI Model RP-SY lab pump to achieve flow rates of 2 mL/min, and a Pharmacia UV-1/214 single path monitor connected to a Linear 1200 recorder for detection of peaks. Unless indicated otherwise, peptide–resins (5–10 mg) were hydrolyzed in 12 N HCl–HOAc (1:1) (1 mL) plus 2 drops of liquefied phenol, in vacuo, 130 °C, 24 h; free peptides were hydrolyzed in 6 N HCl plus 2 drops of liquefied phenol, in vacuo, 110 °C, 18 h. Hyrolyzed samples were concentrated to dryness, redissolved in pH 2.2 citrate buffer, and applied onto the 0.6 × 20 cm column (W-3H sulfonated resin) of a Beckman Model 118BL amino acid analyzer. Fast atom bombardment mass spectra[21] were obtained on instrumentation at the University of Nebraska described by Gross et al.[20] and involved the use of 8-keV xenon atoms to bombard the sample dissolved in an acidified glycerol matrix.

General Procedure for Photolytic Cleavage of *o*-Nitrobenzyl Ester Anchoring Linkages of Peptide–Resins. The resin sample (10–150 mg) was placed in a 15-mL glass vial and then suspended in trifluoroethanol–CH_2Cl_2 (7:3) (12 mL). The microtip of a Branson Model W185 Cell Disruptor Ultrasonicator was then immersed into this suspension, which was externally chilled with an ice bath, and sonication was applied (5 × 6 min, with 3-min pauses to allow recooling). A serum cap was wired on the vial, which was again chilled in ice and purged with nitrogen (30 min) while being magnetically stirred. Irradiation was then carried out, without external cooling but with magnetic stirring, in a Rayonet RPR reactor with 3500-Å lamps, for 9 h (longer irradiation generally does not improve yields). The irradiated suspension was then transferred into a solid-phase reaction vessel (1 × 6 cm), and the filtrate was expressed from the vessel with positive nitrogen pressure. The cleaved peptide–resin, which had taken on a red coloration, was washed with trifluoroethanol–CH_2Cl_2 (7:3) (2 × 5 mL), CH_2Cl_2 (2 × 5 mL), and acetonitrile (2 × 5 mL), and the combined filtrate and washings were evaporated to dryness at 25 °C and 2 mm. Often, white powders were obtained by redissolving in water with just enough acetonitrile added to ensure solubility, filtration through glass wool, and lyophilization.

Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-ONb–Resin (2). Aminomethyl–resin (400 mg, 0.24 mmol) was placed in a solid-phase reaction vessel (1.5 × 8 cm) and was washed (all wash volumes = 15 mL) with TFA–CH_2Cl_2 (3:7) (2 × 5 min), CH_2Cl_2 (5 × 1 min), DIEA–CH_2Cl_2 (1:19) (2 × 5 min), and CH_2Cl_2 (5 × 1 min). Next, **3b** (123 mg, 0.29 mmol) in CH_2Cl_2 (4 mL) was added and agitated for 5 min, followed without filtering by DCC (59 mg, 0.29 mmol) in CH_2Cl_2 (2 mL). After shaking for 12 h at 25 °C, the resultant Dts-Leu-ONb–resin was washed with CH_2Cl_2 (5 × 1 min) and gave a negative ninhydrin test.[18]

Each appropriate Dts-amino acid (**1**) of the leucine-enkephalin sequence was quantitatively incorporated as follows: Deprotection was achieved with β-mercaptoethanol–DIEA (0.5/0.5 M) in CH_2Cl_2 (2 × 2 min), after which the peptide–resin was washed with CH_2Cl_2 (5 × 1 min). Meanwhile, the Dts-amino acid DCHA salt (0.72 mmol) was dissolved or suspended in CH_2Cl_2 (5 mL) in a separate tube, and Dowex 50X8-400 ion-exchange resin in the hydrogen form (1 g, 2.1 mequiv) was added. After vigorous agitation (1 min) on a Vortex mixer, the CH_2Cl_2 phase of this heterogeneous neutralization mixture was withdrawn by a glass-wool plugged pipet, the ion-exchange resin was washed with more CH_2Cl_2 (1–2 mL), and the combined soluble extract was refiltered through glass wool and partially concentrated by a gentle stream of nitrogen. The resultant solution[28a] of Dts-amino acid (free carboxyl form) in CH_2Cl_2 (final volume 2.5 mL) was added to the deprotected and washed peptide–resin, which was now briefly shaken (3 min) prior to adding DCC (148 mg, 0.72 mmol) in CH_2Cl_2 (2 mL) to effect coupling (90 min). Finally, washes[28b] with CH_2Cl_2 (5 × 1 min) followed by a ninhydrin test[18] (which was in all cases negative) completed this deprotection/coupling cycle.

