Selective Catalysis of Ester Aminolysis: An Approach to Peptide Active Esters

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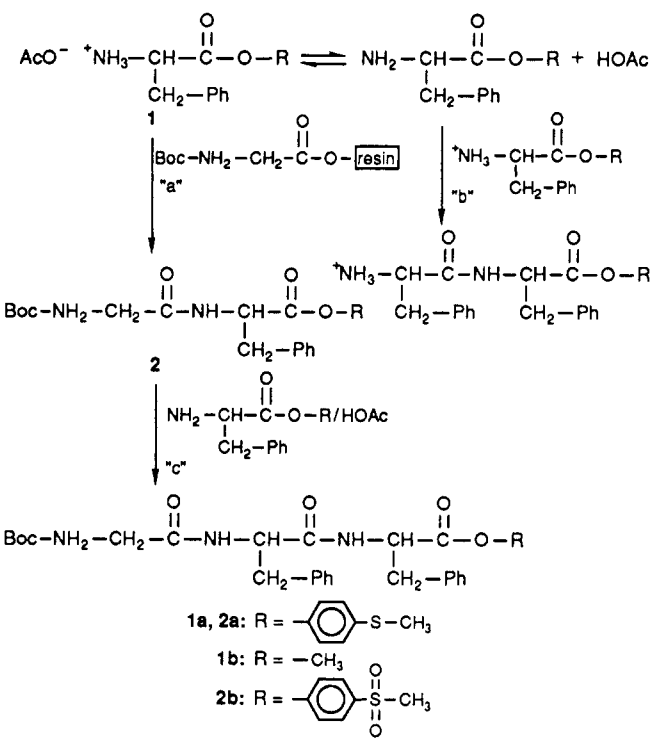
Model studies have been carried out in which the Kaiser–DeGrado procedure for solid-phase synthesis has been combined with the use of reactants with a latent activation feature as a potential route to peptide active esters. The rates of reaction of the 4-(methylthio)phenyl ester of L-phenylalanine (**1a**) with itself, as well as those of a close analogue with *tert*-butyloxycarbonylglycine bound to a benzophenone oxime functionalized polystyrene resin have been measured in the presence of varying concentrations of the aminolysis catalyst, acetic acid. These studies indicate that under appropriate conditions, it is possible to generate the 4-(methylthio)phenyl esters of suitable peptides, synthesized by solid-phase methods on the oxime-functionalized resin. These peptide esters can then be oxidized to the more strongly electrophilic sulfone derivatives. The preparations of the 4-(methylsulfonyl)phenyl esters of *N*-*tert*-butyloxycarbonylglycyl-L-phenylalanine and *N*-carbobenzyloxy-*O*-carbobenzyloxy-L-tyrosylglycylglycyl-L-phenylalanine are described as examples of the use of this method of preparing peptide active esters.

Introduction

Although the chemical synthesis of peptides is a field of study well-enough developed that the preparation of a variety of small- to medium-sized peptides is now routine, there remain a number of unsolved problems in this area, many of which would profit from the attention of organic chemists involved in basic research. Prominent among these difficulties is that of developing effective methodology for the coupling of medium- to large-sized peptide fragments.[1] One approach to this problem is embodied in new strategies for fragment condensation, such as Kemp's use of "prior thiol capture".[2] Another avenue of attack would involve the use of activated derivatives of peptide chains, such as peptide active esters.

The use of reactive esters of N-protected amino acids represents one of the popular classic modes for the formation of the amide bond in peptide synthesis.[3] In general, practical applications of this class of active acyl compounds have utilized carboxyl groups esterified with aryloxy moieties; the step-by-step use of such amino acid esters has been employed in the synthesis of a variety of peptides, including the octapeptide oxytocin[4] and a 27-residue long portion of porcine secretin.[5] Although such examples of the practical use of the active esters of simple protected amino acids are common, similar examples for the use of the analogous active esters of peptides are much more rare, despite the obvious utility of the latter materials in such important applications as fragment condensation procedures, protein semisynthesis, and peptide augmentation of non-protein materials. The basis for this limitation in the use of the active ester methodology lies in the usual requirement that such derivatives be generated from activated acyl forms of the parent carboxylic acid. In the case of the acylated C-terminal residues of peptides, such activation carries with it a severe risk of racemization; for the corresponding urethane-protected amino acids, loss of chiral integrity is much less likely to be a problem.[6] Hence, the direct preparation of peptide active esters is in general limited to those chains terminating in glycine or proline residues. A procedure for the preparation of peptide active esters which involved the acylation of only urethane-protected amino acid derivatives would thus provide a possible means of side-stepping this limitation. The "backing-off" procedure of Goodman[7] illustrates one approach to this problem; here we describe another, related strategy, based on a solid-phase method for the preparation of the bulk of the peptide chain.