Once chain assembly of protected leucine-enkephalin–resin **2** (610 mg; compare to theoretical weight of 625 mg, corrected for ca. 25 mg of total aliquots for ninhydrin test) was finished, a portion was hydrolyzed and subjected to amino acid analysis: Gly 2.06, Leu 0.9, Tyr[29] 0.6, Phe 0.97. The loading was 0.36 mmol/g of peptide–resin (theory = 0.39, with correction for weight gain due to growing peptide).

Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH. Peptide–resin **2** (150 mg, 54 μmol) was subjected to photolysis; the general procedure already described provided 33 μmol (62%) of crude peptide which was about 92% pure by analytical HPLC (conditions of Figure 1). Half of this material (16.5 μmol) was applied to reversed-phase MPLC (convex gradient formed from 175 mL each of 1:4 and 4:1 mixtures of CH_3CN and 0.1% TFA in H_2O) to provide 7.9 μmol (48%) of the pure protected peptide (see Figure 1A): white powder, mp 137–140 °C; composition Gly 2.02, Leu 0.99, Tyr[29] 0.34, Phe 0.99; ^{1}H NMR Figure 2A; FABMS, *m/z* 768 [$(M + K)^+$, 1%], 752 [$(M + Na)^+$, 1%], 744 (1%), 730 (MH^+, 5%), 674 ($MH^+ - C_4H_8$, 2%), 612 [$(MH - 2\,COS + 2\,H)^+$, 2%], 485 (5%), 393 (3%), 336 (4%), 279 (17%), 185 (50%), 120 (100%).

Dts-Tyr-Gly-Gly-Phe-Leu-OH. A. The purified protected peptide described in the preceding (2.5 μmol) was dissolved in TFA–CH_2Cl_2 (1:1) (1 mL). After 1 h at 25 °C, solvent was removed by rotary evaporation, and the sample was redissolved in CH_3CN–H_2O and lyophilized prior to further characterization by HPLC (Figure 1B) and FABMS: *m/z* 718 [$(M + 2\,Na)^+$, 6%], 712 [$(M + K)^+$, 3%], 704 (7%), 696 [$(M + Na)^+$, 24%], 626 (3%), 604 [$(M + Na - COS_2)^+$, 8%], 401 (10%), 254 (12%), 182 (11%), 120 (18%), 102 (100%). **B.** Peptide–resin **2** (30 mg, 11 μmol) was exposed to TFA–CH_2Cl_2 (1:1) (2 × 30 min), washed with CH_2Cl_2 (5 × 1 min), and photolyzed. The resultant peptide (6 μmol, 55%) was >96% pure by analytical HPLC and used directly for ^{1}H NMR (Figure 2B) composition Gly 2.02, Leu 1.03, Tyr[29] 0.28, Phe 0.95.

H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH. A. Purified Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH (4.5 μmol) was fully dissolved in a mixture (1 mL) of β-mercaptoethanol–DIEA (0.5/0.5 M) in CH_2Cl_2, which was maintained at 25 °C for 5 min following which solvents and most of the reagents were removed by rotary evaporation at 25 °C, 1 mm, 15 min (chased 3 times with CH_2Cl_2). The resultant film was then immediately passed through an MPLC column (convex gradient formed from 175 mL each of 1:19 and 3:2 mixtures of CH_3CN and 0.1% TFA in H_2O) to provide pure title peptide (Figure 1C): composition Gly 2.06, Leu 1.06, Tyr 0.92, Phe 0.96; ^{1}H NMR Figure 2C; FABMS, *m/z* 634 [$(M + Na)^+$, 5%], 612 (MH^+, 36%), 556 ($MH^+ - C_4H_8$, 10%), 397 (7%), 334 (6%), 279

(25) The protected peptides related to the leucine-enkephalin sequence that were obtained in this work were readily soluble in acetonitrile–water mixtures and could be purified without difficulty by reversed-phase MPLC.