Scheme I



In 1980, Kaiser and DeGrado reported the use of a benzophenone oxime modified polystyrene as a support for the solid-phase synthesis of peptides.[8] One intriguing aspect of their procedure is that it allows the use of C-protected amino acid nucleophiles (although generally not larger peptide nucleophiles) as reactants for the removal of the peptide chain from the solid support. As a result, this procedure permits a number of features unique among

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(6) Kemp, D. S. In *The Peptides: Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; pp 342–351.
(7) Goodman, M.; Steuben, K. C. *J. Am. Chem. Soc.* **1959**, *81*, 3980–3983.
(8) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295–1300.

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solid-phase methods, features which have not been widely exploited. Among the most important of these are the following: the Kaiser–DeGrado procedure allows one to introduce at the peptide C-terminus an amino acid residue containing functionality which would not ordinarily withstand the conditions of solid-phase procedures; and the peptide product ultimately liberated from the support remains fully protected, and thus amenable to a wide variety of chemical modifications. These characteristics provide the opportunity to utilize the solid-phase method for the preparation of peptide active esters.

Our general strategy for the synthesis of a peptide active ester utilizes an aryloxy ester of an amino acid as the reactant for cleaving a peptide from an oxime resin; the procedure is shown in Scheme I. As this scheme illustrates, cleavage of peptide from the resin support (pathway a) competes with a self-condensation reaction of the amino acid nucleophile (pathway b). While the latter reaction in and of itself is harmful only in that it acts to deplete the cleaving agent, it does serve to model a much more deleterious process, illustrated by pathway c, which would lead to the generation of troublesome error-peptides. Since the resin-cleavage reaction at the oxime ester linkage requires the use of a weak acid catalyst (typically acetic acid), it is the susceptibility of pathways a and b (or c) to acetic acid catalysis which ultimately determines the feasibility of this strategy. Accordingly, we have investigated the rate of a resin-cleavage reaction and the rate of the self-condensation reaction for the 4-(methylthio)phenyl ester of L-phenylalanine and have utilized the findings of these studies to prepare two simple peptide active esters.

Results

Kinetics of Model Reactions. Preliminary studies on the use of this strategy for the preparation of peptide active esters employed the *p*-nitrophenyl esters of several amino acids as the agents used to cleave peptides from an oxime resin; the appearance of the yellow color of the *p*-nitrophenoxide ion during the cleavage procedure led us to evaluate alternative active ester structures. In particular, the 4-(methylthio)phenyl esters initially studied by Johnson[9] provided several attractive properties: in the thioether form, they would be expected to be less reactive to self-condensation processes than the *p*-nitrophenyl esters, yet they could be oxidized to the very reactive sulfone analogues to obtain derivatives of substantial acylating ability. Although peptide 4-(methylsulfonyl)phenyl active esters have not been widely used in peptide synthesis, they have found practical application in a procedure for the polymerization of peptides,[10] and in the preparation of a family of cyclic peptides.[11]

The specific reaction scheme we focused on was the cleavage by the 4-(methylthio)phenyl ester of L-phenylalanine (**1a**) of an N-protected glycine residue esterified to an oxime resin, with acetic acid present as a catalyst. The relative importance of the self-condensation process (pathway b in Scheme I) was assessed by studying the rate of disappearance of the phenylalanine ester under the conditions of the reaction, in the absence of the resin electrophile, with varying amounts of catalyst. The concentration of **1a** was followed by HPLC and was quantified by comparison to an internal standard. A plot of $1/[\mathbf{1a}]$ vs time gave a straight line from which the rate constants and half-lives shown in Table I could be extracted.

Table I. Reaction Rates for the Self-Condensation Reaction of Ester 1a

solvent	HOAc, equiv	k,[a] M^{-1} min^{-1}	half-life, min
CH_2Cl_2	0	3.8×10^{-3}	2.6×10^3
CH_2Cl_2	1.0	2.0×10^{-2}	5.0×10^2
CH_2Cl_2	2.0	4.0×10^{-2}	2.5×10^2
CH_2Cl_2	4.0	3.8×10^{-2}	2.6×10^2
CH_3CN	0	6.3×10^{-3}	1.6×10^3
CH_3CN	1.0	2.0×10^{-2}	5.0×10^2
CH_3CN	2.0	4.3×10^{-2}	2.3×10^2
CH_3CN	4.0	4.5×10^{-2}	2.2×10^2

[a] Reaction at 19 °C, with 0.10 M **1a**.