(26) Barany, G.; Schroll, A. L.; Mott, A. W.; Halsrud, D. A. *J. Org. Chem.* **1983**, *48*, 4750–4761.

(27) Stewart, J. M.; Young, J. D. "Solid Phase Peptide Synthesis"; W. H. Freeman: San Francisco, CA, 1969; (a) p 59, (b) pp 31–32.

(28) (a) With regard to amino acids in the leucine-enkephalin sequence, the following should be noted: (i) the DCHA salt of Dts-phenylalanine is not entirely soluble in CH_2Cl_2, but solution of the free acid is complete at the end of the neutralization procedure. (ii) Dts-glycine liberated from its DCHA salt by the described procedure forms a supersaturated solution which is suitable for use in the coupling reaction. The preparation of sufficiently concentrated solutions starting from free Dts-glycine can only be achieved by dissolving in minimal acetonitrile followed by addition of 3 volumes of CH_2Cl_2. (b) Specifically, when Dts-glycine was incorporated, the wash cycle at this point was modified to be CH_2Cl_2 (3 × 1 min), CH_3CN (2 × 1 min), and CH_2Cl_2 (3 × 1 min).

(29) The tyrosine value is always less than theory because the Dts group partially survives acid hydrolysis; see ref 8 and 10. Good tyrosine values were obtained whenever this residue had a free N^{α}-amino group. Note the presence of phenol as a scavenger and antioxidant for the hydrolysis.

Table II. Dts-Amino Acyl *o*-Nitrobenzyl Ester Handle Intermediates[a]

compd	yield, %	mp, °C	chemical shift of α-H, δ[b]
3a	90	139–141	4.70 (d, J = 9.2 Hz)
3b	90	133	5.13 (dd, J = 5.5, 10.3 Hz)
3c	94	128–130	5.50 (dd, J = 5.9, 10.6 Hz)
4a	60	85	4.68 (d, J = 9.2 Hz)
4b	47	97–98	5.11 (dd, J = 5.5, 10.2 Hz)
4c	40	112–113	5.31 (dd, J = 6.6, 10.0 Hz)

[a] Abstracted from Table IV in Supplementary Material, which includes TLC data (all compounds gave single spots in two systems) and elemental analysis data (in accord with theory for all compounds). [b] Abstracted from complete data in Table IV in Supplementary Material; recorded at 80 MHz in $CDCl_3$ except for **3c** in $CD_3(C{=}O)CD_3$. Reported here are the very characteristic resonances due to the α-H of the Dts-amino acid. Derivatives **3** all showed resonances at 8.81 (d, J = 1.7 Hz, 1 H), 8.37 (dd, J = 1.6, 8.0 Hz, 1 H), 7.66 (d, J = 8.0 Hz, 1 H), 5.66 (s, 2H, benzyl CH_2). Derivatives **4** all showed resonances at about 8.7 (d, J = 1.5 Hz, 1 H), 8.3 (dd, J = 1.5, 8.0 Hz, 1 H), 7.6 (d, J = 8.0 Hz, 1 H), 5.65 (s, 2 H, benzyl CH_2), and 1.62 (t, 9 H, *tert*-butyl).

(17%), 136 (80%), 120 (100%). **B.** Peptide–resin **2** (30 mg, 11 μmol) was treated with β-mercaptoethanol–DIEA (0.5/0.5 M) in CH_2Cl_2 (2 × 2 min), washed with CH_2Cl_2 (5 × 1 min), and photolyzed to provide the title peptide (5.9 μmol, 54%), 96% pure by analytical HPLC.