Table II. Reaction Rates for the Reaction of 1b with Resin-Bound Boc-Glycine

concn of **1b**, M	HOAc, equiv	half-life,[a] min
0.060	0	1.2×10^3
0.060	1.0	5.0
0.060	2.0	5.0
0.060	4.0	4.0
0.10	1.0	3.5
0.10	1.5	3.0
0.10	2.0	4.0
0.10	4.0	7.0

[a] Ester **1b** present in 100% excess relative to equivalents of Boc-glycine on resin; reaction at 19 °C in CH_2Cl_2.

In order to approximate the rate of the resin cleavage reaction (pathway a in Scheme I), we carried out model studies with the methyl ester of L-phenylalanine (**1b**) as a way to avoid any complications due to concomitant self-condensation processes. We thus assumed that any differences between the nucleophilicity of the amino group of the methyl ester as compared to the 4-(methylthio)-phenyl ester would be small. Accordingly, samples of an oxime-modified polystyrene resin which had been esterified with a known amount of *N-tert*-butyloxycarbonylglycine were reacted with **1b** in the presence of an internal standard as well as varying amounts of acetic acid catalyst. The amounts of Boc-glycyl-L-phenylalanine methyl ester present at any point in time were determined by removal of aliquots from the heterogenous reaction mixture and analysis by HPLC. In this case, the rate of product formation followed no simple kinetic scheme; the half-lives shown in Table II were obtained by examination of a plot of concentration of dipeptide formed vs time and assessment of the period of time needed to reach half the amount of maximum conversion. The values thus obtained are considerably cruder than those obtained for the self-condensation reaction, and the total conversion to product dipeptide determined by this method typically varied between 80% and 110% of that expected. Nonetheless, these data clearly indicate that resin-cleavage is exceedingly sluggish in the absence of catalyst (a requirement for the use of this linkage in the solid-phase method) and that acetic acid has a proportionally much greater effect on the rate of the resin-cleavage aminolysis than it does on that of the self-condensation process. Furthermore, the difference in rate is large enough that the efficient generation of peptide ester, uncontaminated by significant amounts of error-peptide, seemed reasonable.

Besides providing quantitative evidence that acetic acid is a much more effective catalysis for aminolyses of oxime esters as compared to aryloxy esters, the data in Tables I and II suggested optimal reaction conditions for the use of this strategy for the preparation of peptide active esters. In particular, these data indicated that we should be able to observe the maximum preference for resin-cleavage by carrying out reactions with as high a concentration of amino acid nucleophile as practical, with 1–2 equiv of

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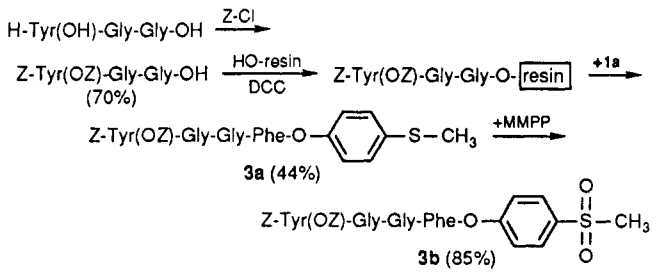
Scheme II



acetic acid catalyst, and with as short a reaction time as possible.

Preparation of Peptide Active Esters. The preparation of dipeptide active ester **2b** was demonstrated on a practical scale by carrying out the resin-cleavage along the lines suggested by the studies described above. The cleavage reaction used to generate precursor **2a** was performed with dichloromethane as the solvent, since it is especially effective at swelling the resin matrix and since the self-condensation appeared to be modestly impeded in less polar solvents. The cleavage reaction was carried out with 0.06 M **1a**, in 25% excess relative to resin-bound glycine, in the presence of 1.4 equiv of acetic acid, over a time period of 18 min. Extractive workup, followed by purification by flash column chromatography, gave **2a** in 64% yield as a white solid; analysis by ^{1}H NMR spectroscopy at 300 MHz confirmed the identity of the expected product, with no indication of the presence of any error tripeptide.

Oxidation of **2a** was carried out under conditions of phase-transfer catalysis using the magnesium salt of monoperoxyphthalic acid. This procedure gave crude **2b** as a thick oil in 91% yield; this material could be readily recrystallized to provide a solid whose structure was confirmed by ^{1}H NMR spectroscopy at 300 MHz. This material was an effective acylating agent: reaction of the ester with an excess of the 4-nitroanilide of L-leucine in DMF gave the expected tripeptide anilide in 82% yield.

In an additional illustration of the use of this strategy, the tetrapeptide active ester **3b** was prepared by the sequence illustrated in Scheme II. In this example, the tripeptide electrophile was attached to the resin support by direct coupling rather than stepwise synthesis for reasons of expediency. The structures of the tetrapeptide 4-(methylthio)phenyl ester **3a**, as well as the corresponding 4-(methylsulfonyl)phenyl ester **3b**, were established by 2D-COSY analysis.

Discussion

The results of these initial investigations suggest that the Kaiser–DeGrado procedure for solid-phase peptide synthesis can be exploited to yield peptide active esters, although the strategy does have a number of important limitations. It has been established that the extent of β-branching at the amino acid side chain is the key steric feature which determines the relative reaction rates in acyl substitutions involving activated acyl derivatives such as *p*-nitrophenyl esters.[12] Thus, it is likely that the resin-cleavage procedure described here will occur more slowly for peptides attached to the support at the amino acids more hindered than glycine and that self-condensation (and the generation of error peptides) will be more of a problem with the use of cleaving esters of amino acids less hindered than phenylalanine. The use of catalytic additives other than acetic acid which show a greater differential effect in the acceleration of the resin-cleavage reaction may help alleviate this problem.[13]

A second difficulty limiting the generality of this procedure involves the requirement of an oxidation to activate the (methylthio)phenyl ester to its sulfone form, which precludes the use of cysteine, methionine, or tryptophan residues as components of any peptide active esters so generated. In this context, the development of new active ester systems incorporating latent activation would be of considerable value.

In addition, the chiral integrity of the C-terminal residue of these active esters has not been established; however, the individual operations which make up the procedure—preparation of the (methylthio)phenyl ester, peptide cleavage from an oxime resin by the acetate salt of an amino acid ester, and oxidation of the (methylthio)phenyl ester—have all been reported to be free of problems of racemization.[8,9] Moreover, in preliminary studies, the cleavage of a hexapeptide from an oxime resin by use of the *p*-nitrophenyl ester of L-isoleucine gave a product which was hydrolyzed and then submitted for amino acid analysis; less than 1% of allo-isoleucine was detected in the hydrolysis mixture.

In summary, these investigations indicate that the Kaiser–DeGrado strategy for solid-phase peptide synthesis can be used as a basis for preparing the active esters of peptides, albeit with several limitations. Nevertheless, this strategy is amenable to improvement in both the step leading to the liberation of the peptide ester and the activation of that ester; as such, it may provide a first step to the design of a completely general scheme for the convenient preparation of peptide active esters by use of the solid-phase method.

Experimental Section

General Methods. ^{1}H NMR spectra were obtained at 80 MHz using a Varian FT-80A spectrometer and at 300 MHz on a Varian VXR-300S; chemical shifts are reported in ppm relative to TMS. Thin-layer chromatography was performed using silica gel sheets with fluorescent indicator (Kodak Chromagram). Flash column chromatography was carried out with Merck silica gel grade 60. Diisopropylethylamine (DIEA, Aldrich) was distilled from ninhydrin prior to use. Chromatographic solvents used included acetonitrile (A), ethyl acetate (EA), petroleum ether (PE), and water (W). Dichloromethane was purified by distillation from P_2O_5; other solvents were HPLC grade and were used without further purification. The *p*-nitrobenzophenone oxime resin used was prepared by the modified procedure described by Kaiser and DeGrado.[14] Elemental analyses were performed by Midwest Microlabs.

Kinetics Procedures. Self-condensation reactions were studied using a Varian 5000 liquid chromatograph equipped with a 250 mm × 4.6 mm Alltech column packed with 10 μm Econosil C18. The solvent program used was: isocratic at 20% A/80% W, 0–4 min; linear gradient to 30% A/70% W, 4–6 min; isocratic at 100% A, 6–15 min; flow rate, 1.0 mL/min. Benzamide, with a retention time of 6.2 min, was used as the internal standard; under those conditions, 4-methylmercaptophenol (MMP) eluted at 10.9 min and L-phenylalanine-4-(methylthio)phenyl ester (H-Phe-OMMP) at 12.7 min. The UV detector was set at 265 nm when dichloromethane was used as the reaction solvent, and 254 for reactions in acetonitrile. In a typical reaction, 36.8 mg (0.100 mmol) of H-Phe-OMMP·HBr and 20.0 mg of benzamide were placed in a 1.0-mL volumetric test tube. Most of the volume of solvent required was added, and the suspension was sonicated

(12) Kemp, D. S.; Choong, S. H.; Pekaar, J. *J. Org. Chem.* **1974**, *39*, 3841–3847.

(13) For example, 2-hydroxypyridine seems to be substantially less effective than acetic acid in catalyzing the self-condensation procedure, but still reasonably active in the catalysis of the resin-cleavage: Trzupek, L. S.; Ervin, C. E.; Rhodes, A. L., unpublished.