Leucine-Enkephalin. A. The starting peptide–resin **2** (200 mg aminomethyl–resin yielding 320 mg of peptide–resin) for this experiment was assembled exactly as in the text description but with a different choice of the deprotective reagents.[17] The amino acid analysis was Gly 1.98, Leu 0.99, Tyr[29] 0.55, and Phe 1.03 at a loading of 0.40 mmol/g (theory = 0.39, calculated as explained earlier). This peptide–resin was treated in the standard way to remove the Dts group, washed with CH_2Cl_2 (5 × 1 min), and then exposed to TFA–CH_2Cl_2 (1:1) (1-min prewash; 2 × 30 min) to remove the *t*-Bu group. After further washing with CH_2Cl_2 (5 × 1 min), the peptide–resin was dried over P_2O_5 at 15 mm, and a portion (180 mg, 72 μmol) was subjected to photolysis by the general procedure described earlier. The yield of cleavage as calculated by back-hydrolysis of the recovered resin was 65%, as compared to 58% based on peptide (42 μmol) in the filtrate: Gly 1.96, Leu 1.07, Tyr 0.91, Phe 0.96. The crude peptide was analyzed by analytical HPLC (CH_3CN–0.1% TFA in H_2O (1:4), 1.1 mL/min) and showed several non-peptide peaks early in the chromatogram, an impurity (ca. 10%) at 7.9 min, and the major peak at 10.3 min. After preparative reversed-phase MPLC (14 μmol scale; eluted with same isocratic solvent used for HPLC), the desired leucine-enkephalin was obtained in 70% recovery and >99.8% purity by HPLC: Gly 2.01, Leu 1.01, Tyr 1.01, Phe 0.98; FABMS, m/z 1112 ($M_2H_2^+$, 1%), 578 [(M + Na)$^+$, 2%], 556 (MH^+, 37%), 399 (3%), 279 (12%), 120 (100%). **B.** Purified Dts-Tyr-Gly-Gly-Phe-Leu-OH was thiolytically deprotected in solution and purified exactly as described in method A for H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH to provide for this case HPLC-pure leucine-enkephalin. C. Purified H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH was acidolytically deprotected in solution exactly as described in method A for Dts-Tyr-Gly-Gly-Phe-Leu-OH to provide without further purification in this case HPLC-pure leucine-enkephalin.

Dts-Glycyl-Valine. By the same protocols explained in detail in connection with the protected enkephalin experiments, **3a** was coupled onto aminomethyl–resin (100 mg, 0.06 mmol), Dts-glycine was incorporated, and the resultant Dts-Gly-Val-ONb–resin was photolyzed (58% yield by back-hydrolysis of cleaved resin). The protected dipeptide thus released was 97% pure by reversed-phase HPLC [t_R 9.6 min in CH_3CN–0.1% TFA in H_2O (3:17), 1.1 mL/min]; ^1H NMR [(CD_3CN) δ 7.0 (br, 1 H, NH), 4.39 (s, 2 H, α-H of Dts-Gly), 4.31 (dd, J = 5.3, 8.5 Hz, 1 H, α-H of Val), 2.14 (m, 1 H, β-H of Val), 0.93 (d, J = 6.8 Hz, 3 H), 0.92 (d, J = 6.9 Hz, 3 H)], plus *very trace* (ca. 0.5%) peaks in aromatic region (7.3–8.0) which may be resin-breakdown products, and no indication (<0.5%) of any upfield doublets that would suggest photochemical cleavage[30] of the Dts-glycyl residue. The HPLC and NMR data cited were identical with those for pure Dts-glycyl-valine,[11b] mp 178–180 °C.

4-(N^{α}-Dithiasuccinoylaminoacyloxymethyl)-3-nitrobenzoic Acids (3). The corresponding *t*-butyl esters **4** (2 mmol) were dissolved in TFA–CH_2Cl_2 (1:1) (50 mL) and stirred for 3 h at 25 °C. After evaporation to dryness in vacuo, including chasing (5 times) with CH_2Cl_2, the resultant title products (Table II, abstracted from more detailed information with Supplementary Material) were obtained in 90–95% crystalline yields, pale yellow needles, by dissolving in minimal ethyl acetate at 25 °C, adding pentane to incipient turbidity, and chilling to −20 °C.