(14) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1982**, *47*, 3258–3261.

to effect as complete dissolution as possible. The appropriate volume of acetic acid was added by microliter syringe, followed by 18 μL of DIEA (0.100 mmol) to start the reaction. The vessel was diluted to the mark with solvent and shaken briefly. Aliquots of ca. 5 μL were withdrawn at regular intervals and were diluted 1:100 with 0.023 M DIEA before analysis. These reactions were carried out at ambient temperature, 19 ± 1 °C.

Kinetics of the resin-cleavage reaction were determined with a Hewlett-Packard 1060 liquid chromatograph, equipped with a 60 mm × 4.6 mm H-P 3 μm Hypersil reversed-phase column. The solvent program used was: isocratic at 35% A/65% W, 0–2.5 min; linear gradient to 50% A/50% W, 2.5–2.6 min; linear gradient to 60% A/40% W, 2.6–5.5 min; isocratic at 60% A/40% W, 5.5–7.5 min; flow rate, 1.0 mL/min; and detector wavelength, 254 nm. Under these conditions, the internal standard, toluene, had a retention time of 7.2 min; L-phenylalanine methyl ester (H-Phe-OMe), 2.4 min; and *tert*-butyloxycarbonylglycyl-L-phenylalanine methyl ester (Boc-Gly-Phe-OMe), 5.5 min. Reactions were carried out in 1.0-mL volumetric tubes essentially as described above, except that the heterogeneous mixture in this case was stirred vigorously with a Teflon-coated magnetic flea. Typical amounts of reactants used were: H-Phe-OMe·HCl, 21.6 mg, 0.10 mmol; Boc-Gly resin, 99.0 mg, 0.49 mequiv/g loading, 0.049 mmol; DIEA, 18 μL, 0.103 mmol. Acetic acid was added in amounts appropriate to the run under study. For rapid reactions, aliquots were withdrawn every 0.5–1.0 min, diluted 1:100 with dichloromethane, and analyzed as soon as possible.

Boc-Glycyl Ester of the *p*-Nitrobenzophenone Oxime Resin (Boc-Gly-Resin). In a typical procedure, a suspension of 3.00 g of oxime resin was treated with a solution of 263 mg (1.50 mmol) of Boc-glycine (Sigma) in 32 mL of CH_2Cl_2. To the cooled (0 °C) suspension was added a solution of 310 mg of dicyclohexylcarbodiimide (DCC) in 5 mL of CH_2Cl_2. The reaction vessel was shaken mechanically for 18 h, and the reaction mixture was filtered and washed with 100 mL of CH_2Cl_2, 100 mL of DMF, 100 mL of i-PrOH, and 100 mL of CH_2Cl_2. After drying in a vacuum, a weighed portion was analyzed to determine the extent of functionalization: the glycine Boc group was removed by reaction with excess trifluoroacetic acid; the washed and neutralized free-amino-containing resin was treated with a solution of picric acid; and the picrate was eluted from the washed resin with a solution of DIEA in CH_2Cl_2. The amount of the picrate ion liberated was determined by spectrophotometric analysis at 358 nm.

L-Phenylalanine 4-(Methylthio)phenyl Ester Hydrobromide (H-Phe-OMMP·HBr). To a cooled (0 °C) solution of 0.70 g (5.0 mmol) of 4-(methylthio)phenol (Fluka) and 1.65 g (5.0 mmol) of *N*-carbobenzyloxy-L-phenylalanine (Aldrich) in 9 mL of CH_2Cl_2 was added 1.13 g (5.5 mmol) of DCC. After being stirred for 15 h, the mixture was filtered, and the filtrate extracted twice with 5% $NaHCO_3$ and once with saturated brine. The dried (Na_2SO_4) organic layer was concentrated to give 2.83 g of a white solid, which was recrystallized from 10–12 mL of boiling i-PrOH. Two crops of solid gave a total of 1.48 g (3.50 mmol, 70%) with mp 114.5–116 °C: IR (KBr) 3320, 1750, and 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 2.45 (s, 3 H), 3.23 (d, 2 H, J = 6 Hz), 4.6–4.9 (m, 2 H), 5.11 (s, 2 H), 5.27 (d, 2 H), 6.7–7.3 (m, 14 H).

Anal. Calcd for $C_{24}H_{23}NO_4S$: C, 68.38; H, 5.51; S, 7.61. Found: C, 68.10; H, 5.44; S, 7.54.

A 1-mL suspension in acetic acid of 0.422 g (1.00 mmol) of the Z-Phe-OMMP described above was prepared in a 10-mL flask equipped with a Teflon-coated magnetic stirring bar and covered with a septum stopper. To the stirred suspension was added by syringe 1.75 mL of 30% hydrogen bromide in acetic acid (Aldrich). The mixture was allowed to stir for 30 min, with occasional venting by syringe needle. The resulting tan material was diluted with ca. 10 mL of dry ether, and the mixture was added slowly to a 250-mL flask containing ca. 100 mL of stirring ether. After a few minutes, the resulting white solid was allowed to settle, and the orange supernatant was drawn off. The solid remaining was stirred up with similar amounts of dry ether three more times, and after drawing off the final batch of supernatant, it was dried in a slow stream of nitrogen to give 0.364 g (0.99 mmol, 99%) of H-Phe-OMMP·HBr; IR (KBr) 2900, 1770, 1200 cm^{-1}.