***tert*-Butyl 4-(N^{α}-Dithiasuccinoylaminoacyloxymethyl)-3-nitrobenzoates (4).** The appropriate Dts-amino acid (**1**) (7.2 mmol) was dissolved in CH_2Cl_2 (40 mL), and then alcohol **8** (1.5 g, 6 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (1.5 g, 7.2 mmol) were added by using additional CH_2Cl_2 (total 10 mL) for dissolving these substances and rinsing them into the reaction mixture. Within 5 min, a precipitate of *N*,*N*′-dicyclohexylurea appeared, but stirring was continued for 2 days at 25 °C. The mixture was then chilled to 4 °C for 2 h, filtered to remove urea, diluted with ethyl acetate (150 mL), and washed with pH 9.5 carbonate[16a] (3 × 75 mL) and saturated NaCl (3 × 40 mL). Drying ($MgSO_4$) and concentration gave oils which were dissolved in chloroform and applied for chromatography on columns (2 × 35 cm) containing 50 g of silica gel 60 (200 mesh), eluted with $CHCl_3$–pentane (65:35) at ca. 4 mL/min. The desired title products were cleanly separated from unreacted **8** which eluted later; crystallizations were achieved similar to those of **3**, by use of ethyl acetate–pentane. Overall yields of pale yellow needles were 40–60% (Table II, abstracted from more detailed information with Supplementary Material).

4-Bromomethyl-3-nitrobenzoic Acid (5). First, 4-(bromomethyl)-benzoic acid, pale yellow stars, mp 220–222 °C [lit.[12a] mp 224–226 °C], was prepared as follows: A mixture of *p*-toluic acid (60 g, 0.44 mol) and benzoyl peroxide (450 mg, 1.7 mmol) in dry benzene (800 mL) was brought into solution by strong reflux, and then *N*-bromosuccinimide (78.3 g, 0.44 mol) combined with more benzoyl peroxide (450 mg) was added portionwise over 0.5 h through the top of a Liebig condenser. A solid precipitate formed immediately; reflux was continued for a further 1.5 h. After cooling to 25 °C, the precipitate was collected by filtration (removing polybromination products that remain in the filtrate) and extracted with water (400 mL) at 60–80 °C for 1 h to remove succinimide. The desired acid (68 g, 72%) was then obtained by filtering, washing with hot water (3 × 50 mL), drying in vacuo over P_2O_5, and recrystallizing from hot methanol; NMR (CD_3COCD_3) δ 8.03 (d, J = 8.3 Hz), 7.59 (d, J = 8.3 Hz), 4.70 (s, benzyl CH_2), *no* 2.67 (succinimide); R_f (CA), 0.54; R_f (BPAW), 0.70. Next, the 4-(bromomethyl)-benzoic acid (68 g, 0.32 mol) was added over 1 h to fuming HNO_3 (400 mL) at −10 °C. After 1 h at 0 °C, by which time everything was in solution, the reaction mixture was poured onto crushed ice (65 g). The title product (73 g, 89%) was obtained by filtering, washing with cold water, drying in vacuo over P_2O_5, and recrystallizing by dissolving in hot CH_2Cl_2, adding pentane at 25 °C, and chilling to 4 °C; pale yellow stars; mp 128–130 °C [lit.[12a] mp 125–126 °C]; NMR (CD_3COCD_3) δ 8.60 (d, J = 1.7 Hz), 8.32 (dd, J = 1.7 Hz, 8.0 Hz), 7.91 (d, J = 8.0 Hz), 5.00 (s, benzyl CH_2); R_f (CA), 0.52; R_f (BPAW), 0.69.

4-(Acetoxymethyl)-3-nitrobenzoic Acid (6). Sodium acetate (252 g, 3.1 mol) was dissolved in acetic acid (1.1 L) at 100 °C, following which 4-(bromomethyl)-3-nitrobenzoic acid (**5**) was added and stirring was continued for 16 h at 100 °C. The reaction mixture was diluted with water (1.8 L) and evaporated to dryness in vacuo. The resultant oil was redissolved in 0.5 N aqueous HCl (2 L) and extracted with ethyl acetate (3 × 1 L). The combined organic phases were washed repeatedly with water (8 × 500 mL) to remove all acetic acid, dried ($MgSO_4$), and concentrated to give an oil which was crystallized by dissolving in hot ethyl acetate, adding petroleum ether at 25 °C, and chilling to 4 °C. This gave the title product (32 g, 94%): yellow stars; mp 128–130 °C; NMR (CD_3COCD_3) δ 8.67 (d, J = 1.5 Hz, 1 H), 8.36 (dd, J = 1.5, 7.9 Hz, 1 H), 7.90 (d, J = 7.9 Hz, 1 H), 5.56 (s, 2 H), 2.16 (s, 3 H); R_f (CA), 0.49; R_f (BPAW), 0.67.