***N*-*tert*-Butyloxycarbonylglycyl-L-phenylalanine 4-(Methylthio)phenyl Ester (2a).** In a 25-mL round-bottomed flask equipped with a stirring bar were placed 1.00 g of Boc-Gly-Resin (0.382 mequiv Boc-Gly/g, 0.382 mmol), a solution of 0.176 g of H-Phe-OMMP·HBr (0.478 mmol, 25% excess) in 10 mL of CH_2Cl_2, 31 μL of acetic acid (32.8 mg, 0.541 mmol, 40% excess), and 85 μL of DIEA (63.1 mg, 0.488 mmol), in that order. The reaction mixture was vigorously stirred for 18 min after the addition of the DIEA, and then filtered through a sintered-glass funnel into a flask containing 60 mL of CH_2Cl_2. The filter cake was quickly washed with 3 mL of DMF, 3 mL of methanol, and 3 mL of CH_2Cl_2. The combined filtrates were immediately washed with two 50-mL portions of cold 5% citric acid and two 50-mL portions of brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated to give 134 mg of crude product. This material was applied to a 45 mm × 36 mm flash column and eluted with four 50-mL portions of 20% EA in PE, and 12 65-mL portions 25% EA in PE. Fractions 7–14 contained material with R_f = 0.15 by TLC (elution solvent: 20% EA in PE); these fractions were combined and concentrated to yield 114 mg of **2a** (0.246 mmol, 64%). Recrystallization from ethyl acetate/hexane gave an analytical sample with mp 87–89 °C: NMR ($CDCl_3$, 300 MHz) δ 1.35 (s, 9 H), 2.36 (s, 3 H), 3.14 (d, J = 6.3 Hz, 2 H), 3.71 (m, 2 H), 4.98 (dt, J = 7.7, 6.3 Hz, 1 H), 5.20 (t, J = 5.4 Hz, 1 H), 6.77 (d, J = 7.8 Hz, 1 H), 6.81 (d, J = 8.8 Hz, 2 H), 7.11–7.27 (m, with d, J = 8.8 Hz,7 H); exact mass (FAB/CI) calculated for $C_{23}H_{28}N_2O_5S$ + H^+ 445.1797, found 445.1801.

***N*-*tert*-Butyloxycarbonylglycyl-L-phenylalanine 4-(Methylsulfonyl)phenyl Ester (2b).** A 55-mg (0.124-mmol) portion of **2a** was oxidized with the magnesium salt of monoperoxyphthalic acid (MMPP; Aldrich) under conditions of phase-transfer catalysis using the general procedure described by Brougham et al.[15] The dipeptide and ca. 2 mg of methyltricaprylammonium chloride were dissolved in 3.25 mL of chloroform and treated with a solution of 400 mg of 80% MMPP (0.647 mmol of active reagent, 5.2-fold excess) in 4 mL of water. The two-phase system was stirred vigorously for 6 h; the layers were separated, and the chloroform layer was washed with four 3-mL portions of water. Drying (Na_2SO_4) and concentration using a rotary evaporator gave a crude solid which was treated with 4 mL of CH_2Cl_2 and sonicated. The undissolved solid remaining was removed by filtration; the filtrate was concentrated to 53.6 mg (0.112 mmol, 91%) of a thick oil, which could be crystallized using ethyl acetate/hexane to give 26.2 mg of a white solid: mp 144–145 °C; NMR ($CDCl_3$, 300 MHz) δ 1.36 (s, 9 H), 2.97 (s, 3 H), 3.18 (dd, 2 H), 3.75 (m, 2 H), 4.97 (dt, J = 7.2, 6.5 Hz, 1 H), 5.18 (t, J = 5.8 Hz, 1 H), 6.84 (d, J = 6.5 Hz, 1 H), 7.07 (d, J = 8.7 Hz, 2 H), 7.12–7.30 (m, 5 H), 7.86 (d, J = 8.7 Hz, 2 H).

Anal. Calcd for $C_{23}H_{28}N_2O_7S$: C, 57.96; H, 5.93; N, 5.86. Found: C, 57.87; H, 5.94; N, 5.79.

***N*-*tert*-Butyloxycarbonylglycyl-L-phenylalanyl-L-leucine *p*-Nitroanilide.** A solution of 64 mg (0.256 mmol) of L-leucine *p*-nitroanilide (Sigma) and 100 mg of **2b** (0.210 mmol) in 1 mL of dimethylformamide (DMF) was stirred for 16 h. The DMF was removed on a vacuum line, and the residue was recrystallized from ethyl acetate/hexane to yield 95.3 mg (0.172 mmol, 82%) of the tripeptide *p*-nitroanilide; mp 205–206 °C; NMR (DMSO-d_6, 300 MHz) δ 0.87 (dd, 6 H), 1.31 (s, 9 H), 1.56 (m, 3 H), 2.78 (dd, 1 H), 2.99 (dd, 1 H), 3.46 (d, 2 H), 4.42 (m, 1 H), 4.52 (m, 1 H), 6.97 (t, 1 H), 7.08–7.19 (m, 5 H), 7.86 (d, 2 H), 7.94 (d, 1 H), 8.21 (d, 2 H), 8.33 (d, 1 H), 10.52 (s, 1 H).

Anal. Calcd for $C_{28}H_{37}N_5O_7·H_2O$: C, 58.62; H, 6.87; N, 12.20. Found: C, 58.45; H, 6.49; N, 11.76.

***N*-Carbobenzyloxy-*O*-carbobenzyloxy-L-tyrosylglycylglycine.** The procedure used for the preparation of this compound was modeled on that described by Katchalsky for the analogous protection of tyrosine.[16] A 1.00-g (3.39-mmol) portion of L-tyrosylglycylglycine (Sigma) was suspended in a mixture of 9 mL of water and 1.8 mL of dioxane and treated with 4 N NaOH until the pH of the solution reached 10. The stirred mixture was cooled in an ice bath and alternately treated with 0.10-mL portions of benzyl chloroformate (ca. 2.0 mL total) and enough 4 N NaOH to keep the pH between 9 and 11. After addition was complete, the solution was acidified to pH 4.5 with 4 N HCl. The solid

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remaining at this point was mixed with 30 mL of ethyl acetate, and the resulting suspension was extracted with three 25-mL portions of 1 N HCl and one 25-mL portion of brine. The dried ($MgSO_4$) organic layer was concentrated using a rotary evaporator, the material remaining was once again taken up in ethyl acetate, extracted thrice with 1 N HCl, dried, concentrated, and recrystallized (isopropyl alcohol) to give 1.33 g (2.36 mmol, 70%) of a white solid: IR (KBr) 3300, 1760, 1700, 1650, 1240 cm^{-1}; NMR (DMSO-d_6, 300 MGz) δ 2.77 (dd, J = 10.9, 13.8 Hz, 1 H), 3.06 (dd, J = 3.8, 13.8 Hz, 1 H), 3.78 (d, J = 5.9, 4 H), 4.30 (m, 1 H), 4.94 (s, 2 H), 5.26 (s, 2 H), 7.12–7.46 (m, 14 H), 7.58 (d, J = 8.5 Hz, 1 H), 8.14 (t, J = 5.9 Hz, 1 H), 8.37 (t, J = 5.7 Hz, 1 H), 12.59 (s, 1 H).

Anal. Calcd for $C_{29}H_{29}N_3O_9 \cdot 1.5H_2O$: C, 58.97; H, 5.47; N, 7.11. Found: C, 59.35; H, 5.29; N, 7.05.

This material, as a solution in 2 mL of DMF, was loaded onto 2.00 g of oxime resin using 0.281 g (1.36 mmol) of DCC in 20 mL of CH_2Cl_2, by a procedure analogous to that described for the Boc-Gly-Resin above.

***N*-Carbobenzyloxy-*O*-carbobenzyloxy-L-tyrosylglycyl-glycyl-L-phenylalanine 4-(Methylthio)phenyl Ester (3a).** A suspension of 1.00 g of the (N-Z,O-Z)-Tyr-Gly-Gly-Resin described above (ca. 0.50 mequiv) and 0.208 g of H-Phe-OMMP·HBr (0.56 mmol) in 9 mL of CH_2Cl_2 was stirred and treated with 35 μL of acetic acid (0.62 mmol) and 100 μL of DIEA. After 10 min, the reaction mixture was filtered through a medium-porosity sinter into 60 mL of CH_2Cl_2, and the resin mass was washed with 5-mL portions of DMF, methanol, and CH_2Cl_2. The combined filtrate was washed twice with 50-mL portions of 1 N HCl and once with a 50-mL portion of brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated to yield 0.160 g (0.233 mmol, 44%) of a slightly yellow solid which could be recrystallized from isopropyl alcohol: NMR (DMSO-d_6, 300 MHz) δ 2.75 (dd, 1 H), 3.05 (dd, 1 H), 3.02 (m, 2 H), 3.33 (s, 3 H), 3.99 (d, 4 H), 4.28 (m, 1 H), 4.62 (m, 1 H), 4.94 (s, 2 H), 5.26 (s, 2 H), 6.87 (d, 2 H), 7.12 (d, 2 H), 7.18–7.48 (m, with d, 17 H), 7.42 (d, 2 H), 7.58 (d, 1 H), 8.11 (t, 1 H), 8.37 (t, 1 H), 8.56 (d, 1 H); the assumed structure of the product was consistent with the 2D-COSY spectrum of this material.