Anal. Calcd for $C_{10}H_9NO_6$ (M_r 239.18): C, 50.22; H, 3.79; N, 5.86. Found: C, 50.38; H, 3.83; N, 5.78.

***tert*-Butyl 4-(Acetoxymethyl)-3-nitrobenzoate (7).** Using a 80 mL high-pressure glass tube with fittings as described by Hoye and Caruso,[31] acid **6** (9.0 g, 40 mmol) was suspended in $CHCl_3$–dioxane (3:1) (30 mL), and isobutylene (ca. 40 mL) was condensed in at −78 °C. Concentrated sulfuric acid (0.4 mL, 14 mmol) was then added, and the tube was sealed. The reaction mixture was stirred for 3 days under a hot plate–stirrer at the lowest setting, providing an external temperature of 40–50 °C. At the end of this time, everything was in solution which indicated completion of the reaction. The tube was then chilled, opened, and partially concentrated in vacuo to remove the isobutylene. Ethyl acetate (200 mL) was added, and the organic phase was washed with pH 9.5 carbonate buffer[16a] (3 × 100 mL) and saturated NaCl (3 × 50 mL), followed by drying ($MgSO_4$) and concentrating in vacuo. The resultant solid was recrystallized from ethyl acetate–pentane to give title product (10.6 g, 95%); pale orange needles; mp 103–105 °C; NMR ($CDCl_3$) δ 8.65 (d,

(30) Irradiation of Dts-glycine in methanol was described earlier: see ref 8, to provide methoxycarbonylglycine.

(31) Hoye, T. R.; Caruso, A. J. *Tetrahedron Lett.* **1978**, *47*, 4611–4614.

J = 1.6 Hz, 1 H), 8.25 (dd, J = 1.6, 8.1 Hz, 1 H), 7.66 (d, J = 8.1 Hz, 1 H), 5.55 (s, 2 H), 2.19 (s, 3 H), 1.62 (s, 9 H); R_f (CA), 0.62; R_f (BPAW), 0.81.

Anal. Calcd for $C_{14}H_{17}NO_6$ (M_r 295.29): C, 56.95; 5.80; N, 4.74. Found: C, 57.07; H, 5.81; N, 4.78.

***tert*-Butyl 4-(Hydroxymethyl)-3-nitrobenzoate (8).** The corresponding acetoxymethyl derivative **7** (10.9 g, 37 mmol) was dissolved in $CHCl_3$ (150 mL), and hydrazine hydrate (4.7 g, 150 mmol) was added dropwise at 25 °C over 0.5 h. After an additional 2-h stirring at 25 °C, ethyl acetate (300 mL) was added and the organic phase was washed with saturated NaCl (3 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo to give a solid which was recrystallized from ethyl acetate–pentane by the method used for **6** and **7**. The title product (8.8 g, 94%) comprised pale orange needles: mp 68–70 °C; NMR ($CDCl_3$) δ 8.63 (d, J = 1.6 Hz, 1 H), 8.26 (dd, J = 1.7, 6.3 Hz, 1 H), 7.86 (d, J = 6.3 Hz), 5.05 (s, 2 H), 1.62 (s, 9 H); R_f (CA), 0.48; R_f (BPAW), 0.77.

Anal. Calcd for $C_{12}H_{15}NO_5$ (M_r 253.25): C, 56.91; H, 5.97; N, 5.53. Found: C, 56.79; H, 5.82; N, 5.42.

Protected Dipeptidyl-Resins (9, 10, and 11) for Experiments on Formation of Diketopiperazine 12 (Scheme II; Table I). By conventional solid-phase methodologies [deprotection with TFA–CH_2Cl_2 (3:7) (2 + 15 min); neutralization with DIEA–CH_2Cl_2 (1:19) (2 × 5 min); single coupling with DCC (3 equiv, 90 min)], Boc-leucine as an "internal reference" amino acid was coupled onto an aminomethyl–resin, followed by 4-(bromomethyl)-3-nitrobenzoic acid (**5**) introduced[12b] via its performed symmetrical anhydride. Next, the cesium salt of Boc-L-proline was prepared[32] and coupled (95% yield) onto this resin by reaction in *N*,*N*-dimethylformamide as the solvent at 50 °C for 14 h. After washings according to the standard procedures, the resultant Boc-Pro-ONb-Leu–resin was divided into three portions, and the appropriate N^{α}-protected derivatives of D-valine were each quantitatively incorporated by DCC-mediated couplings. Details on experiments to treat these resins with deblocking agents and study retention of chains on the resin are in the notes to Table I. The filtrates after treatments of resins 9 and **10** were evaporated to dryness and redissolved for reversed-phase HPLC analysis [CH_3OH–H_2O (1:4), 1.0 mL/min] to reveal a major peak (t_R 4.4 min) comigrating with an authentic standard of *cyclo*[D-Val-L-Pro] (**12**).

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Registry No. 1 (R = $CH(CH_3)_2$), 96965-23-0; **1** (R = $CH_2CH(CH_3)_2$), 70824-58-7; **1** (R = $CH_2C_6H_5$), 64724-53-4; **1** (R = H), 64724-51-2; **1** (R = CH_2-*p*-$C_6H_4OC(CH_3)_3$), 96998-89-9; **3a**, 96965-24-1; **3b**, 96965-25-2; **3c**, 96965-26-3; **4a**, 96965-27-4; **4b**, 96965-28-5; **4c**, 96965-29-6; **5**, 55715-03-2; **6**, 96965-30-9; **7**, 96965-31-0; **8**, 65276-91-7; **12**, 27483-18-7; Dts-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH, 96998-93-5; Dts-Tyr-Gly-Gly-Phe-Leu-OH, 96998-94-6; H-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-OH, 66912-84-3; Boc-D-Val-OH, 22838-58-0; Fmoc-D-Val-OH, 84624-17-9; Dts-D-Val-OH, 96965-36-5; leucine-enkephalin, 58822-25-6; Dts-glycylvaline, 70711-37-4; 4-(bromomethyl)benzoic acid, 6232-88-8; Boc-leucine, 13139-15-6; Boc-L-proline cesium salt, 42538-66-9; *p*-toluic acid, 99-94-5.

Supplementary Material Available: Table III (^{1}H NMR parameters of leucine-enkephalin and protected derivatives), Table IV (analytical and spectral data for Dts-aminoacyl-*o*-nitrobenzyl ester handle intermediates), and figures showing expanded NMR data (ref 19) and full FABMS spectra (ref 21) (12 pages). Ordering information is given on any current masthead page.

Disilathiiranes: Synthesis and Crystal Structure

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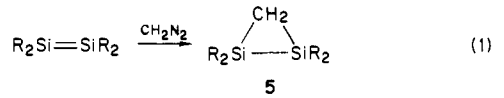
Abstract: Reaction of elemental sulfur with disilene **1** or **2** produces respectively disilathiirane 1,1,2,2-tetramesityl-1,2-disilathiirane (**3**) or 1,2-di-*tert*-butyl-1,2-dimesityl-1,2-disilathiirane (**4**). The crystal structure of **3** is reported; this molecule has a short Si–Si distance of 228.9 (2) pm and a nearly planar arrangement of C, C, and Si atoms around each silicon. These results are interpreted in terms of partial π bonding between the silicon atoms. The molecular geometry of **3** is compared with those of other three-membered-ring compounds containing two silicon atoms.

Several kinetically stable disilenes have now been synthesized,[1] and some of their chemical reactions have been investigated.[2] Disilenes readily undergo addition to the Si–Si π bond; for instance, tetramesityldisilene (**1**) reacts with certain alkynes and ketones to give 1,2-disilacyclobutenes and 1,2-disilaoxetones, respectively. Two addition reactions of disilenes to give three-membered-ring products have been published. Treatment of 1,1,2,2-tetrakis(2,6-dimethylphenyl)disilene with diazomethane yields disilacyclopropane **5**[3] (eq 1), and *trans*-1,2-di-*tert*-butyl-



(1)

5

R = 2,6-dimethylphenyl

1,2-dimesityldisilene (**2**) reacts with oxygen to give, among other products, disilaoxirane **6**[4] (eq 2). Two other three-membered rings containing two silicon atom are known, compounds **7** and **8**, synthesized by a different procedure.[5] In this paper we report

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