Anal. Calcd for $C_{45}H_{44}N_4O_{10}S$: C, 64.88; H, 5.34; N, 6.72. Found: C, 64.95; H, 5.42; N, 6.68.

***N*-Carbobenzyloxy-*O*-carbobenzyloxy-L-tyrosylglycyl-glycyl-L-phenylalanine 4-(Methylsulfonyl)phenyl Ester (3b).** A 45-mg (0.056-mmol) portion of **3a** was taken up in 1.0 mL of chloroform and mixed with ca. 1 mg of methyltricaprylammonium chloride. This material was mixed with a solution of 200 mg of 80% MMPP (0.32 mmol active reagent, 5.7-fold excess) in 2 mL of water, and the resulting two-phase system was stirred vigorously for 6 h. The organic layer was separated and extracted with three 2-mL portions of 5% $NaHCO_3$, two 2-mL portions of water, and one 2-mL portion of brine. After drying ($Na_2SO_4/MgSO_4$), the chloroform solution was concentrated using a rotary evaporator to give 41 mg (0.047 mmol, 85%) of a white solid. Recrystallization from ethyl acetate/hexane gave 18 mg of a crystalline material; NMR ($CDCl_3$, 300 MHz) δ 2.90 (s, 3 H), 2.86–3.04 (m, 2? H), 3.13 (dd, 2 H), 3.66–3.91 (m, 4 H), 4.30 (q, 1 H), 4.85–4.96 (m, 3 H), 5.14 (s, 2 H), 5.58 (d, 1 H), 6.96–7.07 (m, with d, J = 8.8 Hz, 6 H), 7.13–7.35 (m, 15? H), 7.78 (d, J = 8.7 Hz, 2 H), 7.99 (t, 1 H).

Anal. Calcd for $C_{45}H_{44}N_4O_{12}S$: C, 62.48; H, 5.14; N, 6.47. Found: C, 62.92; H, 5.33; N, 6.50.

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N-(9-Phenylfluoren-9-yl)-α-amino Ketones and *N*-(9-Phenylfluoren-9-yl)-α-amino Aldehydes as Chiral Educts for the Synthesis of Optically Pure 4-Alkyl-3-hydroxy-2-amino Acids. Synthesis of the C-9 Amino Acid MeBmt Present in Cyclosporin

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Serine-derived *N*-(9-phenylfluoren-9-yl)-α-amino ketones were prepared by acylation of primary organometallic reagents with amino acid isoxazolidides. When the amino and hydroxyl functions of serine were constrained in oxazolidine and oxazolidinone rings, alkylation of these ketones as their lithium enolates proceeded regiospecifically with good to excellent diastereoselectivity. Reduction of the oxazolidine and oxazolidinone ketones diastereoselectively led to N-protected 4-alkyl-branched 2-amino 1,3-diols that were subsequently oxidized in two steps, via the *N*-(9-phenylfluoren-9-yl)-α-amino aldehyde, to produce 4-alkyl-β-hydroxy-α-amino acids. In this way, L-(+)-MeBmt (1), the C-9 amino acid of cyclosporin, and its D-(−) enantiomer were prepared in 12 steps from D- and L-serine, respectively, with 22% overall yield and >99% enantiomeric purity. *N*-(9-Phenylfluoren-9-yl)-MeBmt triple-bond and 6*Z* double-bond analogues **37** and **39** were also prepared. This synthetic route requires only a single chiral source (serine) and provides for configurational choice and control at all four diastereomeric centers.

Introduction

(2*S*,3*R*,4*R*,6*E*)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoic acid (MeBmt (1)) is the unique C-9-amino acid constituent of the immunosuppressive drug cyclosporin.[1] Structurally, **1** is related to (2*S*,3*S*,4*S*)-3-hydroxy-4-methylproline (Hmp), which is found in the antifungal lipopeptide echinocandin D.[2] MeBmt may be classified among both β-hydroxy-α-amino acids[3] and γ-alkyl-

(1) MeBmt is the IUPAC/IUB three-letter notation for (4*R*)-4-((*E*)-2-butenyl)-4,*N*-dimethyl-L-threonine.

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